E1

Section 6 Report Review

Attachment to letter dated ______ 24 May 2002 ______

Project: Monitoring Effects of a Renovation Project on Endangered Fish and Invertebrates in Diamond Y Draw

Final or interim report? FINAL

Job #: WER 38

Reviewer's Station: Austin ES

Lead station was contacted and concurs with the following comments:
___ Yes ___ No _X_ Not applicable (reviewer is from lead station)

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Report: _X_ is acceptable as is

____ is acceptable as is for an interim report, but the following comments are made for future reference

____ needs revision (listed below)

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Comments: (Note to commenter: If you make comments directly on a copy of the report, write legibly and dark so comments will reproduce well when photocopied.)

The authors have completed a comprehensive monitoring study to allow for an important restoration project for Leon Springs pupfish at Diamond Y Draw. The report is well written and documents that all of the project objectives were fully accomplished. In large part due to the work of this project, Leon Springs pupfish is now occurring as a wild population again. We encourage the author's to publish as much of this report as possible in peer-reviewed scientific journals as an important example of practical applications of conservation biology.

**FINAL REPORT**

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. E-1-13

Endangered and Threatened Species Conservation

# Project WER38: Monitoring Effects Of A Renovation Project On Endangered Fish And Invertebrates In Diamond Y Draw

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TEXAS PARKS & WILDLIFE

John Herron
Program Director, Wildlife Diversity

Robert Cook
Executive Director

March 31st, 2002

# FINAL REPORT

**STATE:** Texas **GRANT NO:** E – 1 – 13

**PROGRAM TITLE:** Endangered and Threatened Species Conservation

**PERIOD COVERED:** September 1, 1997 – August 31, 2001

**PROJECT NUMBER:** WER38

**PROJECT TITLE:** Monitoring Effects Of A Renovation Project On Endangered Fish And Invertebrates In Diamond Y Draw

**SEGMENT COST:**
**TOTAL –** $20,000
**FEDERAL SHARE -** $13,442.64

**PROJECT OBJECTIVES:**

1. To obtain the following baseline information:
   a) Genetic structure of the Diamond Y Draw population of Pecos gambusia.
   b) Distribution of the known endemic taxa, including invertebrates, in the two watercourses and their associated springs in Diamond Y Draw.
2. To monitor effects of renovation on:
   a) Genetic structure and morphological characteristics of the restored pupfish population in Diamond Y Draw.
   b) Genetic structure and status of the Diamond Y Draw population of Pecos gambusia.
   c) Status of the known endemic invertebrates.
3. To monitor surface waterflow, to document seasonal changes and episodic flash flood events that sometimes link the two watercourses supporting Leon Springs pupfish.
4. To incorporate knowledge from objectives 1 and 2 into an adaptive management strategy for Diamond Y Draw.

**PREPARED BY:** A. A. Echelle 12/15/01

**APPROVED BY:** Neil E. Carter 03/21/02
Federal Aid Coordinator Date

## FINAL REPORT

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|---|---|
| STATE: | Texas (Contract #388-0622) |
| PROJECT NUMBER: | E-1-13; Code WER38 |
| PROJECT TITLE: | Monitoring effects of a renovation project on endangered fish and invertebrates in Diamond Y Draw |
| DATE: | 12 December 2001 |
| PERIOD COVERED: | 1 September 1997 to 31 August 2001 |


EXECUTIVE SUMMARY:

Diamond Y Draw, a flood tributary of the Pecos River area of Trans-Pecos Texas, contains two short, (< 4 km) spring- and seepage-fed watercourses ("upper" and "lower" watercourses) separated by approximately 3 km of dry land. This is an area of special concern to conservationists because of the presence of two federally listed, endangered fishes, Leon Springs pupfish (*Cyprinodon bovinus*) and Pecos gambusia (*Gambusia nobilis*) and several geographically restricted invertebrate species, including two endemic springsnails (*Tryonia adamantina* and *T. circumstriata*), and an endemic amphipod (*Gammarus pecos*).

In the early 1990s it appeared that the entire wild population of the endangered Leon Springs pupfish (*Cyprinodon bovinus*) had been genetically introgressed by a non-native pupfish (*C. variegatus*). This led to efforts to restore the genetic structure of the pupfish by using genetically pure captive stock that had been held in captivity at Dexter National Fish Hatchery and Technology Center (DNFH) since 1976. This report describes the restoration efforts and assesses effects on the genetic structure of *C. bovinus* (assessed with allozymes and mtDNA) and, for an area treated with ichthyocide (Antimycin A), effects on fish and invertebrate abundances and the genetic structure of *G. nobilis* (allozymes). We also describe the distributions of the native fishes and invertebrates of concern and we present a qualitative description of observed changes in surface water in Diamond Y Draw during our survey from 1997 to 2001. Finally, the report discusses various other factors affecting the long-term status of the fauna, and it provides management recommendations.

Two approaches were used in the restoration efforts. The Diamond Y Spring headpool and approximately 500 m of its outflow channel were treated with the ichthyotoxin to eliminate all pupfish from this section of water and this was followed by releases of pupfish from a captive stock of genetically pure *C. bovinus* that has been maintained at Dexter National Fish Hatchery and Technology Center (DNFH) since 1976. In most other areas supporting pupfish, restoration involved physically removing all pupfish encountered by seining and dipnetting and then releasing large numbers of DNFH pupfish in an attempt to dilute the non-native genetic material.

The efforts to deplete the non-native genetic material were generally successful. In pre-renovation samples, the average frequency of non-native markers across 6 diagnostic marker systems (5 allozyme loci and mtDNA) ranged from 3.6% to 20%, except in one extremely small population where no non-native markers were detected. Post-renovation frequencies of these markers were 0.0% in the Diamond Y Spring headpool and its outflow channel and 0.042 in a small population ("observation platform" population). In post-renovation samples from the lower watercourse, non-native markers were either absent or they occurred at <1%, except in one area where the frequency was 2.9%. For various reasons, we recommend additional releases of the captive DNFH stock into the observation platform population of the upper watercourse and no further releases in the lower watercourse.

One purpose of treating Diamond Y Spring and its outflow with Antimycin was to eliminate two non-native fishes, largespring gambusia (*G. geiseri*) and common carp (*Cyprinus carpio*) from Diamond Y Draw, and this attempt was successful. Possibly because of the removal of carp, pupfish appear considerably more abundant in the headpool of Diamond Y Spring than they were in the past. This coincided with the disappearance of dense growths of filamentous algae and pond weed, an effect that increased the amount of open, shallow-water habitat preferred by the pupfish. Prior to their elimination, the carp may have maintained the vegetation as a result of their feeding activity, which is known to pump nutrients from the sediments back into the water column.

There was an almost complete kill of the amphipods (*Gammarus pecos* and *Hyallela azteca*) in the area treated with Antimycin. However, these species are abundant elsewhere in the watercourse and they returned to pre-renovation abundances within 7 months.

Large numbers of *G. nobilis* and the rainwater killifish (*Lucania parva*) were removed prior to treatment and returned afterwards. As a result, there was no detectable direct effect on them. However, post-renovation observations indicate that *G. nobilis* and pupfish may be less abundant in the outflow channel of Diamond Y Spring than they were prior to renovation. We have no explanation for this, or for a persistently low post-renovation abundance of one of the snail *Tryonia circumstriata* in this outflow channel. As reported in this report, however, *T. circumstriata* apparently is not native to this part of Diamond Y Draw; it is endemic to Euphrasia Spring of the lower watercourse, where it remained abundant in post-renovation samples.

Major recommendations in addition to those previously mentioned regarding further releases of DNFH pupfish include 1) consideration of the possibility and feasibility of using herbicide and/or construction of shade in small, local areas to retard encroachment of the water channel by bulrush, thereby improving habitat conditions for pupfish, 2) continued monitoring of the pupfish, 3) monitoring of the possibility that the introduced population of *T. circumstriata* in the outflow of Diamond Y Spring is replacing *T. adamantina*, and 4) development of a management plan for the invertebrates.

## OBJECTIVES

1. Obtain the following baseline information:
   - a) Genetic structure of the Diamond Y Draw population of Pecos gambusia.
   - b) Distribution of the known endemic taxa, including invertebrates, in the two watercourses and their associated springs in Diamond Y Draw.
2. Monitor effects of renovation on:
   - a) Genetic structure and morphological characteristics of the restored pupfish population in Diamond Y Draw.
   - b) Genetic structure and status of the Diamond Y Draw population of Pecos gambusia.
   - c) Status of the known invertebrates.
3. Monitor surface waterflow, to document seasonal changes and episodic flash flood events that sometimes link the two watercourses supporting Leon Springs pupfish.
4. Incorporate knowledge from objectives 1 and 2 into an adaptive management strategy for Diamond Y Draw.

## I. INTRODUCTION:

A unique aquatic fauna occurs in Diamond Y Draw, a small spring-fed system in the Chihuahuan Desert near Fort Stockton, Pecos County, Texas. The fauna includes two federally listed endangered species of fish, the Leon Springs pupfish (*Cyprinodon bovinus*), which is endemic to Diamond Y Draw, and the Pecos gambusia (*Gambusia nobilis*) which occurs in four isolated spring systems in the Pecos River Basin of New Mexico and Texas. The waters of Diamond Y Draw also support several invertebrates of special concern regarding conservation (Williams et al. 1985). These include two hydrobiid springsnails (*Tryonia adamantina* and *T. circumstriata*) and an amphipod (*Gammarus pecos*), all of which are endemic to Diamond Y Draw, and disjunct populations of a third hydrobiid snail (*Assiminea pecos*) and the limnaeid snail (*Stagnicola caperata*), both of which have their nearest populations in central New Mexico (Bequaert and Miller 1973, Taylor 1987). *Tryonia circumstriata* and *T. adamantina* are candidates for the federal list of endangered species (Federal Register 61:7596), and *A. pecos* is also being considered for listing (N. Allan, pers. comm.). In this report we describe the distributions of the various species of concern and we examine the effects of an attempt to restore the native genetic structure of the pupfish after it had been genetically introgressed by an introduced, non-native pupfish (*C. variegatus*). Published studies on the invertebrates of this system include a taxonomic review of the extant and fossil molluscs in the area (Taylor 1987), a phylogenetic study that included the two species of *Tryonia* (Hershler et al. 1999), and the description of the endemic amphipod (Cole and Bousfield 1970).

Genetic introgression has occurred in several situations in the wild where endemic pupfishes in the Pecos River Basin were exposed to anthropogenic introduction of *C. variegatus*.

By the 1960s, *C. variegatus* was introduced into Lake Balmorhea, about 90 km west of Diamond Y Draw, where it hybridized with the endemic Comanche Springs pupfish *C. elegans* (Stevenson and Buchanan 1973; Echelle and Echelle 1994). Genetic markers indicate that, since then, the Lake Balmorhea population of *C. variegatus* has served as a source for introductions elsewhere in the Pecos River Basin, leading to hybridization with *C. pecosensis* in the Pecos River (Childs et al. 1996) and *C. bovinus* in Diamond Y Draw (Echelle and Echelle 1997).

The first record of *C. variegatus* in Diamond Y Draw was a collection made by R. D. Suttkus at the Highway 18 bridge in 1974 (Kennedy 1977). By 1976 there was evidence of hybridization in the downstream watercourse (Hubbs et al. 1978, Hubbs 1980), but there was no evidence of *C. variegatus* influence in the upper watercourse. Subsequently, two specimens that seemed to have characteristics of *C. variegatus* were taken near the headpool of Diamond Y spring, but this apparently did not result in genetic introgression (Hubbs 1980).

The presence of hybrids in the lower watercourse stimulated an effort in 1976-1978 to renovate the stream with a combination of ichthyotoxins (rotenone and antimycin A), intensive seining, and release of pure *C. bovinus* from the upper watercourse (Hubbs 1980). Hubbs (1980) reported that, based on morphological appearance of the fish, the renovation was successful in removing the hybrid genome, a conclusion that was supported by an allozyme survey of the species at several locations in 1984 (Echelle et al. 1987).

However, a subsequent introduction of *C. variegatus* into Diamond Y Draw apparently occurred in the late 1980s or early 1990s. By 1994, evidence of genetic influence by *C. variegatus* was detected in both of the primary watercourses in Diamond Y Draw (Echelle and Echelle 1997). At that time, frequencies of foreign genetic markers averaged across five diagnostic systems (four allozyme loci and mtDNA) varied from 6.1% to 7.4% in the downstream watercourse to 15.1% in the Diamond Y Spring outflow of the upstream watercourse. This resulted in another effort to restore the native genome in 1998 to 2000. In this report we describe this effort and its effects on the aquatic fauna of Diamond Y Draw. Our objectives were to evaluate the effects of the renovation on the genetic status of the pupfish and to determine if there were any other detectable effects on the aquatic fauna in Diamond Y Draw.

The two watercourses of Diamond Y Draw were subjected to different approaches to restoring the native genome of the pupfish. The effort on the upper watercourse included treatment of a large portion of the surface water with the ichthyocide Antimycim A and release of pupfish from a genetically pure captive stock of *C. bovinus* that had been maintained since 1976 at Dexter National Fish Hatchery and Technology Center (DNFH) in New Mexico. Ichthyocide was not used in the lower watercourse. Instead, efforts were made to swamp out the hybrid population by physical removal of pupfish and release of large numbers of pupfish from the captive DNFH stock. For both watercourses, we examine changes in the genetic structure of the pupfish. For the section treated with ichthyocide, we also examined the effects of the renovation on the genetic structure of another federally listed endangered species, the Pecos gambusia, *Gambusia nobilis*, and we assessed changes in the abundances of selected invertebrates, with emphasis on three endemic species–an amphipod *Gammarus pecos* and two springsnails, *Tryonia adamantina* and *T. circumstriata.*

## II. STUDY AREA:

Diamond Y Draw (Fig. 1) is located about 12.5 km north of Fort Stockton, Pecos County, Texas. Diamond Y Draw includes two watercourses separated by about 3 km of dry land (Fig. 1). The water for the system is primarily from the brackish to saline Rustler aquifer (Boghici 1997). Details of the following description are based primarily on observations made on 19-20 October 1997. Measurements of width (= width of surface water) and stream distances were made with a 100-m measuring tape as we walked the entire watercourse. Stream distances closely follow the course of the stream. Depth of water was measured at about 20-m intervals with a dowel rod marked in centimeters. During the period of this study there was a noticeable decline in the amount of surface water in the area; thus, this describes the maximum extent of water observed during our study period. Descriptions of the upper and lower watercourses are followed by comments on changes observed between October 1997 and September 2001.

*Upper watercourse*:

The primary source of water for the upper watercourse is a spring in the bottom of the headpool of Diamond Y Spring (Fig. 2). The headpool was about 14 m wide and 25 m and Veni (1991) reported a maximum depth of about 3.5 m in maximum depth. A small spring run (10 m long, 2 cm deep, 45 cm wide) emptied into the east side of the headpool. The outflow from the Diamond Y Spring headpool flowed for about 270 m in a small (12 cm to 1.5 m wide, 9 to 30 cm deep) channel lined with bulrush (*Scirpus americanus*). The channel emptied into a deep (1 m), narrow pool that joined a short, dry side-spring channel on its south side. From the pool, water flowed in a bulrush lined channel for about 150 m. From this point, the stream opened into a wide (2.3 m), shallow (16 cm maximally) pool with two partially buried PVC pipes in the channel. The channel (2 m wide maximally, 8 to 20 cm deep) then flowed about 200 m into a wide bulrush marsh at the south side of a caliche surfaced oil-field road. Water then flowed through a metal culvert beneath the road and continued for about 225 m in a 1 to 2-m wide, shallow (8 to 16 cm) channel that emptied into a large bulrush marsh.

The channel (20 cm wide, 4 to 7 cm deep) extended about 240 m into the marsh to an earthen berm across the channel. Water then passed through a metal culvert and entered another bulrush marsh that extended 200 m downstream to an area of shallow open water (2 m wide, 4-7 cm deep, 40 m long) at a fence line crossing just downstream of the confluence with a springfed channel (labelled I, Fig. 2; 140 m long, 40 cm wide, 4 to 7 cm deep) on the east side of the marsh. Downstream of the pool, water flowed another 140 m through a dense bulrush marsh with very little surface flow. This was followed by about 50 m of dry area covered with saltgrass (*Distichlis spicata*). After this, there was a dry, bulrush-covered channel that began at a confluence with a wet side-channel (site F, Fig. 2; 40 m long) and extended 30 m downstream to a confluence with a common channel (77 m long) for two small springs on the east side of the channel (sites E and D, Fig. 2). After this confluence, water extended in a small bulrush-covered channel (1 m wide, 8 cm deep) for about 50 m and then disappeared. About 100 m farther downstream there was a springfed, bulrush-covered, side channel (site C, Fig. 2; 99 m long, 9 m wide, 4-7 cm deep) on the east side of the marsh. A linear sequence of small springs extends upslope from C. Those labelled A and B had water in short channels during our visit. Springs farther upslope from B were dry except for Bennett Spring, which was located outside of the Diamond Y Preserve. Two other dry spring channels (G and H, not shown in the figures) occurred at the south edge of the marsh between springs F and I.

Near the observation platform in the upper watercourse, a small channel originated from a small spring reported by Kennedy (1977) to have about 5 lps of flow. In October of 1997, the spring run was narrow (50 cm) and shallow (1 to 2 cm) and emptied into a small open pool (30 cm wide, 2 cm deep) that also received seepage flow from the marsh fed by Diamond Y Spring. The pool then emptied into a shallow (9 cm maximally), narrow (30 cm to 1 m) channel that extended about 145 m to the confluence with the dry channel of Leon Creek. Water flowed in a channel (30 to 40 cm wide, 2 to 10 cm deep) downstream from the confluence for about 170 m into a dense marsh that ended just upstream from a fence line.

*Lower watercourse:*

In October of 1997, surface water in the lower watercourse (Figs. 1 and 3) began with a spring in a small channel (13 m long, 3-6 cm deep) that emptied into a pool referred to herein as Monsanto Pool (= "Monsanto Well"; Hubbs et al. 1978). Monsanto Pool was 42 m long, maximally about 5 m wide and 50 cm deep, and had a soft-mud bottom and no bulrush except at the downstream end. This was followed by a channel that was dry and devoid of bulrush for about 40 m; then followed a bulrush-covered channel that had surface water derived from seepage and meager flow from a small side spring on the south side. After this, the channel emptied into a wide (4-12 m) marsh that extended for approximately 360 m to an old road crossing with a metal culvert. The marsh had little surface water and was densely covered with bulrush except for a pool (7 m wide, 28 m long, >2 m deep) near its upper end (= "Lower Monsanto Pool" herein). Downstream of the culvert, the watercourse consisted of a narrow (< 1 m) bulrush-lined channel of especially shallow (2-4 cm) water until about 98 m downstream where it joined with the outflow from a spring referred to by Nature Conservancy personnel Euphrasia Spring.

Euphrasia Spring was the largest spring in the lower watercourse. The spring originated with a headpool (7-8 m wide, 15 cm deep) that had a small area of open water surrounded by bulrush. Water from the spring flowed for 35 m in a small channel (maximum width, 1 m; depth 3 cm) to the confluence with Diamond Y Draw. For the next 685 m downstream from the confluence, there was sluggish flow through dense bulrush growths in a narrow (2.5 m, maximum) channel at the bottom of a shallow, sharply incised, gully, often with dense growths of salt cedar (*Tamarix* sp.; mechanically removed by the Texas Nature Conservancy in 2000).

Then followed an open, mud-bottomed pool (11 m long, 1.5 m wide, up to 18 cm deep) where the water was backed up by an old road crossing with a metal culvert. On the north side, this pool had a small connection to a shallow, stagnant, mud-bottomed side-pool (about 10 m wide, 10 cm deep; "John's Pool," herein). Water emerged from the culvert into a short (9 m) open area (maximally 2.5 m wide, 13 cm deep) with discernable flow. From there, the water entered a narrow (<50 cm) channel with dense bulrush. This channel received seepage from a short (12 m) side channel after about 80 m and continued for another 350 m with dense bulrush cover and a water depth of 5-12 cm. From there the channel emptied into an extensive (200 m long, 35 m wide) bulrush marsh with moist soil, no open areas, and scant surface water. This marsh ran parallel with, and on the west side of, Highway 18 and surrounded a 10 x 100 m island of saltgrass. Just past the island of saltgrass, the marsh narrowed and emptied into a channel (2 m wide, maximally) that extended for 45 m to the Highway 18 bridge. There was no surface flow under the bridge. On the northeast side of the bridge, the watercourse began with seepage from under the bridge platform and continued with meager flow in a narrow channel that was 360 m long, 20-60 cm wide, and generally 1-2 cm deep with dense bulrush; this channel terminated with a small, open pool at the upstream end of a metal culvert that passed under a pasture road. At the other end of the culvert, a dense bulrush growth (95 m long, 4 m wide, with wet soil) was followed by a dry channel. At about 250 m downstream of Highway 18 a small spring outflow channel (14 m long, maximally 60 cm wide and 5 cm deep; no discernable flow) joined the primary channel on the south side.

*Changes observed in surface waters during the study period:*

Leon Creek was not included in the detailed description for October 1997 because it appeared dry. However, on 26 May 1999, water from recent rains was flowing through the culvert across the caliche-surfaced road that crosses the upper watercourse. Also, two shallow, semi-permanent, seepage fed pools with dense bulrush occur approximately 150-200 m upstream of that road. These pools appear to have water during the cooler months but dry out in the summer. On 27 August 2001 these pools were completely dry and the surrounding sedges were brown, indicating that the pools had been dry for some time. Thorough exploration at that time revealed no water in the Leon Creek portion of the Diamond Y Preserve.

