THE CHEMICAL RESTORATION OF DIAMOND Y DRAW, TEXAS: EFFECTS ON FISHES AND MACROINVERTEBRATES

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CHAPTER ONE

PREFACE

In the late 1980s or early 1990s the sheepshead minnow, *Cyprinodon variegatus*, was introduced into Diamond Y Draw, a small watercourse in west Texas, resulting in genetic introgression of the endemic Leon Springs pupfish, *C. bovinus*, throughout its restricted geographic range (Echelle and Echelle 1997). This led to an effort to restore the native genetic structure in one portion of the system by restocking with genetically pure captive stock after using a chemical toxicant (Antimycin A) to eradicate all fishes.

The use of Antimycin A generated considerable concern by biologists for the aquatic biota of Diamond Y Draw. This small, isolated ecosystem supports several endemic taxa (Williams et al. 1985), including the federally endangered Leon Springs pupfish, two hydrobiid springsnails (*Tryonia circumstriata* and *T. adamantina*), and an amphipod (*Gammarus pecos*). In addition, Diamond Y Draw is one of four locations supporting the Pecos gambusia (*Gambusia nobilis*), another federally endangered species, and it supports a widely disjunct population of each of two gastropods, a limnaeid (*Stagnicola caperata*) and a third hydrobiid (*Assiminea pecos*). In addition to the aquatic fauna, the puzzle sunflower (*Helianthus paradoxus*) occurs only in hydric soils of Diamond Y Draw and two locations in New Mexico (Van Auken and Bush 1998).

This dissertation is presented in the form of two separate papers. Chapter Two describes the distribution of and historic changes in the status of various macroinvertebrate species of concern in Diamond Y Draw. Chapter Three describes the renovation and examines its effect on the genetic structure of the two endangered fishes and on abundances of various species of concern, with emphasis on the macroinvertebrates. Pre-renovation monitoring provided needed information for development of a renovation plan that minimized effects on species of concern and provided a baseline of information for analysis of effects on the aquatic community. Pre- and post-renovation monitoring also will help provide important perspectives for future restorations of a similar nature.

LITERATURE CITED

Echelle, A. A., and A. F. Echelle. 1997. Genetic introgression of endemic taxa by non-natives: a case study with Leon Springs pupfish and sheepshead minnow. Conservation Biology 11:153-161.

Van Auken, O. W., and J. K. Bush. 1998. Spatial relationships of *Helianthus paradoxus* (Compositae) and associated salt marsh plants. Southwestern Naturalist 43:313-320.

Williams, J. E., D. B. Bowman, J. E. Brooks, A. A. Echelle, R. J. Edwards, D. A. Hendrickson, and J. J. Landye. 1985. Endangered aquatic ecosystems in North American deserts with a list of vanishing fishes of the region. Journal of the Arizona-Nevada Academy of Science 20:1-62.

CHAPTER TWO

DISTRIBUTION AND STATUS OF AQUATIC MACROINVERTEBRATES IN DIAMOND Y DRAW

INTRODUCTION

A unique aquatic fauna occurs in Diamond Y Draw, a small spring-fed system located in the Chihuahuan Desert near Fort Stockton, Pecos County, Texas. The fauna includes three endemic invertebrate species, all of which are species of concern to conservationists, primarily because of their restricted distributions (Williams et al. 1985). These include two small hydrobiid springsnails, *Tryonia adamantina* and *T. circumstriata*, and an amphipod, *Gammarus pecos*. The area also supports the southern-most populations of a third hydrobiid, *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* also is being considered for listing (N. Allan, pers. comm.).

The objective of this study was to describe the distribution of the invertebrates of concern in Diamond Y Draw. 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).

STUDY AREA

Diamond Y Draw (Figure 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 (Figure 1). The primary source of water for the upper watercourse is the headpool of Diamond Y Spring, which is about 14 m wide, 25 m long, and 3.5 m in maximum depth. The source of water for the headpool comes from the Rustler aquifer, which is brackish to saline (Boghici 1997).

Details of the following descriptions are based on observations made in October 1997. A small spring (10 m long, 2 cm deep, 45 cm wide) emptied into the east side of the headpool of Diamond Y Spring. The outflow of Diamond Y Spring 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 olneyi*) and emptied into a deep (1 m), narrow pool that joined a short dry, bulrush-lined 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 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 old dirt track. 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 (140 m long, 40 cm wide, 4 to 7 cm deep) at the south side of the marsh. Downstream of the pool, water flowed another 140 m through a dense bulrush marsh with very little surface flow. That was followed by about 50 m of dry area covered with *Distichlis*. After that, there was a dry, bulrush-covered channel that began at a confluence with a wet side-channel (40 m long) and extended 30 m downstream to a confluence with a common channel (77 m long) for two small springs. After that 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 side channel (99 m long, 9 m wide, 4-7 cm deep) that was covered in bulrush.

Near the observation tower, there was a channel that originated from a small spring. That spring was reported by Kennedy (1977) to have about 5 lps of flow. In October 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 for about 145 m to the confluence with Leon Creek. Leon Creek was dry upstream from the confluence, but downstream, the channel (30 to 40 cm wide, 2 to 10 cm deep) flowed for about 170 m into a dense marsh that ended just upstream from the fence line.

In October 1997, surface water in the lower watercourse began with seepage in a small channel (13 m long, 3-6 cm deep) that emptied into a large pool ("Monsanto Well";

Hubbs et al.1978). Monsanto well 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. That was followed by a channel that was dry and devoid of bulrush for about 40 m; it 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 that, 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. Downstream of the culvert, the watercourse consisted of a narrow (< 1 m) bulrush-lined channel with shallow (2-4 cm) water until it joined with the outflow from Euphrasia Spring about 98 m downstream.

Euphrasia Spring was the largest spring in the lower watercourse; G. Veni reported a discharge of 30-40 lps from the spring in 1990 (unpublished report, Texas Chapter of the Nature Conservancy, San Antonio). 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, but sharply incised, gully, often with dense growths of exotic salt cedar (*Tamarix* sp.; mechanically removed by the Nature Conservancy in 2000).

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 followed. On the north side, that pool had a small connection to a shallow, stagnant, mud-bottomed side-pool (about 10 m wide, 10 cm deep). 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. That 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 east 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; that channel terminated with a small, open pool just upstream 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.

MATERIALS AND METHODS

To describe the distribution of the invertebrates of concern in the study area, I did two longitudinal surveys of the upper and lower watercourses of Diamond Y Draw, one in 1998 on 11-12 May (upper water course) and 17 June (lower watercourse) and one on in 1999 on 20-21 May 1999 (both watercourses). Forty-eight sample sites were established at 20-m intervals in the upper watercourse (starting at the head of the outflow from Diamond Y Spring), and 64 sites were established at 20-m intervals (starting at Mansanto Pool) in the lower watercourse (Figures 2 and 3). Two additional sample sites were located in the channel near the observation platform (sites 49 and 50, Figure 2). Two collections also were taken along the length of Euphrasia Spring (sites 24 and 25, Figure 3). Collections were made with a fine mesh (2 mm) 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. On 9 - 10 March 1998, a collection was taken from each of seven small springs (sites A-F, and I, Figure 2) near the lower end of the upper watercourse. Those collections were made with a tea strainer at several sites at each seep or spring, and all collections from within each spring were combined. Samples from seep locations were used only to determine presence/absence of the endemic forms and were not used in the statistical analyses.

In addition, collections were made on a seasonal basis at eight sites (Figure 1) used in reports to the Texas Chapter of The Nature Conservancy (San Antonio, Texas) on studies of Diamond Y Draw (unpublished reports by G. Veni in 1991 on hydrology and R. Fullington and R Goodloe in 1993 on macroinvertebrates). Seasonal sample locations were sampled on 18 August 1997, 20 October 1997, 9 March 1998, 17 June 1998, 14 October 1998, 18 December 1998, 15 March 1999, 21 May 1999, and 10 August 1999. To ensure sampling consistency, I 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. 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 for identification. 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.

Species' distributions were plotted on maps created with ArcView from digitized aerial photographs (Diamond Y Spring, NHAP 85, 169-147; 10/28/84). Using the CANOCO computer package (ter Braak and Smilauer 1998) detrended correspondence analysis (DCA) was used to ordinate samples on the basis of number of individuals collected for each taxon. Samples made with a tea strainer were not included in the analysis because of possible sampling bias. 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. Finally, Spearman rank correlations were used to examine the following pairwise correlations in abundances (number of individuals collected) in the following selected pairs of species: *Gammarus* versus *Hyallela*, *T. circumstriata* vs *T. adamantina*, and *Melanoides* vs each of the *Tryonia* species. These comparisons were based on collections containing one or the other or both taxa of concern.

RESULTS

Taxa collected in the longitudinal stream survey of invertebrates included 11 insect families (Table 1), two amphipods, *Gammarus pecos* and *Hyallela azteca*, and representatives of all molluscs previously reported (Taylor 1987, Bequaert and Miller 1973) from the system. Water quality was similar within the two watercourses but differed between watercourses (Table 2). Conductivity, salinity, and total dissolved solids were considerably higher in the lower watercourse, with minimum values in the lower watercourse being higher than the maximum value for the upper watercourse. Temperature and pH values were similar between the two watercourses.

The first DCA axis explained 38.4% of the variance in abundance of species, 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 reflected 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, primarily those in the upper watercourse and Euphrasia Spring. Correspondingly, the various measurements of water chemistry (pH, conductivity, total dissolved solids), which were higher in the lower watercourse (Table 2), were positively correlated with axis 1. Axis 2 was not associated with any obvious pattern of sites or species in the system.

Plots of sample and species scores on DCA axes 1 and 2 (Figures 4 and 5) show 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. The scores for these three species on axis 1 were lower than for all other taxa examined except the introduced snail, *Melanoides tuberculata*. Samples containing two or more of these four species were primarily taken from Euphrasia Spring and the upper 430 m of Diamond Y Spring outflow. The remaining samples were taken from the mainstem of the lower watercourse.

Tryonia adamantina and *T. circumstriata* exhibited complementary distributions in the upper watercourse (Figure 6) ($r_s = -0.63$, $P = 0.002$ and $r_s = -0.60$, $P = 0.02$ in 1998 and 1999, respectively). 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 A), 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 oil-field 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 (Figures 7 and 8) 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 positive correlation in the upper watercourse (r_s = 0.28, $P = 0.012$), but no correlation in either the lower watercourse ($r_s = -0.14$, $P = 0.127$), or in both watercourses combined (r_s = 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. That 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. That 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 A). The species was negatively correlated with *T. adamantina* in both 1998 and 1999 ($r_s = -0.62$; $P = 0.0001$). Abundances of *Melanoides* showed a positive but non-significant correlation with *T. circumstriata* ($r_s = 0.22$; $P = 0.17$).

DISCUSSION

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*). Diamond Y Draw receives considerable legal protection because it lies within the Diamond Y Preserve, which has been owned and managed as a protected natural area, Diamond Y Preserve, by the Texas Chapter of The Nature Conservancy since 1992. The area also 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, there are various causes for concern regarding conservation of individual species and the Diamond Y Draw aquatic ecosystem.

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 sporadically and primarily at sites in the lower watercourse where it occasionally outnumbered *G. pecos* (21 of 128 samples had higher abundance of *Hyalella*).

Hyalella azteca has not previously been reported from Diamond Y Draw, except for an unpublished report received by the Texas chapter of The 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.

A crayfish (*Procambarus sp.*) was collected in this study. The species was common in the Diamond Y Spring headpool, and it was observed or collected at several sites in the outflow of the spring. The species currently is undescribed and is geographically restricted to a few scattered localities elsewhere in the Pecos River Basin (D. L. Hillis, pers. comm.).

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 I 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 my samples; members of this family may burrow and aestivate in response to drying habitat and may emerge in large numbers with the return of surface water (Brown 1991). Correspondingly, I found large numbers of *S. caperata* 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 my survey of the system 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 my 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. An unpublished report received by the Bitter Lakes National Wildlife Refuge in 1985 (D. W. Taylor, in litt.) indicates that this experienced malacalogist had collected Diamond Y Draw "at various times since 1968" and in 1984 when "non-quantative 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 Chapter of The Nature Conservancy by another experienced malacologist (R. Fullington, in litt.) 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 comments in the previously mentioned unpublished report to the Bitter Lakes National Wildlife Refuge 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 oil-field 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 conditions that may have caused depletion of *T. adamantina* prior to the introduction. Regardless, the situation requires close monitoring because the possibility remains that the present situation is 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 small spring near the observation tower, 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 thiarid 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. 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. 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 to try 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 shells of living animals. 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).

