

PARASITES AND HEALTH OF FISHES  
IN THE VERDE RIVER, ARIZONA, AND IMPLICATIONS FOR  
MANAGEMENT OF RAZORBACK SUCKERS, *Xyrauchen texanus*,  
AND COLORADO SQUAWFISH, *Ptychocheilus lucius*

A Final Report

by:

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**Abstract**--Endangered razorback sucker, *Xyrauchen texanus*, and Colorado squawfish, *Ptychocheilus lucius*, reintroduced into the Verde River, Arizona have failed to establish self-sustaining populations. The failure of these reintroductions is often attributed to predation and competition by nonnative fishes and habitat loss, but parasite infections (particularly *Lernaea cyprinacea*) also have been implicated. We assessed the relationships among fish health, macroparasites, and bacterial and viral infections of these endangered fishes collected from two locations (Perkinsville and Childs) on the Verde River, Arizona during February and June of 1996. We also examined surrogate, nonendangered native fishes (Sonora sucker, *Catostomus insignis*, desert sucker, *Catostomus clarki*, and roundtail chub, *Gila robusta*) and nonnative species (common carp, *Cyprinus carpio* and smallmouth bass, *Micropterus dolomieu*) to determine if there were any system wide patterns in parasite infection and health of fishes. All tests for viruses were negative. We identified 19 bacteria (seven known fish pathogens) and 14 macroparasites from examined fishes. Six of the seven dominant (prevalence > 10%) parasite taxa were more prevalent at Perkinsville than at Childs; *Lernaea* occurrence was not related to site. Time of year did not have a consistent effect on parasite prevalence across fish species, and *Lernaea* occurrence was not related to time of year. *Lernaea* were present on 17.3% of the 313 fish examined; 67% of the parasitized fish were infected with a single *Lernaea*. Regression analyses on the calculated Health Assessment Index (HAI), hematocrit, and leukocrit indicated that time of year, site, *Trichodina*, *Ichthyophthirius* (ich), *Ornithodiplostomum* and *Posthodiplostomum* (white grubs), and the cestode *Isoglaridacris hexacotyle*, negatively affected the health of fishes. To establish razorback sucker and Colorado squawfish populations in the Verde River, we recommend manipulations of the stocking regime: (1) increase the number of stockings each year and decrease the number of fish per stocking; (2) increase the number of stocking sites on the Verde River; and (3) discontinue stocking at Perkinsville and upstream sites. In addition, fish should be chemically treated to remove parasites prior to stocking.

### Introduction

Parasites cause harm to their hosts (Begon et al. 1986) and can limit host populations (Crofton 1971a, 1971b; Wilson 1971; May and Anderson 1982; Lemly and Esch 1984), and thus, they should be a serious management concern

for endangered fish populations. Under 'stable' environmental conditions, the persistence of both host and parasite species is insured due to evolutionary interplay between parasite virulence and host resistance. Environmental conditions, such as drought, that stress and overcrowd fish species may allow parasites to flourish and diminish the density of the host species (Post 1987). In addition to direct mortality, parasites can indirectly affect fish mortality by making the host more susceptible to predation (Hoogenboom and Dijkstra 1987; Lafferty and Morris 1996) and secondary infections (Post 1987).

The decline in the native fish fauna of the American Southwest has been attributed to altered flow regimes (due to damming, water diversions, and land use practices) of riverine systems, and to the predatory and competitive effects of introduced nonnative fishes (Miller 1961; Minckley and Deacon 1968, 1991). Nonnative parasites were inadvertently introduced into the region via introductions of nonnative fishes (Hoffman and Schubert 1984). Some of these parasites (e.g., *Lernaea cyprinacea* and *Bothriocephalus acheilognathi*) are generalists and have parasitized many of the Southwest's native fish species (James 1968; Mpoame and Rinne 1983; Heckman et al. 1986, 1987; Brouder and Hoffnagle 1997). Native fishes may be particularly susceptible to introduced parasites (since they have not coevolved with these parasites), which may pose a threat to their existence.

Razorback sucker, *Xyrauchen texanus*, and Colorado squawfish, *Ptychocheilus lucius*, are two native fish species endemic to the Colorado River system. Both are federally listed as endangered (U.S. Fish and Wildlife Service [USFWS] 1989 and 1991). The two species occupied the Gila River drainage in Arizona (Minckley 1973), but were extirpated prior to 1960 (Hendrickson 1993). Attempts to reestablish these endangered species into the Gila River Basin by the Arizona Game and Fish Department (AGFD) and U.S. Fish and Wildlife Service (USFWS) began in 1981 and have been unsuccessful (Hendrickson 1993). Poor survival of initial reintroductions was largely attributed to predation by nonnative fishes (Minckley 1983, Marsh and Brooks

1989, Minckley et al. 1991, Hendrickson 1993). In an effort to alleviate the high mortality rates, larger (>300 mm total length) razorbacks and squawfish were reintroduced into the Verde and Salt rivers (tributaries to the Gila River) during 1991 and 1992 (Creef et al. 1992). However, the larger fish still experienced low survivorship. This low survivorship was partly attributed to *Lernaea cyprinacea* infestations (Creef et al. 1992; Hendrickson 1993). A study (AGFD 1995) of non-predator mortality of razorback suckers and Colorado squawfish in the Verde River indicated that high infection rates (>30 individuals per fish) of *Lernaea* were correlated with fish mortality.