The small springs on the south side of the downstream reach of the upper watercourse (Fig. 2) showed considerable fluctuation depending on season. On 10 March 1998 there was a wide area of seepage in the area between the fenceline and springs D, E, and F, and four springs that were dry in October 1997 had water in their small headpools and channels. However, by 17 June 1998, there was no such area of seepage and conditions had returned to those seen in October 1997. Also, during the cooler months, areas downstream of the bulrush marshes often have shallow, temporary water over ground covered with saltgrass.

During our study period, the lower watercourse showed an overall decline in amount of surface water. In October of 1997, there was flow, albeit meager, from a small spring that emptied into the head of Monsanto Pool and the pool was more than a meter deep. But on 18 June 1998, this spring was dry and the water level in Monsanto Pool was noticeably lower. The maximum depth of the water was only about 30 cm on 10 September 2000 and 25 August 2001.

The pool designated herein as Lower Monsanto Pool was receiving noticeable flow from the upstream channel during all of our visits except the last one on 25 August 2001, when the pool appeared stagnant and was apparently anoxic except at the surface where the fish, including *Cyprinodon*, *Gambusia nobilis*, *G. affinis*, and *Lucania parva*, were concentrated. Six of 11 pupfish were found dead in minnow traps that had been left overnight in about 50 cm of water near the head of the pool. Such deaths were not observed by Clark Hubbs (pers. comm.) during repeated overnight trapping of this pool in the 1990s.

Finally, the downstream reach of the lower watercourse receded during our study. In August and October of 1997, surface water, albeit meager, extended to the upstream side of a metal culvert at a pasture road crossing, and, in March of 1998, it extended 110 m farther downstream. However, on 6 September 1999 there was no surface water for 30 to 100 m upstream of the culvert and this was true for all subsequent visits.

*Water quality measurements:*

Water quality was similar among sites within the upper and lower watercourses but differed between watercourses (Table 1). Conductivity, salinity, and total dissolved solids were all higher in the lower watercourse, with minimum values being higher than the maximum value for the upper watercourse. Other workers also report higher values for these variables in the lower watercourse (Veni 1991; C. Hubbs, pers. comm.). Temperature was slightly warmer in the lower watercourse, possibly because it was measured in June and the upper watercourse was measured about a month earlier. Values for pH were similar between the two watercourses.

## III. RESTORATION EFFORTS:

*Upper watercourse:*

On 3-5 August 1998, the portion of the Diamond Y Spring outflow from the headpool to site 30 (Fig. 2) at the crossing of a caliche-surfaced road was treated with Antimycin A to eliminate all pupfish from the reach. Two upper-watercourse areas, each supporting small, isolated populations of pupfish, were not treated with Antimycin A: one at the fenceline crossing in the lower reach of the outflow from Diamond Y Spring and another in the watercourse near the observation tower.

Before applying Antimycin A, large numbers of *Gambusia nobilis* (~1000) and another indigenous fish, the rainwater killifish, *Lucania parva* (~400) were collected in seines and minnow traps and transported to DNFH in 150-gallon aerated tanks for temporary holding until completion of the project. Also, large samples of springsnails and *Gammarus pecos* were placed in four 5-gallon plastic buckets that were then placed in the streamflow as on-site refugia. Samples of these invertebrates were also transported to the San Marcos National Fish Hatchery, San Marcos, Texas, and DNFH for temporary holding to guard against possible extirpation of these species. In addition, general samples of invertebrates were set aside in two plastic "kiddie" pools (1 m in radius with water about 10 cm deep) and about 100 crayfish (*Procambarus* sp.) were placed in styrofoam coolers for reintroduction after completion of the project. Individuals of the endangered puzzle sunflower, *Helianthus paradoxus*, were marked to avoid trampling them.

The application of Antimycin A began at 1700 hours on 3 August. The headpool of Diamond Y Spring was treated by broadcasting two units of Antimycin A-coated sand with a plastic scoop. Elsewhere, Antimycin A was administered using drip stations and backpack sprayers. Drip stations were set up at downstream distances of 32, 84, 140, 178, 220, and 257 meters and were set to maintain minimum concentrations of about 10 ppb in spring flow until dawn of 4 August. Backpack sprayers were used to apply antimycin in areas of low flow and along banks and marsh areas throughout the treatment reach.

On the morning of 4 August, drip stations were set up at downstream distances of 32, 257, 300, and 350 meters from the headpool, and backpack sprayers were again used in marshy areas of low flow. A potassium permanganate detoxification station was established at about 400 m downstream of the headpool and left running until early morning 5 August. Drip stations were removed in the morning of 5 August.

On 6 August, to test for complete detoxification before reintroduction of fish and invertebrates, *Gambusia nobilis* collected from an untreated portion of Diamond Y Draw were placed in minnow traps in the headpool and its immediate outflow, and *Gammarus* collected from the untreated area were placed in 5 gallon buckets with 10 cm of treated water. Minnow traps and buckets were left overnight. On 7 August 1998 there was no sign of mortality in the test animals and the invertebrates held in onsite refugia were released into the outflow of Diamond Y Spring headpool. That afternoon a total of 550 *Cyprinodon bovinus* from DNFH were released into the Diamond Y Spring headpool and two sites in its outflow (Fig. 4). One week later, the live collections of *G. nobilis* (n = 879) and *L. parva* (n = 309) being held temporarily at DNFH were released in the headpool and its immediate outflow. The hatchery-maintained samples of springsnails and *Gammarus* were eventually disposed of at the hatcheries.

*Lower watercourse:*

Renovation of the lower watercourse included efforts at physical removal of pupfish on two separate occasions. During the first effort (13-14 March 2000), a group of 10 people from a variety of state and federal agencies attempted to remove as many pupfish as possible with a variety of methods, including traps (both plastic and metal minnow traps), seines, and dipnets. Trapping and seining were most effective in Lower Monsanto Pool. Seines and dipnets were used elsewhere. In areas where the channel was covered by dense bulrush growth, nets were set downstream of one or more people who drove fish into the net by moving downstream vigorously kicking the water and vegetation.

The pupfish removal effort was concentrated in Monsanto Pool, Lower Monsanto Pool, Euphrasia Spring downstream for approximately 350 m to a widened part of the channel ("Johns Crossing"), and John's Pool and the adjacent flowing water channel. These areas were the only parts of the watercourse with enough water to be workable for pupfish. During this two-day effort, 165 pupfish were removed from the following sites: Lower Monsanto Pool (n = 38), a narrow channel from the confluence of Euphrasia Spring downstream to "John's Crossing" (n = 43), and John's Pool and the adjacent water channel (n = 84). No pupfish were found in Monsanto Pool. In the second removal effort, C. Hubbs, A. F. Echelle, and A. A. Echelle trapped continuously for two nights and two days (16-18 March 2001) with a minimum of 10 minnow traps and removed 69 pupfish. Other species taken during these removal efforts were released back to the water immediately after capture; these included *Gambusia affinis* (the predominant species in Monsanto Pool), *Gambusia nobilis* (the prevalent species in the remainder of the system), and *Lucania* (rare to common except in Monsanto Pool). Releases of pupfish (standard lengths 18 to 35 mm) from the genetically pure captive stock of *C. bovinus* were made immediately after both removal efforts (see Fig. 4 for sites of release): approximately 2300 on15 March 2000 and 3,370 fish on 22 March 2001.

During the time of the second release of pupfish in the lower watercourse, 1630 pupfish were also released in the upper watercourse: 800 in the small springfed channel near the observation platform and 830 in various temporary pools of water in Leon Creek and Diamond Y Draw downstream of the observation platform.

During the first removal effort, an attempt was also made to improve habitat for pupfish by clearing areas of bulrush. Bulrush was uprooted by hand in three relatively wide, shallow areas: the water channel at the head of Lower Monsanto Pool, John's Crossing, and the water channel adjacent to John's Pool. About 30 $m^2$ was cleared in first two sections and about 60 $m^2$ in the third. Within six months the bulrush densities appeared to have returned to previous levels, but the effort was an attempt to at least temporarily increase the amount of open, shallow-water habitat preferred by pupfish, thereby enhancing the effort to swamp out the hybrid genome with captive stock.

## IV. MATERIALS AND METHODS:

*Analysis of fish occurrences:*

On and 18 June 1998 and 26 May 1999 prior to, and following the application of Antimycin A (3 August 1998), we sampled for fish at 20 sites in the upper watercourse from a site 30 m downstream of the Diamond Y Spring headpool to the fenceline that crosses the outflow of the stream. Sampling was done with a small seine or a fine-mesh dipnet or both. Effort and method varied between samples depending on the situation, but, for each sample site, we spent 5 to 22 minutes and made a special attempt to collect pupfish. Usually the net was held in place while someone attempted to drive fish downstream to the net. All fishes were identified at the time of collection and released alive at the site. For each species occurring in sufficient samples, Wilcoxon signed-rank tests were used to compare abundances between the two surveys; only sites where the species was taken in one or the other survey were used in the analysis.

For the headpool of Diamond Y Spring, the number of pupfish present prior to treatment with Antimycin A was based on numbers of dead pupfish observed following the treatment. Crude assessments of post-treatment numbers of pupfish in the headpool were based on numbers of adults estimated from visual surveys of a shallow area (about 25 $m^2$) at the outlet end of the pool. Scattered information on occurrences of fishes in the lower watercourse was obtained at various times during the study, but there was no intensive survey except for records of numbers of pupfish removed during the attempt to deplete their numbers prior to the initial release of captive stock on 13-14 March 2000.

The only other native fishes encountered in Diamond Y Draw during this study were the western mosquitofish, *G. affinis*, and the plains topminnow, *Fundulus zebrinus*. Only a single specimen of *F. zebrinus* was taken from the area treated with Antimycin A during this study, and *G. affinis* is not a common in this part of the watercourse.

*Genetic survey of* Cyprinodon bovinus *and* Gambusia nobilis:

Reference samples of *C. bovinus* and *C. variegatus* were taken from, respectively, the captive stock at DNFH, and Lake Balmorhea, Reeves Co., Texas. The latter location appears to be the source of the stock of *C. variegatus* introduced into Diamond Y Draw (Echelle and Echelle 1997). Appendix A shows the collections used in the genetic analyses of *C. bovinus* and *G. nobilis* before and after efforts to restore the genetic structure of the pupfish.

We used standard methods of horizontal starch-gel electrophoresis (Murphy et al. 1990) to assay allozyme products for the pupfish and *Gambusia*. Proteins, buffer systems, and tissues used for resolution of each of the seven loci examined in the pupfish are given in Appendix B. Five of these (Est-1, Gda-A, Gpi-B, Pep-D, and Pgdh-A) were chosen because *C. bovinus* and *C. variegatus* previously exhibited fixed or nearly fixed allelic differences (Fig. 5). The other two loci, Adh-A and Gpi-A, were included because they are polymorphic in *C. bovinus* (Echelle and Echelle 1997), thereby contributing additional information regarding the genetic structure of the pupfish population. For both loci, *C. bovinus* is polymorphic and has an allele that is absent in *C.*

*variegatus*, and conversely, *C. variegatus* has an allele for Gpi-A that is absent in the endemic species.

Restriction-fragment-length-polymorphism analysis was used to determine whether individuals carried the mitochondrial DNA (mtDNA) of *C. bovinus* or *C. variegatus*. Sequences made available from a survey of the mitochondrial control region in *C. variegatus* and *C. bovinus* (unpublished data) were screened for diagnostic restriction enzymes using the McVector computer program. This search revealed a single diagnostic enzyme, *Hin*fI, that produces diagnostic fragment patterns for the two species (Figure 6). To assess the mtDNA for individual specimens, the DNeasy Tissue Kit (Qiagen, Cat. no. 69506) was used to extract genomic DNA from muscle. Then a 360-bp portion of the control region was amplified by the polymerase chain reaction (PCR) using primers L15926 (Kocher et al. 1989) and H16498 (Meyer et al. 1990). The amplifications were carried out in 50-µl reactions under the following conditions: 93C, 3 min; 30 cycles of 94C, 1 min, and 72C, 2 min; 72C, 30 min. The PCR reaction was then subjected to the *Hin*fI digestion protocol recommended by the manufacturer (Promega). The resulting fragments were electrophoresed through 0.8% agarose gels containing ethidium bromide and visualized with ultraviolet light.

A broad pre-treatment genetic survey of *Gambusia nobilis* included 23 loci and 138 fish from five upper watercourse sites and one lower watercourse site (Appendix A). Proteins, buffer systems, and tissues used for resolution of these loci are given in Appendix C. Post-treatment, a sample of 33 *Gambusia nobilis* from Diamond-Y headpool was analyzed for the only two loci (Adh-A and Gpi-A) that were polymorphic in the initial survey.

For the allozyme data, BIOSYS-1 (Swofford and Selander 1981) was used to compute Nei's (1978) unbiased estimate of heterozygosity (*H*), exact probability chi-square tests of divergence of genotypic frequencies from Hardy-Weinberg expectation (with Levene's correction for small sample size), and heterogeneity chi-square analyses of among-sample differences in allele frequencies.

*Pupfish morphology*:

Because of relatively small sample sizes and problems with distortion in preparation of specimens for allozyme analysis (freezing and dissection), we did not do morphometric measurements to compare the effects of the restoration on pupfish morphology. Instead, during the dissection of specimens for allozyme analysis, specimens were qualitatively scored for pattern of lateral pigmentation (0 = irregular lateral blotches tending to form a broken horizontal band with little barring; 1 = three or more vertical bars extending almost completely to the ventral profile on both sides of the body, sometimes with single "secondary bars" inserted ventrolaterally between long bars). Scores of 0 indicate color pattern typical of *C. bovinus*, scores of 1 indicate a pattern that indicates influence by *C. variegatus*.

*Collection of invertebrates*:

To describe major patterns of invertebrate distributions and to assess the effects of Antimycin A treatment in the upper watercourse, we performed two longitudinal surveys of invertebrates in the two watercourses of Diamond Y Draw, one on 11-12 May (upper watercourse) and 17 June 1998 (lower watercourse) prior to application of Antimycin A, and one on 20-21 May 1999 (both watercourses) subsequent to the Antimycin A treatment. Forty-eight sample sites were established at 20-m intervals in the upper watercourse (starting at the head of the outflow from the headpool of Diamond Y Spring) and 64 sites were established at 20-m intervals (starting at Mansanto Pool) in the lower watercourse (Figs. 2 and 3). Additional sample sites included in this survey were two sites in the channel near the observation platform (sites 49 and 50, Fig. 2) and two collections from the outflow stream of Euphrasia Spring (sites 24 and 25). Collections were made with a 2-mm mesh, D-frame net or a circular tea strainer. Sampling effort consisted of three to four bottom scraping sweeps of the D-frame net or, at sites too small or too densely crowded with bulrush to use the D-frame net, five bottom-scraping passes with a tea strainer. Finally, on 9 to 10 March of 1998, a collection was taken from each of seven small springs (sites A-F, and I, Fig. 2) near the lower end of the upper watercourse. These collections were made with a tea strainer at several places from each spring channel and all collections from within each spring were combined. Samples from these springs were used only to determine presence/absence of the endemic forms; they were not used in the statistical analyses.

In the verbal descriptions of species occurrences and in the analysis of the effects of exposure to Antimycin A, we included collections made on a seasonal basis at eight sites used in unpublished reports of invertebrates in Diamond Y Draw (Veni 1991, Fullington and Goodloe 1993). These included four sites (1-4, Fig. 1) in the section of the upper watercourse that was treated with Antimycin A and four sites (5-8, Fig. 1) in untreated areas; all of these were at or near sites used in the longitudinal survey except for sites 1 and 2, which were located, respectively, in the small spring run on the east side of the Diamond Y Spring headpool and on the southwest side of the headpool itself. Seasonal locations were sampled four times before application of ichthyotoxin (18 August 1997, 20 October 1997, 9 March 1998, and 17 June 1998) and five times after treatment (14 October 1998, 18 December 1998, 15 March 1999, 21 May 1999, and 10 August 1999). The same individual (Lisa Kiner) made all collections using three to four bottom scraping sweeps with a D-frame net.

Collections of invertebrates were preserved in ethanol and identified in the lab. The amphipods and molluscs were identified to species; insects were identified to family except for some dipterans identified only to order. Specimens of *Tryonia* were sent to R. Hershler of the Smithsonian Institution to verify our identifications. Environmental variables (dissolved oxygen, total dissolved solids, temperature, pH, conductivity, and salinity) were measured with each sample by using a portable water quality meter (Yellow Springs Instruments, model 600XL), and habitat of the channel was recorded as either open (greater than 5% of the water surface was visible and exposed to direct, overhead sunlight) or covered by bulrush.

*Analyses of invertebrate occurrences:*

For the analysis of invertebrate distributions in Diamond Y Draw, the CANOCO computer package (ter Braak and Smilauer 1998) was used to ordinate samples on the basis of number of individuals collected for each taxon. Detrended correspondence analysis (DCA), with rare taxa downweighted, was performed on data from the longitudinal samples from both watercourses for both years. Year of collection was used as a covariable, and all environmental data were included as supplementary variables in the analysis. Spearman rank correlations were used to examine pairwise correlations in abundances (number of individuals collected) in selected pairs of species.

Six species were chosen to represent the invertebrate community in the analysis of the effects of the Antimycin A treatment: two amphipods, *Gammarus pecos* and *Hyallela azteca*, and four gastropods, *Tryonia adamantina*, *T. circumstriata*, *Physella* (= *Physa*) *mexicana*, and *Melanoides tuberculata*. Wilcoxin sign-rank tests of paired collection sites from the two longitudinal surveys were used to assess effects on the abundance and distribution of the six species of concern in the portion of the watercourse (sites 1-30, Fig. 2) exposed to ichthyotoxin. These tests included only those sites where the species of concern was collected in one or the other of the two surveys. For each of the six species, Mann-Whitney U-tests were used to compare abundances in the four seasonal samples made before Antimycin A treatment with the five seasonal samples made after the treatment (Sites 1-4, Fig. 1); only those seasonal sites where the species was collected at least once were included in these analyses.

## IV. RESULTS:

*Pre- vs Post-renovation abundance of fishes in the area treated with Antimycin A:*

*G. nobilis* occurred in all 20 collections in the pre-renovation survey (18 June 1998) of the upper watercourse, and represented 96% of the fish collected; it occurred in all but one of the 20 collections made on 26 May 1999 at similar sites approximately 10 months following treatment and represented 87% of the fish collected. Only four other species were taken in these two surveys. Their occurrences were as follows (number collected and percent in, respectively, the first and second surveys): *C. bovinus* (7, 2.4%; 5, 3.9%); *Gambusia affinis* (2, 0.3%; 2, 1.1%); *G. geiseri* (5, 1.8%; 0, 0%); and *Lucania parva* (1, 0.2%; 1, 1.1%). The abundances of three species (*G. nobilis*, *C. bovinus*, and *G. geiseri*) were significantly lower in the post-treatment survey, assuming that there was no consistent bias in sampling effort. The results of the Wilcoxon signed ranks test for these species were as follows: *G. nobilis* $Z = -2.86$, $P = 0.004$); *G. geiseri* $Z = -2.53$, $P = 0.011$; *C. bovinus* $Z = -1.97$, $P = 0.049$.

Two non-native fishes, the largespring gambusia (*G. geiseri*) and the common carp (*Cyprinus carpio*) were apparently eliminated from the upper watercourse of Diamond Y Draw.

Intensive post-treatment minnow-trap collecting by Hubbs (2001; pers. comm.) convincingly demonstrated that *G. geiseri* had been eliminated from the system. During the treatment with Antimycin A, a total of 19 adult *C. carpio* (19 individuals, 3-5 lbs each) were removed from the headpool, and subsequent observations indicated that this species was eliminated from the pool.

Our post-treatment observations, and those reported by Hubbs (2001) indicate that *Gambusia nobilis* and *Lucania parva*, the two most abundant native fishes other than the pupfish, were thriving in the headpool of Diamond Y Spring and elsewhere in Diamond Y Draw. Both of these species also occur elsewhere in the Pecos River Basin in Texas and New Mexico. The only other species collected from the upper watercourse during our study wasthe plains killifish (*Fundulus zebrinus*). In the pre-renovation survey we collected a single female in the relatively deep pool that occurs approximately 270 m downstream of the Diamond Y Spring headpool.

The post-renovation abundance of pupfish in the headpool of Diamond Y Draw appeared to be several times greater than it was prior to the renovation. We counted 52 dead adult pupfish around the edges of the headpool following application of Antimycin A, and 40 others were frozen for genetic analysis for a total of 90 fish. Pre-renovation observations made from shore and by snorkeling indicated a sparse population, and numbers seemed low subsequent to renovation during visits on 14 October 1998 and 26 May 1999. However, by 5 September 1999, about 15 months post-renovation, we conservatively estimated that there were more than 200 pupfish in the shallow north end of the headpool. At the same time, we noted that the ordinarily dense growths of filamentous algae and pond weed (*Potamogeton*) in this area had disappeared. In all of our previous visits, this area was densely covered with growths of these plants. This situation seems to have remained essentially the same since then, and the pupfish has remained at high levels of abundance in the headpool.

It appears, however, that pupfish abundances are very low elsewhere in the upper watercourse. On 28 August 2001, we failed in an intensive effort to collect pupfish in a 200-m reach of the Diamond Y Spring outflow from the road crossing downstream to the marsh. Previously, on 5 September 1999, we easily made a sample of 20 specimens in less than 50 m of the same reach of stream. During the August 2001 visit, a previously shallow, open-water area at the fence-line (site 40, Fig. 2) had very little water and was more overgrown with bulrush than we had ever seen it, and we failed to find pupfish at this site. During the same visit, we searched intensively for pupfish in the watercourse downstream of the observation platform and were able to collect only four specimens. However, pupfish were never abundant in this area during our visits, and on each of two previous trips we were able to collect only a single specimen.