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 G. Veni (in litt.) 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. Regardless, a reduced extent of surface waters in both watercourses is evident from a comparison of Figure 1 with maps and descriptions of the area in the 1970's when surface waters in both watercourses extended 0.5-1.0 km farther downstream (Hubbs et al. 1978, Kennedy 1977, Echelle and Echelle 1980). In 1979, 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 on record (National Climate Data Center Records, 1940-2000). The present low flow also may 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. Allen, 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).

LITERATURE CITED

Bequaert, J. C., and W. B. Miller. 1973. The mollusks of the arid southwest with an Arizona check list. University of Arizona Press, Tucson.

Boghici, R. 1997. Hydrogeological investigations at Diamond Y Springs and surrounding area, Pecos County, Texas. Unpublished Master's Thesis, University of Texas, Austin.

Brown, K. M. 1991. Mollusca: Gastropoda. *In*: J. H. Thorp and A. P. Covich, eds., Ecology and Classification of North American Freshwater Invertebrates. Academic Press, San Diego, 911 pp.

Brune, G. 1981. Springs of Texas, Vol I. Branch-Smith Inc., Fort Worth, Texas.

Cole, G. A., and E. L. Bousfield. 1970. A new freshwater *Gammarus* from western Texas. Southwestern Naturalist 83:89-95.

Contreras-B, S., and M. L. Lozano-V. 1996. Extinction of most Sandia and Potosi valleys (Nuevo Leon, Mexico) endemic pupfishes, crayfishes, and snails. Ichthyolocial Explorations of Freshwaters 7:33-40.

Duan, Y., S. I. Guttman, J. T. Oris, and A. J. Bailer. 2000. Genetic structure and relationships among populations of *Hyalella azteca* and *H. montezuma* (Crustacea: Amphipoda). Journal of North American Benthological Society 19:308-320.

Echelle, A. A., and A. F. Echelle. 1980. Status of the Pecos gambusia. U. S. Fish and Wildlife Service, Albuquerque, NM.

Echelle, A. A., and A. F. Echelle. 1997. Genetic introgression of endemic taxa by non-natives: a case study with Leon Springs pupfish and sheepshead minnow. Conservation Biology 11:153-161.

Echelle, A. A., A. F. Echelle, and D. R. Edds. 1989. Conservation genetics of a spring-dwelling desert fish, the Pecos gambusia (*Gambusia nobilis*, Poeciliidae). Conservation Biology 3:159-169.

Hershler, R., H. P. Liu, and M. Mulvey. 1999. Phylogenetic relationships within the aquatic snail genus *Tryonia*: implications for biogeography of the North American southwest. Molecular Phylogenetics and Evolution 13:377-391.

Hubbs, C., T. Lucier, E. Marsh, G. P. Garrett, R. J. Edwards, and E. Milstead. 1978. Results of an eradication program on the ecological relationships of fishes in Leon Creek, Texas. Southwestern Naturalist 23:487-496.

Kennedy, S. E. 1977. Life history of the Leon Springs pupfish, *Cyprinodon bovinus*. Copeia 1977:93-103.

McDermott, B. S. 2000. Distribution and infection relationships of an undescribed digenetic trematode, its exotic intermediate hose, and endangered fishes in springs of west Texas. Unpublished Master's Thesis, Southwest Texas State University, San Marcos, Texas.

Miller, R. R. 1961. Man and the changing fish fauna of the American southwest. Papers of the Michigan Academy of Sciences, Arts, and Letters 46:365-404.

Neck, R. W. 1985. *Melanoides tuberculata* in extreme south Texas. Texas Conchologist 21:150-152.

Pointer, J. P., and F. McCullough. 1989. Biological control of the snail hosts of *Schistosoma mansoni* in the Caribbean area using *Thiara* spp. Acta Tropica 46:147-155.

Roessler, M. A., C. L. Beardsley, and D. C. Tabb. 1977. New records of the introduced snail, *Melanoides tuberculata* (Mollusca: Thiaridae), in South Florida. Florida Scientist 40:87-94.

Taylor, D. W. 1987. Freshwater molluscs from New Mexico and vicinity. New Mexico Bureau of Mines and Mineral Resources, Bulletin 116:1-51.

ter Braak, C. J. F., and P. Smilauer. 1998. Canoco 4. Reference manual and user's guide to Canoco for Windows: software for canonical community ordination. Microcomputer Power, Ithaca.

Williams, J. E., D. B. Bowman, J. E. Brooks, A. A. Echelle, R. J. Edwards, D. A. Hendrickson, and J. J. Landye. 1985. Endangered aquatic ecosystems in North American deserts with a list of vanishing fishes of the region. Journal of the Arizona-Nevada Academy of Science 20:1-62.

Table 1. List of invertebrate taxa collected from Diamond Y Draw with detrended correspondence analysis species scores for axes 1 and 2.

Taxa	Axis 1	Axis 2
Amphipoda		
Gammarus pecos	0.8480	1.3008
Hyalella azteca	3.0731	1.6585
Coleoptera		
Dytiscidae	2.0039	1.0483
Hydrophilidae	2.6054	0.8985
Diptera		
Ceratopogonidae	2.3093	1.0157
Chironomidae	1.9775	-0.3301
Culicidae	2.0553	2.2653
Stratiomyidae	2.1999	1.1228
Unknown	2.2015	1.5156
Hemiptera		
Belostomatidae	1.5374	1.9745
Odonata		
Aeshnidae	2.0615	2.2512
Coenagrionidae	2.1461	2.2816
Libellulidae	1.7432	2.1917
Gastropoda		
Tryonia adamantina	-0.2689	3.7934
Tryonia circumstriata	-0.2063	0.1691
Assiminea pecos	n/a	n/a
Stagnicola caperata	n/a	n/a
Physa virgata	1.4981	2.6762
Melanoides tuberculata	-0.6580	1.7894

Taxa not used in the analysis are designated with n/a

Table 2. 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 (^{0}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 (^{0}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

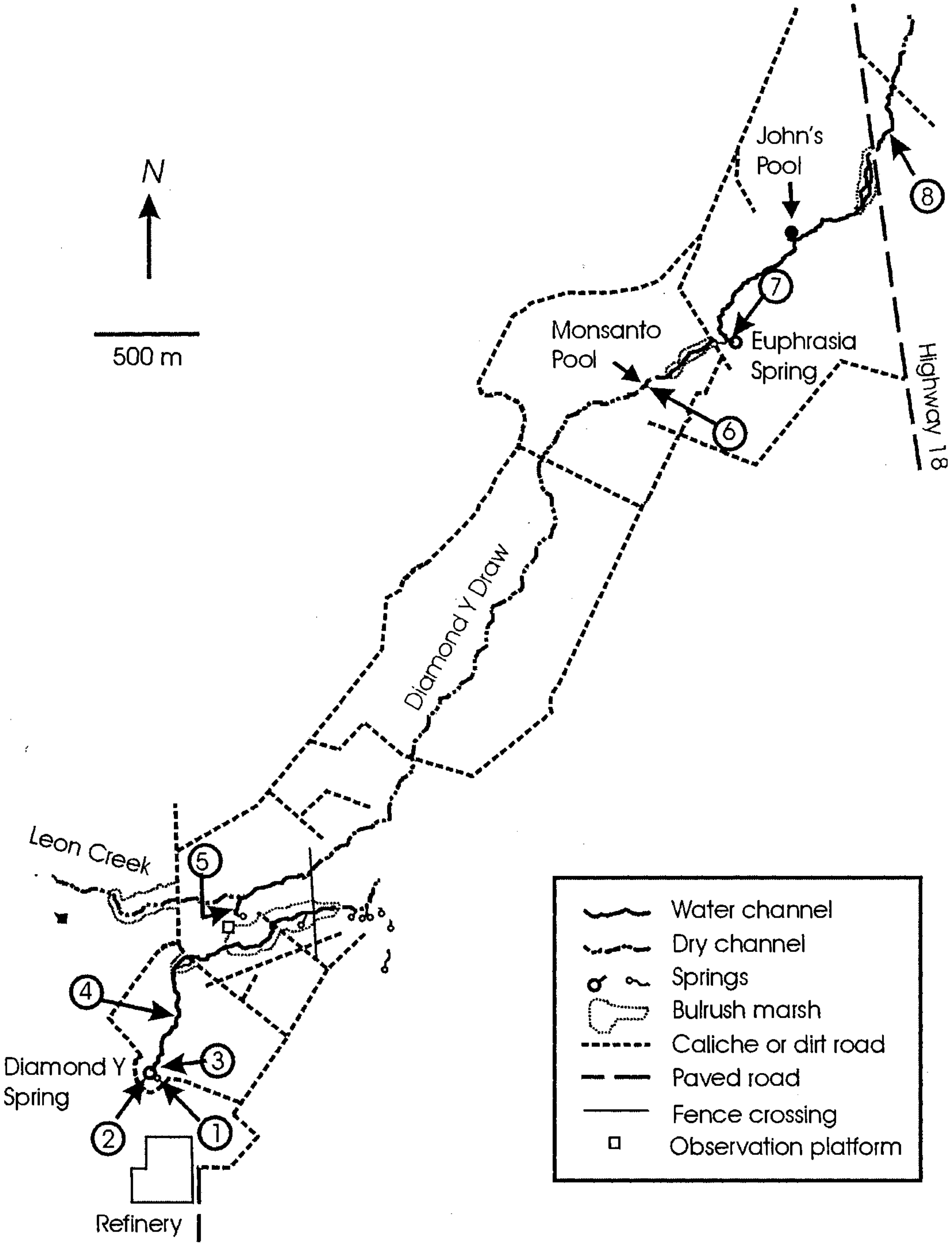

Figure 1. Diamond Y Draw, Pecos County, Texas. Encircled numbers represent seasonal sampling sites mentioned in the text.

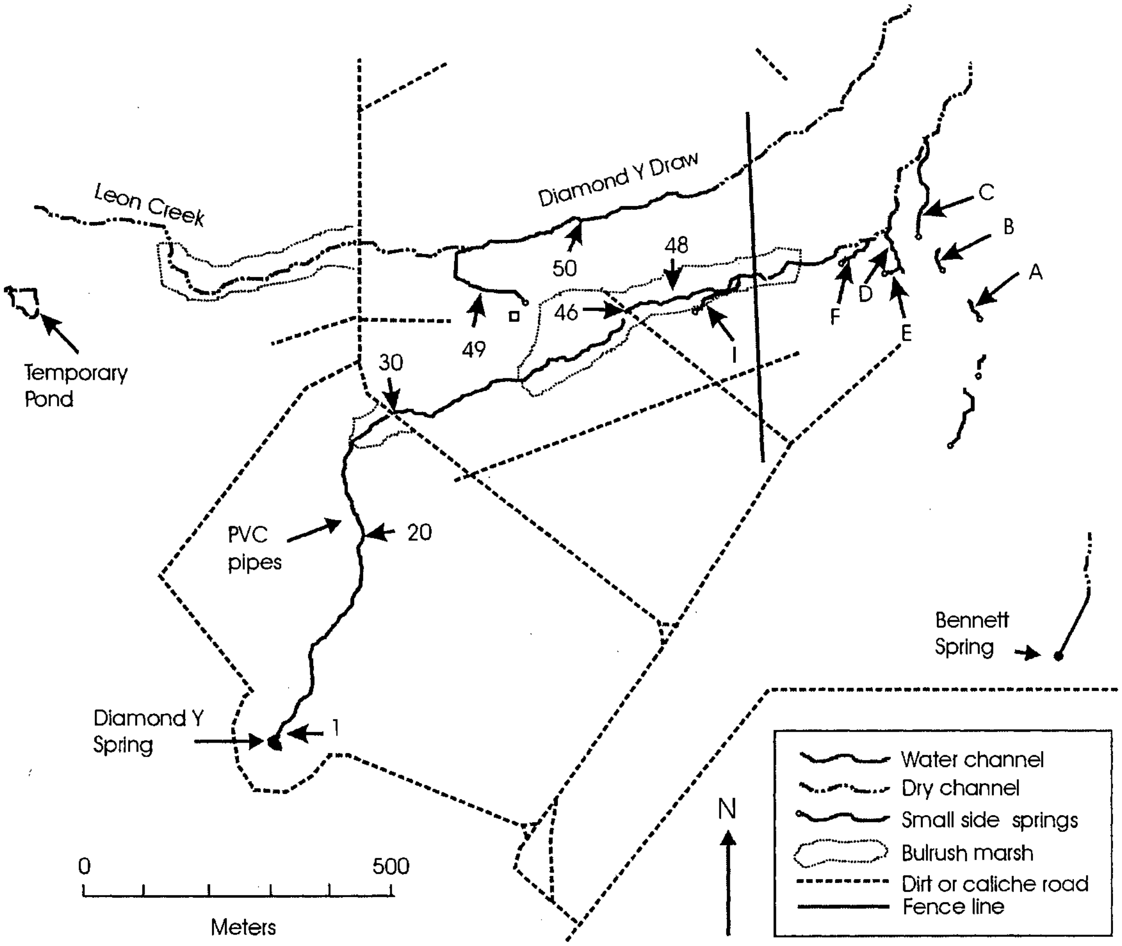

Figure 2. Upper watercourse of Diamond Y Draw. Numbers show positions of selected sites among the 50 sampling sites for the longitudinal surveys of invertebrates in 1998 and 1999. Except for numbers 49 and 50, samples were made at 20-m intervals starting at the head of the Diamond Y Spring outflow stream.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