*Lernaea* is an exotic parasitic copepod, which is thought to have originated in Asia and spread throughout the world via the commercial goldfish trade (Hoffman 1970). This parasite, also known as 'anchor worm', has been reported from many species of freshwater fishes (Hoffman and Schubert 1984). The male is not parasitic, however, the female has the potential of causing large-scale mortality of host species via consumption of blood and secondary infections at sites of attachment (Hoffman 1976). Reports of mortality for free-ranging fishes from *Lernaea* infestation are rare, but McNeil (1957) observed significant losses of rainbow trout, *Oncorhynchus mykiss*, infected with this parasite in Arizona hatcheries. Similar losses of Colorado squawfish and razorback suckers have been observed at Bubbling Ponds Hatchery, Arizona (Phil Hines, personal observation).

A number of other organisms, in addition to *Lernaea*, have been reported to parasitize native fishes in the Gila River basin (Amin 1969, Mpoame and Rinne 1983). Several of the parasites are generalists, and have been reported to infect several of the native fish species. For instance, Mpoame and Rinne (1983) reported the protozoan *Ichthyophthirius multifiliis*, and the trematodes *Ornithodiplostomum ptychocheilus* and *Clinostomum marginatum* on roundtail chub, *Gila robusta*, Sonora sucker, *Catostomus insignis*, and desert sucker, *Catostomus clarki*. The intestinal cestode *Isoglaridacris hexacotyl*, a parasitic leech, *Illinobdella moorei*, and the acanthocephalan

*Neoechinorhynchus* sp. have been observed on both desert and Sonora suckers in the Gila River Basin (Amin 1969, Mpoame and Rinne 1983). These generalist parasites may infect and affect populations of reintroduced razorback suckers and Colorado squawfish.

Our study was initiated to assess the effects of parasite infection, particularly *Lernaea*, on the health of razorback sucker and Colorado squawfish stocked in the Verde River. Due to the scarcity of these endangered species, the scope of the study was broadened to include other native and nonnative species. We hoped to utilize the nonendangered species as surrogates to infer the effects of parasites on the two endangered species. The objectives of this study were to:

- (1) collect baseline data on the health and parasitology (micro- and macroparasites) of native fishes in the Verde River;
- (2) determine if health of Verde River fishes is related to parasite infection, season, and river location;
- (3) determine if parasite infections are related to season and river location.

#### Study Area

The study was conducted on the Verde River, a tributary to the Salt River, in Arizona (Figure 1). Two 1 km-long sites were sampled; one in the 'upper' Verde River near Perkinsville, and the other approximately 120 km downstream in the 'lower' Verde River near Childs. These sites were chosen because they were stocking sites for razorback suckers and Colorado squawfish, and they differed in flow regimes, habitat, and physicochemical parameters. Perkinsville is upstream from, and Childs is downstream from, the urban areas of the Verde Valley. Base flow discharge at Perkinsville is approximately 0.7 to 0.8 m<sup>3</sup>/s (Paulden gage), and habitat is dominated by low velocity waters such as pools and glides, interconnected by shallow riffles. At the Childs

site, base flows vary from 1.8 to 5.4 m<sup>3</sup>/s, and habitat consist of rapids, riffles, runs, glides and pools.

#### Methods

We sampled February 11-13 and June 23-26, 1996, at the Childs site, and February 14-16 and June 27-30, 1996, at the Perkinsville site.

A maximum of 20 fish (>100 mm TL) per species were collected at each site during each sampling month. Fish were captured with trammel nets, angling, and via electroshocking. Fish were anesthetized with Tricaine Methanesulfonate (MS222) prior to necropsy. Total length ( $\pm 1$  mm) and weight ( $\pm 1$  g) of each fish was recorded. The condition of external (fins, skin, body deformities, eyes, opercula, gills, thymus, and pseudobranchs) and internal (spleen, kidney, liver, hindgut, gall bladder, and mesenteric fat) anatomy of fishes was qualitatively rated using the Health Condition Profile technique of Goede and Barton (1990). Blood hematocrit, leukocrit, and plasma protein levels also were determined. The caudal peduncle, caudal fin, or gills were cut and blood was collected in a heparinized 1.1 mm diameter capillary tube. Blood samples were centrifuged for 5 min. Each sample was then placed in a hand-held microhematocrit tube reader and the percent volume of hematocrit and leukocrit were recorded. The hematocrit tube was then broken just above the 'buffer' zone to obtain the clear plasma fraction only. The plasma was blown onto a hand-held refractometer, and the plasma protein concentration (g/dl) was recorded.

Fish also were examined for numbers and locations of macroscopic and microscopic external parasites. The entire external surface and the gills were examined visually for macroparasites. Location and numbers of adult female *Lernaea* and other visible macroparasites were recorded. Skin mucous and gill samples were then collected to determine the presence and identity of microscopic external parasites. Skin under a pectoral fin was scraped with a cover slip, and the resultant material placed on a slide and examined with a

compound microscope. Gill filaments were cut from each fish, placed on a slide and examined with a compound microscope. Organisms were identified to the lowest possible taxonomic category and quantified.

During the internal health examination, presence and type of internal macroparasites on or in the viscera were recorded. In addition, the stomach of each fish was excised, preserved in ethanol, and later examined in the laboratory for the presence and type of helminth parasites. For catostomids without a morphologically distinct stomach, we examined the portion of the intestine anterior to the first loop.

We sampled for the presence of microparasites (viruses and bacteria) from kidney and spleen tissue of each fish. To collect viral pathogens, we excised tissue from the kidney and spleen of each fish, and then stored the tissue in vials of minimal essential medium (MEM) at approximately 4°C. Samples were cultured using standardized techniques (Thoesen 1994), and the presence and type of virus were determined.

Bacterial samples were collected aseptically, using two inoculating needles consecutively inserted into and withdrawn from the kidney of each fish. One needle was swabbed onto tryptone yeast extract (TYE) culture media, and the other onto brain-heart infusion agar (BHIA). For Colorado squawfish and razorback suckers that were not sacrificed (fish captured that were stocked <2 months prior to sampling), samples were collected from the anus and around the eye and swabbed onto culture media. Bacterial isolates were identified using Gram stains, Cytochrome/Oxidase test strips, hanging drop motility tests, differential medias, and the Minitek™ Gram negative bacterial identification system.