*Fish distributions in the lower watercourse of Diamond Y Draw*:

In the following, we describe observations on the distributions of fishes in the lower watercourse with an emphasis on the pupfish. This account proceeds in the downstream direction. When we initiated our study in 1997, there were no *C. bovinus* in Monsanto Pool, but the species was abundant in this pool during all visits following the release of individuals from the captive DNFH stock. *Gambusia affinis* was the only other fish species observed in this pool, and it was abundant during every visit.

Lower Monsanto Pool supported *C. bovinus* throughout this study. During our last visit in August 2001, however, the species was relatively scarce, probably as a result of the loss of inflowing shallow water in the channel upstream of this relatively deep, steep-sided pool, and the apparent anoxia mentioned earlier for this pool. Other species present in this pool were *L. parva*, *G. affinis*, and *G. nobilis*.

Downstream of Lower Monsanto Pool, the next site where we encountered pupfish in this study was the stretch of stream extending from Euphrasia Spring downstream to the John's Pool area. Because of dense bulrush growth, pupfish were difficult to collect in most of this stretch, but they seemed to occur throughout most of this stretch, with highest densities in the relatively open-water, channel/pool situation in the John's Pool area. *Gambusia nobilis* was the most abundant species in areas of this stretch with dense bulrush growth; other species encountered included an occasional *L. parva* and, *G. affinis*. The last two were abundant in John's Pool and the associated area of open water channel.

We rarely found pupfish downstream of the John's Pool area, except in the immediate vicinity at the downstream side of a metal culvert. After this, point, most of the channel was choked with bulrush, and the most abundant species was *G. nobilis*. A minor exception was underneath the Highway 18 bridge where there was no bulrush and, during two visits (17 August 1997; 5 September 1999), a thin film of flowing water. On these visits we collected *C. bovinus* (2 specimens, one in each of two visits), *F. zebrinus* (1 specimen) and *G. affinis* (10 specimens). From here downstream, there was only a trickle of water in a bulrush covered channel during our visits. *Gambusia nobilis* was the only fish taken in this stretch except early in the study when a shallow, open pool was present at the extreme terminus of the lower watercourse (at a metal culvert crossing for a pasture road). At this site we collected 306 *G. affinis*, 3 *F. zebrinus*, and 1 *C. bovinus* on 17 August 1997; on 18 October 1997, we collected 46 *G. affinis*, 1 *G. nobilis*, and 2 *C. bovinus*. As described earlier, the watercourse subsequently dried up in this area.

*Genetic structure of* C. bovinus:

Appendix D shows allozyme genotypic frequencies and haplotype frequencies in *C. bovinus*. For allozymes, there were no statistically significant deviations from Hardy-Weinberg expectations. Only one pre-treatment collection of pupfish from Diamond Y Draw was free of introduced genetic markers (Tables 2 and 3, Figs. 7 and 8). This was the collection (n = 13) from a small, isolated population from the fenceline crossing of the Diamond Y Spring outflow.

Frequencies of introduced markers for both mtDNA and allozymes declined from pre- to post-treatment sample periods in both watercourses of Diamond Y Draw (Tables 2 and 3; Figs. 7 and 8). The only post-treatment evidence of introduced markers in the upper watercourse occurred in the small population near the observation platform, where the pre- and post-treatment frequencies for such markers were, respectively, 0.200 and 0.050 for allozymes (for mtDNA, post-treatment frequency of the introduced haplotype was 0.000–not assayed pre-treatment). However, these estimates were based on extremely small sample sizes (respectively, n = 1 and 4). Pre-treatment frequencies of introduced markers in the headpool of Diamond Y Spring were 0.067 for mtDNA and ranged from 0.017 to 0.083 ($\bar{x}$ = 0.030) for allozymes. Such markers were absent in two samples taken from this locality approximately one and three years after the restoration effort, and they were absent from the one post-treatment sample from the Diamond Y Spring outflow immediately downstream of the first road crossing.

For the four pre-treatment collections from the lower watercourse, frequencies of introduced mtDNA ranged from zero to 0.067 ($\bar{x}$ = 0.017) and the average frequencies of introduced allozyme alleles in each collection ranged from 0.039 to 0.085 ($\bar{x}$ = 0.053). For the eight post-treatment samples there was no evidence of introduced mtDNA and the frequencies of introduced allozyme alleles ranged from zero to 0.035 ($\bar{x}$ = 0.007).

The heterogeneity chi-square analysis indicated no significant differences in comparisons of allozyme allele frequencies in the captive DNFH stock of *C. bovinus* with those of the three post-treatment samples from the Diamond Y Spring headpool and its outflow stream ($P > 0.11$). However, two loci showed statistically significant differences (Est-1, $P = 0.002$; Gda-A, $P < 0.00001$) between the DNFH stock and the eight post-treatment collections from the lower watercourse. For Est-1, this reflects the presence of the allele (b) typical of *C. variegatus* (Table 3). The frequency of this allele was relatively high (0.087) in pupfish from a segment of stream that extends from John's Crossing to approximately 200 m upstream; the frequency ranged from 0.000 to 0.033 in the remaining post-treatment samples. Excluding the John's Crossing collection from the analysis resulted in non-significant heterogeneity for Est-1, both when the DNFH sample was included ($P = 0.20$), and when the DNFH sample was excluded ($P = 0.32$).

For Gda-A, the statistically significant heterogeneity for the lower watercourse reflects the occurrence of an allele (c) known only from the lower watercourse. This allele was not detected in our reference samples of *C. bovinus* and *C. variegatus* or in any samples from the upper watercourse. Perhaps because a different electrophoretic buffer system was used, the allele also went undetected in a previous survey of *C. bovinus* in both watercourses (Echelle and Echelle 1997). In the collections made prior to the restoration effort, this allele occurred at frequencies of 0.100 to 0.125 at all sites sampled from the lower watercourse where pupfish were taken (pupfish were absent from Monsanto Pool at that time). In September 2000, after the first release of captive DNFH stock in March of that year, the allele occurred only at John's Draw (frequency = 0.197). On August 2001, after the second release of captive stock (22 March 2001), the allele was absent in all collections, including the one from John's Draw, and the heterogeneity chi-square analysis for Gda-A indicated no significant differences among samples from the lower watercourse or between these and the captive DNFH stock ($P = 0.31$).

*Pupfish Morphology versus genetics*:

No specimen collected from Diamond Y Draw during this study had the color pattern typical of *C. variegatus*, and only 20 specimens (4.6% of 439) had patterns that, based on our qualitative judgment, deviated from those expected of *C. bovinus*. However, 30% (6 of 20) of these had one or more genetic elements (allozyme alleles or mitochondrial DNA) diagnostic of *C. variegatus*, whereas only 13% of 419 individuals with the pattern typical of *C. bovinus* had alleles diagnostic of *C. variegatus*. A 2 X 2 chi-square analysis rejected the null hypothesis of no relationship between color pattern and presence/absence of genetic elements typical of *C. variegatus* ($\chi^2 = 4.42$, 1 d.f., $P < 0.05$). There was, however, no significant difference in frequency of color pattern indicative of hybrids between the pre- and post-renovation samples from Diamond Y Draw (frequency = 4.5% and 4.6% in the two groups of samples).

*Genetic structure of* Gambusia nobilis *pre- and post-renovation*:

The analysis of 23 loci in seven samples of *G. nobilis* revealed only two variable loci (Adh-A and Gpi-A). Allele frequencies for those loci are shown in Appendix E. There were no statistically significant deviations from Hardy-Weinberg expectations. The heterogeneity chi-square analysis for the set of seven samples indicated no significant among-sample differences in allele frequencies for the two polymorphic allozyme loci (Adh-A, $P = 0.80$; Gpi-A, $P = 0.13$).

*Distribution of invertebrates*:

The first DCA axis explained 38.4% of the variance in abundance of invertebrate taxa, whereas the second axis explained only 9.9%. The lengths of the gradient for DCA axes 1 and 2 were 3.02 and 2.34 standard deviation units, respectively. This reflects less than one complete turnover in species composition for both axes (ter Braak and Smilauer 1998).

Sample scores on axis 1 tended to separate sites in the mainstem of the lower watercourse from all other sites sampled, which were primarily in the upper watercourse, but included the three sites in the Euphrasia Spring outflow stream(Fig. 9). Correspondingly, the various measures of water chemistry (pH, conductivity, total dissolved solids), which were higher in the lower watercourse (Table 1), showed positive correlations with axis 1. Axis 2 was not associated with any obvious pattern of sites in the system.

Samples with low scores on axis 1 tended to include one or the other of the endemic snails, *T. adamantina* and *T. circumstriata* together with the endemic amphipod, all of which had low scores on axis 1. The scores for these three on axis 1 were lower than for all other taxa examined, except for the introduced snail, *M. tuberculata* (Fig. 10). Samples containing two or more of these four species were primarily taken from Euphrasia Spring and the upper watercourse. The remaining samples were from the mainstem of the lower watercourse.

*Tryonia adamantina* and *T. circumstriata* exhibited complementary distributions in the upper watercourse (Fig. 11): $r_s = -0.63$ ($P = 0.002$) and $-0.60$ ($P = 0.02$), respectively for 1998 and 1999. The former species was restricted to the upper watercourse where, except for the small side spring entering the Diamond Y Spring headpool, it was essentially restricted to areas downstream of the oil field road (Appendix F), including the spring and channel near the observation platform and the side springs on the south side of the upper watercourse. In contrast, *T. circumstriata* was the most abundant springsnail in collections from the first 430 m of the Diamond Y Spring outflow channel and few individuals were collected downstream of the oilfield road. *Tryonia circumstriata* was the only springsnail found in the lower watercourse, where it was restricted to the outflow channel of Euphrasia Spring.

*Gammarus pecos* was abundant in most samples (Figs. 12 and 13) and was collected at all sites except Monsanto Pool in the lower watercourse and at the edge of a deep (1.5 m) pool 120 m downstream from there. Abundances of *G. pecos* and the other amphipod, *Hyallela azteca* showed a significant positive correlation in the upper watercourse ($r = 0.28$, $P = 0.012$), but no significant correlation in either the lower watercourse ($r = -0.14$, $P = 0.127$), or in both watercourses combined ($r = 0.13$ and $-0.03$ for 1998 and 1999, respectively; $P > 0.19$). The significant positive correlation in the upper watercourse may be a spurious result of the scarcity of *Hyalella* in that watercourse (48 individuals in 20 collections).

*Assiminea pecos* was rarely collected in my samples. Specimens were collected from scattered locations in both watercourses and the side springs of the upper watercourse. The sampling method was not well-designed to collect this semi-aquatic species.

*Stagnicola caperata* was collected at only three sites, one in Leon Creek, one in the outflow of Diamond Y Spring, and one in Diamond Y Draw. Abundance was low (single specimens) at the last two sites, but it was high (214 individuals in three dip net sweeps) at the Leon Creek site. This site, which was immediately downstream of the first road crossing Diamond Y Draw, had water only during the May 1998 sampling period, which followed a recent rain event. This site was dry by June of that year.

*Melanoides tuberculata*, an exotic snail from Asia, occurred only in the first 425 m of the outflow of Diamond Y Spring (Appendix F). This species was negatively correlated with *T. adamantina* in both 1998 and 1999 ($r = -0.62$; $P = 0.0001$). Abundances of *Melanoides* showed a positive but non-significant correlation with *T. circumstriata* ($r = 0.22$; $P = 0.17$).

*Occurrences of invertebrates in pre- vs post-treatment samples from the upper watercourse:*

The two amphipods, *Gammarus pecos* and *Hyallela azteca*, were depleted for some time after the application of Antimycin A. On the day following the treatment, all amphipods observed in the treated area were dead, including those taken in scattered dipnet samples. In addition, despite releases of the five collections maintained at stream-side after detoxification of the Antimycin A, both amphipod species extremely rare in samples from the treated reach on 14 October 1998, 42 days following treatment, and numbers remained low until March of 1999 (Figure 14). The populations had recovered by the time of the longitudinal survey of the upper watercourse on 20 May 1999. A comparison of numbers collected in the treated reach between that survey and the pre-treatment survey indicated no significant difference for *G. pecos*, and *H. azteca* was significantly more abundant in the post-treatment survey of the upper watercourse (Table 4; Fig. 12).

The crayfish (*Procambarus* sp.) was rarely collected in our samples. Dead crayfish were observed in the headpool of Diamond Y Spring immediately following the application of Antimycin. The species was, however, commonly observed in the headpool of Diamond Y Draw in 1999 and 2000, and we collected it at two of the 20 sites in the 1999 survey of fishes in the upper watercourse.

Visual examination of *Tryonia* and *Melanoides* indicated no mortality of snails as a direct result of the chemical treatment. Individuals collected as late as three days after treatment in the immediate outflow of Diamond Y Spring emerged from their shells within seconds and began moving about when left untouched in a white enamel pan with water. Nonetheless, numbers for both species of *Tryonia* showed a marked reduction in all five of the seasonal surveys made following the treatment (Table 5; Figure 14), producing a statistically significant difference in pre- versus post-treatment numbers of *T. circumstriata* ($Z = -3.0$; $p = 0.003$). No such effect was observed for *T. adamantina* ($Z = -0.9$; $p = 0.350$) or *Melanoides* ($Z = -1.9$; $p = 0.059$).

The comparison of longitudinal surveys (Table 4) indicated a significantly reduced abundance of *Tryonia circumstriata* in the upper watercourse approximately one year following the chemical treatment ($P = 0.003$), with a lower incidence of occurrence (13 pre-treatment samples vs 4 post-treatment samples) and a markedly lower average abundance of individuals (38.7 vs 2.6) in samples from the 15 sites of occurrence for this species. *Melanoides tuberculata* showed a similar, but marginally significant pattern ($P = 0.06$), occurring in 10 pre-treatment and 6 post-treatment samples, with number of specimens averaging, respectively, 16.9 and 10.3 across the 10 sites of occurrence. The analyses of *T. adamantina* and *Physella mexicana* indicated no statistically significant difference in the two longitudinal surveys, and the latter species also showed no significant variation based on the seasonal surveys.

## V. DISCUSSION:

*Genetic structure of* C. bovinus:

Our results indicate that the present population of Leon Springs pupfish (*C. bovinus*) in the segment of Diamond Y Draw treated with Antimycin A now is free of genetic material from the introduced non-native *C. variegatus*. The comparison of this renovated population with the captive source-stock at DNFH indicated that the transfer of captive stock was accomplished without severe bottlenecking and resulting change as a result of genetic drift.

Another possibly uncontaminated upper-watercourse population occurs in a small area of open surface-water near the fenceline crossing the outflow of Diamond Y Draw. No effort was made to restore this population because the pre-renovation genetic assays revealed no evidence of genetic contamination. It is possible, however, that contamination could have been detected with other genetic markers. The lack of such evidence for the markers we used could reflect random losses as a result of genetic drift in a small population. Genetic drift might explain the relatively low level of variability in this population ($H = 0.088$) compared with the minimum (0.152) observed in all other samples from Diamond Y Draw except for the population near the observation platform (0.000 pre-renovation). Nonetheless, the data indicate that this population is free of genetic contamination and, because of this and the small size of this population, it seemed advisable to treat it as an uncontaminated population.

The remaining upper watercourse area known to support pupfish at the end of our study was the springfed channel downstream of the observation platform. This population was treated by simply releasing DNFH stock. The single pre-renovation specimen that we were able to collect from this area was homozygous for the introduced Est-1 allele, making it the only specimen in the entire study that was homozygous for an introduced allele at any of the five diagnostic allozyme loci. This suggests genetic drift in an extremely small population and reinforces the conclusion from sampling that this is an especially small population. The four specimens examined from population after release of captive stock included two individuals that were heterozygous for the Est-1 allele; there was no other evidence of genetic contamination, but the average level of introgression across all five diagnostic allozymes and mtDNA is 4.2%, and if this is a random sample of the genome, then the genetic material of this population is 4.2% non-native.

The populations in the lower watercourse were subjected to physical removal of the original, genetically contaminated pupfish and/or releases of the captive DNFH stock of genetically pure *C. bovinus*. Of these, the Monsanto Pool population in the lower watercourse is the only one that presently seems to carry no non-native genes. This population apparently was devoid of pupfish at the time of the release of captive stock.

Among the remaining populations in the lower watercourse, levels of non-native genetic material were highest in the population between Euphrasia Spring and the John's Draw area. Post-renovation frequencies of non-native alleles in this area ranged from 0.000 for mtDNA to 0.087 for Est-1 (mean = 0.029). The corresponding frequencies for all markers in the other post-renovation samples from the lower watercourse (Lower Monsanto Pool and John's Pool and nearby channel) ranged from 0.000 to 0.033 (means = 0.000 to 0.008).

In the absence of management in the lower watercourse, and assuming selective neutrality, frequencies of non-native genes should eventually approach homogeneity among sites, with the ultimate frequencies depending roughly on the overall frequency of non-native genes in the entire population of the lower watercourse. At the time our study ended, the population in the water channel downstream from the Euphrasia Spring to the John's Pool area probably represented less than half of the total population. If we assume that it represents half of the total (probably an overestimate), then at equilibrium, the minimum frequencies for the markers we examined would be roughly 1%, the maximum would be about 5%, and the average across all markers would be about 2%.

Various considerations lead us to suggest that the present level of genetic introgression throughout most of Diamond Y Draw are acceptable, with the possible exception of the small population near the observation platform. The levels of genetic introgression that might be acceptable to conservationists concerned with genetic introgression of native genomes are debatable and somewhat arbitrary. Allendorf and Leary (1988) suggested a value of 1%, whereas others suggest that somewhat higher levels can be acceptable, depending on circumstances (Campton 1987, Dowling and Childs 1992).

For *C. bovinus*, the captive DNFH stock is the only available source of large numbers of genetically pure stock that can be used in attempts to further reduce levels of genetic variability, and there is reason to believe that this should not be done indiscriminately. The DNFH stock has been maintained in captivity for nearly 25 years with no infusion of genes from the wild, and there is some evidence of losses of rare alleles (Edds and Echelle 1989). Thus, there may have been some loss of potential for variability that might still remain in the areas of Diamond Y Draw not treated with Antimycin A. Another consideration is that there seems to be little morphological effect of the present level of introgression in the population. This is based on the qualitative assessment of color pattern presented here, and our general observations on the overall body form and fin characteristics of the present population. For these reasons, it seems advisable to avoid further swamping of the wild genome in the lower watercourse, even though it retains small levels of genetic introgression.

We do, however, recommend further releases of the DNFH stock of *C. bovinus* in the small area at, and downstream of the observation platform. This population apparently has the highest level of non-native genetic material (4.2%), and, as previously discussed, it appears to have undergone a severe bottleneck that reduced its level of variability. Because of the small size of this population, it should be relatively easy to dilute the non-native genetic material and at the same time increase its level of genetic variability. The threat posed by this population for the remainder of the upper watercourse is disproportionately large because it lies in the mainstem of Diamond Y Draw and could expand considerably if surface waters in this area ever approach levels observed in earlier studies (e.g., Kennedy 1977, Echelle and Echelle 1980).

*Effects of the Antimycin treatment on the upper watercourse:*

The renovation of Diamond Y Draw with Antimycin A had at least two beneficial effects on the fish fauna. First, it eliminated two introduced species, the largespring gambusia (*G. geiseri*) and the common carp (*C. carpio*), both of which had occupied the upper watercourse since at least the 1970s (Kennedy, 1977). Second, the renovation was followed by increased numbers of pupfish in the headpool of Diamond Y Draw. Our pre-renovation observations and comments by workers in the 1970s (Kennedy 1977; Echelle and Echelle 1980) indicated that the pupfish was uncommon in the headpool prior to the renovation, whereas, within 15 months of the renovation, hundreds of adult pupfish could be observed from the shore. This change in abundance coincided with the disappearance of dense growths of algae and *Potamogeton* from the shallow, north end of the headpool, creating a wide area of the shallow, open-water habitat preferred (Kennedy 1977) by the pupfish. The observed change may be a secondary effect of the elimination of carp. Removal of carp and other benthic-feeding fishes is a method sometimes used to control excessive algal growth in ponds (R. Drenner, pers. comm.); carp appear to act as "nutrient pumps" that consume sedimented detritus and then excrete nutrients in dissolved form into the water column (Lamarra 1975).

Negative effects of the Antimycin A renovation included an immediate and dramatic decline in the abundance of amphipods. However, their abundances returned to pre-renovation levels within seven months of the renovation. In contrast, the renovation seemed to have no immediate effect on the snail assemblage, but the abundance of one of the two springsnails (*Tryonia circumstriata*) was markedly and persistently lower following the renovation. We have no explanation for this decline or for the possibly reduced post-renovation densities of *G. nobilis* and *C. bovinus* in the outflow channel and marshes fed by Diamond Y Spring. *Tryonia circumstriata* is, however, introduced into this area from Euphrasia Spring where it has replaced *T. adamantina* (see below), and present levels scarcity of the former in this area seem comparable to that of the latter in the early 1980s (Taylor 1985). Regarding the fishes, *G. nobilis* remains abundant in the channels and marshes of both the upper and the lower watercourse. The possibly reduced abundance of pupfish in the outflow channel of Diamond Y Spring is offset by the increased abundance of the species in the headpool of the spring.