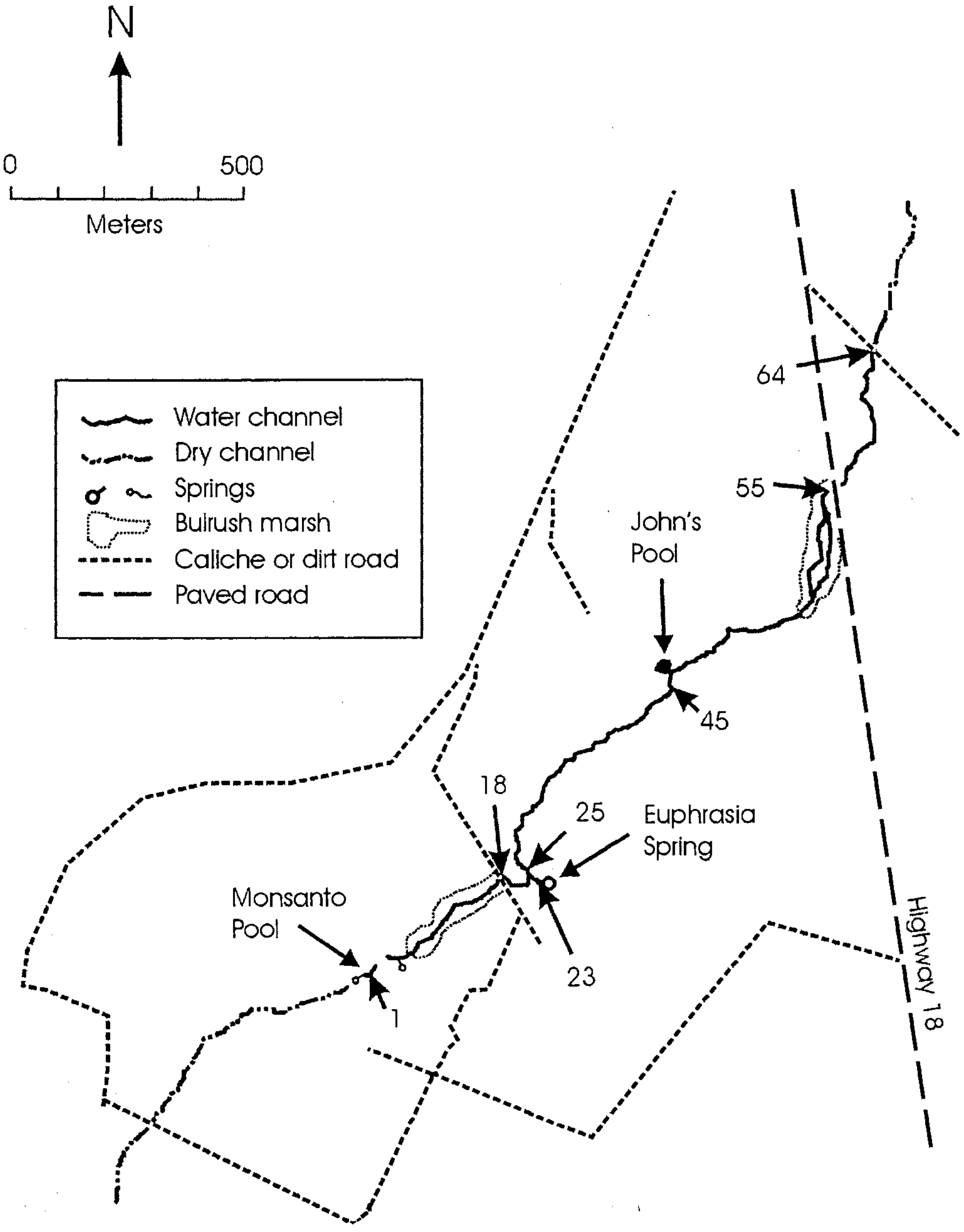

Figure 3. Lower watercourse of Diamond Y Draw. Numbers show positions of selected sites among the 64 sampling sites for the longitudinal surveys of invertebrates in 1998 and 1999.

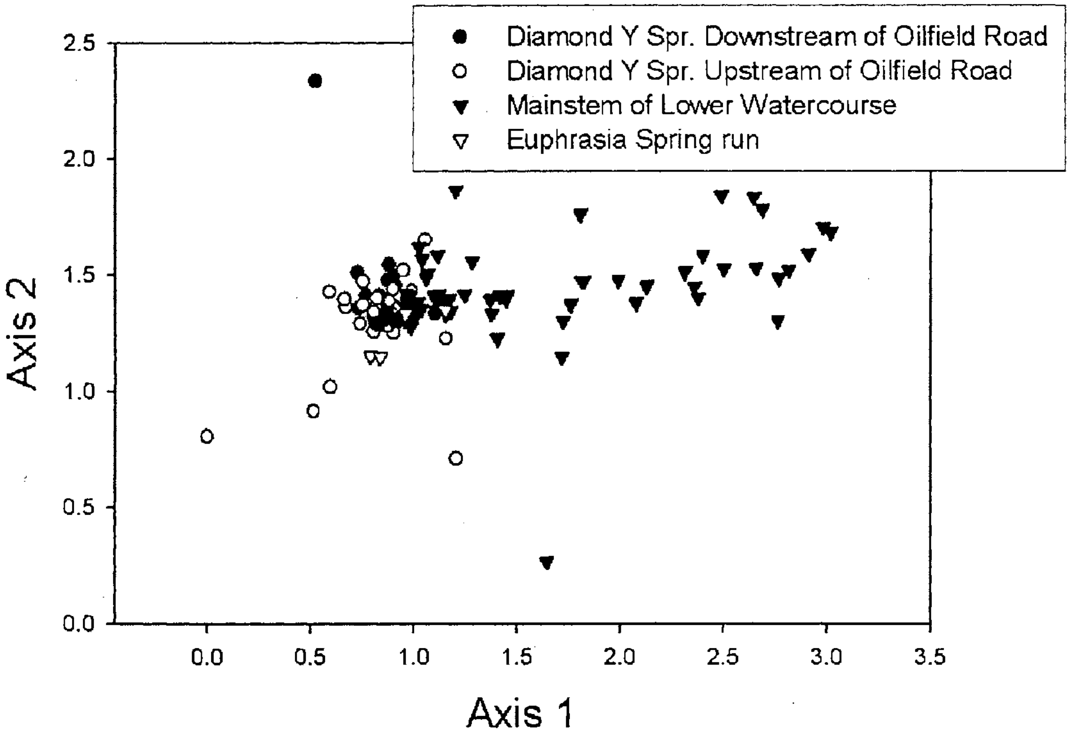

Figure 4. Site scores for the first two DCA axes derived for invertebrate collections in the longitudinal surveys of Diamond Y Draw in 1998 and 1999.

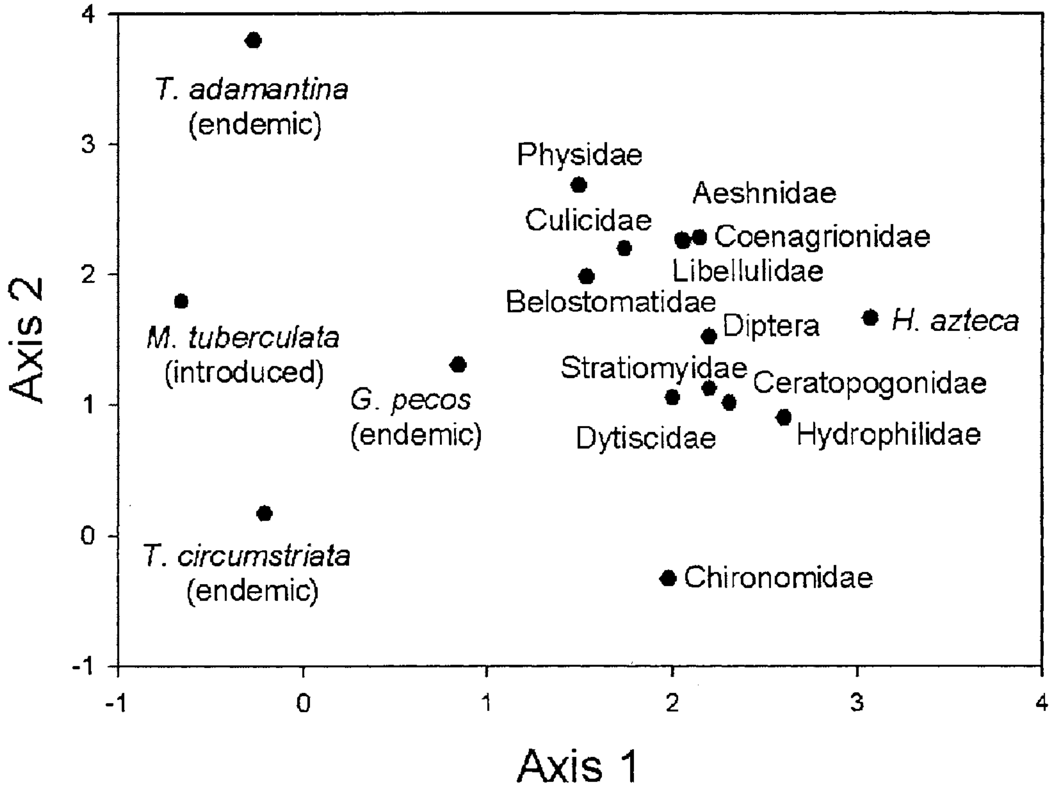

Figure 5. Species scores for the first two DCA axes derived for invertebrate collections in the longitudinal surveys of Diamond Y Draw in 1998 and 1999.