To assess potential water quality effects on parasites and fish health we measured physicochemical parameters during each sampling period at each site. We used a Hydrolab Surveyor 3 datalogger and H2O transmitter to measure water temperature (°C), pH, dissolved oxygen (mg/L and % saturation), and conductivity (μS) at 4 h intervals for a 24 h period. In addition, we

measured nitrate-nitrite nitrogen (cadmium reduction method) and soluble reactive phosphate (ascorbic acid method) with a Drel 2000 spectrophotometer; three replicate samples, consecutively collected during a single day at each site, were measured.

#### *Statistical analysis*

Presence of each of the dominant (present on more than 10% of the fishes) parasite taxa was tested for independence among sites and months with loglinear analysis (independence model; Feinberg 1980). These tests were performed without regard to fish species, since the main objective was to determine any temporal and spatial differences in parasite occurrences.

For each individual fish we calculated a Health Assessment Index (HAI) similar to that described by Adams et al. (1993). Our HAI differed from that of Adams et al. (1993) in that ratings for hematocrit, leukocrit, and plasma protein were not included, since the normal range of these parameters for the native fishes was not known. In addition, ratings for parasite infestation were not included. Finally, condition of the opercula (0 = no shortening, 10 = mild shortening and 30 = severe shortening) was also included as a category. Our modified index therefore represents the sum of ratings for 11 anatomical features, and ranged from 0 (most healthy) to 330 (most unhealthy). The HAI was compared to hematological parameters (hematocrit, leukocrit, and plasma protein) and condition factor (k<sub>tl</sub>) with Pearson's correlation analysis. Forward stepwise multiple linear regression analyses were used to determine relationships of the HAI, hematocrit, leukocrit, and plasma protein levels to parasite abundances, site, and month. The HAI data were log+1 transformed prior to analysis. For these analyses, month (1 = February, 2 = June) and site (1 = Childs, 2 = Perkinsville) were categorical variables. The white grub variable was categorical (0 = absent, 1 = present), because abundances were not recorded for this macroparasite during June at Perkinsville.

Variables were added to the equation only if the probability associated with the F test was  $\leq 0.05$ .

### Results

We necropsied 79 Sonora suckers (223-452 mm total length [TL]), 64 desert suckers (167-401 mm TL), six (11 captured) razorback suckers (242-478 mm TL), one (three captured) Colorado squawfish (312 mm TL), 44 roundtail chub (107-371 mm TL; two <200 mm TL), 79 common carp (163-672 mm TL), and 40 smallmouth bass (138-307 mm TL). Condition factors, HAI, hematocrit, leukocrit, and plasma protein levels of the necropsied fishes are summarized in Appendix 1. All tests for viruses were negative. We identified 19 bacteria taxa (seven are known fish pathogens) and 13 macroparasites from examined fishes (Tables 1-6). Although we did not identify any bacteria from the one sacrificed Colorado squawfish, we did identify seven types of bacteria (gram positive, *Aeromonas* sp., *Flexibacter* sp., *Proteus mirabilis*, *Plesiomonas shigelloides*, *Pseudomonas* sp., and *P. putrefaciens*) from the two Colorado squawfish not sacrificed. No macroparasites were found on the one Colorado squawfish necropsied; nonsacrificed fish were not examined. *Trichodina*, white grubs (identified as *Ornithodiplostomum* spp. and *Posthodiplostomum* spp. by O. Amin of the Institute of Parasitic Diseases, Phoenix, Arizona), and *Lernaea* were present on all fish species except Colorado squawfish. The three catostomid species had four parasite taxa (*Trichodina*, white grubs, *Lernaea*, and piscicolid leeches) in common. The cyprinids (carp and roundtail chub) had six parasite taxa (*Trichodina*, *Gyrodactylus*, *Neascus* [black grubs], white grubs, and *Lernaea*) in common.

Eleven parasite taxa were identified from fishes captured at Childs and 10 from fishes captured at Perkinsville (Tables 1-6). *Ichthyobodo* (costia), *Cleidodiscus*, and piscicolid leeches were only found at Childs, whereas *Ichthyophthirius* (ich) and *Chilodonella*, were only found at Perkinsville. Presence of five of the six dominant (occurrence > 10%) parasite taxa was

dependent on site and month (*Trichodina*  $\chi^2 = 21.0$ ,  $p < 0.001$ ; *Gyrodactylus*  $\chi^2 = 97.1$ ,  $p < 0.001$ ; *Neascus*  $\chi^2 = 37.5$ ,  $p < 0.001$ ; white grubs  $\chi^2 = 25.4$ ,  $p < 0.001$ ; Cestoda  $\chi^2 = 68.1$ ,  $p < 0.001$ ). All five of these taxa occurred more frequently at Perkinsville than at Childs. *Trichodina*, *Gyrodactylus*, and Cestoda were more prevalent in February than June, whereas black grubs and white grubs were more frequent during June than February. Ich, although not a 'dominant' taxa (9% occurrence), were more prevalent in February than June ( $\chi^2 = 289.58$ ,  $p < 0.001$ ). The occurrence of *Lernaea* was independent of site and month ( $\chi^2 = 7.6$ ,  $p = 0.11$ ).

*Lernaea* were present on 17.3% (54) of the fish examined (Tables 1-6). The greatest infestation of *Lernaea* was 30 individuals on a razorback sucker captured at Childs during February. However, that maximum was unusual in that the next greatest infestation was five *Lernaea* on both desert and Sonora suckers. Sixty seven percent (36/54) of the *Lernaea* infested fish were infected with one *Lernaea*. *Lernaea* abundance was not related to HAI, hematocrit, leukocrit, plasma protein levels, or bacteria (known pathogens) presence for any of the fish species.