*Status of invertebrates of concern*:

Nearly all sites sampled in Diamond Y Draw supported one or more of the five invertebrate species that are of concern to conservationists because of either being restricted to the area (*Gammarus pecos, Tryonia circumstriata*, and *T. adamantina*) or occurring as a broadly disjunct population of a more widespread species (*Assiminea pecos* and *Stagnicola caperata*). This area receives considerable legal protection because it lies within the Diamond Y Preserve, which, since, has been owned and managed as a protected natural area, Diamond Y Preserve, by the Texas Nature Conservancy. In addition, the area supports three species that are federally listed as threatened or endangered, Pecos gambusia, *Gambusia nobilis*, Leon Springs pupfish, *Cyprinodon bovinus*, and puzzle sunflower, *Helianthus paradoxus*. The area from Diamond Y Spring downstream "to a point 1 mile northeast of the Texas Highway 18 crossing," is federally designated critical habitat for the Leon Springs pupfish (Federal Register: 45 FR 54678), providing additional protection under the Endangered Species Act. However, as presented in the following discussion, there are various causes for concern regarding conservation of individual species.

Previous information on the distribution of the endemic amphipod *Gammarus pecos* was based only on the description of the species from specimens taken from the outflow of Diamond Y Spring and Diamond Y Draw at the Highway 18 bridge (Cole and Bousfield 1970). My results indicate that the species is widespread and abundant at most sites in Diamond Y Draw. The other amphipod of the system, *Hyalella azteca*, occurred primarily at sites in the lower watercourse where it occasionally outnumbered *G. pecos* (21 of 128 samples had higher abundances of *Hyalella*).

*Hyalella azteca*, has not previously been reported from Diamond Y Draw, except for an unpublished report received by the Texas Nature Conservancy in 1992 (B. Henry in litt.). This widespread form comprises an unknown number of cryptic species (G. Wellborn, pers comm, Duan et al. 2000). This, together with the high level of endemicity in the fauna of Diamond Y Draw, suggests that the population in the system might eventually be recognized as a geographically restricted taxon.

The crayfish (*Procambarus* sp.) in Diamond Y Draw is an undescribed, geographically restricted species that otherwise occurs only in scattered localities elsewhere in the Pecos River Basin (D. L. Hillis, pers. comm.). The status of this species was not emphasized in this study, but it was common in Diamond Y Spring headpool, and it was observed or collected at several sites in the outflow of this spring.

The gastropod species with disjunct populations in Diamond Y Draw, *Assiminea pecos* and *Stagnicola caperata*, probably are more widespread in the system than indicated in my collections. The former species is common in both watercourses of Diamond Y Draw, but not in the strictly aquatic situations that we sampled (B. Lang, pers. comm.). Taylor (1987:9) noted that *A. pecos* occurs in "moist earth beside seepages or spring-brooks, never beside standing water. They occur beneath salt grass or sedges, less often on exposed surfaces." Another source of sampling bias apparently explains the rarity of *S. caperata* in our samples. Members of this family may burrow and aestivate in response to drying of the habitat and may emerge in large numbers with the return of surface water (Brown 1991). Correspondingly, we found large numbers of the species in a recently flooded pool in a normally dry area of Leon Creek.

The present distributions of the two endemic springsnails of the genus *Tryonia* indicate that substantial changes have occurred in the 1990's. Taylor (1987) reported that *T. circumstriata* (= *T. stocktonensis*) was restricted to Euphrasia Spring and its outflow stream in the lower watercourse, whereas *T. adamantina* was the only species of the genus in the upper watercourse and associated springs. The distribution of the genus in our survey was essentially as described by Taylor (1987), except that *T. circumstriata* appears to have largely replaced *T. adamantina* in the first 500 m of the outflow stream from Diamond Y Spring in the upper watercourse. Based on our collections and a collection made by J. Landye in 1995 (Smithsonian Institution catalogue number, NMNH 883960), *T. adamantina* remains the sole species of the genus in the small spring-run that empties into the east side of the Diamond Y Spring headpool.

The effective replacement of *T. adamantina* by *T. circumstriata* in a portion of the upper watercourse of Diamond Y Draw appears to have occurred sometime between 1991 and 1995. Taylor's (1987) summary of the original distributions of the two species of *Tryonia* in Diamond Y Draw is well-substantiated. Taylor (1985) indicated that he had collected Diamond Y Draw "at various times since 1968" and in 1984 when "non-quantitative samples were made repeatedly" as he walked along the entire watercourse of Diamond Y Spring to Highway 18. Subsequently, an unpublished report to the Texas Nature Conservancy by another experienced malacologist (Fullington and Goodloe 1993) indicated that, in 1991, the distributions of the two species were as reported by Taylor (1987). By 1995, however, *T. circumstriata* was present in the immediate outflow of Diamond Y Spring in sufficient numbers that R. Hershler and J. Landye were able to make samples of 50+ animals in each of two separate visits (NMNH 883960 and 892020). Because of the intervening population of *T. adamantina* and the poor dispersal abilities of these small snails, it appears that *T. circumstriata* was transported from Euphrasia Spring to the Diamond Y Spring outflow. This apparently occurred sometime between 1991 and 1995.

The introduced population of *T. circumstriata* in the upper watercourse of Diamond Y Draw is a potential threat to *T. adamantina*. The sympatric occurrence of two congeneric species is unusual for *Tryonia* (Taylor 1987, Hershler et al. 1999), and Taylor (1987) commented that competitive exclusion may explain the original, mutually exclusive distributions of the two species in Diamond Y Draw. Such competition may also explain the present negative relationship between the two species in the outflow of Diamond Y Spring, but Taylor's (1985) comments indicate that the introduction of *T. circumstriata* may have occurred after *T. adamantina* had already declined in abundance in the Diamond Y Spring outflow. In 1968, he found the latter species abundant upstream of the oilfield road that crosses the Diamond Y Spring outflow, whereas, in 1984, it was almost absent from that stretch but remained moderately abundant elsewhere in the upper watercourse.

Thus, the pattern of abundance of *T. adamantina* at present appears similar to that in 1984, prior to the introduction of *T. circumstriata*. It is possible that *T. circumstriata* is simply tolerant of some habitat variable that may have caused depletion of *T. adamantina* prior to the introduction. Regardless, the situation requires close monitoring. The present situation may represent a transient stage in a progressive replacement of *T. adamantina* by *T. circumstriata* in the outflow of Diamond Y Spring. *Tryonia adamantina* occurs in several other localities, including the small side spring entering the headpool of Diamond Y Spring, the spring near the observation platform, and various small springs on the south side of the large marsh fed by Diamond Y Spring. The fragmented nature of this distribution renders the species susceptible to incremental decline as a result of local extirpation and barriers to colonization from elsewhere in the system.

The introduction of *T. circumstriata* into the upper watercourse apparently occurred at about the same time as an introduction of the exotic snail, *Melanoides tuberculata*, into the system. The first specimens of this large, conspicuous species in Diamond Y Draw were taken along with the first samples of *T. circumstriata* from the upper watercourse (R. Hershler, pers. comm.), and the present distributions of these two closely coincide in this area. The introductions of these species into the upper watercourse apparently represent independent events because *T. circumstriata* was originally restricted to the Euphrasia Spring area where *M. tuberculata* has not been found. The nearest known source for *M. tuberculata* is an introduced population about 90 km away in an isolated system of springs and associated canals near Balmorhea, Reeves County, Texas, but the species also occurs at a number of other localities in south Texas (McDermott 2000) that could have served as source populations. The introductions of both species probably represent accidental transport by humans, possibly during various studies of the aquatic fauna that were being conducted on springs of the region in the early 1990's.

Competition with the introduced population of *M. tuberculata* is a potential threat to the status of the other snails in the system. Introductions of thiarid snails have been used in attempts to suppress the abundance of native snail populations in areas where the latter serve as intermediate hosts for the parasite responsible for schistosomiasis (Pointer and McCullough 1989). Presently, *Melanoides* appears confined to the first 425 m of the Diamond Y Spring outflow, where, in places, much of the substratum is covered with this species. Expansion into other parts of the system seem inevitable given the hardiness of this species and its colonizing abilities (Roessler et al. 1977; Neck 1985; McDermott 2000).

*Overall threats to the ecosystem:*

Oil and gas extraction and refinery activities pose various threats for the Diamond Y Spring ecosystem. The study area is part of the Fort Stockton Oil and Gas Field, an area of intense petrochemical activity since the 1950's. Related operations include pump installations, roads, pipelines, and a large refinery less than 300 m from Diamond Y Spring headpool. The major companies involved have been cooperative in various efforts to mitigate effects of their activities (J. Karges, pers. comm.), but the level of activity is a continuing threat to the system.

Loss of spring-flows is the ultimate threat to the long-term persistence of the unique aquatic community of Diamond Y Draw. Although requiring further investigation, springs and surface waters in the system may be declining. From 1943 to 1976, discharge measurements for Diamond Y Spring ranged from 11 to 41 lps (Brune 1981). Measurements by Veni (1991) in 1990 ranged from 14 to 42 lps, indicating little change. However, an electronic gage recently installed in the Diamond Y Spring outlfow gave a reading of only 1 lps on 7 September 2000 (Nathan Allen, pers. comm.), which is well below any previous record for the spring. It remains to be seen whether the low flows continue.

A reduced extent of surface waters in both watercourses of Diamond Y Draw is evident from a comparison of Figure 1 with published maps and descriptions of the area in the 1970's (Hubbs et al. 1978, Kennedy 1977, Echelle and Echelle 1980). In those years, surface waters in both watercourses extended 0.5-1.0 km farther downstream, and surface waters supporting endemic fishes also were more extensive in upstream areas in both watercourses (Echelle and Echelle 1980). The present situation may reflect the severe drought that the area has experienced since 1992. Rainfall averages in Fort Stockton for the past eight years were well below average for seven years (1992-1996, 1998-2000) and barely above average for the remaining year (1997), making this the most extended drought of record (National Climate Data Center Records, 1940-2000). However, the present low abundance of surface waters in Diamond Y Draw may also be part of a more permanent pattern of declines and failures of spring-flows in the region as a result of over-mining of groundwater (Brune 1981, N. Allan, pers. comm.). This factor threatens existing spring faunas elsewhere in west Texas (Echelle et al. 1989) and throughout arid regions of southwestern United States and Mexico (Miller 1961, Williams et al. 1985, Contreras and Lozano 1996).

## VI. RECOMMENDATIONS FOR MANAGEMENT:

1. Consider developing a program of periodic local application of herbicide to control bulrush in small areas as a means of habitat enhancement for the pupfish. Because local pupfish densities can be extremely high, a few, relatively small areas of suitable habitat can be adequate to maintain relatively large numbers of pupfish. The shallow, open water habitat preferred by the pupfish declines as surface flow declines, allowing encroachment of bulrush. The use of herbicide may be preferred over physical modification with heavy machinery because, by killing the root system, herbicide is likely to have a longer lasting effect, and it is less physically disruptive of the habitat.

2. An alternative to herbicide is to consider 1) the possible effectiveness of artificial shading to retard bulrush growth in local spots and 2) the feasibility of constructing and maintaining such structures.

3. Continue the culture of the present captive pupfish stocks at DNFH, and develop a plan to protect the present level of genetic variability in that stock.

4. The population of pupfish near the observation platform in Diamond Y Draw should receive additional releases of captive DNFH stock. Releases of small pupfish (< 20 mm) in early spring before reproduction peaks in the population in Diamond Y Draw seems likely to have the largest effect. This population is small enough that this is likely to be especially effective in reducing the level of non-native genetic material. As described above, the position of this population in the mainstem of the upper watercourse of

Diamond Y Draw makes it a serious risk for the remainder of the pupfish population.

5. Discontinue releasing captive DNFH pupfish in the lower watercourse unless justified on the basis of future genetic monitoring. The present frequencies of non-native alleles appear sufficiently low that further releases seem unnecessary. As discussed above, this will help preserve any remnants of the original elements of genetic variation that may have been lost during the 25+ years of captivity for the DNFH stock.

6. Periodically monitor the genetic status of the pupfish in Diamond Y Draw to detect future releases of non-native pupfish.

7. Establish a program of monitoring the abundances of the invertebrates of concern. There is some indication that the introduced upper watercourse population of *T. circumstriata* might be replacing *T. adamantina* in that area.

8. Monitor the various springflows for springsnails. This includes the Diamond Y Spring outflow, the small spring on the SE side of Diamond Y Spring, Euphrasia Spring, and the series of small springs on the southeast side of the upper watercourse, which are crucial for *T. adamantina*.

9. Develop a management plan for the endemic invertebrates.

## VII. ACKNOWLEDGMENTS:

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Table 1. Water quality measurements for the upper (May 1998; n = 50) and lower (June 1999; n = 64) watercourses of Diamond Y Draw.

| | Mean | Standard Error | Minimum | Maximum |
|---|---|---|---|---|
| Upper Watercourse | | | | |
| Temperature (°C) | 21.06 | 0.45 | 17.22 | 26.74 |
| Conductivity (µmho) | 6.18 | 0.08 | 5.49 | 7.94 |
| Salinity (ppt) | 3.67 | 0.03 | 3.4 | 5.15 |
| Total Dissolved Solids (ppt) | 4.35 | 0.04 | 4.04 | 5.95 |
| pH | 7.46 | 0.04 | 6.75 | 7.95 |
| Lower Watercourse | | | | |
| Temperature (°C) | 24.67 | 0.45 | 20.54 | 33.13 |
| Conductivity (µmho) | 11.63 | 0.15 | 9.60 | 13.73 |
| Salinity (ppt) | 6.69 | 0.08 | 5.75 | 8.17 |
| Total Dissolved Solids (ppt) | 7.63 | 0.09 | 6.61 | 9.10 |
| pH | 7.56 | 0.04 | 6.91 | 8.53 |

Table 2. Frequency of non-native alleles and mtDNA typical of *C. variegatus* (≥0.99 frequency in *C. variegatus* from Lake Balmorhea) in pupfish collected from upper watercourse, Diamond-Y Draw. None of the alleles listed were shared between *C. bovinus* and *C. variegatus* in our recent reference samples except for Adh-A-b. This allele is also the predominant allele (frequency = 0.890) in hatchery samples of *C. bovinus*. Bold type indicates the five allozyme loci diagnostic of *C. bovinus* and *C. variegatus* and frequencies of the corresponding non-native alleles at sites where they occurred, and mtDNA typical of *C. variegatus*. For site designations, DYS = Diamond Y Spring headpool; numbers = site numbers in upper watercourse (Fig. 2).

| | Pre-restoration Sample site numbers/dates | | | Post-restoration Sample sites numbers/dates | | | |
|---|---|---|---|---|---|---|---|
| Allele | DYS 8/98 (30) | 46 8/97 (13) | 49 6/98 (1) | DYS 9/99 (28) | DYS 8/01 (23) | 30 9/99 (20) | 49 8/01 (4) |
| Adh-A-b | 1.000 | 1.000 | — | 0.875 | 0.761 | 0.875 | 0.750 |
| Gpi-A-a | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Est-1-b** | **0.083** | 0.000 | **1.000** | 0.000 | 0.000 | 0.000 | **0.250** |
| **Gda-A-a** | **0.017** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Gpi-B-b** | **0.017** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Pep-D-a** | **0.017** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Pgdh-A-a** | **0.017** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Mean** | **0.030** | 0.000 | **0.200** | 0.000 | 0.000 | 0.000 | **0.050** |
| mtDNA | **0.067** | 0.000 | — | 0.000 | 0.000 | 0.000 | 0.000 |
| **Grand Mean** | **0.036** | 0.000 | **0.200** | 0.000 | 0.000 | 0.000 | **0.042** |

Table 3 . Frequency of alleles and mtDNA typical of *C. variegatus* (≥0.99 frequency in *C. variegatus* from Lake Balmorhea) in pupfish from lower watercourse, Diamond-Y Draw. Numbers = site numbers in lower watercourse (Fig. 3); 25-36 = a 200-m stretch of Diamond Y Draw just downstream from Euphrasia Spring. 45a = 45 on Fig. 3, 45b = John's Pool in Fig. 3, 45-ab = a combined sample from these locations. See Table 2 for rest of legend.

| | Pre-restoration Sample site numbers/dates | | | | Post-restoration Sample site numbers/dates | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 25-36 | 45a | 45ab | 1 | | 20 | 25-36 | 45a | | 45b | |
| Allele | 99/00 (30)* | 99/00 (30)* | 9/99 (13) | 3/00 (18) | 9/00 (27) | 8/01 (25) | 8/01 (30) | 8/01 (23) | 9/00 (38) | 8/01 (30) | 9/00 (36) | 8/01 (25) |
| Adh-A-b | 1.000 | 0.983 | 0.962 | 1.000 | 0.741 | 0.860 | 0.817 | 0.978 | 0.987 | 0.800 | 0.820 | 0.880 |
| Gpi-A-a | 0.083 | 0.083 | 0.077 | 0.028 | 0.000 | 0.000 | 0.017 | 0.022 | 0.000 | 0.017 | 0.000 | 0.020 |
| **Est-1-b** | **0.083** | **0.083** | **0.154** | **0.139** | 0.000 | 0.000 | **0.017** | **0.087** | 0.000 | **0.033** | 0.000 | **0.020** |
| **Gda-A-a** | **0.017** | **0.033** | **0.115** | 0.000 | 0.000 | 0.000 | 0.000 | **0.022** | 0.000 | 0.000 | 0.000 | 0.000 |
| **Gpi-B-b** | **0.033** | **0.033** | **0.038** | **0.028** | 0.000 | 0.000 | 0.000 | **0.022** | 0.000 | 0.000 | 0.000 | 0.000 |
| **Pep-D-a** | **0.017** | **0.033** | **0.038** | 0.000 | 0.000 | 0.000 | 0.000 | **0.022** | 0.000 | 0.000 | **0.014** | 0.000 |
| **Pgdh-A-a** | **0.050** | **0.050** | **0.080** | **0.028** | 0.000 | 0.000 | **0.017** | **0.022** | 0.000 | **0.017** | 0.000 | 0.000 |
| **Mean** | **0.040** | **0.046** | **0.085** | **0.039** | **0.000** | **0.000** | **0.007** | **0.035** | **0.000** | **0.010** | **0.003** | **0.004** |
| **mtDNA** | **0.067** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Grand Mean** | **0.045** | **0.038** | **0.071** | **0.033** | 0.000 | 0.000 | **0.006** | **0.029** | 0.000 | **0.008** | **0.003** | **0.003** |

*Combined samples from September 1999 and March 2000.