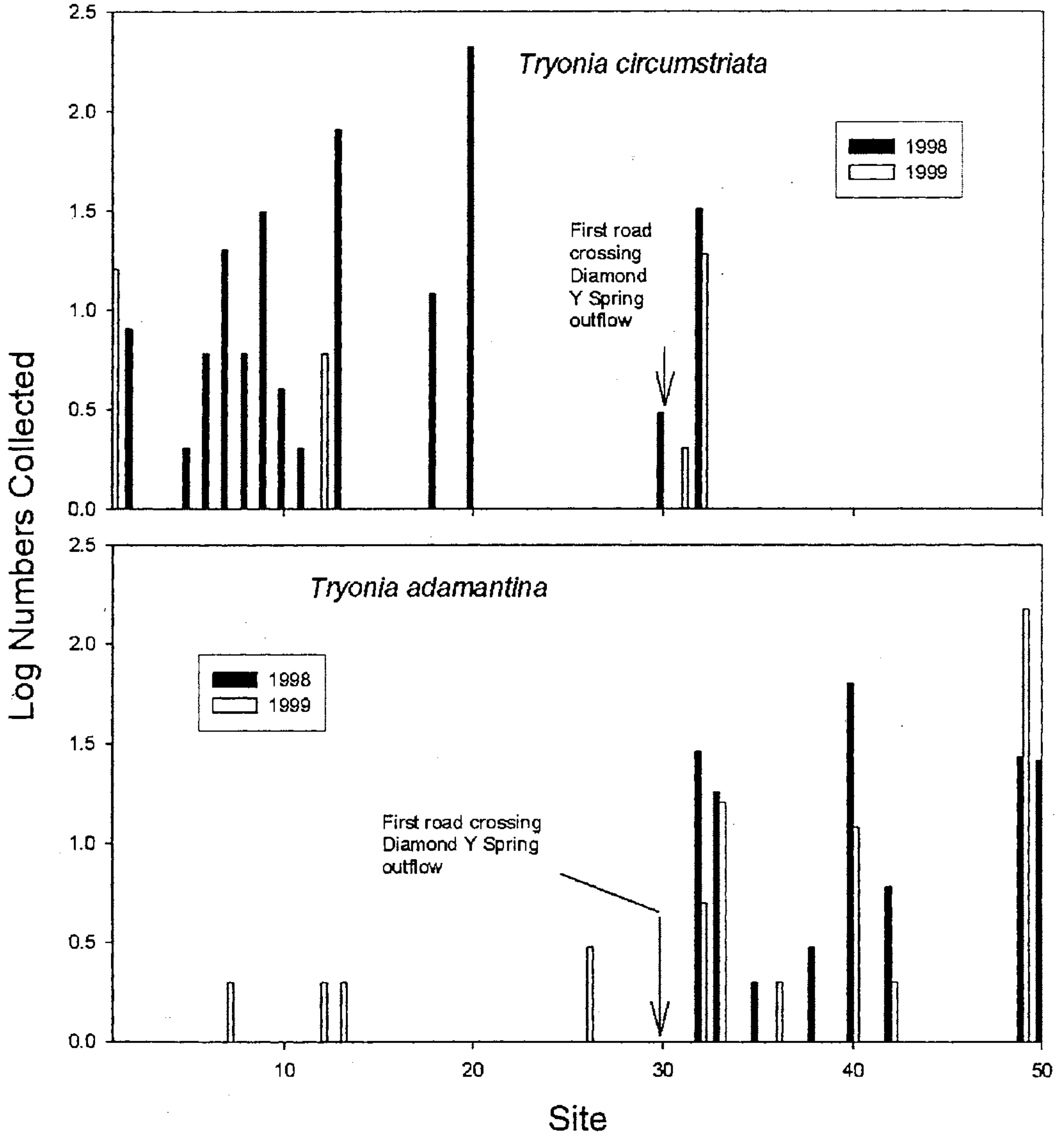

Figure 6. Abundance as log (n+1) of two springsnails in the upper watercourse of Diamond Y Draw for 1998 and 1999. Sites are numbers 1-50 as depicted in Figure 2.

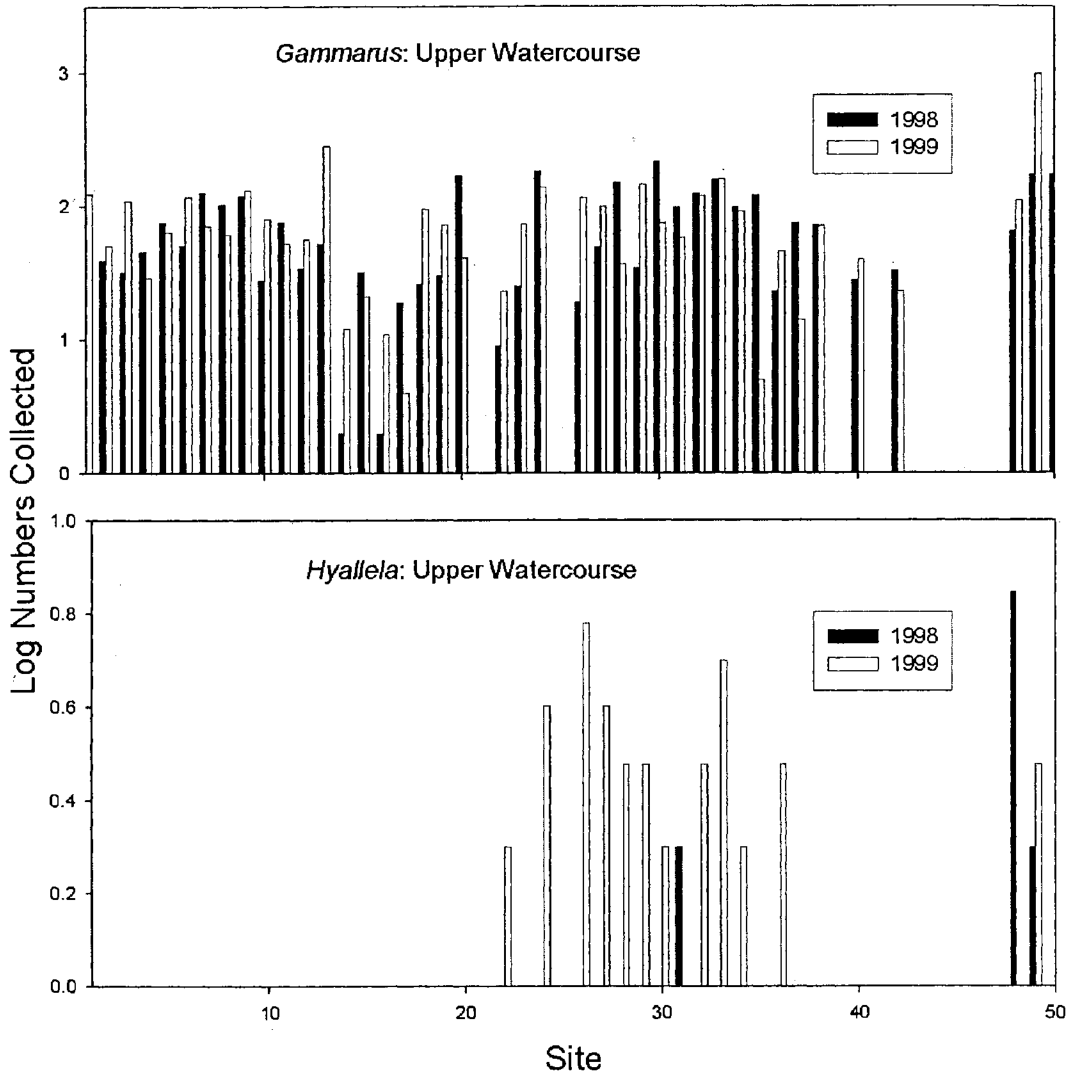

Figure 7. Abundance as log(n+1) of two amphipods in the upper watercourse of diamond Y Draw for 1998 and 1999. Sites are numbers 1-50 as depicted in Figure 2.

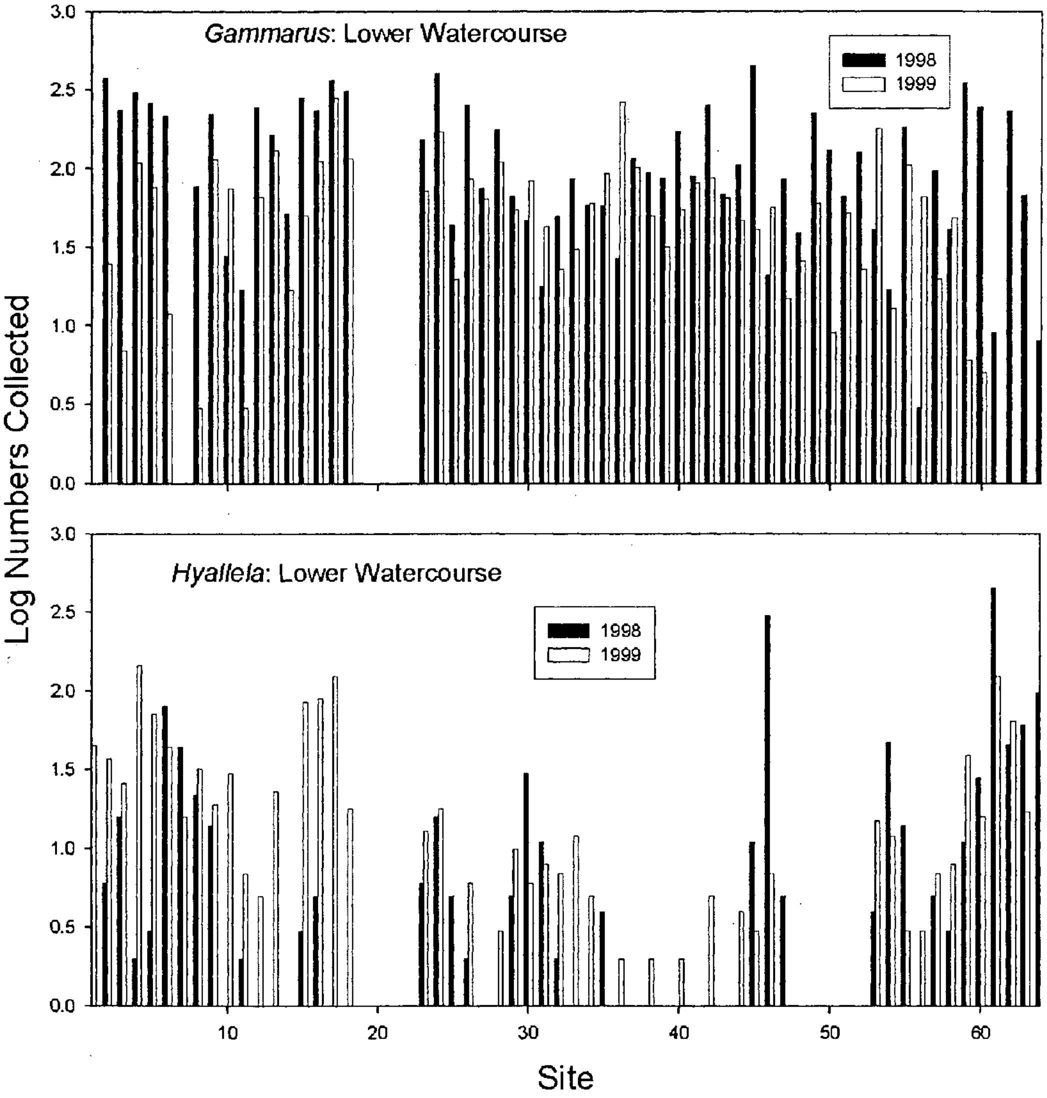

Figure 8. Abundance as log(n+1) of two amphipods in the lower watercourse of diamond Y Draw for 1998 and 1999. Sites are numbers 1-64 as depicted in Figure 3.

CHAPTER THREE

A CHEMICAL FISH-ERADICATION AND RESTOCKING EFFORT TO RESTORE THE NATIVE GENETIC STRUCTURE OF A SPRING-DWELLING PUPFISH: EFFECTS ON FISHES AND MACROINVERTEBRATES

INTRODUCTION

Habitat restoration is a holistic approach for the re-establishment of pre-disturbance physical, chemical, and biological functions to obtain a self-regulating ecosystem (National Research Council [NRC] 1992). Restoration can involve habitat improvement or modification and biological manipulation for maintenance of gamefish stocks or removal of exotic species. Most restorations of aquatic systems have been sponsored by organizations such as Trout Unlimited and government agencies to improve habitat for game species, typically salmonids (NRC 1992, Rinne and Turner 1991). However, Winemiller and Anderson (1997) reported a successful response of a non-game species, the Comanche Springs pupfish, *Cyprinodon elegans*, to construction of a semi-natural refuge at Phantom Lake Spring in west Texas implying that such approaches have value for non-native fishes as well.

In this paper, I examine effects of an effort to restore the native genetic structure of Leon Springs pupfish, *C. bovinus*, a local endemic to Diamond Y Draw, a small, desert stream in the Pecos River drainage of west Texas. In 1994, the entire natural population of this species carried genetic material from the non-native sheepshead minnow, *C.*

variegatus, a species that apparently was introduced to the system in the late 1980s or early 1990s (Echelle and Echelle 1997). This was one of several situations of introgressive hybridization involving this non-native species and the three pupfishes endemic to the Pecos River Basin.