The HAI was significantly affected by three macroparasite taxa, month, and site (Table 7). For Sonora and desert suckers, the HAI increased as cestode abundance increased. A similar positive relationship was found for ich on desert suckers and for white grubs on roundtail chubs. Month had a positive effect on the HAI of Sonora sucker and common carp, indicating that the HAI increased from February to June. The HAI was negatively related to site for common carp, indicating that the health index decreased from Childs to Perkinsville.

Three macroparasite taxa, month and site significantly affected hematocrit, whereas only month affected leukocrit (Table 7). For desert suckers, hematocrit increased as cestode abundance increased. For roundtail chub, hematocrit decreased from February to June. Hematocrit increased as *Trichodina* abundance decreased for carp. Smallmouth bass hematocrit decreased

from Childs to Perkinsville and when white grubs were present. For three fish species, month had an affect on leukocrit levels; leukocrit decreased from February to June. Plasma protein levels were not significantly impacted by parasite taxa, month, or site.

The HAI for desert suckers was positively correlated with hematocrit ( $r = 0.29$ ,  $p = 0.02$ ). No other significant correlations among the HAI, ktl, and hematological parameters were found for any of the fish species.

Water quality differed among sites and months (Table 8). Water temperature and conductivity were greater at Childs than at Perkinsville, and were greater during June than February. Dissolved oxygen (mg/L) also was greater at Childs than Perkinsville, but was greater during February than June. Water at Perkinsville had greater pH than that at Childs, and at both sites pH was greater during June than February. Nitrate and orthophosphate levels were not statistically compared among sites or months due to low sample size; however, no pattern was evident among sites or months (Table 8) for these two nutrients.

#### Discussion

Five of six dominant macroparasite taxa were more prevalent in the upper than the lower Verde River which was opposite the expectation of greater prevalence with warmer water temperatures. It may be that the differences in temperature and chemical parameters between sites were statistically, but not biologically, significant.

The observed greater prevalence of macroparasites at Perkinsville compared to Childs may be partly attributed to flow conditions. The fishes examined in this study tend to occupy slow-flowing habitats (Minckley 1973; Clarkson et al. 1993; Barrett and Maughan 1995), and the Verde River near Perkinsville is dominated by such habitats (Creef et al. 1992; Clarkson et al. 1993). Fishes may become concentrated in these pool habitats when flows decrease to levels where movement out of the pool is restricted. Infection of

fishes by free-living life stages of parasites is accentuated by slow or stagnant water (Post 1987). Fishes in the Verde River near Childs (where base flows are at least two times greater than at Perkinsville) may rarely become restricted to pool habitats.

In addition to host availability, abiotic environmental conditions can also limit parasite populations (Kennedy 1994). Water temperature is probably the most important abiotic environmental factor that can limit fish parasites (Wedemeyer et al. 1976; Post 1987; Kennedy 1994). Although temperature differences between sites were slight, temperatures during June were 10°C greater than during February. Summer water temperatures in the Verde River are near optimal for some parasite species to complete their life cycles. For example, *Lernaea cyprinacea* require 23-40°C (Hoffman 1976), *Gyrodactylus* 22-24°C (Post 1987), and *Posthodiplostomum* 21-29°C (Post 1987) to complete their life cycles. We therefore expected parasite prevalence to be greater in June than February, and this was true for white and black grubs. However, three of the other dominant parasites were more prevalent during February than June, when water temperatures should have been less than optimal for completion of their life cycles. It may be that the native fishes were more susceptible to infection by these parasites during the winter due to spawning related stress (57% of the native fishes examined during February had mature gonads compared to 11% in June).

Although parasites tended to be more prevalent at Perkinsville than Childs, the HAI did not differ among sites, except for carp. Based on the HAI regressions, carp tended to be healthier at Perkinsville than at Childs, and healthier in February than in June. Other factors are apparently affecting carp health more than parasites. The month-site pattern may be explained by spawning stress (100% of the carp examined at Childs had mature gonads, whereas at Perkinsville 95% of the carp in February and 55% in June had mature gonads). Carp leukocrit also was greater in February than in June, further indicating that the fish were more stressed during winter.

Results of regression analysis also indicate that Sonora sucker were healthier in February (lower HAI) than in June. If true, then leukocrit levels during this month could be considered 'normal' and the decrease in leukocrit in June may indicate environmentally related stress. Cestode infection in Sonora suckers apparently was not related to the high HAI in June, since infection by this parasite was less in June than in February.

Four parasite taxa (*Ichthyophthirius multifiliis*, *Trichodina*, *Isoglaridacris hexacotyle*, and white grubs) were found to negatively impact fish health (based on the HAI, hematocrit, and leukocrit regressions) in the Verde River. Although parasite effects on health varied among the surrogate fish species, we cautiously suggest that these four parasites may negatively impact any razorback suckers and Colorado squawfish reintroduced into the Verde River. Although *Isoglaridacris hexacotyle* has not been reported to infect these two endangered fishes, *Trichodina* and *Ornithodiplostomum* infect Colorado squawfish (Flagg 1981) and razorback sucker (present study), and *Ich* infestations of both fish species have been noted at Bubbling Ponds Hatchery (Phil Hines, personal observation). Of these four parasite species, *Ich* may be the most dangerous since epizootics of this introduced parasite (Hoffman and Schubert 1984) seem to be common in Arizona streams (Mpoame and Rinne 1983). Drought, which can result in increased proportions of slow or no velocity pools, increased localized fish densities, and high water temperatures probably favors outbreaks of this parasite (Mpoame and Rinne 1983). Similar conditions would favor epizootics of *Trichodina*, the other likely introduced (Hoffman and Schubert 1984) protozoan parasite.