Table 4. Comparison of Pre- and Post-treatment longitudinal surveys for six species in the outflow stream from Diamond Y Spring. Values for pre- and post-treatment are number of sites of occurrence in the survey and (in parentheses) mean ± one standard deviation for number of individuals across all sites of occurrence for both surveys. Asterisks signify statistical significance

| | | | | Wilcoxin signed-rank test | |
|---|---|---|---|---|---|
| Species | Number of Sites of Occurrence | Pre-Treatment | Post-Treatment | Z | P |
| *T. adamantina* | 13 | 11 (11.9 ± 21.6) | 10 (9.3 ± 20.6) | -0.9 | 0.350 |
| *T. circumstriata* | 16 | 13 (38.7 ± 66.3) | 4 (2.6 ± 5.8) | -3.0 | 0.003* |
| *M. turberculata* | 10 | 10 (16.9 ± 21.1) | 6 (10.3 ± 21.1) | -1.9 | 0.059 |
| *P. mexicana* | 45 | 39 ( 3.3 ± 3.2) | 34 ( 4.3 ± 11.8) | 0.7 | 0.476 |
| *G. pecos* | 47 | 47 (59.0 ± 53.6) | 47 (69.9 ± 53.1) | 1.3 | 0.197 |
| *H. azteca* | 20 | 4 (0.2 ± 0.8) | 16 (0.2 ± 0.9) | 2.8 | 0.005* |

Table 5. Comparison of pre- and post-treatment abundances of six invertebrate species in seasonal samples from four sites exposed to ichthyotoxin. Values shown are ranges and means across the four sites. Mann-Whitney U-tests were based on total numbers collected across the four sites. Asterisks signify statistical significance.

| | Species | | | | | |
|---|---|---|---|---|---|---|
| Date of Collection | *T. adamantina* | *T. circumstriata* | *M. turberculata* | *P. mexicana* | *G. pecos* | *H. azteca* |
| Pre-Treatment | | | | | | |
| 18 Aug. 1997 | | 0-529 (241.3) | 0-106 (26.5) | 0-9 (2.3) | 0-349 (105.5) | 0-2 (0.5) |
| 20 Oct. 1997 | | 0-364 (106.0) | | 0-32 (8.0) | 6-177 (62.2) | 0-52 (16.0) |
| 9 Mar. 1998 | | 0-508 (141.0) | 0-54 (13.5) | 1-3 (2.5) | 4-204 (104.5) | 0-9 (2.5) |
| 17 Jun. 1998 | | 0-627 (220.3) | 0-140 (35.0) | 0-4 (1.5) | 0-72 (32.0 ) | |
| Post-Treatment | | | | | | |
| 14 Oct. 1998 | | 0-53 (14.0) | 0-201 (50.2) | 2-21 (9.8) | 0-1 (0.5) | |
| 18 Dec. 1998 | 0-3 (0.8) | 0-16 (5.5) | 0-38 (9.5) | 2-23 (8) | 3-32 (14.8) | 0-2 (0.5) |
| 15 Mar. 1999 | 0-8 (2.3) | 0-5 (2.0) | 0-8 (2.0) | 0-7 (3) | 26-100 (50.3) | 0-12 (3.0) |
| 21 May 1999 | | 0-4 (1.8) | 0-41 (10.2) | 0-5 (2.3) | 40-169 (113.5) | 0-1 (0.3) |
| 10 Aug. 1999 | | 0-1 (0.3) | 0-61 (15.3) | 0-6 (1.8) | 25-137 (74.8) | |
| Mann-Whitney U | 6.0 | 20.0 | 11.0 | 7.5 | 13.0 | 13.5 |
| *P* | 0.18 | 0.005* | 0.061 | 0.54 | 0.46 | 0.38 |

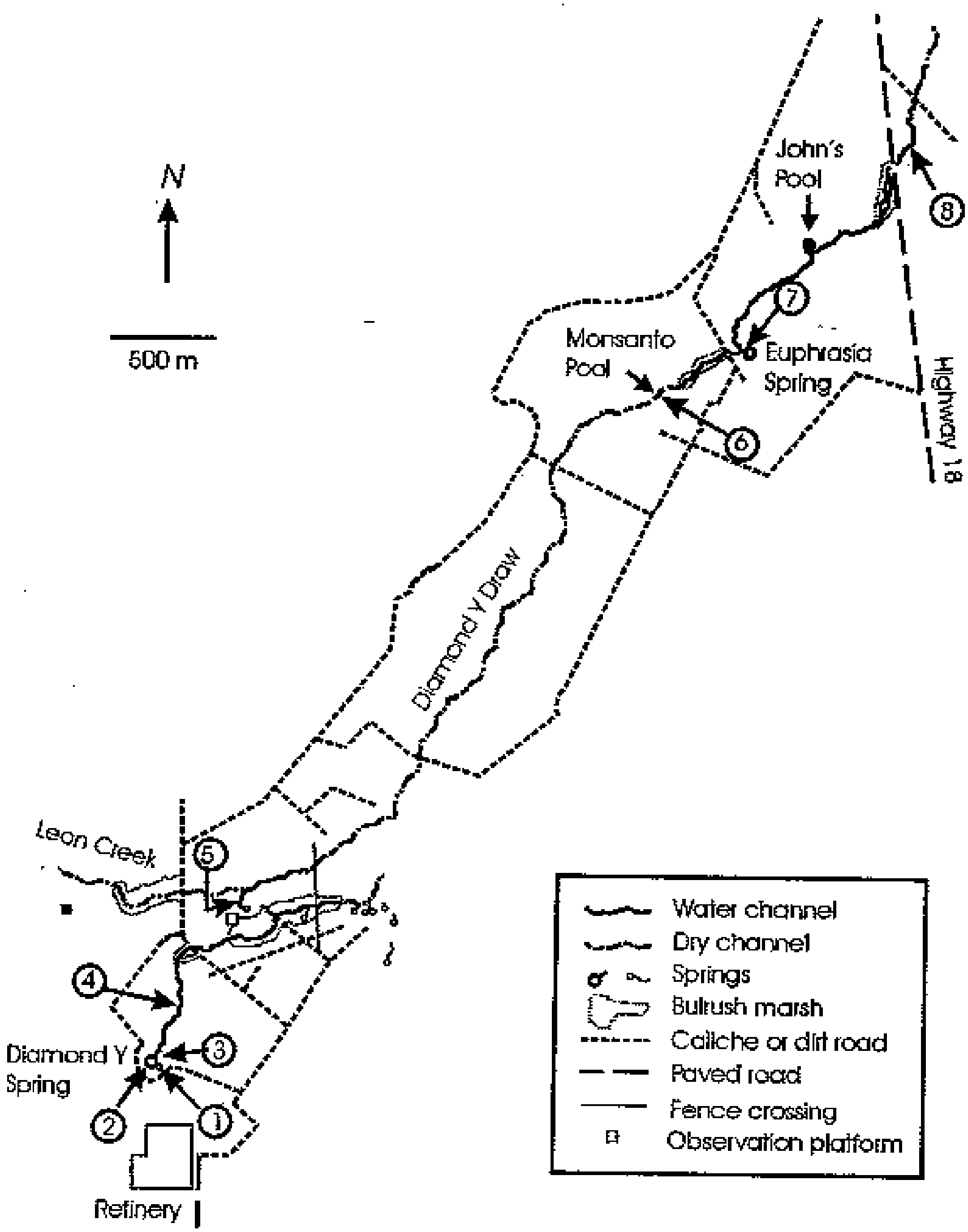


Figure 1. Diamond Y Draw, Pecos County, Texas. Encircled numbers represent seasonal sampling sites mentioned in the text.

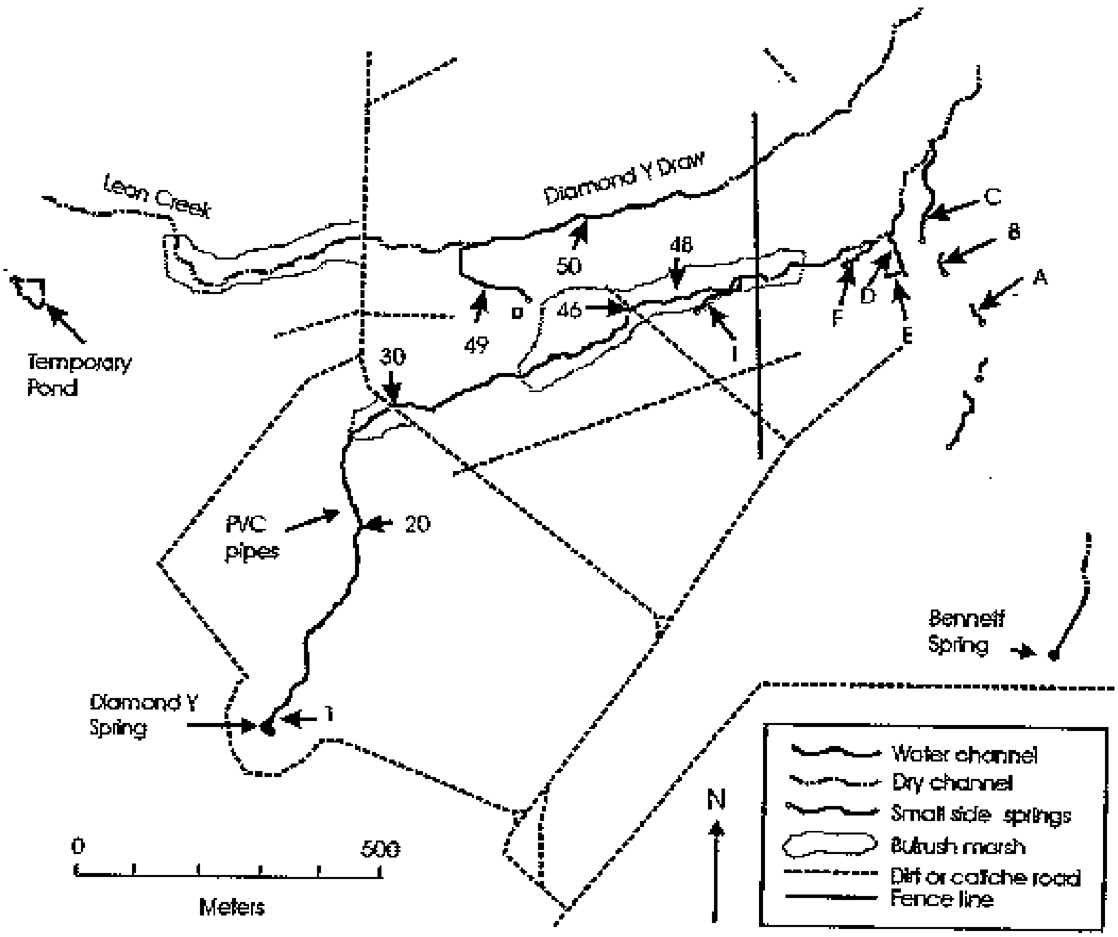


Figure 2. Upper watercourse of Diamond Y Draw. Numbers show positions of selected sites among the 50 sampling points for the longitudinal surveys of invertebrates in 1998 and 1999. Except for numbers 49 and 50, samples were made at 20-m intervals starting at the head of the Diamond Y Spring outflow stream. Letters represent the 7 sites sampled in small springs in the downstream reach of the watercourse.

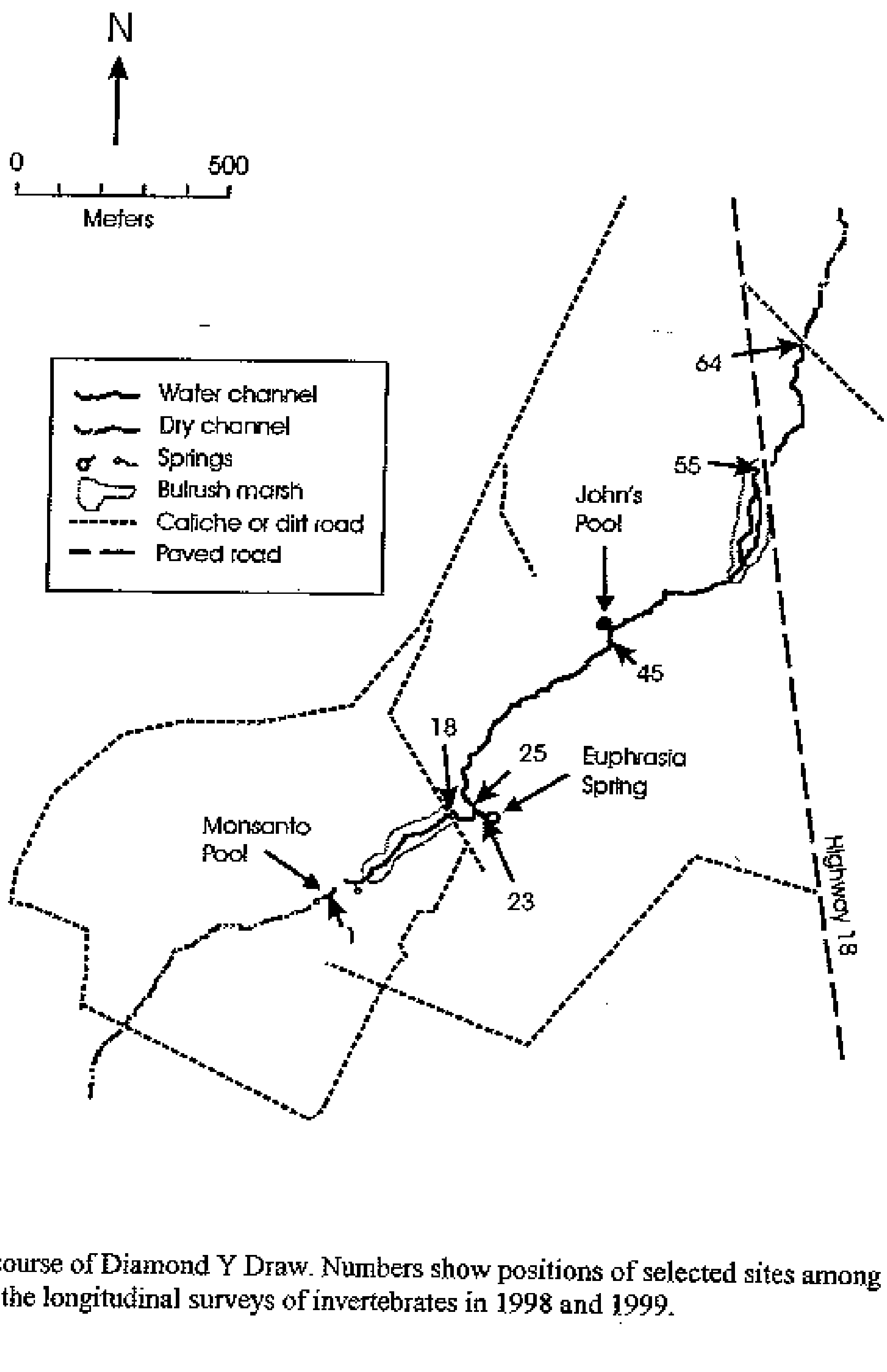


Figure 3. Lower watercourse of Diamond Y Draw. Numbers show positions of selected sites among the 64 sampling points for the longitudinal surveys of invertebrates in 1998 and 1999.

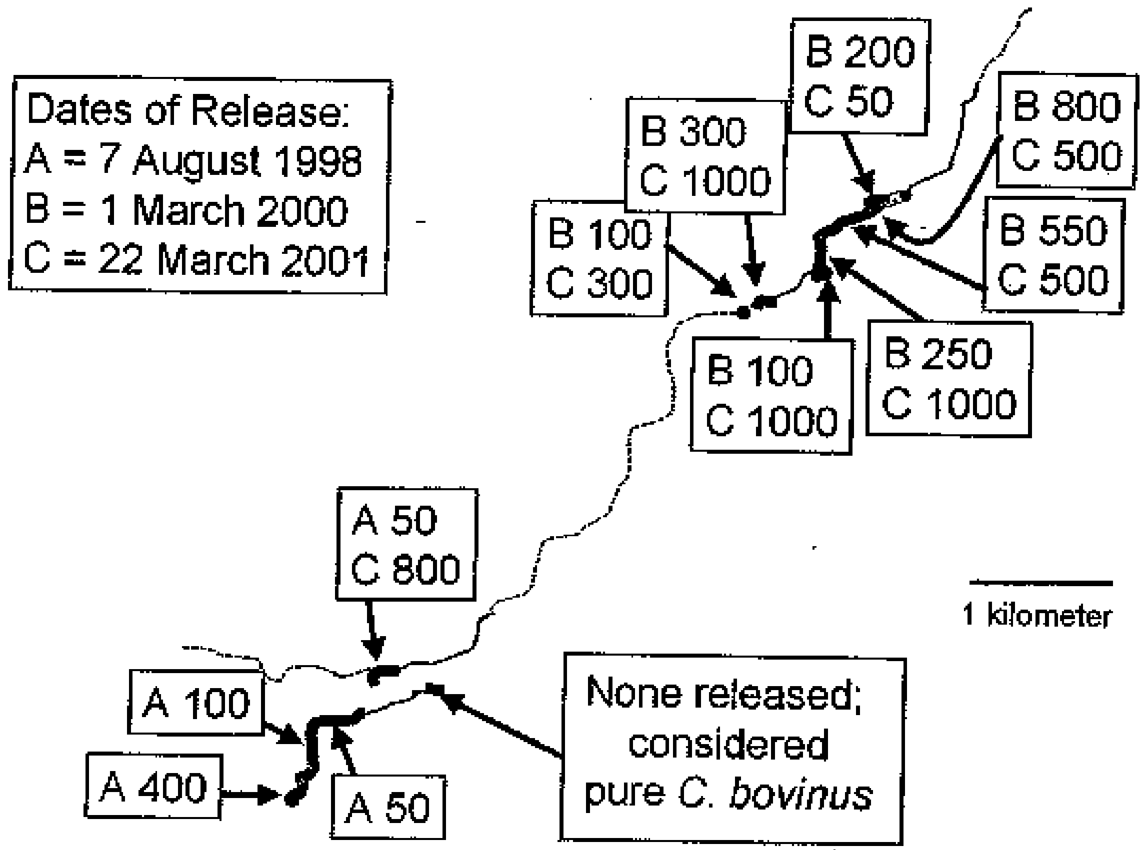


Figure 4. Dates, sites, and numbers released for the releases of the captive DNFH stock of Leon Springs pupfish in Diamond Y Draw. The thick lines indicate areas of the watercourse where pupfish were collected in this survey.

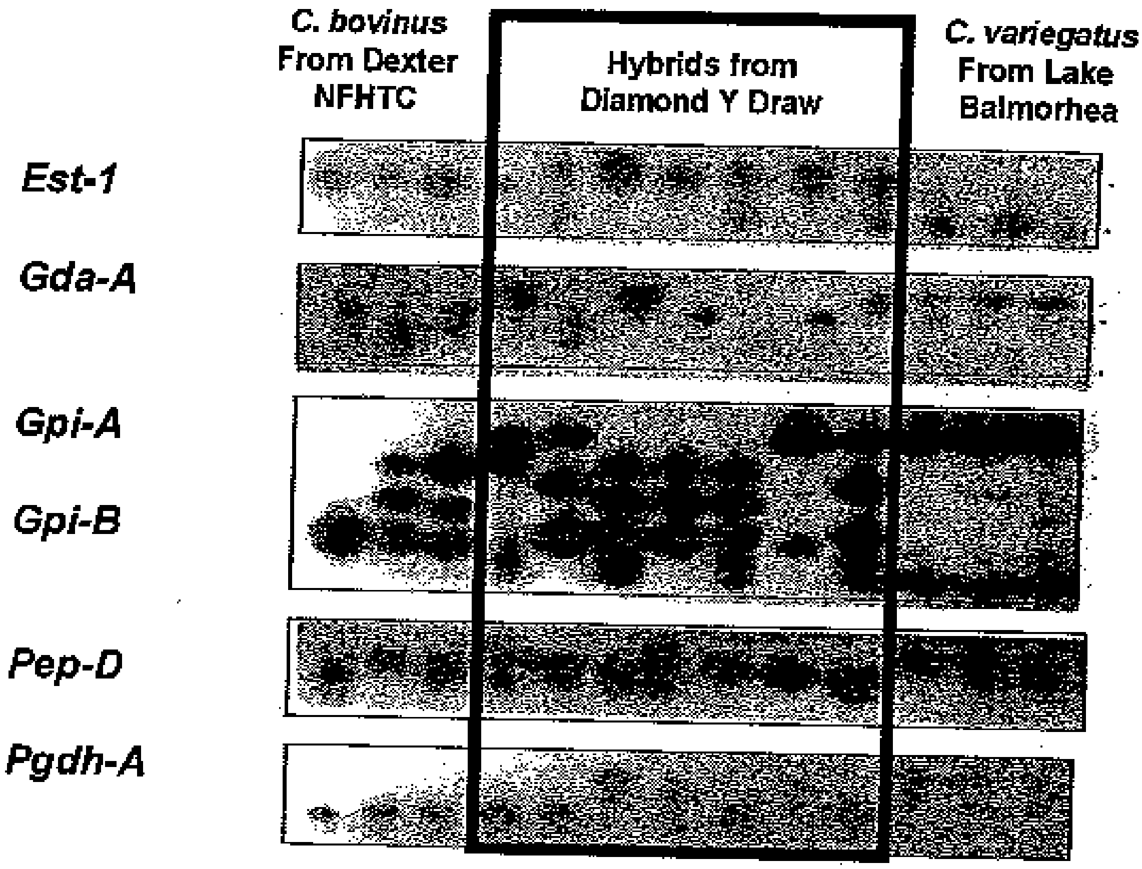


Figure 5. Allozymes used in the genetic survey of pupfish in Diamond Y Draw. From Echelle and Echelle (1997).

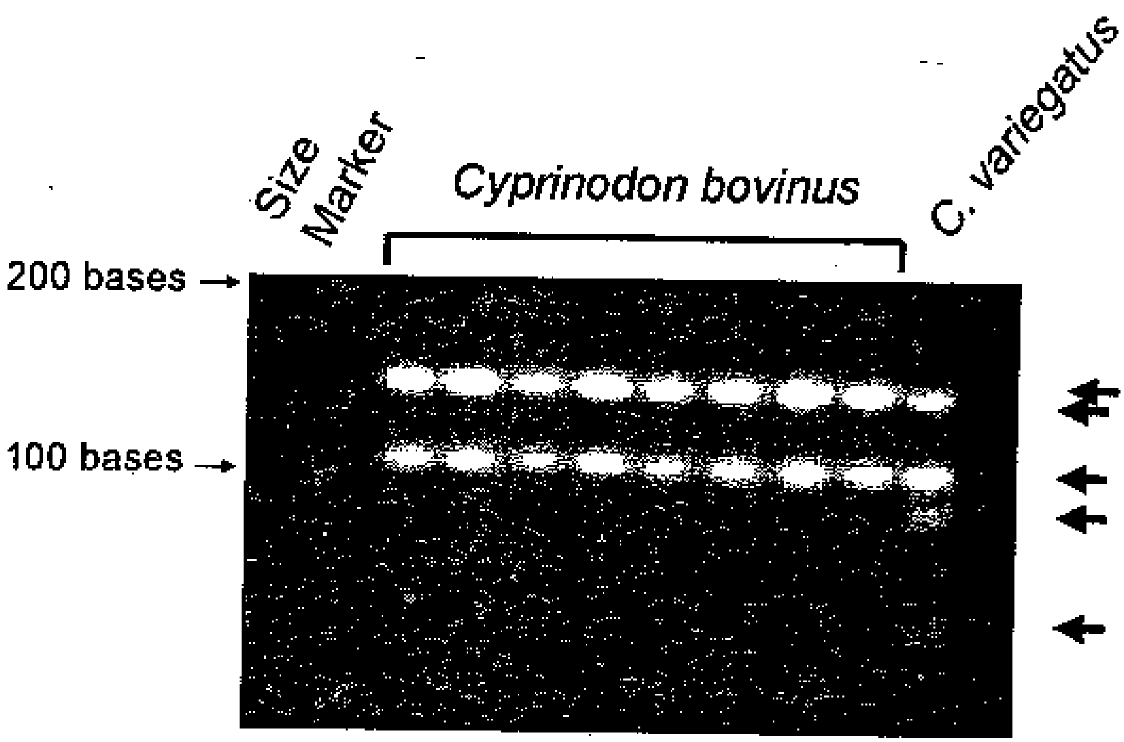


Figure 6. RFLP patterns obtained with the *HinfI* restriction enzyme for an amplified segment of the mitochondrial DNA control region in *Cyprinodon bovinus* and *C. variegatus*. Note two bands in *C. bovinus* and four in *C. variegatus*.