By the 1960s, *C. variegatus* was introduced into Lake Balmorhea, about 90 km west of Diamond Y Draw, where it hybridized with the locally 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 with *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 in 1974 (Kennedy 1977). By 1976, hybrids were widespread in one of the two primary watercourses of the system ("lower watercourse"), but there was no evidence of *C. variegatus* in the upper watercourse (Hubbs et al. 1978, Hubbs 1980). Subsequently, two specimens having characteristics of *C. variegatus* were taken in the upper watercourse, 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 rotenone, 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 supported by an allozyme survey of the species at several locations in 1984

(Echelle et al. 1987).

Subsequently, however, there was a second introduction of *C. variegatus* into Diamond Y Draw. By 1994, evidence of genetic influence by *C. variegatus* was detected in both the upper and the lower watercourse (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 15.1%. In response, the U.S. Fish and Wildlife Service approved and with the help from other agencies, implemented a plan developed by the Rio Grande Fishes Recovery Team to restore the native pupfish genome in Diamond Y Draw. As part of this plan, a portion of the upstream watercourse of Diamond Y Draw was restocked with a genetically pure, captive stock of *C. bovinus* after eradicating all fishes with the toxin Antimycin A.

My objectives were to assess the success of the effort to restore the native pupfish genome in the upper watercourse of Diamond Y Draw and to assess effects on the remainder of the aquatic community in the area, including changes in the genetic structure of another federally listed endangered species, the Pecos gambusia, *Gambusia nobilis*, and possible 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*.

There has been little documentation of stream restorations aimed at removal of unwanted fishes. Rinne and Turner (1991:226) noted that "the literature is woefully lacking in documentation of extent, results, or even the techniques used" in ichthyocide treatment of streams. Exceptions for non-game fishes include reports of unsuccessful renovation efforts (Meffe 1983, Rinne et al. 1981) and the apparently successful attempt in 1976 to remove hybrid pupfish from the lower watercourse of Diamond Y Draw (Hubbs et al. 1978, Echelle et al. 1987).

Additionally, there have been only meager attempts to document effects of chemical stream renovations on aquatic organisms other than fishes. In such renovations, the objective usually is complete elimination of all fishes. Thus, the stream fauna generally is exposed to higher concentrations of the toxicant than the minimum required to kill fish in the laboratory or field trials. In studies where macroinvertebrates were monitored, there were no apparent long-term effects of renovation (Minckley and Mihalick 1981, Rinne and Turner 1991), although in one of these instances there was an immediate reduction in abundance and diversity of macroinvertebrates (Minckley and Mihalick 1981).

STUDY AREA

Diamond Y Draw supports a small spring-fed system near Fort Stockton, Pecos County, Texas. The aquatic 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 elsewhere in the Pecos River Basin of New Mexico and Texas. The other fishes observed in the study area included two indigenous species that are widely distributed in the Pecos River and elsewhere (rainwater killifish, *Lucania parva*, and plains killifish, *Fundulus* *zebrinus*) and three introduced non-native species (large spring gambusia, *Gambusia geiseri*, western mosquitofish *G. affinis*, and common carp, *Cyprinus carpio*.

The aquatic fauna of Diamond Y Draw includes 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* also is being considered for listing (N. Allan, pers. comm.).

Diamond Y Draw (Figure 9) includes two watercourses separated by about 3 km of dry land (Figure 9). The upper watercourse was the focus of this study. The primary source of water for the upper watercourse (Figure 10) is the headpool of Diamond Y Spring, which is about 14 m wide, 25 m long, and 3.5 m in maximum depth. The source of water for the headpool comes from the Rustler aquifer, which is brackish to saline (Boghici 1997).

Details of the following descriptions are based on observations made in October 1997. A small spring (10 m long, 2 cm deep, 45 cm wide) emptied into the east side of the headpool of Diamond Y Spring. The outflow of Diamond Y Spring 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 olneyi*) and emptied into a deep (1 m), narrow pool that joined a short dry, bulrush-lined 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 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 old dirt track. 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 (140 m long, 40 cm wide, 4 to 7 cm deep) at the south side of the marsh. Downstream of the pool, water flowed another 140 m through a dense bulrush marsh with very little surface flow. That was followed by about 50 m of dry area covered with *Distichlis*. After that, there was a dry, bulrush-covered channel that began at a confluence with a wet side-channel (40 m long) and extended 30 m downstream to a confluence with a common channel (77 m long) for two small springs. After that 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 side channel (99 m long, 9 m wide, 4-7 cm deep) that was covered in bulrush.

Near the observation tower, there was a channel that originated from a small spring. That spring was reported by Kennedy (1977) to have about 5 lps of flow. In

October 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 for about 145 m to the confluence with Leon Creek. Leon Creek was dry upstream from the confluence, but downstream, the channel (30 to 40 cm wide, 2 to 10 cm deep) flowed for about 170 m into a dense marsh that ended just upstream from the fence line.

MATERIALS AND METHODS

Renovation

On 3-5 August 1998, the portion of the Diamond Y Spring outflow from the headpool to site 30 (Figure 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: one at the fenceline crossing in the lower reach of the outflow of Diamond Y Spring and another in the watercourse near the observation tower.

Renovation of the upper watercourse of Diamond Y Draw began on 3 August 1998. On the previous evening, samples of *Tryonia* and *Gammarus* were placed in 5 gallon buckets in the stream channel to assure that the organisms would survive in streamside refugia. Before applying Antimycin A, at least 1300 individuals of *G. nobilis* and 400 individuals of another indigenous fish, *Lucania parva,* the rainwater killifish, were collected using seines and minnow traps and were transported to Dexter National Fish Hatchery and Technology Center (DNFHTC), Dexter, New Mexico, in 150-gallon aerated tanks for temporary holding until completion of the project. Also, large samples of springsnails and *Gammarus pecos* samples representing hundreds of individuals were placed in four 5 gallon plastic buckets as on-site refugia. Samples of these invertebrates were also transported to the San Marcos National Fish Hatchery, San Marcos, Texas, and DNFHTC for temporary holding to mitigate against possible extirpation of these species. In addition, general samples of invertebrates and 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 renovation 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 m 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 m 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 on the morning on 5 August.

To test for complete detoxification before reintroduction of fish and invertebrates, *Gambusia nobilis* collected from an untreated portion of Diamond Y Draw on 6 August 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, all fish in minnow traps and invertebrates in buckets were still alive. Invertebrates held in onsite refugia were then released into the outflow of Diamond Y Spring headpool. That afternoon 500 *Cyprinodon bovinus* were transported from DNFHTC and stocked into Diamond Y Spring headpool and two sites in its outflow. One week later, the live collections of *G. nobilis* (n = 879) and *L. parva* (n = 309) being held temporarily at DNFHTC were released in the headpool and its immediate outflow. Because the gastropods survived the initial treatment, and *Gammarus* were abundant in the untreated portion of Diamond Y Draw, springsnails and *Gammarus* samples held at the hatcheries were subsequently disposed of.

Invertebrate Abundances

To assess effects of the renovation on invertebrates, I conducted two longitudinal surveys of the upper watercourse, one on 11-12 May 1998, prior to the renovation, and one on 20-21 May 1999, subsequent to the renovation. Forty-eight sample sites were established at 20-m intervals starting at the head of the outflow from the headpool of Diamond Y Spring (Figure 10). Additional sample sites included in the survey were two sites in the channel near the observation platform (Figure 10). Collections were made with a D-frame net (25 cm wide, 1-mm mesh) or in smaller habitats, a 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. Collections were preserved in ethanol and identified to at least the family level in the lab. Specimens of *Tryonia* were sent to R. Hershler of the Smithsonian Institution for identification.

Collections also were made on a seasonal basis at eight sites used for unpublished reports to The Nature Conservancy (San Antonio) by G. Veni in 1991 and R. Fullington and R. Goodloe in 1993 on, respectively, hydrology and macroinvertebrates. These included four sites (1-4, Figure 9) in the section of the upper watercourse that was treated with Antimycin A and four sites (5-8, Figure 9) in untreated areas; all of those 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 time before renovation (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). I made all collections using three to four bottom scraping sweeps with a D-frame net.

Six species were chosen to represent the invertebrate community. These included two amphipods, *Gammarus pecos* and *Hyallela azteca*, and four gastropods, *Tryonia adamantina, T. circumstriata, Physella mexicana,* and *Melanoides tuberculata.* I used Wilcoxon signed rank tests of paired collection sites from the two longitudinal surveys to assess effects on the abundance and distribution of the six species of concern in the portion of the watercourse (sites 1-30, Figure 10) exposed to ichthyotoxin. These analyses considered only sites where the species 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, Figure 9); only those seasonal sites where the species was collected at least once were included in these analyses.

Genetic Structure of the Fishes of Concern

Reference samples of *C. bovinus* and *C. variegatus* were taken from, respectively, DNFHTC, and Lake Balmorhea. The latter location appears to be the source of the stock of *C. variegatus* introduced to 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.

Standard methods of horizontal starch-gel electrophoresis (Murphy et al. 1990) were used 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 (Figure 11). 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.

I used a restriction-fragment-length-polymorphism (RFLP) approach 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* (A. A. Echelle, pers. comm.) were screened for diagnostic restriction enzymes using the MacVector computer program. That search revealed a single diagnostic enzyme, *Hin*fl, that produces diagnostic fragment patterns for the two species (Figure 12). For mtDNA identification of individual specimens, I used the Dneasy Tissue Kit (QIAGEN, Cat. no. 69506) 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*fl 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 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 Spring headpool was analyzed for the only two loci (Ada-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.

RESULTS

Invertebrate Abundances

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 live collections maintained at stream-side after detoxification of the Antimycin A, both amphipod species were extremely rare in samples from the treated reach on 14 October 1998, 42 days following treatment, and numbers remained low until March 1999 (Figure 13). 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 3; Figure 13).

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 it was collected 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 of both species of *Tryonia* showed a marked reduction in all five seasonal surveys made following the treatment (Table 4; Figure 13), producing a statistically significant difference in pre- versus post-treatment numbers of *T. circumstriata* ($Z = -3.0$; $P = 0.003$; Table 3). 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 3) 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 difference in the two longitudinal surveys, and the latter species also showed no significant variation based on the seasonal surveys.

Fishes

At 0730 hours on 4 August, there were live but clearly stressed *Gambusia geiseri* in the head pool. No other live fish were observed in the treated area and intensive post-treatment minnow-trap collecting by C. Hubbs (pers. comm.) convincingly demonstrated that *G. geiseri* was eliminated from Diamond Y Draw. Nineteen dead common carp (about 3-5 lbs each) were removed from the headpool, and subsequent observations indicated that this species was also eliminated from the system. Although not quantified in my study, all other fishes observed during pre-renovation sampling were observed during post-treatment visits except *Fundulus zebrinus*, which was rare (only 1 specimen taken) before the treatment.

Allozyme genotypic frequencies and haplotype frequencies in *C. bovinus* are found in Appendix D. 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 (Table 5, Figures 14 and 15). 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 mtDNA and allozymes declined from pre- to post-treatment sample periods (Table 5; Figures 14 and 15). 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. All such markers were absent in two samples taken from this locality approximately one year 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. Also, 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 ($P > 0.11$ for each of the five diagnostic loci).

Levels of polymorphism in *G. nobilis* were 66.7% before treatment and 100% after treatment, and heterozygosity values were 0.161 and 0.185, respectively (Table 3). For the two polymorphic loci (Adh-A and Gpi-A), no significant deviations from Hardy-Weinberg expectations for individual samples, and no significant difference in allele frequencies among samples before and after treatment.

DISCUSSION

Concentrations of Antimycin A used in renovating the Diamond Y Spring outflow probably were well above the levels necessary to kill many aquatic organisms. The drip stations were set to maintain 10 ppb in flowing waters, and levels of toxicant probably were heightened by the addition of Antimycin A to the headpool and the marshy areas of slower-moving water adjacent to the spring outflow. In field trials by Gilderhus et al. (1969), *Gambusia affinis* showed 99 percent mortality in a reservoir at concentrations of 10 ppb Antimycin A, there was partial mortality of amphipods at 4 ppb, and crayfish showed no effect at concentrations of 10 ppb. My results indicate that the Antimycin A treatment in Diamond Y Draw killed all fish, most if not all amphipods, and at least some crayfish in the treated area.