The tapeworm, *Isoglaridacris hexacotyle*, that infected the sucker species, and the white grubs, *Ornithodiplostomum ptychocheilus* and *Posthodiplostomum minimum*, are likely native to Arizona (Mpoame and Rinne 1983), and therefore may pose less of a threat to the survivorship of reintroduced razorback suckers and Colorado squawfish. Although high fish densities and high water temperatures favor these helminth parasites,

sufficient populations of their intermediate hosts are also necessary for epidemics. Annelids, the likely intermediate hosts for the tapeworm (Amin 1969), tend to inhabit fine grained substrates typical of pools and slow velocity habitats; fish become infected after ingesting the annelids. Although molluscs are the intermediate host for fish digenetic trematodes, fish become infected by direct penetration of the body surface. Therefore, slow velocity waters tend to enhance transmission of white grubs from snails to fish hosts.

*Lernaea* parasitism at the levels we observed did not affect the health (as measured) of native fishes in the Verde River. However, we did not capture fish with *Lernaea* infections as great as those reported by Hendrickson (1993; up to 80-100 *Lernaea*). It is possible that the infrapopulations of the parasite during our field study were too low to significantly impact the health of Verde River fishes. Even the one razorback sucker that was parasitized with 30 *Lernaea* was in healthy condition based on the HAI. However, it is also possible that slight declines in fish health, resulting from low levels of *Lernaea* infection, were not detected with the HAI.

Except for the one razorback sucker infected with 30 *Lernaea*, intensity of infection by this parasite was low and prevalence moderate to high indicating a relatively even distribution among razorbacks. Parasites with even distributions and low to moderate pathogenicity have the potential to effect density declines in the host (Anderson 1979). Therefore, although we did not detect a negative impact of *Lernaea* on the health of razorback suckers, this parasite may affect reintroduced populations of razorback suckers. The observed pattern of *Lernaea* prevalence and low intensity of infection on razorback suckers could occur for the following reasons: 1) mortality of hosts with high intensities of infection; 2) density-dependent mortality among parasites; or 3) acquired resistance to reinfection (Anderson 1982). A larger sample of wild razorback suckers and Colorado squawfish is

necessary to better determine the effects of *Lernaea* on the reintroduced populations of these endangered fishes.

Conditions that stress a host can make it more susceptible to parasitism (Wedemeyer et al. 1976). Hatchery reared razorbacks and squawfish that are subsequently stocked into natural riverine environments may be stressed due to handling and introduction to a 'new' environment, and thus be more susceptible to parasite infection. However, except for the one razorback sucker infected with 30 *Lernaea*, intensity of infection by this parasite was low (<5 per fish), lending little evidence to support this hypothesis.

The Health Condition Profile technique enabled us to detect impacts of macroparasites on health of fishes in the Verde River, but effects were most noticeable for macroparasites with high infection rates. Most fish appeared to be in good condition suggesting that parasites were not causing serious fish health problems in the Verde River. Additional collections and assessments of fishes heavily infected with parasites may increase the chances of detecting health impacts due to parasites such as *Lernaea*. We therefore recommend targeting heavily infected fishes in any future field studies of fish parasites and health in the Gila River Basin.

#### Management Options

1. Since past stocking regimes have not resulted in the establishment of razorback sucker and Colorado squawfish populations in the Verde River, we recommend experimental manipulation of stocking practices to attain the goal of self-sustaining populations. One suggested manipulation is to increase the number of stockings each year, but decrease the number of fish per stocking. This could decrease predation and parasitism risks due to lower post-stocking fish densities. This regime also may result in more constant fish densities throughout the year, which may improve the chances of reproduction. Another manipulation would be to stock at more sites within the Verde Valley and downstream to Childs, thereby

resulting in more even fish distributions in this reach of the river. This might increase the chances for fish to occupy habitats with low predation and parasitism pressures.

2. Stock fish >300 mm TL. Although larger fish will still face the risk of parasite induced mortality, the risk of predation will be decreased.
3. Discontinue stocking of razorback suckers and Colorado squawfish at the Perkinsville site, or any upstream site; stock sites downstream from Sycamore Creek, Yavapai County, where greater flows may hinder parasite dispersal.
4. Treat all fish with appropriate parasiticides prior to stocking. Emphasis should be placed on treating for the exotic parasites *Ich* and *Trichodina*.
5. When and if, species specific, economically feasible, and environmentally safe chemotherapies are developed for riverine environments, they should be used to treat the river to remove selected parasite species.

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Appendix 1. Means (numerator) and sample sizes (denominator) of condition index (k<sub>tl</sub>), health assessment index (HAI), hematocrit, leukocrit, and plasma protein levels for Verde River fishes among sites and months, 1996. Parameter ranges are in parentheses.

Species and factor	K <sub>tl</sub>	HAI	Hematocrit (%)	Leukocrit (%)	P.protein (g/dL)
<i>Catostomus insignis</i>	1.1/79 (0.4-2.6)	9.0/79 (0-90)	42.0/75 (20.0-64.0)	0.7/74 (0.1-2.5)	4.9/74 (2.6-8.0)
February					
Childs	1.2/19	3.2/19	42.6/17	0.8/16	5.8/17
Perkinsville	1.1/20	14.0/20	41.7/19	0.8/19	5.1/19
June					
Childs	1.2/20	10.5/20	43.1/20	0.6/20	4.4/20
Perkinsville	1.1/20	8.0/20	40.7/19	0.5/19	4.4/18
<i>Pantosteus clarki</i>	1.2/64 (0.6-1.6)	13.3/64 (0-70)	41.4/62 (26.5-58.5)	0.7/62 (0.1-2.0)	5.2/63 (3.0-7.4)
February					
Childs	1.3/4	0.0/4	35.9/4	0.8/4	6.8/4
Perkinsville	1.2/20	26.5/20	44.8/20	0.9/20	5.1/20
June					
Childs	1.2/20	10.0/20	42.7/18	0.7/18	5.4/19
Perkinsville	1.2/20	6.0/20	37.7/20	0.5/20	4.9/20
<i>Xyrauchen texanus</i>	1.1/12 (0.8-1.3)	26.7/6 (10-40)	34.8/5 (19.0-32.0)	0.7/5 (0.5-1.0)	3.5/4 (2.9-4.3)
February					
Childs	1.1/7	10.0/1	19.0/1	1.0/1	--
June					
Childs	1.0/5	30.0/5	38.8/4	0.7/4	3.5/4