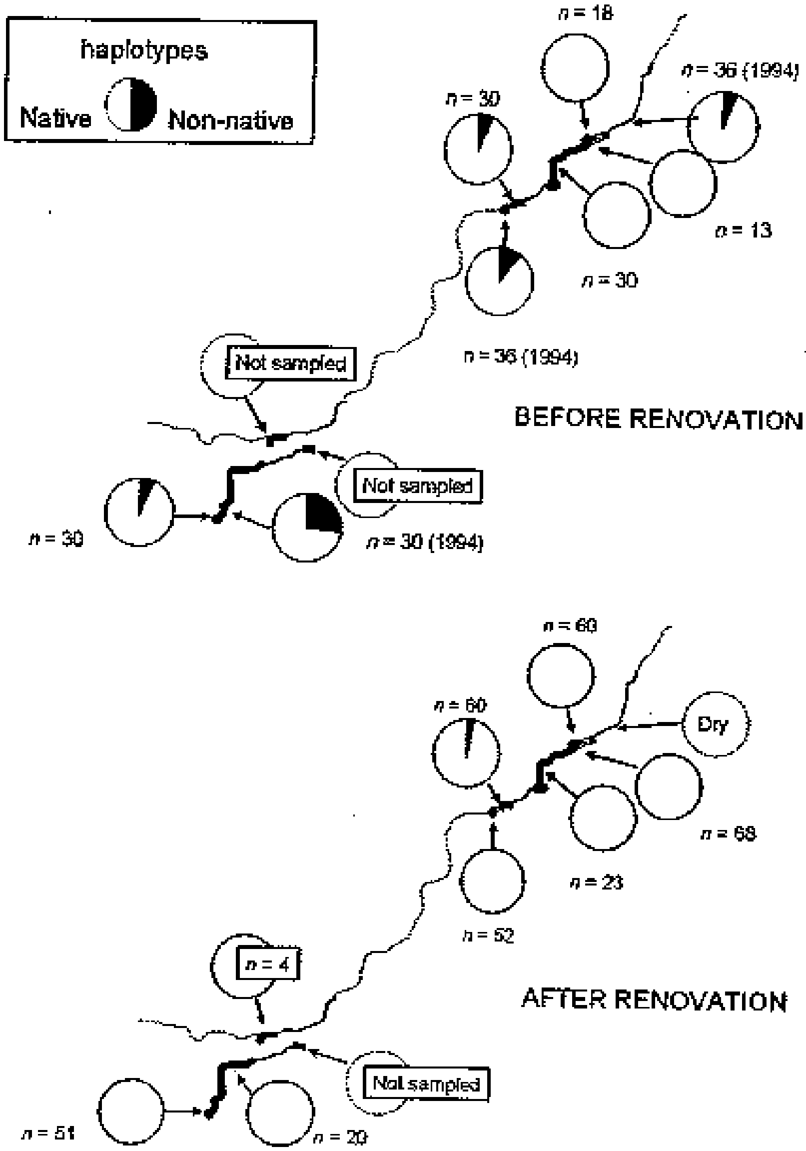


Figure 7. Frequencies of native and non-native pupfish haplotypes for mitochondrial DNA in Diamond Y Draw before and after attempts to restore the native genome.

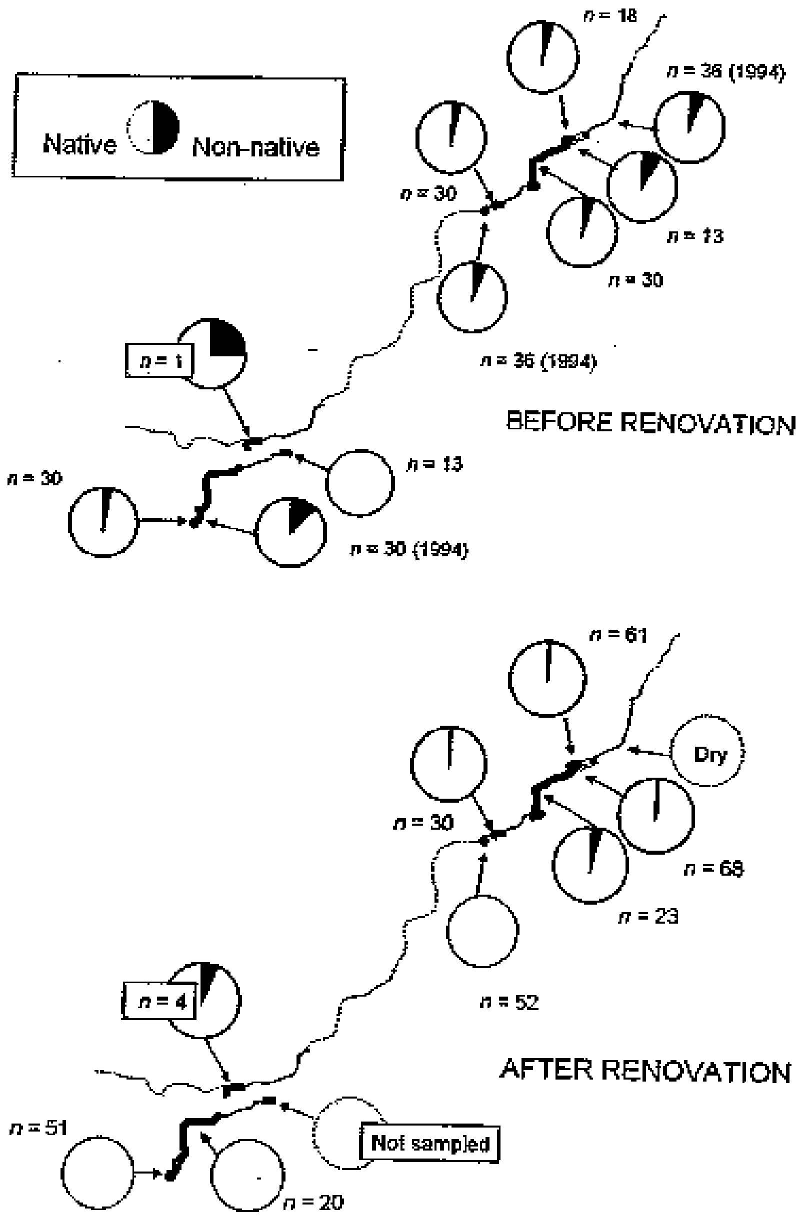


Figure 8. Frequencies of native and non-native alleles for pupfish allozymes in Diamond Y Draw before and after attempts to restore the native genome.

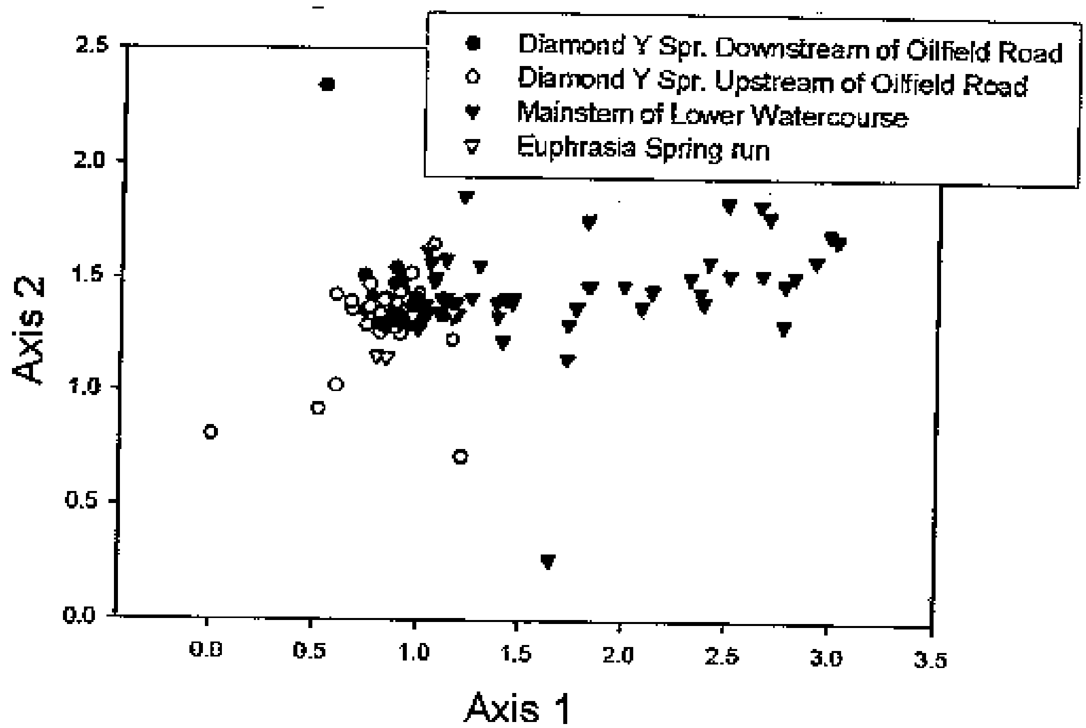


Figure 9. Site scores for the first two DCA axes derived for invertebrate collections in the longitudinal surveys of Diamond Y Draw in 1998 and 1999.

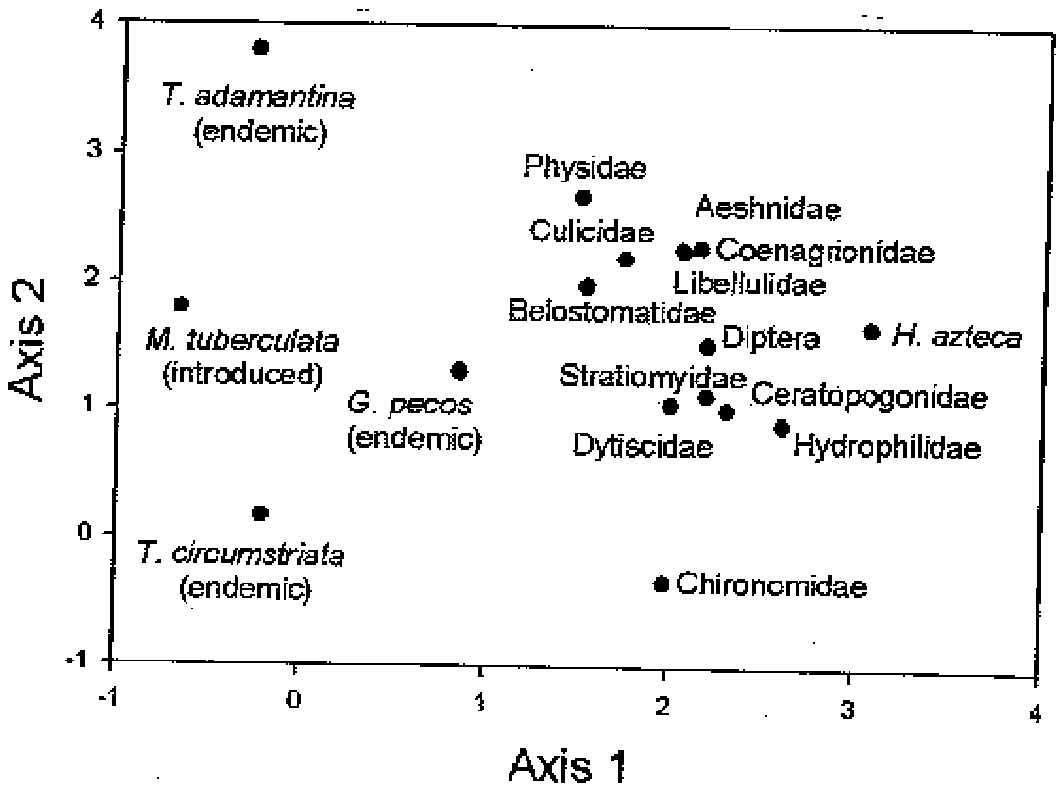


Figure 10. Species scores for the first two DCA axes derived for invertebrate collections in the longitudinal surveys of Diamond Y Draw in 1998 and 1999.

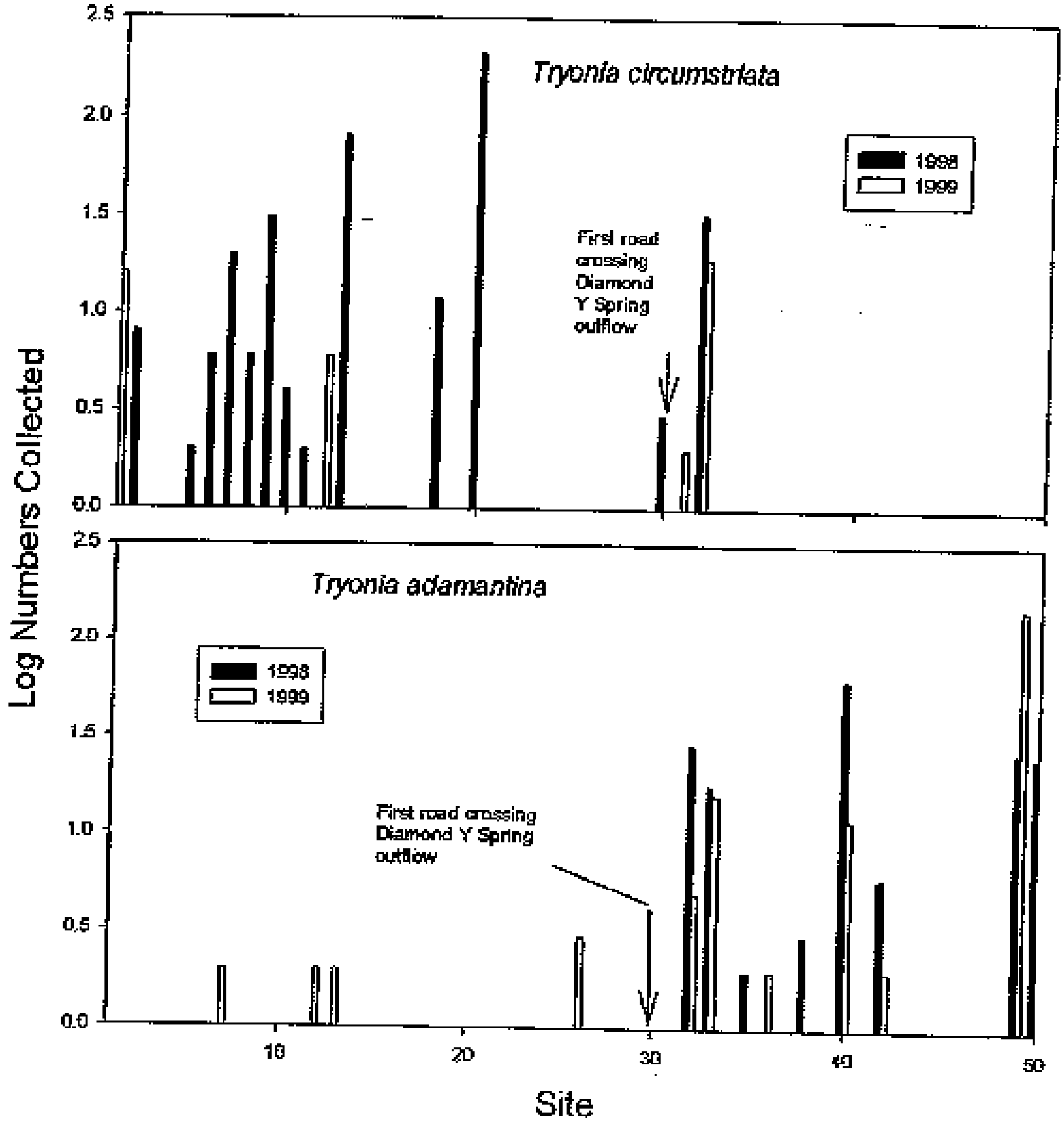


Figure 11. Abundance as log (n+1) of two springsnails in the upper watercourse of Diamond Y Draw for 1998 and 1999. Sites are numbers 1-50 as depicted in Figure 2.

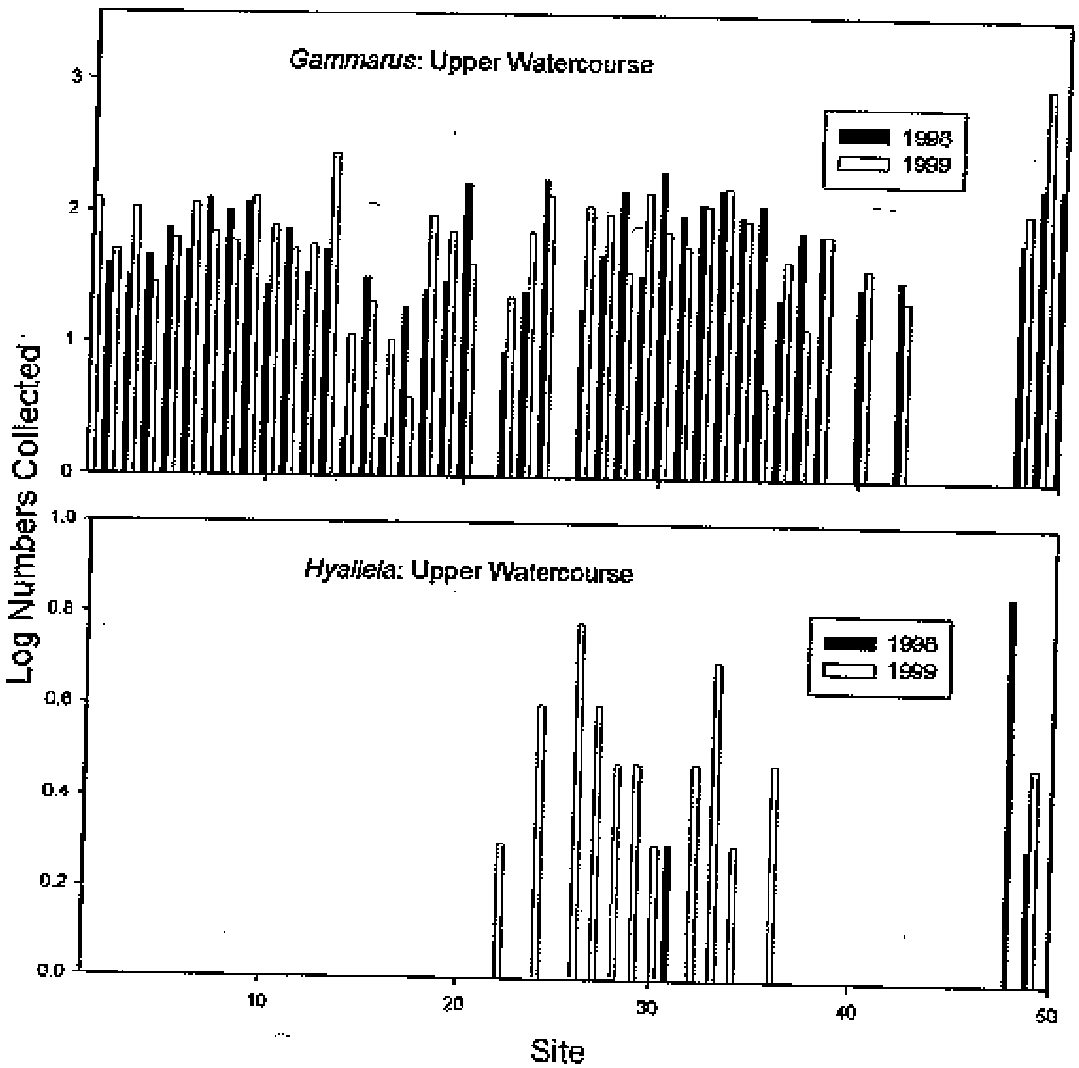


Figure 12. Abundance as log(n+1) of two amphipods in the upper watercourse of diamond Y Draw for 1998 and 1999. Sites are numbers 1-50 as depicted in Figure 2.