The renovation was successful in restoring the native genome of Leon Springs pupfish (*C. bovinus*) in the segment of Diamond Y Draw treated with Antimycin A. The comparison of this renovated population with the captive source-stock at DNFH indicated that the transfer of captive stock to Diamond Y Draw was accomplished without a notable change in genetic structure. Similarly, the renovation had no obvious effect on the genetic structure of the threatened fish *G. nobilis*, samples of which were removed, held at DNFH, and released back to the site after degradation of the toxin.

Two small populations (probably < 100 fish) of pupfish occur in the upper watercourse outside the area treated with Antimycin A: one in a small area of open surface-water on the east side of the upper watercourse of Diamond Y Draw near the fenceline crossing ("fenceline population") and the other in a small stretch of water near the observation platform ("platform population"). These areas were not treated with Antimycin A, both because of the small size of the pupfish populations and the desire to avoid exposing the entire watercourse to the toxicant.

The pre-renovation genetic assays revealed no evidence of genetic contamination in the fenceline population. It is possible, however, that contamination could have been detected with additional genetic markers. The lack of such evidence for the markers I used could reflect random losses as a result of genetic drift in a small population. Regardless, it seemed prudent to treat this population as a remnant of the original native population of the species. Thus, this population was not subjected to any restoration effort.

The platform population was treated by releasing DNFH stock in an effort to swamp out introduced genetic material. The single pre-renovation specimen collected from this location 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 this population three years 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%. If this is a random sample of the genome, then the genetic material of this population is 4.2% non-native.

Allendorf and Leary (1988) suggested that, in instances of genetic introgression, less than 1% introgression might be an acceptable goal for efforts to restore native genomes. Other workers suggest that higher levels of introgression might be acceptable, depending on circumstances (Campton 1987, Dowling and Childs 1992). By the 1% criterion, the native genetic structure has been restored to the area treated with Antimycin A. However, the post-treatment level of 4.2% introgression for the small platform population seems to require action. If surface waters expand following the present drought for the area, then the platform population, which is in the mainstem of Diamond Y Draw, could expand and influence the genetic structure of the entire system. Because of the small size of this population, the level of non-native alleles probably could be reduced to acceptable levels by simply releasing additional pupfish from the captive stock at DNFHTC.

The beneficial effects of the renovation include the elimination of two fishes that are not native to the Pecos River Basin, the largespring gambusia (*G. geiseri*) and the common carp (*C. carpio*), both of which had occupied the upper watercourse of Diamond Y Draw since at least the 1970s (Kennedy, 1977). *Gambusia geiseri* seems to have been introduced to the Pecos River drainage from farther east sometime after the U.S. Mexican Boundary Survey in the 1800s (Hubbs and Springer 1957), and the carp is a widespread exotic species in North America.

Another beneficial effect of the renovation was an apparent increase in numbers of pupfish in the headpool of Diamond Y Draw. My 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 following the renovation. Similarly, dead individuals of the undescribed crayfish (*Procambarus* sp.) were seen in the headpool of

Diamond Y Draw immediately following the application of Antimycin A, but there seems to have been no long-term effect, because live individuals were seen in this pool on several occasions following treatment.

In contrast, the renovation seemed to have no immediate effect on the snail assemblage, but the abundance of one of the two springsnails (*T. circumstriata*) was markedly and persistently lower following the renovation. This scarcity of springsnails in the immediate outflow of Diamond Y Spring is similar to that of the early 1980s (D. Taylor, in litt.) when *T. adamantina* occurred there in the absence of *T. circumstriata* (Taylor 1987). The latter was introduced into the Diamond Y Spring outflow from Euphrasia Spring of the lower watercourse of Diamond Y Draw (see Chapter Two). One possible explanation for the pattern of pre- and post-treatment abundances of the two springsnails is that *T. circumstriata* was better at exploiting the pre-treatment filamentous algal growths, and its post-treatment decrease in abundance was a result of the decline in those growths. Prior to treatment, it was easy to collect *T. circumstriata* by simply removing them from handfuls of algae taken from the stream.

Except for the persistently reduced abundance of *T. circumstriata*, the Antimycin A treatment had no apparent long-term effect on the invertebrate taxa examined in this study. For the amphipods, there was an immediate and marked reduction in numbers followed, over a period of several months, by a return to previous levels of abundance. These results parallel the pattern observed for a variety of macroinvertebrates identified to family in a small stream in Arizona that was treated with Antimycin A (Minckley and Mihalick 1981). In that study, a sample made three years following the renovation indicated that the general abundances of invertebrates had rebounded to pre-renovation levels. In Diamond Y Draw, all taxa included in my analysis except the introduced snail (*Melanoides tuberculatae*) are known from locations in the upper watercourse that were not treated with Antimycin A (see Chapter Two), reducing the probability of a catastrophic loss of at least the known endemic taxa.

LITERATURE CITED

Allendorf, F. W., and R. F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conservation Biology 2:170-184.

Bequaert, J. C., and W. B. Miller. 1973. The mollusks of the arid southwest with an Arizona check list. University of Arizona Press, Tucson.

Boghici, R. 1997. Hydrogeological investigations at Diamond Y Springs and surrounding area, Pecos County, Texas. Unpublished Master's Thesis, University of Texas, Austin.

Campton, D. E. 1987. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations. Pp. 161-192 *in* N. Ryman and F. Utter (eds.), Population genetics and fishery management. University of Washington Press, Seattle.

Childs, M. R., A. A. Echelle, and T. E. Dowling. 1996. Development of the hybrid swarm between Pecos pupfish (Cyprinodontidae: *Cyprinodon pecosensis*) and sheepshead minnow (*C. variegatus*): a perspective from allozymes and mtDNA. Evolution 50:2014-2022.

Dowling, T. E., and M. R. Childs. 1992. Impact of hybridization on a threatened trout of the southwestern United States. Conservation Biology 6:355-364.

Echelle, A. A., and A. F. Echelle. 1994. Assessment of genetic introgression between two pupfish species, *Cyprinodon elegans* and *C. variegatus* (Cyprinodontidae), after more than 20 years of secondary contact. Copeia 1994:668-681.

Echelle, A. A., and A. F. Echelle. 1997. Genetic introgression of endemic taxa by non-natives: a case study with Leon Springs pupfish and sheepshead minnow. Conservation Biology 11:153-161.

Echelle, A. A., and A. F. Echelle. 1980. Status of the Pecos gambusia. U. S. Fish and Wildlife Service, Albuquerque, New Mexico.

Echelle, A. F., A. A. Echelle, and D. R. Edds. 1987. Population structure of four pupfish species (Cyprinodontidae: *Cyprinodon*) from the Chihuahuan Desert region of New Mexico and Texas: allozymic variation. Copeia 1987:668-681.

Gilderhus, P. A., B.L. Berger, and R.E. Lennon. 1969. Field trials of Antimycin A as a fish toxicant. Investigations in Fish Control 27:17-21.

Hubbs, C. 1980. Solution to the *C. bovinus* problem: eradication of a pupfish genome. Proceedings of the Desert Fishes Council 10:9-18.

Hubbs, C., and V. G. Springer. 1957. A revision of the *Gambusia nobilis* species group, with descriptions of three new species, and notes on their variation, ecology, and evolution. Texas Journal of Science 9:279-327.

Hubbs, C., T. Lucier, E. Marsh, G. P. Garrett, R. J. Edwards, and E. Milstead. 1978. Results of an eradication program on the ecological relationshipe of fishes in Leon Creek, Texas. Southwestern Naturalist 23:487-496.

Kennedy, S. E. 1977. Life history of the Leon Springs pupfish, *Cyprinodon bovinus*. Copeia 1977:93-103.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci., U.S.A. 86:6196-6200.

Lamarra, V. A., Jr. 1975. Digestive activities of carp as a major contributor to the nutrient loading of lakes. Verh. Int. Ver. Theor. Angew. Limnol. 19:2461-2468.

Meffe, G. K. 1983. Attempted chemical renovation of an Arizona springbrook for management of the endangered Sonoran topminnow. North American Journal of Fisheries Management 3:315-321.

Meyer, A. T., D. Kocher, P. Basasibwaki, and A. C. Wilson. 1990. Monophyletic origin of Lake Victoria fishes suggested by mitochondrial DNA sequences. Nature 347:550-553.

Minckley, W. L., and P. Mihalick. 1981. Effects of chemical treatment for fish eradication on stream-dwelling invertebrates. Journal of the Arizona-Nevada Academy of Science 16:79-82.

Murphy, R. W., J. W. Sites, Jr., D. G. Buth, and C. H. Haufler. 1990. Proteins I: isozyme electrophoresis. Pages 45-126 in D. M. Hillis and C. Moritz, editors. Molecular systematics. Sinauer Associates, Sunderland, Massachusetts.

National Research Council. 1992. Restoration of aquatic ecosystems, science, technology, and public policy. National Academy Press, Washington, D.C.

Rinne, J. N., W. L. Minckley, and J. N. Hanson. 1981. Chemical treatment of Ord Creek, Apache County, Arizona, to re-establish Arizona trout. Journal of the

Arizona-Nevada Academy of Science 16:74-78.

Rinne, J. N., and P. R. Turner. 1991. Reclamation and alteration as management techniques, and a review of methodology in stream renovation, pp. 219-244. *In*: Battle against extinction: native fish management in the American West. W. L. Minckley and J. E. Deacon (eds.). University of Arizona Press, Tucson.

Stevenson, M. M., and T. M. Buchanan. 1973. An analysis of hybridization between the cyprinodont fishes *Cyprinodon variegatus* and *C. elegans*. Copeia 1973:682-692.

Taylor, D. W. 1987. Freshwater molluscs from New Mexico and vicinity. New Mexico Bureau of Mines and Mineral Resources, Bulletin 116:1-51.

Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281-283.

Williams, J. E., D. B. Bowman, J. E. Brooks, A. A. Echelle, R. J. Edwards, D. A. Hendrickson, and J. J. Landye. 1985. Endangered aquatic ecosystems in North American deserts with a list of vanishing fishes of the region. Journal of the Arizona-Nevada Academy of Science 20:1-62.

Winemiller, K. O., and A. A. Anderson. 1997. Response of endangered desert fish populations to a constructed refuge. Restoration Ecology 5:204-213.

Table 3. 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 4. 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.

Date of Collection	Species					
	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