## Appendix 1 continued.

Species and factor	Ktl	HAI	Hematocrit (%)	Leukocrit (%)	P.protein (g/dl)
<i>Gila robusta</i>	1.0/44 (0.6-2.0)	37.6/42 (0-70)	36.9/43 (12.5-48.5)	0.9/43 (0.1-3.0)	4.6/43 (3.0-6.4)
February					
Childs	0.9/4	0.0/2	35.0/4	1.1/4	6.1/4
Perkinsville	1.1/20	35.0/20	41.1/20	1.3/20	4.5/20
June					
Perkinsville	0.9/20	44.0/20	32.8/19	0.5/19	4.4/19
<i>Ptychocheilus lucius</i>	0.8/3	30.0/1	37.0/1	1.0/1	9.0/1
February					
Childs	0.8/3	30.0/1	37.0/1	1.0/1	9.0/1
<i>Cyprinus carpio</i>	1.3/79 (1.1-2.1)	9.6/79 (0-50)	39.6/79 (16.8-66.3)	0.9/79 (0.1-3.0)	3.5/77 (1.5-5.2)
February					
Childs	1.3/19	5.3/19	38.3/19	1.0/19	3.5/18
Perkinsville	1.4/20	1.5/20	38.1/20	1.2/20	4.1/20
June					
Childs	1.3/20	22.0/20	42.2/20	0.8/20	3.4/19
Perkinsville	1.3/20	9.5/20	39.9/20	0.7/20	2.9/20
<i>Micropterus dolomieu</i>	1.3/40 (1.1-1.7)	36.0/40 (0-70)	39.4/38 (23.0-52.0)	0.3/37 (0.1-1.5)	5.0/38 (3.0-7.1)
June					
Childs	1.2/20	42.5/20	42.7/18	0.4/18	4.6/18
Perkinsville	1.4/20	29.5/20	36.4/20	0.2/19	5.4/20

TABLE 1. Prevalence (percent occurrence; numerator) of organisms and mean intensity of infection (mean number of parasites per fish; denominator) of macroparasites found on and in *Catostomus insignis* at two sites on the Verde River during February and June, 1996. Number of fish sampled is in parentheses. Collection locations for eukaryotic organisms were: b = external body surface; g = gills; c = coelomic cavity; I = gastrointestinal tract.

Organism	February		June		Total (79)
	Childs (19)	Perkins. (20)	Childs (20)	Perkins. (20)	
<b>Bacteria</b>					
*Gram positive	0	0	5	25	8
* <i>Acinetobacter lwoffii</i>	5	0	0	0	1
* <i>Aeromonas</i> sp.	11	10	0	0	5
* <i>Pasteurella haemolytica</i>	0	5	0	0	1
<i>Pseudomonas fluorescens</i>	0	0	0	5	1
* <i>Pseudomonas multifilia</i>	0	5	0	0	1
* <i>P. paucimobilis</i>	0	0	0	5	1
* <i>P. putrefaciens</i>	5	0	0	0	1
<i>Plesiomonas shigelloides</i>	0	5	0	5	3
* <i>Shigella</i> sp.	5	0	0	0	1
* <i>Yersinia pseudotuberculosis</i>	0	0	0	5	1
<b>Macroparasites</b>					
<b>Protozoa</b>					
<i>Ichthyobodo</i> <sup>b</sup>	0	0	5/0.05	0	1/0.01
<i>Ichthyophthirius multifiliis</i> <sup>g</sup>	0	5/0.05	0	0	1/0.01
<i>Trichodina</i> sp. <sup>b</sup>	5/0.05	0	0	0	1/0.01
* <i>Epistylis</i> sp. <sup>g</sup>	0	5/0.05	0	0	1/0.01
<b>Platyhelminthes</b>					
<i>Gyrodactylus</i> sp. <sup>b,g</sup>	5/0.05	80/1.55	0	30/0.30	29/0.48
<i>Neascus</i> sp. <sup>b</sup>	0	20/1.05	15/0.30	15/0.80	11/0.54
'White grub' <sup>c</sup>	63	10	20	40	30
Unknown Trematoda <sup>g</sup>	0	0	0	5/0.05	1/0.01
<i>Isoglaridacris hexacotyle</i> <sup>i</sup>	11/0.21	65/4.35	0	30/2.45	27/1.77
<b>Crustacea</b>					
<i>Lernaea cyprinacea</i> <sup>b</sup>	5/0.05	5/0.05	10/0.30	0	5/0.10
<b>Annelida</b>					
Piscicolidae <sup>b</sup>	0	0	15/0.20	0	4/0.10

\* not known to be a fish parasite

<sup>i</sup>*Ornithodiplostomum ptychocheilus* or *Posthodiplostomum minimum*

TABLE 2. Prevalence (numerator) or organisms and mean intensity of infection (mean number of parasites per fish; denominator) of macroparasites found on and in *Pantosteus clarki* at two sites on the Verde River during February and June, 1996. Number of fish examined is in parentheses. See Table 1 for explanation of superscripted letters.