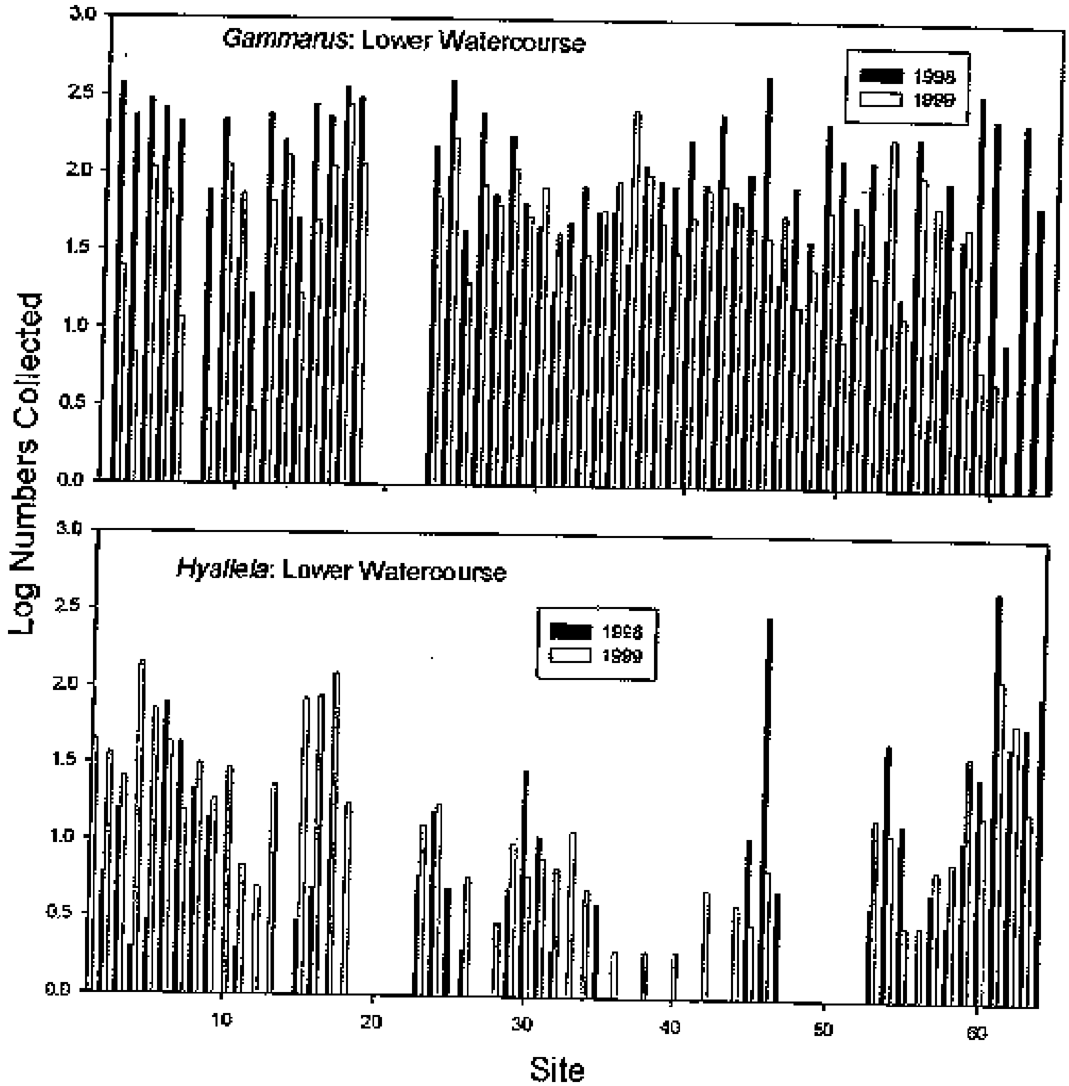


Figure 13. Abundance as log(n+1) of two amphipods in the lower watercourse of diamond Y Draw for 1998 and 1999. Sites are numbers 1-64 as depicted in Figure 3.

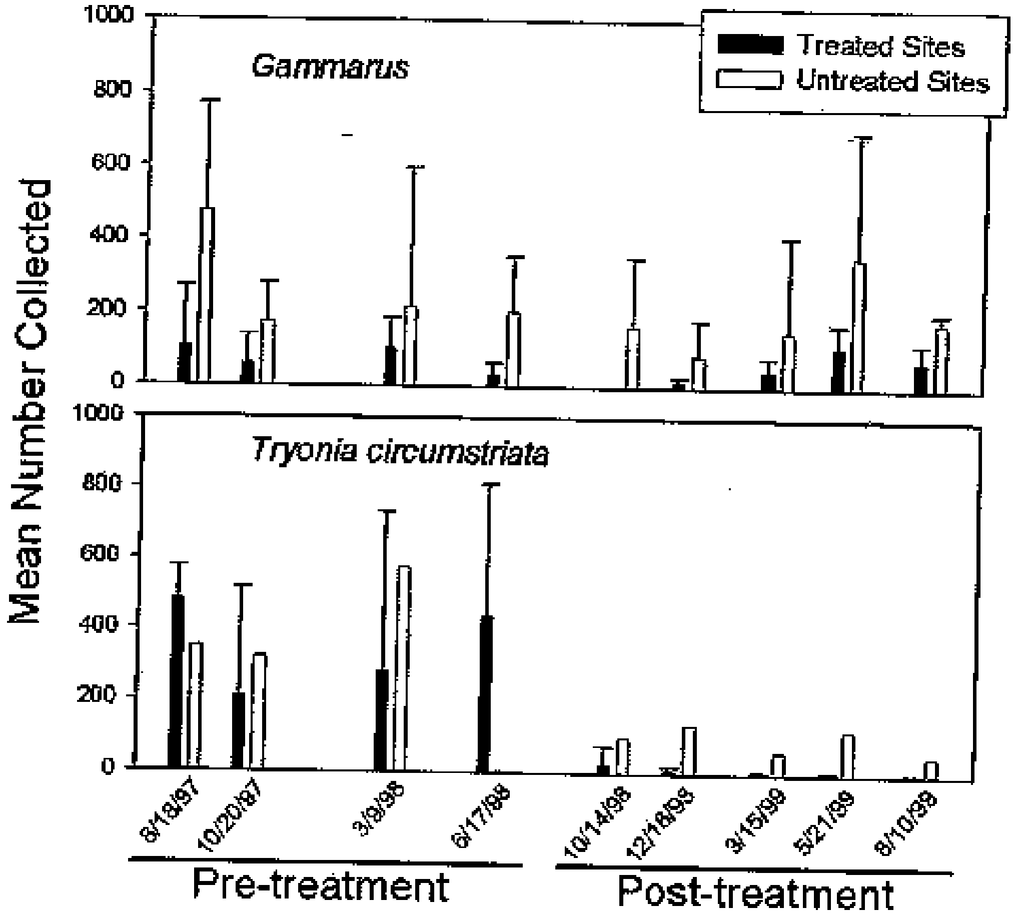


Figure 14. Abundance of two endemic species in the portion of the upper watercourse treated with Antimycin A.

Appendix A. Collection sites, dates, and sample sizes of *Cyprinodon* and *Gambusia nobilis* used in genetic analyses. Post-treatment dates in the respective watercourses are marked with an asterisk. DNFH = Dexter National Fish Hatchery and Technology Center, Dexter, NM; For designations, U = upper watercourse, L = lower watercourse, numbers refer to site numbers in Figures 2 and 3.

| Site | Date | Species | Allozymes (N) | mtDNA (N) |
|---|---|---|---|---|
| Lake Balmorhea, Reeves Co., Texas | 14 Oct. 98<br>5 Sept. 99 | *C. variegatus* | 23 | 6 |
| DNFH | 4 Sept. 99 | *C. bovinus* | 56 | 2 |
| Headpool, Diamond-Y Spring (Fig. 1) | 17-18 Aug. 97 | *G. nobilis* | 25 | – |
| | 5 Sept. 99* | *G. nobilis* | 33 | – |
| | 3 Aug. 98 | *C. bovinus* | 30 | 30 |
| | 5 Sept. 99* | *C. bovinus* | 28 | 28 |
| | 28 Aug. 01* | *C. bovinus* | 23 | 23 |
| 30 m downstream of Diamond Y Spring Headpool (1U) | 17 Aug. 97 | *G. nobilis* | 24 | -- |
| 100 m downstream of Diamond Y Spring Headpool (5U) | 17 Aug. 97 | *G. nobilis* | 24 | – |
| Diamond-Yspring outflow downstream of oilfield road crossing (30U) | 5 Sept. 99* | *C. bovinus* | 20 | 20 |
| Observation Tower (49U) | 18 Aug. 97 | *G. nobilis* | 23 | – |
| | 18 Aug. 97 | *C. bovinus* | 1 | – |
| | 28 Aug. 01* | *C. bovinus* | 4 | 4 |
| Marsh at Fenceline (46U) | 18 Aug. 97 | *G. nobilis* | 22 | – |
| | 18 Aug. 97 | *C. bovinus* | 13 | – |
| Monsanto Pool (1L) | 10 Sept. 00* | *C. bovinus* | 27 | 27 |
| | 27 Aug. 01* | *C. bovinus* | 25 | 25 |
| Lower Monsanto Pool (20L) | 6 Sept. 99 | *C. bovinus* | 14 | 14 |
| | 13-14 Mar. 00 | *C. bovinus* | 16 | 16 |
| | 27 Aug. 01* | *C. bovinus* | 30 | 30 |
| Euphrasia Spring downstream to pipeline crossing (25L-36L) | 17 Aug. 97 | *G. nobilis* | 20 | – |
| | 6 Sept. 99 | *C. bovinus* | 10 | 10 |
| | 13-14 Mar 00 | *C. bovinus* | 20 | 20 |
| | 26 Aug 01* | *C. bovinus* | 23 | 23 |
| Diamond-Y Draw adjacent to John's Pool (45L-a) | 6 Sept 99 | *C. bovinus* | 13 | 13 |
| | 10 Sept. 00* | *C. bovinus* | 38 | 38 |
| | 26 Aug. 01* | *C. bovinus* | 30 | 30 |
| John's Pool and adjacent draw (45L-a&b) | 13-14 March 00 | *C. bovinus* | 18 | 18 |
| John's Pool (45L-b) | 10 Sept. 00* | *C. bovinus* | 36 | 36 |
| | 26 Aug. 01* | *C. bovinus* | 25 | 24 |
| Diamond-Y Draw at Highway 18 bridge crossing (55L) | 6 Sept. 99 | *C. bovinus* | 1 | 1 |

Appendix B. Proteins, presumptive loci, tissue sources, and buffer systems used in the genetic monitoring of *Cyprinodon bovinus*. Enzyme nomenclature follows recommendations of the International Union of Biochemistry (1984). Locus designations follow Buth's (1983) recommendations.

| Protein (EC number) | Locus | Tissue scored | Analytical system[a] |
|---|---|---|---|
| Alcohol dehydrogenase (1.1.1.1) | Adh-A | liver | 1 |
| Esterase (3.1.1.1) | Est-1 | eye | 1 |
| Glucose-6-phosphate isomerase (5.3.1.9) | Gpi-A | eye | 1 |
| | Gpi-B | muscle | 1 |
| Guanine deaminase (3.5.4.3) | Gda-A | liver | 1 |
| Dipeptidase-D (3.4.13.11) | Pep-D[b] | eye | 1 |
| Phosphogluconate dehydrogenase-A (1.1.1.44) | Pgdh-A | eye | 2 |

[a]Analytical systems as follows: (1) after Turner (1983)-- stock solution: 0.9 M Tris-hydroxymethylaminomethane (= "Tris"), 0.5 M boric acid, 0.1 M disodium EDTA, pH 8.6; electrode buffer: 1 vol stock solution + 6.9 vols $H_2O$; gel buffer: 1 vol stock solution + 24 vols $H_2O$; (2) after Stein et al. (1985) except adjust pH with 10 N NaOH-- electrode buffer: 0.1 M Tris, 0.03 M citric acid; gel buffer: 1 vol electrode solution + 6 vols $H_2O$.

[b]Substrate used for this peptidase = phe-pro (Sigma # P-6258)

Appendix C. Proteins, presumptive loci, tissue sources, and buffer systems used in the initial genetic survey of *Gambusia nobilis*. Enzyme nomenclature follows recommendations of the International Union of Biochemistry (1984). Locus designations follow Buth's (1983) recommendations.

| Protein (EC number) | Locus | Tissue scored | Analytical system[a] |
|---|---|---|---|
| Adenylate kinase (EC 2.7.4.3) | Ak-A | eye | 3 |
| Alcohol dehydrogenase (1.1.1.1) | Adh-A* | liver | 1 |
| Aspartate aminotransferase (EC 2.6.1.1) | m-Aat-A | eye | 2 |
| | s-Aat-A | liver | 1 |
| Creatine kinase (EC 2.7.3.2) | Ck-A | eye | 1 |
| | Ck-B | eye | 1 |
| | Ck-C | eye | 1 |
| Fumarate hydratase (EC 4.2.1.2) | Fum-A | liver | 1 |
| Glucose-6-phosphate isomerase (5.3.1.9) | Gpi-A* | eye | 1 |
| | Gpi-B | eye | 1 |
| Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) | Ga3pdh-B | eye | 2 |
| Isocitrate dehydrogenase (EC 1.1.1.42) | m-Icdh-A | eye | 2 |
| | s-Icdh-A | liver | 2 |
| L-Lactate dehydrogenase (EC 1.1.1.27) | Ldh-A | eye | 1 |
| | Ldh-B | eye | 1 |
| | Ldh-C | eye | 1 |
| Malate dehydrogenase (NAD) (EC 1.1.1.37) | m-Mdh-A | eye | 3 |
| | s-Mdh-A | eye | 3 |
| | s-Mdh-B | eye | 3 |
| Malate dehydrogenase ($NADP^+$) (EC 1.1.1.40) | mMdhp-A | eye | 3 |
| Phosphoglucomutase (5.4.2.2) | Pgm-A | eye | 2 |
| Phosphogluconate dehydrogenase-A (1.1.1.44) | Pgdh-A | eye | 1 |
| Superoxide dismutase (EC 1.15.1.1) | s-Sod-A | liver | 1 |

[a]Analytical systems as follows: (1) after Turner (1983)-- stock solution: 0.9 M Tris-hydroxymethylaminomethane (= "Tris"), 0.5 M boric acid, 0.1 M disodium EDTA, pH 8.6; electrode buffer: 1 vol stock solution + 6.9 vols $H_2O$; gel buffer: 1 vol stock solution + 24 vols $H_2O$; (2) after Stein et al. (1985) except adjust pH with 10 N NaOH-- electrode buffer: 0.1 M Tris, 0.03 M citric acid, pH 7.5; gel buffer: 1 vol electrode solution + 6 vols $H_2O$; (3) after Shaw and Prasad (1970)--electrode buffer: 0.69 M Tris, 0.16 M citric acid, ph8.0; gel buffer: 0.02 M Tris, 0.005 M citric acid, pH 8.0

Appendix D. Genotype and mtDNA haplotype frequencies and mean heterozygosity (*H*) in *C. variegatus* from Lake Balmorhea, *C. bovinus* from DNFH, and 19 pupfish samples from upper watercourse, Diamond-Y Draw. Asterisks = post-treatment dates. For mtDNA, "B" and "V" = haplotypes, respectively, of *C. bovinus* and *C. variegatus*. Numbers followed by U = site numbers in upper watercourse (Fig. 2), L = site numbers in lower watercourse (Fig. 3). 45aL = 45 on Fig. 3, 45bL = John's Pool in Fig. 3, 45a-b = a combined sample from these locations.

| | Reference samples | | Headpool, Diamond Y Spring | | | 30U | 49U | | 46U |
|---|---|---|---|---|---|---|---|---|---|
| Locus | *C. variegatus* (24) | *C. bovinus* (56) | (8/98) (30) | (9/99) (28) | (8/01)* (23) | (9/99)* (20) | (6/98) (1) | (8/01)* (4) | (8/97) (13) |
| Adh-A | | aa(2) | | | | | — | aa(1) | |
| | | ab(8) | | ab(7) | ab(11) | ab(5) | — | | |
| | bb(22) | bb(45) | bb(30) | bb(21) | bb(12) | bb(15) | — | bb(3) | bb(13) |
| Est-1 | | aa(56) | aa(25) | aa(28) | aa(23) | aa(20) | | aa(2) | aa(13) |
| | | | ab(5) | | | | | ab(2) | |
| | bb(23) | | | | | | bb(1) | | |
| Gda-A | aa(23) | | | | | | | | |
| | | | ab(1) | | | | | | |
| | | bb(40) | bb(29) | bb(22) | bb(16) | bb(12) | | bb(4) | bb(13) |
| | | bd(13) | | bd(6) | bd(5) | bd(7) | | | |
| | | dd(1) | | | dd(2) | dd(1) | dd(1) | | |
| Gpi-A | aa(11) | | | | | | | | |
| | ab(1) | | | | | | | | |
| | ac(8) | | | | | | | | |
| | cc(2) | cc(2) | | cc(4) | cc(2) | | cc(1) | cc(1) | |
| | | cd(22) | cd(10) | cd(14) | cd(11) | cd(9) | | cd(2) | cd(2) |
| | | dd(32) | dd(20) | dd(10) | dd(10) | dd(11) | | dd(1) | dd(11) |
| Gpi-B | | aa(56) | aa(29) | aa(28) | aa(23) | aa(20) | aa(1) | aa(4) | aa(13) |
| | | | ab(1) | | | | | | |
| | bb(22) | | | | | | | | |
| Pep-D | aa(22) | | | | | | | | |
| | | | ab(1) | | | | | | |
| | | bb(20) | bb(5) | bb(14) | bb(7) | bb(5) | | bb(2) | bb(2) |
| | | bc(29) | bc(19) | bc(8) | bc(12) | bc(11) | | bc(2) | bc(5) |
| | | cc(7) | cc(4) | cc(6) | cc(4) | cc(4) | cc(1) | | cc(6) |
| Pgdh-A | aa(24) | | | | | | | | |
| | | | ab(1) | | | | | | |
| | | bb(56) | bb(29) | bb(28) | bb(23) | bb(20) | bb(1) | bb(4) | bb(13) |
| *H* | 0.063 | 0.182 | 0.152 | 0.196 | 0.235 | 0.207 | 0.000 | 0.114 | 0.088 |
| mtDNA | | B(2) | B(28) | B(28) | B(23) | B(20) | — | B(4) | |
| | V(6) | | V(2) | | | | — | | |

Appendix D continued (2 of 3)

| Locus | 1L | | 20L | | 25L-36L | | 45aL | | | 45a-bL | 45bL | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 9/00* (27) | 8/01* (25) | 99/00 (30) | 8/01* (30) | 99/00 (30) | 8/01* (23) | 9/99 (13) | 9/00* (38) | 8/01* (30) | 3/00 (18) | 9/00* (36) | 8/01* (25) |
| Adh-A | aa(2) | | | aa(2) | | | | | aa(1) | | | |
| | ab(10) | ab(7) | | ab(7) | ab(1) | ab(5) | ab(1) | ab(13) | ab(10) | | ab(13) | ab(6) |
| | bb(15) | bb(18) | bb(30) | bb(21) | bb(29) | bb(18) | bb(12) | bb(25) | bb(19) | bb(18) | bb(23) | bb(19) |
| Est-1 | aa(27) | aa(25) | aa(25) | aa(29) | aa(25) | aa(19) | aa(9) | aa(38) | aa(28) | aa(13) | aa(36) | aa(24) |
| | | | ab(5) | ab(1) | ab(5) | ab(4) | ab(4) | | ab(2) | ab(5) | | ab(1) |
| Gda-A | | | ab(1) | | ab(2) | ab(1) | ab(3) | | | | | |
| | bb(18) | bb(19) | bb(20) | bb(19) | bb(20) | bb(16) | bb(9) | bb(16) | bb(20) | bb(14) | bb(22) | bb(18) |
| | | | bc(7) | | bc(5) | | | bc(13) | | bc(2) | | |
| | bd(8) | bd(6) | | bd(8) | bd(2) | bd(6) | bd(1) | bd(7) | bd(8) | bd(1) | bd(14) | bd(7) |
| | | | | | | | | | | cc(1) | | |
| | | | | | cd(1) | | | cd(2) | | | | |
| | dd(1) | | | dd(3) | | | | | dd(2) | | | |
| Gpi-A | | | aa(1) | | | | | | | | | |
| | | | | | ac(1) | ac(1) | ac(1) | | | | | |
| | | | ad(3) | ad(1) | ad(4) | | ad(1) | | ad(1) | ad(1) | | ad(1) |
| | cc(1) | cc(3) | cc(2) | cc(2) | | | | cc(3) | | cc(2) | cc(2) | |
| | cd(10) | cd(7) | cd(8) | cd(11) | cd(12) | cd(8) | cd(5) | cd(9) | cd(12) | cd(5) | cd(13) | cd(9) |
| | dd(16) | dd(15) | dd(16) | dd(16) | dd(13) | dd(14) | dd(6) | dd(26) | dd(17) | dd(10) | dd(21) | dd(15) |
| Gpi-B | aa(27) | aa(25) | aa(28) | aa(30) | aa(28) | aa(22) | aa(12) | aa(38) | aa(30) | aa(17) | aa(36) | aa(25) |

ab(2) ab(2) ab(1) ab(1) ab(1)

## Appendix D continued (3 of 3)

| | 1L | | 20L | | 25L-36L | | 45aL | | | 45a-bL | 45bL | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | 9/00* (27) | 8/01* (25) | 99/00 (30) | 8/01* (30) | 99/00 (30) | 8/01* (23) | 9/99 (13) | 9/00* (38) | 8/01* (30) | 3/00 (18) | 9/00* (36) | 8/01* (25) |
| Pep-D | | | ab(1) | | ab(1) | ab(1) | ab(1) | | | | | |
| | | | | | ac(1) | | | | | | ac(1) | |
| | bb(12) | bb(7) | bb(14) | bb(9) | bb(11) | bb(7) | bb(8) | bb(13) | bb(13) | bb(12) | bb(14) | bb(10) |
| | bc(11) | bc(16) | bc(11) | bc(15) | bc(14) | bc(10) | bc(3) | bc(14) | bc(10) | bc(6) | bc(15) | bc(11) |
| | cc(4) | cc(2) | cc(4) | cc(6) | cc(3) | cc(5) | cc(1) | cc(11) | cc(7) | | cc(6) | cc(4) |
| Pgdh-A | | | | | | | aa(1) | | | | | |
| | | | ab(3) | ab(1) | ab(3) | ab(1) | | | ab(1) | ab(1) | | |
| | bb(27) | bb(25) | bb(27) | bb(29) | bb(27) | bb(22) | bb(11) | bb(38) | bb(29) | bb(17) | bb(36) | bb(25) |
| | | | | | | | bc(1) | | | | | |
| *H* | 0.217 | 0.192 | 0.212 | 0.235 | 0.235 | 0.229 | 0.255 | 0.228 | 0.227 | 0.189 | 0.212 | 0.188 |
| mtDNA | B(27) | B(25) | B(28) | B(30) | B(30) | B(23) | B(13) | B(38) | B(30) | B(18) | B(36) | B(24) |
| | | | V(2) | | | | | | | | | |

Appendix E. Genotype frequencies for the polymorphic loci in *Gambusia nobilis* from Diamond-Y Draw. Samples include six from the Upper Water Course, five collected 17-18 August 1997 (pre-treatment = "pre"), and one collected 5 September 1999 (post-treatment = "post"), and one from the Lower Watercourse, collected 17 August 1997 (pre-treatment). Samples sizes are in parentheses. Asterisk signifies the post-treatment sample from the headpool of Diamond Y Spring.

| | Upstream Watercourse | | | | | | Downstream Watercourse |
|---|---|---|---|---|---|---|---|
| Locus | Headpool of Diamond-Y Spring | | 30 m downstream of headpool(1U) | 100 m downstream of headpool (5U) | Near observation platform (49U) | Marsh at fenceline (46U) | Euphrasia Spring (25L) |
| | n = 25 | n = 33* | n = 24 | n = 24 | n = 23 | n = 22 | n = 20 |
| Adh-A | ab(1) | ab(2) | ab(2) | ab(1) | ab(2) | ab(3) | ab(3) |
| | bb(22) | bb(31) | bb(22) | bb(23) | bb(21) | bb(19) | bb(17) |
| Gpi-A | ab(1) | ab(3) | ab(4) | ab(1) | ab(1) | ab(1) | |
| | ac(5) | | ac(2) | ac(3) | ac(2) | ac(2) | ac(1) |
| | | bb(1) | | | | | |
| | bc(5) | bc(13) | bc(3) | bc(1) | bc(5) | bc(5) | bc(8) |
| | cc(14) | cc(16) | cc(15) | cc(19) | cc(14) | cc(14) | cc(10) |

Appendix F. Collections of six invertebrates of concern during 1997-1999. Numbers for each year are arranged as follows: number of samples at the site/number producing the species (number of individuals of the species) total number of invertebrates collected.