Table 5. 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**

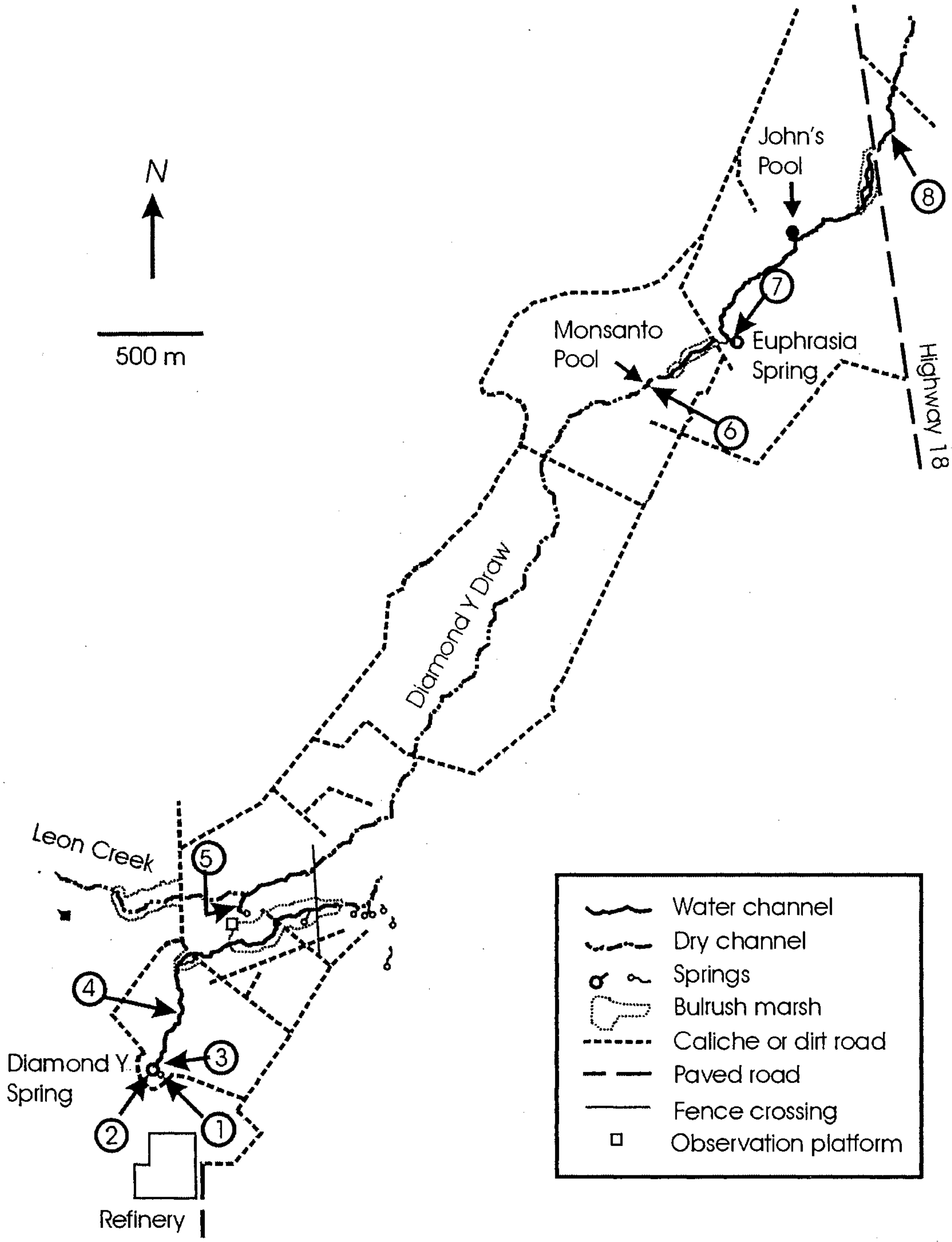

Figure 9. Diamond Y Draw, Pecos County, Texas. Encircled numbers represent seasonal sampling sites mentioned in the text.

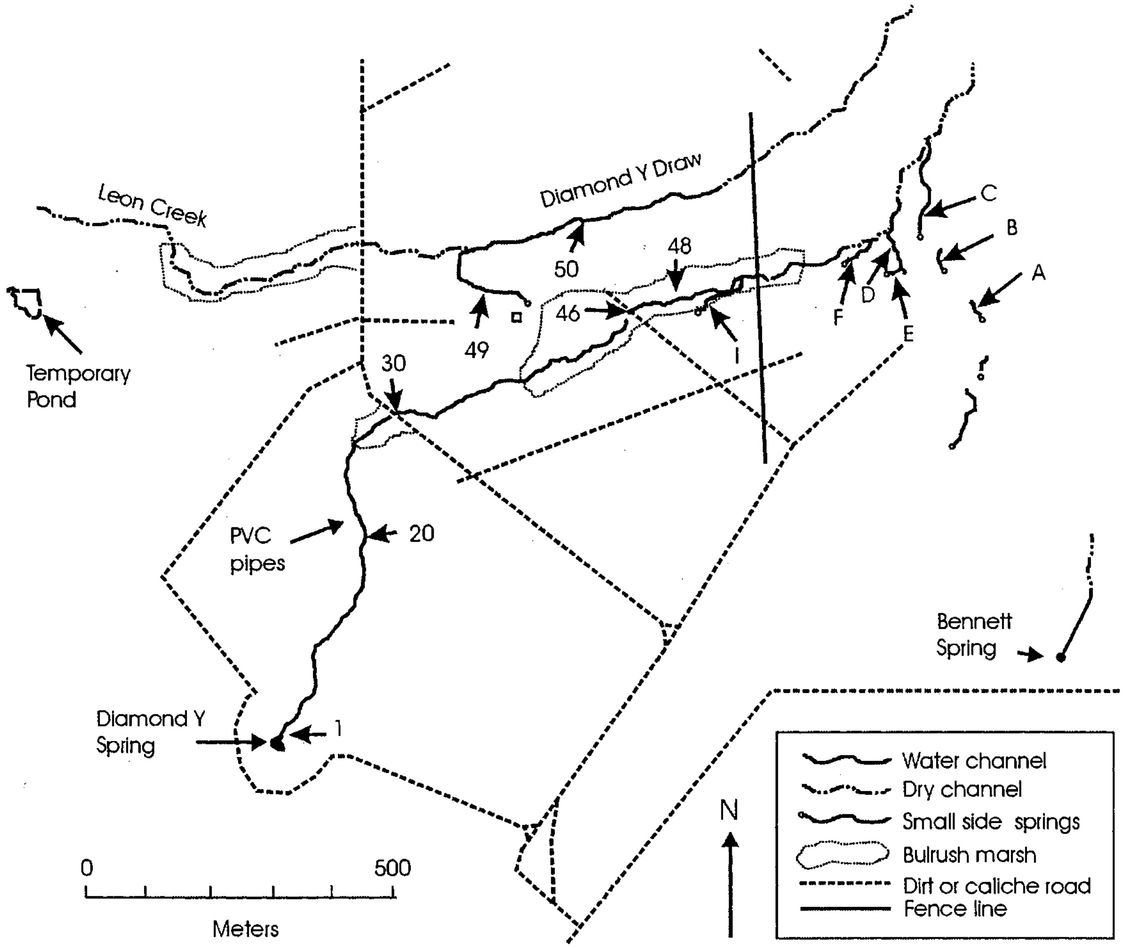

Figure 10. Upper watercourse of Diamond Y Draw. Numbers show positions of selected sites among the 50 sampling sites for the longitudinal surveys of invertebrates in 1998 and 1999. Except for numbers 49 and 50, samples were made at 20-m intervals starting at the head of the Diamond Y Spring outflow stream. Letters represent the 7 sites sampled in small springs in the downstream reach of the watercourse.

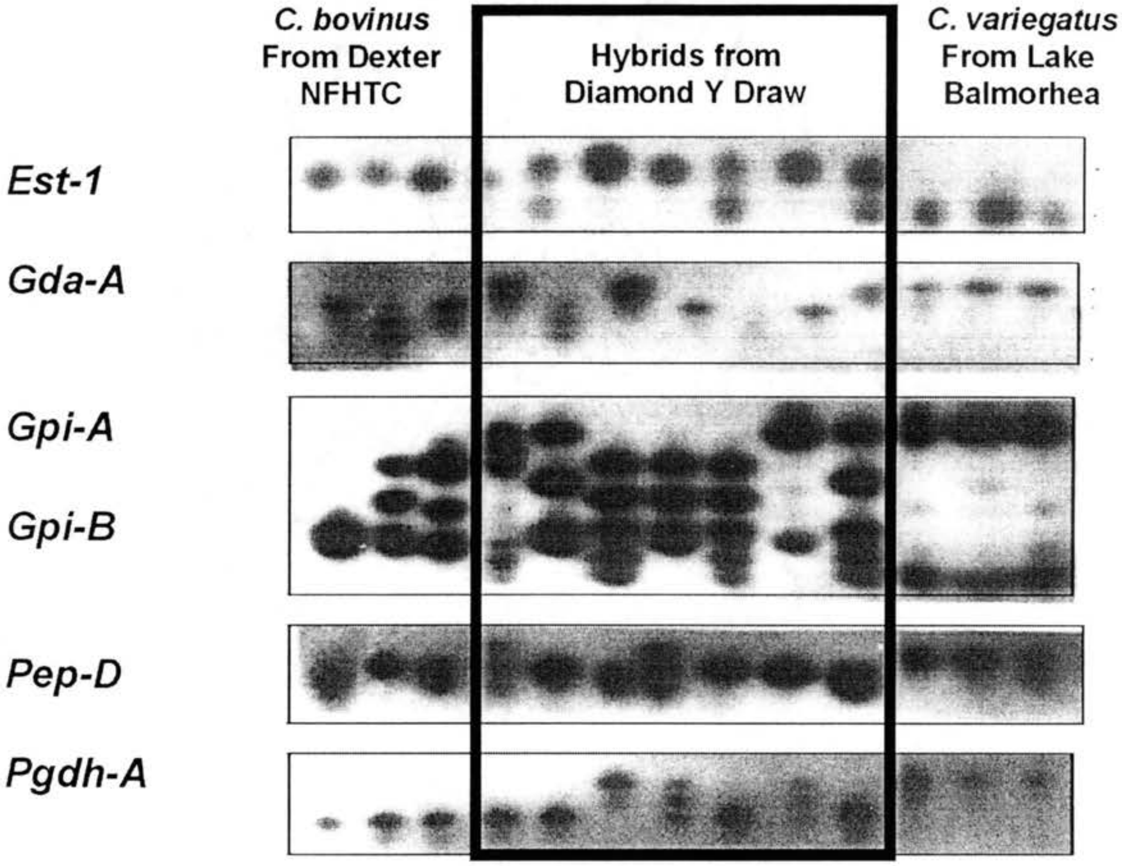

Figure 11. Allozymes used in the genetic survey of pupfish in Diamond Y Draw. From Echelle and Echelle (1997).

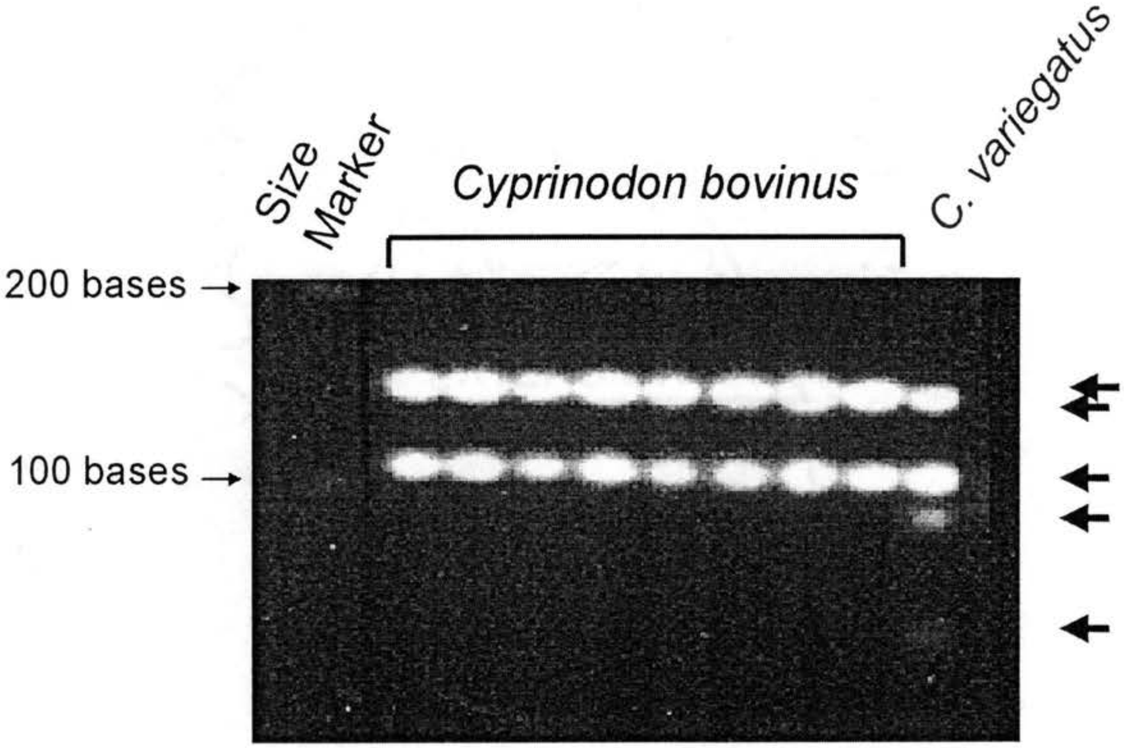

Figure 12. RFLP patterns obtained with the *Hin*fI restriction enzyme for an amplified segment of the mitochondrial DNA control region in *Cyprinodon bovinus* and *C. variegatus.* Note two bands in *C. bovinus* and four in *C. variegatus*.