Organism	February		June		Total (64)
	Childs (4)	Perkins. (20)	Childs (20)	Perkins. (20)	
<b>Bacteria</b>					
* <i>Acinetobacter lwoffii</i>	0	5	0	10	5
<i>Aeromonas</i> sp.	0	5	0	0	2
<i>A. hydrophila</i>	0	0	0	5	2
*Gram positive	0	5	0	15	6
* <i>Klebsiella oxytoca</i>	0	0	0	5	2
<i>Pseudomonas</i> sp.	0	5	0	0	2
<i>P. flourescens</i>	0	0	0	5	2
* <i>P. paucimobilis</i>	0	10	0	0	3
<i>Plesiomonas shigellodius</i>	0	5	5	0	3
<b>Macroparasites</b>					
<b>Protozoa</b>					
<i>Ichthyophthirius multifiliis</i> <sup>b,*</sup>	0	35/1.80	0	5/0.05	13/0.58
<i>Chilodonella</i> sp. <sup>s</sup>	0	0	0	5/0.15	2/0.05
<i>Trichodina</i> sp. <sup>b,*</sup>	0	5/0.15	5/0.02	10/0.10	6/0.09
<b>Platyhelminthes</b>					
<i>Gyrodactylus</i> sp. <sup>b,*</sup>	0	85/1.95	0	15/0.15	31/0.66
Neascus <sup>b</sup>	0	20/1.40	20/0.75	65/7.10	33/2.89
'White grub' <sup>c</sup>	50	75	45	100	72
<i>Isoglaridacris hexacotyle</i> <sup>i</sup>	0	95/16.35	5/0.05	30/2.25	47/6.34
<b>Crustacea</b>					
<i>Lernaea cyprinacea</i> <sup>b</sup>	25/0.50	30/0.35	35/0.50	55/1.00	39/0.61
<b>Annelida</b>					
Piscicolidae <sup>b</sup>	0	0	20/0.35	0	6/0.18
*Unknown <sup>b,*</sup>	0	0	10/0.10	5/0.05	5/0.18

\* not known to be a fish parasite

<sup>i</sup>*Ornithodiplostomum ptychocheilus* or *Posthodiplostomum minimum*

TABLE 3. Prevalence (percent occurrence; numerator) of organisms and mean intensity of infection (mean number of parasites per fish; denominator) of macroparasites found on and in *Gila robusta* at two sites on the Verde River, February and June, 1996. Number of fish examined is in parentheses. See Table 1 for explanation of superscripted letters.

Organism	February		June	Total (44)
	Childs (4)	Perkins. (20)	Perkins. (20)	
<b>Bacteria</b>				
* <i>Acinetobacter anitratus</i>	0	5	0	2
<i>Aeromonas</i> sp.	0	10	0	5
*Gram positive	0	20	20	18
* <i>Moraxella</i> sp.	0	5	0	2
<i>Pseudomonas</i> sp.	0	5	0	2
* <i>Pseudomonas cepacia</i>	0	0	10	5
* <i>P. paucimobilis</i>	0	10	5	7
* <i>Shigella</i> sp.	0	5	5	5
* <i>Yersinia pseudotuberculosis</i>	0	0	5	2
<b>Macroparasites</b>				
<b>Protozoa</b>				
<i>Ichthyophthirius multifiliis</i> <sup>b,†</sup>	0	95/5.40	0	43/2.45
<i>Trichodina</i> sp. <sup>b,†</sup>	0	30/0.35	0	14/0.16
* <i>Epistylis</i> <sup>†</sup>	0	5/0.05	0	2/0.02
<b>Platyhelminthes</b>				
<i>Gyrodactylus</i> <sup>b,†</sup>	25/0.75	45/1.35	5/0.05	25/0.70
<i>Neascus</i> <sup>b</sup>	0	25/0.95	75/5.55	46/2.95
<sup>†</sup> White grub <sup>c</sup>	50	80	100	86
<i>Isoglaridacris</i> sp. <sup>i</sup>	0	5/0.15	10/0.20	7/0.18
<b>Crustacea</b>				
<i>Lernaea cyprinacea</i> <sup>b</sup>	25/0.25	25/0.35	40/0.60	32/0.45

\* not known to be a fish parasite

<sup>†</sup>*Ornithodiplostomum ptychocheilus* or *Posthodiplostomum minimum*

TABLE 4. Prevalence (percent occurrence; numerator) of organisms and mean intensity of infection (mean number of parasites per fish; denominator) of macroparasites found on and in *Cyprinus carpio* at two sites on the Verde River during February and June, 1996. Sample size is in parentheses. See Table 1 for explanation of superscripted letters.

Organism	February		June		Total (79)
	Childs (19)	Perkins. (20)	Childs (20)	Perkins. (20)	
<b>Bacteria</b>					
* <i>Acinetobacter lwoffii</i>	0	0	0	5	1
<i>Aeromonas</i> sp.	11	15	0	0	6
<i>A. hydrophila</i>	0	0	10	5	4
*Gram positive	11	10	15	10	11
* <i>Moraxella</i> sp.	5	0	0	0	1
<i>Pseudomonas fluorescens</i>	0	0	0	25	6
* <i>P. putrefaciens</i>	5	0	0	0	1
<i>Vibrio alginolyticus</i>	5	0	5	0	3
<b>Macroparasites</b>					
<b>Protozoa</b>					
<i>Chilodonella</i> sp. <sup>b</sup>	0	5/0.05	0	0	1/0.01
<i>Trichodina</i> sp. <sup>b,s</sup>	16/0.16	55/2.45	0	20/0.20	22/0.71
* <i>Epistylis</i> sp. <sup>b,s</sup>	16/0.68	5/0.05	0	0	6/0.18
*Unknown protozoa <sup>t</sup>	0	5/0.05	0	0	1/0.02
<b>Platyhelminthes</b>					
<i>Cleidodiscus</i> sp. <sup>t</sup>	0	0	0	10/0.15	3/0.04
<i>Dactylogyrus</i> sp. <sup>b,s</sup>	0	0	10/0.10	0	3/0.03
<i>Gyrodactylus</i> sp. <sup>b</sup>	5/0.05	5/0.05	0	5/0.05	4/0.04
<i>Neascus</i> sp. <sup>b</sup>	0	0	5/0.05	5/0.05	3/0.03
<sup>l</sup> White grub <sup>c</sup>	26	0	30	30	22
<sup>i</sup> Cestoda <sup>i</sup>	5/0.16	50/6.00	15/1.25	15/3.75	22/2.82
<b>Crustacea</b>					
<i>Lernaea cyprinacea</i> <sup>b</sup>	0	5/0.05	5/0.05	0	3/0.03