*Gammarus pecos*

| Site | 1997 | 1998 | 1999 |
|---|---|---|---|
| Upper Watercourse | | | |
| Longitudinal Survey, Diamond Y Spring and Marsh | | | |
| 1 | — | 1(31)282 | 1(122)152 |
| 2 | — | 1(38)64 | 1(50)65 |
| 3 | — | 1(31)40 | 1(108)118 |
| 4 | — | 1(45)62 | 1(28)30 |
| 5 | — | 1(75)90 | 1(63)67 |
| 6 | — | 1(50)83 | 1(117)183 |
| 7 | — | 1(127)188 | 1(70)102 |
| 8 | — | 1(102)129 | 1(60)63 |
| 9 | — | 1(120)193 | 1(131)136 |
| 10 | — | 1(27)36 | 1(80)82 |
| 11 | — | 1(75)78 | 1(51)52 |
| 12 | — | 1(33)35 | 1(56)67 |
| 13 | — | 1(51)132 | 1(281)288 |
| 14 | — | 1(1)2 | 1(11)19 |
| 15 | — | 1(31)37 | 1(20)26 |
| 16 | — | 1(1)5 | 1(10)15 |
| 17 | — | 1(18)18 | 1(3)8 |
| 18 | — | 1(25)54 | 1(94)105 |
| 19 | — | 1(29)33 | 1(71)77 |
| 20 | — | 1(169)388 | 1(40)41 |
| 21 | — | 1(10)11 | 1(168)185 |
| 22 | — | 1(8)11 | 1(22)32 |
| 23 | — | 1(24)26 | 1(72)76 |
| 24 | — | 1(184)187 | 1(139)149 |
| 25 | — | 1(21)24 | 1(40)56 |
| 26 | — | 1(18)26 | 1(115)142 |
| 27 | — | 1(48)56 | 1(99)122 |
| 28 | — | 1(151)153 | 1(36)51 |
| 29 | — | 1(33)49 | 1(145)161 |
| 30 | — | 1(215)220 | 1(73)80 |
| 31 | — | 1(98)102 | 1(57)60 |
| 32 | — | 1(122)189 | 1(120)160 |
| 33 | — | 1(157)177 | 1(159)182 |
| 34 | — | 1(98)101 | 1(90)93 |
| 35 | — | 1(121)127 | 1(4)6 |
| 36 | — | 1(22)25 | 1(45)49 |
| 37 | — | 1(74)80 | 1(13)14 |
| 38 | — | 1(71)74 | 1(70)75 |
| 39 | — | 1(34)50 | 1(52)60 |
| 40 | — | 1(27)93 | 1(39)51 |
| 41 | — | 1(4)9 | 1(49)64 |
| 42 | — | 1(32)37 | 1(22)26 |
| 43 | — | 1(42)118 | 1(22)26 |
| 44 | — | 1(50)72 | 1(37)57 |
| 45 | — | 1(3)3 | 1(27)29 |
| 46 | — | 1(20)25 | 1(48)141 |
| 47 | — | 1(5)5 | 1(58)93 |
| 48 | — | 1(64)73 | 1(108)118 |
| Upper Watercourse, continued | | | |
| Spring at Observation Platform to Confluence with Leon Creek | | | |
| 49 | — | 7/7(172)211 | 7/7(986)1114 |
| Downstream of Confluence with Leon Creek | | | |
| 50 | — | 8/8(172)212 | 6/6(465)600 |
| Seasonal Sample Sites | | | |
| 1 | 2/1(12)19 | 4/3(128)167 | 3/3(332)341 |
| 2 | 2/1(54)125 | 4/3(79)178 | 3/3(101)108 |
| 3 | 2/2(79)1014 | 4/3(120)1832 | 3/3(189)358 |
| 4 | 2/4(349)1191 | 4/4(280)717 | 3/3(332)402 |
| 5 | 2/5(718)875 | 4/4(1435)1491 | 3/3(1197)1230 |
| Seeps | | | |
| C | — | 1(30)79 | — |
| D | — | 1(13)27 | — |
| E | — | 1(26)45 | — |
| F | — | 1(35)63 | — |
| I | — | 1(18)43 | — |

| | | | |
|---|---|---|---|
| Lower Watercourse | | | |
| Longitudinal survey | | | |
| 1 | — | 1(106)128 | 1(0)51 |
| 2 | — | 1(372)378 | 1(24)77 |
| 3 | — | 1(236)254 | 1(6)45 |
| 4 | — | 1(304)307 | 1(108)339 |
| 5 | — | 1(259)261 | 1(75)160 |
| 6 | — | 1(216)310 | 1(11)59 |
| 8 | — | 1(76)104 | 1(2)48 |
| 9 | — | 1(220)236 | 1(114)136 |
| 10 | — | 1(27)40 | 1(74)111 |
| 11 | — | 1(16)47 | 1(2)11 |
| 12 | — | 1(244)261 | 1(65)72 |
| 13 | — | 1(162)162 | 1(129)160 |
| 14 | — | 1(51)51 | 1(16)17 |
| 15 | — | 1(279)285 | 1(50)146 |
| 16 | — | 1(232)239 | 1(111)224 |
| 17 | — | 1(363)366 | 1(280)418 |
| 18 | — | 1(310)311 | 1(115)163 |
| 19 | — | 1(72)73 | 1(43)65 |
| 20 | — | 1(53)54 | 1(68)159 |
| 21 | — | 1(117)117 | 1(81)325 |
| 22 | — | 1(100)100 | 1(66)112 |
| 25 | — | 1(43)51 | 1(19)21 |
| 26 | — | 1(252)254 | 1(85)101 |
| 27 | — | 1(74)74 | 1(63)69 |
| 28 | — | 1(177)183 | 1(109)113 |
| 29 | — | 1(66)73 | 1(54)68 |
| 30 | — | 1(46)103 | 1(83)89 |
| 31 | — | 1(17)34 | 1(42)56 |
| 32 | — | 1(49)58 | 1(22)30 |
| 33 | — | 1(85)86 | 1(30)45 |
| 34 | — | 1(58)67 | 1(59)69 |
| 35 | — | 1(57)61 | 1(92)100 |
| 36 | — | 1(26)26 | 1(261)267 |
| 37 | — | 1(116)121 | 1(101)108 |
| 38 | — | 1(93)94 | 1(49)59 |
| 39 | — | 1(86)93 | 1(31)41 |
| 40 | — | 1(171)197 | 1(54)74 |
| 41 | — | 1(89)103 | 1(81)89 |
| 42 | — | 1(252)279 | 1(86)101 |
| 43 | — | 1(68)101 | 1(64)74 |
| 44 | — | 1(103)175 | 1(46)62 |
| Lower Watercourse, continued | | | |
| Longitudinal Survey | | | |
| 45 | — | 1(10)487 | 1(2)56) |
| 46 | — | 1(20)341 | 1(56)80 |
| 47 | — | 1(85)150 | 1(14)31 |
| 48 | — | 1(38)55 | 1(25)33 |
| 49 | — | 1(224)238 | 1(59)64 |
| 50 | — | 1(129)139 | 1(8)12 |
| 51 | — | 1(66)70 | 1(51)57 |
| 52 | — | 1(127)150 | 1(22)24 |
| 53 | — | 1(40)51 | 1(178)195 |
| 54 | — | 1(16)70 | 1(12)41 |
| 55 | — | 1(182)201 | 1(103)109 |
| 56 | — | 1(2)2 | 1(65)68 |
| 57 | — | 1(96)101 | 1(19)28 |
| 58 | — | 1(40)42 | 1(48)60 |
| 59 | — | 1(349)370 | 1(5)47 |
| 60 | — | 1(244)287 | 1(4)27 |
| 61 | — | 1(8)494 | 1(0)150 |
| 62 | — | 1(228)332 | 1(0)83 |
| 63 | — | 1(67)179 | 1(0)21 |
| 64 | — | 1(7)147 | — |
| Euphrasia Spring | | | |
| 23 | — | 1(152)161 | 1(71)107 |
| 24 | — | 1(400)634 | 1(170)303 |
| 7* | 2/2(958)1973 | 4/4(482)1178 | 3/3(703)1228 |
| Seasonal Sample Sites | | | |
| 8 | 2/2(265)321 | 4/2(101)498 | 3/3(189)813 |

*Also a Seasonal Sample Site

| *Hyallela azteca* | | | |
|---|---|---|---|
| Site | 1997 | 1998 | 1999 |
| Upper Watercourse | | | |
| Longitudinal Survey, Diamond Y Spring Run and Marsh | | | |
| 21 | — | 1(0)11 | 1(5)185 |
| 22 | — | 1(0)11 | 1(1)32 |
| 24 | — | 1(0)187 | 1(3)149 |
| 25 | — | 1(0)24 | 1(2)56 |
| 26 | — | 1(0)26 | 1(5)142 |
| 27 | — | 1(0)56 | 1(3)122 |
| 28 | — | 1(0)153 | 1(2)51 |
| 29 | — | 1(0)49 | 1(2)161 |
| 30 | — | 1(0)220 | 1(1)80 |
| 31 | — | 1(1)102 | 1(0)60 |
| 32 | — | 1(0)189 | 1(2)160 |
| 33 | — | 1(0)177 | 1(4)182 |
| 34 | — | 1(0)101 | 1(1)93 |
| 36 | — | 1(0)25 | 1(2)49 |
| 39 | — | 1(0)50 | 1(1)60 |
| 41 | — | 1(0)9 | 1(3)64 |
| 44 | — | 1(1)72 | 1(0)57 |
| 48 | — | 1(6)73 | 1(0)118 |
| Spring at Observation Platform, to Leon Creek | | | |
| 49 | — | 7/1(1)211 | 7/1(2)1114 |
| Confluence with Leon Creek | | | |
| 50 | — | 8(0)212 | 6(0)600 |
| Seasonal Sample Sites | | | |
| 1 | 2/1(3)19 | 4/1(9)167 | 3(0)341 |
| 2 | 2/1(52)125 | 4/1(1)178 | 3(0)108 |
| 3 | 2/2(11)1014 | 4(0)1832 | 3/1(1)358 |
| 4 | 2(0)1191 | 4/1(2)717 | 3/1(12)402 |
| 5 | 2/2(6)875 | 4/1(2)1491 | 3/3(7)1230 |
| Seeps | | | |
| A | — | 1(40)63 | — |
| B | — | 1(23)36 | — |
| D | — | 1(3)27 | — |
| E | — | 1(5)45 | — |
| F | — | 1(9)63 | — |
| I | — | 1(2)43 | — |
| Lower Watercourse | | | |
| Longitudinal Survey | | | |
| 1 | — | 1(6)128 | 1(44)51 |
| 2 | — | 1(5)378 | 1(36)77 |
| 3 | — | 1(5)125 | 1(25)45 |
| 4 | — | 1(1)307 | 1(144)339 |
| 5 | — | 1(2)261 | 1(71)160 |
| 6 | — | 1(79)310 | 1(43)89 |
| 7 | — | 1(4)147 | 1(15)22 |
| 8 | — | 1(21)104 | 1(3)148 |
| 9 | — | 1(13)236 | 1(18)136 |
| 10 | — | 1(0)40 | 1(29)111 |
| 11 | — | 1(1)47 | 1(6)11 |
| 12 | — | 1(0)761 | 1(4)72 |
| 13 | — | 1(0)162 | 1(72)160 |
| 15 | — | 1(2)285 | 1(34)146 |
| 16 | — | 1(4)239 | 1(11)224 |
| 17 | — | 1(0)366 | 1(23)418 |
| 18 | — | 1(0)311 | 1(7)163 |
| 19 | — | 1(0)73 | 1(18)65 |
| 20 | — | 1(0)54 | 1(89)159 |
| 21 | — | 1(0)117 | 1(236)325 |
| 23 | — | 1(0)100 | 1(42)112 |
| 25 | — | 1(4)51 | 1(0)21 |
| 26 | — | 1(1)254 | 1(5)103 |
| 28 | — | 1(0)183 | 1(2)113 |
| 29 | — | 1(4)73 | 1(9)68 |
| 30 | — | 1(29)103 | 1(5)89 |
| 31 | — | 1(10)34 | 1(7)56 |
| 32 | — | 1(1)58 | 1(6)30 |
| 33 | — | 1(0)85 | 1(11)45 |
| 34 | — | 1(0)67 | 1(4)69 |
| 35 | — | 1(3)61 | 1(0)100 |
| 36 | — | 1(0)26 | 1(1)267 |
| 38 | — | 1(0)84 | 1(1)59 |
| 40 | — | 1(0)197 | 1(1)74 |
| 42 | — | 1(0)279 | 1(4)101 |
| 44 | — | 1(0)175 | 1(3)62 |
| 45 | — | 1(10)487 | 1(2)56 |
| 46 | — | 1(300)341 | 1(6)80 |
| 53 | — | 1(3)51 | 1(14)195 |
| 54 | — | 1(46)70 | 1(11)41 |
| 55 | — | 1(13)201 | 1(2)109 |
| 56 | — | 1(0)2 | 1(2)68 |
| 57 | — | 1(4)101 | 1(6)28 |
| 58 | — | 1(2)42 | 1(7)60 |
| 59 | — | 1(10)370 | 1(38)47 |
| 60 | — | 1(27)287 | 1(15)27 |
| 61 | — | 1(459)494 | 1(124)150 |
| 62 | — | 1(45)332 | 1(63)83 |
| 63 | — | 1(60)179 | 1(16)21 |
| 64 | — | 1(97)147 | — |

| | | | |
|---|---|---|---|
| Euphrasia Spring | | | |
| 23 | — | 1(5)161 | 1(12)107 |
| 24 | — | 1(15)634 | 1(17)303 |
| 7* | 2/1(32)1973 | 4/4(119)1178 | 3/3(92)1228 |
| Seasonal Sample Sites | | | |
| 6 | 2/1(7)47 | 4(0)39 | 3/1(1)18 |
| 8 | 2/2(11)321 | 4/4(306)498 | 3/3(499)813 |

*Also a Seasonal Sample Site

| *Tryonia adamantina* | | | |
|---|---|---|---|
| Site | 1997 | 1998 | 1999 |
| Upper Watercourse | | | |
| Longitudinal survey, Diamond Y Spring and Marsh | | | |
| 7 | — | 1(0)188 | 1(1)102 |
| 11 | — | 1(1)78 | 1(0)52 |
| 12 | — | 1(0)35 | 1(1)67 |
| 13 | — | 1(0)132 | 1(1)288 |
| 21 | — | 1(0)11 | 1(1)185 |
| 26 | — | 1(0)26 | 1(2)142 |
| 32 | — | 1(0)189 | 1(4)160 |
| 33 | — | 1(17)177 | 1(15)182 |
| 36 | — | 1(0)25 | 1(1)49 |
| 38 | — | 1(2)74 | 1(0)75 |
| 39 | — | 1(15)50 | 1(1)60 |
| 40 | — | 1(62)93 | 1(11)51 |
| 41 | — | 1(2)9 | 1(3)64 |
| 42 | — | 1(5)37 | 1(1)26 |
| 43 | — | 1(75)118 | 1(2)26 |
| 44 | — | 1(16)72 | 1(12)57 |
| 45 | — | 1(3)3 | 1(27)29 |
| 46 | — | 1(0)25 | 1(88)141 |
| 47 | — | 1(0)6 | 1(32)93 |
| Spring at Observation Platform, to Confluence with Leon Creek | | | |
| 49 | — | 7/4(25)211 | 7/4(148)1114 |
| Downstream of Confluence with Leon Creek | | | |
| 50 | — | 8/3(26)212 | 6/3(44)600 |
| Seasonal Sample Sites | | | |
| 1 | 2(0)19 | 4/1(3)167 | 3(0)341 |
| 5 | 2/2(55)875 | 4/2(18)1491 | 3/1(2)1230 |
| Seeps | | | |
| C | — | 1(41)79 | — |
| D | — | 1(9)27 | — |
| E | — | 1(10)45 | — |
| F | — | 1(7)63 | — |
| I | — | 1(10)43 | — |

*Tryonia circumstriata*

| Site | 1997 | 1998 | 1999 |
| --- | --- | --- | --- |
| Upper Watercourse | | | |
| Longitudinal survey, Diamond Y Spring and Marsh | | | |
| 1 | — | 1(181)282 | 1(15)152 |
| 2 | — | 1(7)64 | 1(0)65 |
| 5 | — | 1(1)90 | 1(0)67 |
| 6 | — | 1(5)83 | 1(0)183 |
| 7 | — | 1(19)188 | 1(0)102 |
| 8 | — | 1(5)129 | 1(0)63 |
| 9 | — | 1(30)193 | 1(0)136 |
| 10 | — | 1(3)36 | 1(0)82 |
| 12 | — | 1(0)85 | 1(5)67 |
| 13 | — | 1(80)132 | 1(0)288 |
| 18 | — | 1(11)54 | 1(0)105 |
| 20 | — | 1(206)388 | 1(0)41 |
| 30 | — | 1(2)220 | 1(0)80 |
| 31 | — | 1(0)102 | 1(1)60 |
| Seasonal Sample Sites | | | |
| 3 | 2/2(804)1014 | 4/4(1154)1832 | 3/3(10)358 |
| 4 | 2/2(800)1191 | 4/4(369)717 | 3/2(3)402 |
| Euphrasia Spring | | | |
| 23 | — | 1(0)161 | 1(7)107 |
| 24 | — | 1(211)634 | 1(100)303 |
| 7* | 2/2(851)1973 | 4/4(821)1178 | 3/4(240)1228 |

*Also a Seasonal Sample Site

| *Melanoides tuberculata* | | | |
|---|---|---|---|
| Site | 1997 | 1998 | 1999 |
| Upper Watercourse | | | |
| Longitudinal survey, Diamond Y Spring and Marsh | | | |
| 1 | — | 1(70)282 | 1(10)152 |
| 2 | — | 1(16)64 | 1(5)65 |
| 3 | — | 1(2)40 | 1(0)118 |
| 4 | — | 1(6)62 | 1(0)30 |
| 5 | — | 1(6)90 | 1(0)67 |
| 6 | — | 1(20)83 | 1(60)183 |
| 7 | — | 1(33)188 | 1(26)102 |
| 8 | — | 1(12)129 | 1(1)63 |
| 9 | — | 1(2)193 | 1(0)136 |
| 10 | — | 1(2)36 | 1(0)82 |
| Seasonal Sample Sites | | | |
| 3 | 2/1(106)1014 | 4/4(433)1832 | 3/3(110)358 |

| *Assiminea pecos* | | | |
|---|---|---|---|
| Site | 1997 | 1998 | 1999 |
| Upper Watercourse | | | |
| Longitudinal survey, Diamond Y Spring and Marsh | | | |
| 2 | — | 1(1)64 | — |
| 3 | — | 1(2)40 | — |
| 6 | — | 1(1)83 | — |
| 9 | — | 1(1)193 | — |
| 33 | — | 1(1)177 | — |
| 39 | — | 1(1)50 | — |
| 41 | — | 1(1)9 | — |
| 46 | — | 1(4)25 | — |
| Seasonal Sample Sites | | | |
| 3 | 2/2(5)1014 | 4(0)1832 | 3(0)358 |
| 5 | 2/2(42)875 | 4/1(1)1491 | 3(0)1230 |
| Seeps | | | |
| B | — | 1(1)36 | — |
| C | — | 1(5)79 | — |
| D | — | 1(1)27 | — |
| F | — | 1(1)63 | — |
| Lower Watercourse | | | |
| Longitudinal Survey | | | |
| 45 | — | 1(0)487 | 1(1)56 |
| 50 | — | 1(0)139 | 1(1)12 |
| Euphrasia Spring | | | |
| 24 | — | 1(1)634 | 1(0)303 |
| Seasonal Sample Sites | | | |
| 8 | 2/1(6)321 | 4(0)498 | 3(0)813 |

*Also a Seasonal Sample Site