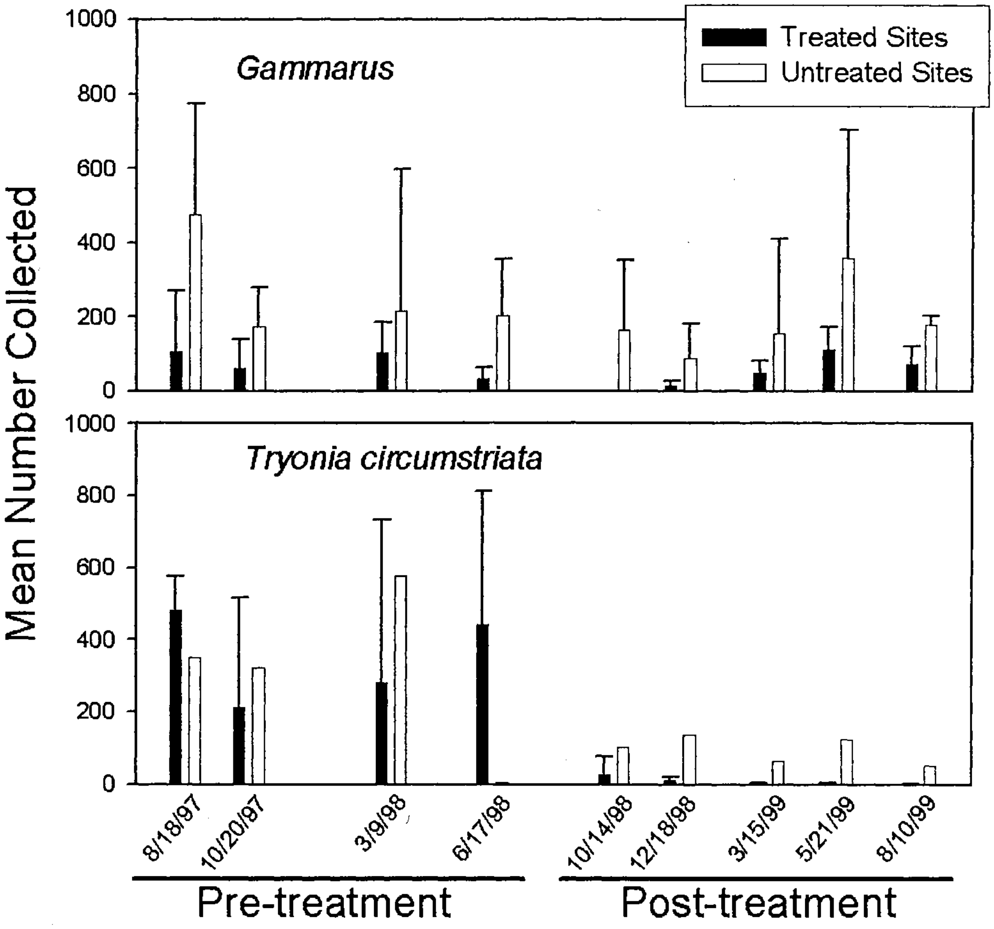

Figure 13. Abundance of two endemic species in the portion of the upper watercourse treated with Antimycin A.

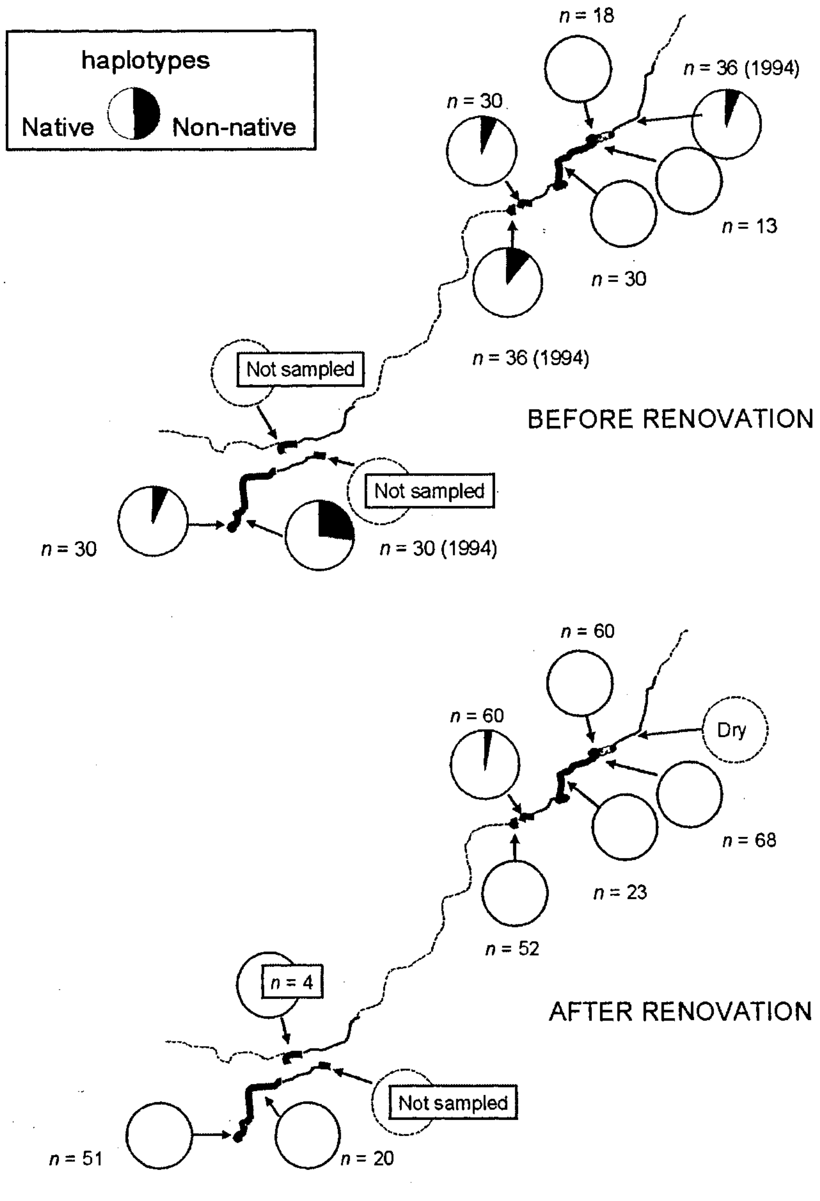

Figure 14. Frequencies of native and non-native pupfish haplotypes for mitochondrial DNA in Diamond Y Draw before and after attempts to restore the native genome.

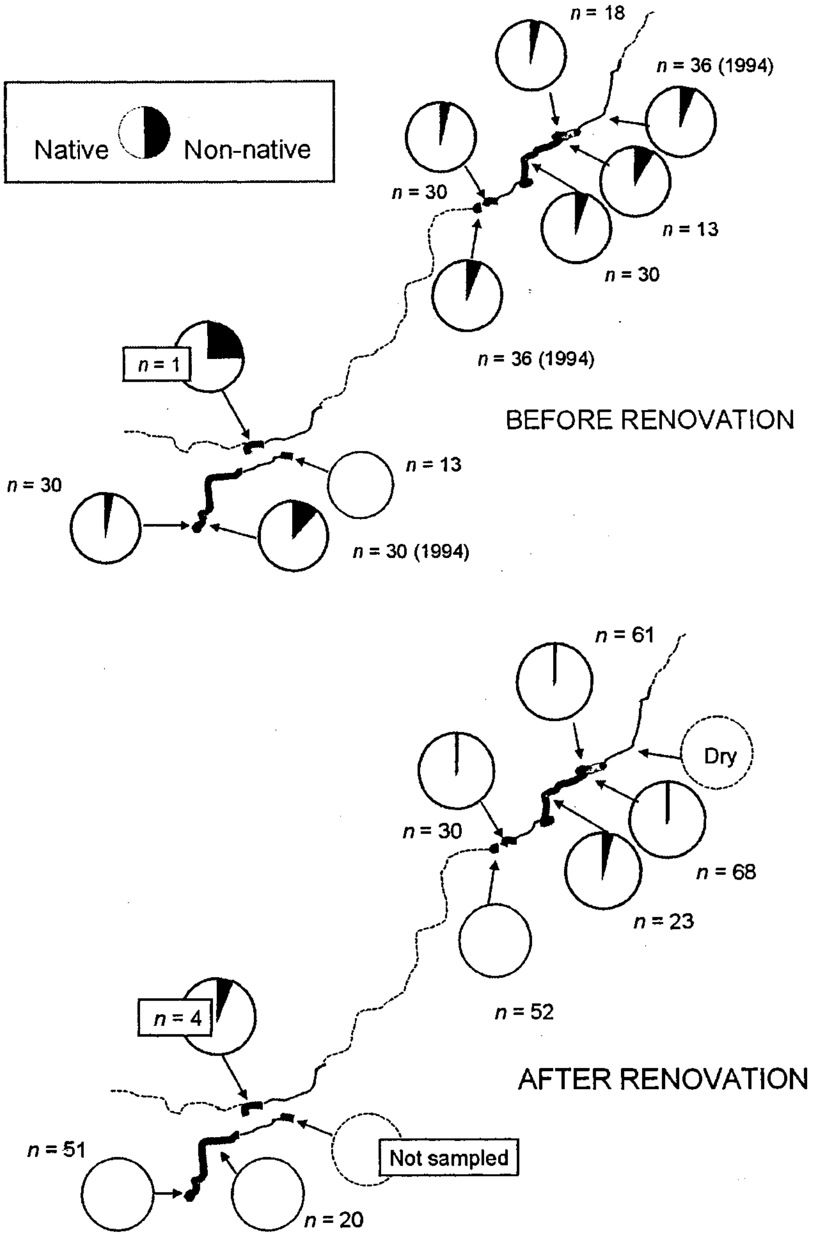

Figure 15. Frequencies of native and non-native alleles for pupfish allozymes in Diamond Y Draw before and after attempts to restore the native genome.

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, 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	*C. variegatus*	23	6
	5 Sept. 99			
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	--

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 7 samples from upper watercourse, Diamond-Y Draw. Asterisks = post-treatment dates. Loci diagnostic of the two species are in boldface, as are the alleles and mtDNA diagnostic of *C. variegatus*. For mtDNA, "bov" and "var" refer, to haplotypes of *C. bovinus* and *C. variegatus*. Numbers followed by U = site numbers in upper watercourse (Fig. 2).

	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		bov(2)	bov(28)	bov(28)	bov(23)	bov(20)	---	bov(4)	
	var(6)		**var(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

Gammarus pecos

Site	1997	1998	1999
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

	Gammarus pecos		
Site	1997	1998	1999
Lower Watercourse, continued			
Longitudinal Survey			
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
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	—

	Hyallela azteca		
Site	1997	1998	1999
Lower Watercourse			
Longitudinal Survey			
1	—	1(6)128	1(44)51
2	—	1(5)378	1(36)77
3	—	1(15)254	1(25)45
4	—	1(1)307	1(144)339
5	—	1(2)261	1(71)160
6	—	1(79)310	1(43)59
7	—	1(43)47	1(15)22
8	—	1(21)104	1(31)48
9	—	1(13)236	1(18)136
10	—	1(0)40	1(29)111
11	—	1(1)47	1(6)11
12	—	1(0)261	1(4)72
13	—	1(0)162	1(22)160
15	—	1(2)285	1(84)146
16	—	1(4)239	1(111)224
17	—	1(0)366	1(123)418
18	—	1(0)311	1(17)163
19	—	1(0)73	1(18)65
20	—	1(0)54	1(89)159
21	—	1(0)117	1(236)325
22	—	1(0)100	1(42)112
25	—	1(4)51	1(0)21
26	—	1(1)254	1(5)101
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)86	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)94	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	—

Hyallela azteca			
Site	1997	1998	1999
Lower Watercourse			
Longitudinal Survey			
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)12
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)5	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)35	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(11)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

VITA

Lisa K Kiner

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE CHEMICAL RESTORATION OF DIAMOND Y DRAW, TEXAS: EFFECTS ON FISHES AND MACROINVERTEBRATES

Major Field: Zoology

Biographical:

Education: Graduated from Southwest Missouri State University, Springfield, Missouri where I received a Bachelor of Science in 1992 and a Master of Science in 1996. Completed the requirements for the Doctor of Philosophy degree with a major in Zoology at Oklahoma State University in May 2002.

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