\* not known to be a fish parasite

<sup>l</sup>*Ornithodiplostomum ptychocheilus* or *Posthodiplostomum minimum*

TABLE 5. Prevalence (percent occurrence; numerator) of organisms and mean intensity of infection (mean number of parasites per fish; denominator) of macroparasites found on and in *Micropterus dolomieu* at two sites on the Verde River, June 1996. Number of fish examined is in parentheses. See Table 1 for explanation of superscripted letters, except l = liver, and s = spleen.

Organism	Childs (20)	Perkinsville (20)	Total (40)
<b>Bacteria</b>			
<i>Aeromonas hydrophila</i>	0	15	8
*Gram positive	0	25	13
<i>Plesiomonas shigelloides</i>	0	15	8
* <i>Pseudomonas paucimobilis</i>	0	5	3
* <i>Yersinia pseudotuberculosis</i>	0	5	3
<b>Macroparasites</b>			
<b>Protozoa</b>			
<i>Trichodina</i> sp. <sup>b</sup>	5/0.05	10/0.10	8/0.08
* <i>Epistylis</i> sp. <sup>a</sup>	5/0.10	0	3/0.05
<b>Platyhelminthes</b>			
<i>Cleidodiscus</i> sp. <sup>a</sup>	5/0.10	0	3/0.05
'White grub' <sup>a,c</sup>	70	90	80
Cestoda <sup>i</sup>	5/0.05	0	3/0.03
<b>Crustacea</b>			
<i>Lernaea cyprinacea</i> <sup>b</sup>	5/0.05	20/0.20	13/0.13

\* not known to be a fish parasite

<sup>i</sup>*Ornithodiplostomum ptychocheilus* or *Posthodiplostomum minimum*

TABLE 6. Prevalence (percent occurrence; numerator) of organisms and mean intensity of infection (denominator) of macroparasites found on and in *Xyrauchen texanus* during February and June, Verde River at Childs, 1996. Number of fish sampled is in parentheses. Bacteria samples were collected from the anus of the 6 fish not sacrificed (nonlethal) in February; these fish were not examined for macroparasites.

Organisms	Nonlethal	Lethal		Grand	
	February (6)	February (1)	June (5)	Total (6)	Total (12)
<b>Bacteria</b>					
* <i>Acinetobacter lwoffii</i>	17	0	0	0	8
<i>Aeromonas</i> sp.	83	0	0	0	33
<i>A. hydrophila</i>	0	0	20	17	8
*Gram positive	50	0	0	0	25
* <i>Pasteurella haemolytica</i>	17	0	0	0	8
<i>Pseudomonas</i> sp.	50	100	0	17	33
* <i>P. cepacia</i>	17	0	0	0	8
<i>P. fluorescens</i>	17	0	0	0	8
* <i>P. paucimobilis</i>	17	0	0	0	8
<i>Plesiomonas shigelloides</i>	17	0	0	0	8
<b>Macroparasites</b>					
<b>Protozoa</b>					
<i>Trichodina</i> sp. <sup>b</sup>	--	100/1.00	0	17/0.17	--
*Unknown protozoa <sup>c</sup>	--	0	20/0.20	17/0.17	--
<b>Platyhelminthes</b>					
'White grub' <sup>c</sup>	--	0	60	50	--
<b>Crustacea</b>					
<i>Lernaea cyprinacea</i> <sup>b</sup>	--	100/30.0	60/1.80	50/6.50	--
<b>Annelida</b>					
<i>Piscicolidae</i> <sup>b</sup>	--	0	20/0.20	17/0.17	--

\* not known to be a fish parasite

<sup>b</sup>*Ornithodiplostomum ptychocheilus* or *Posthodiplostomum minimum*

Table 7. Significant multivariate regression models for the health assessment index (HAI), hematocrit, and leukocrit of Verde River fishes. M = month, S = site, C = *Isoglaridacris hexacotyle* abundance, I = *Ichthyophthirius multifiliis* abundance, T = *Trichodina* abundance, W = white grub presence. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

Health index and species	Model	R <sup>2</sup>	F	p
HAI (log+1 transformed)				
<i>Catostomus insignis</i>	$Y = 0.02 + 0.34(M) + 0.03(C)$	0.13	6.81	**
<i>Catostomus clarki</i>	$Y = 0.58 + 0.02(C) + 0.10(I)$	0.19	7.23	**
<i>Gila robusta</i>	$Y = 0.75 + 0.74(W)$	0.13	6.80	*
<i>Cyprinus carpio</i>	$Y = -0.07 + 0.78(M) - 0.33(S)$	0.42	28.90	***
Hematocrit				
<i>Catostomus clarki</i>	$Y = 40.62 + 0.17(C)$	0.07	4.07	*
<i>Gila robusta</i>	$Y = 49.50 - 8.37(M)$	0.32	17.04	***
<i>Cyprinus carpio</i>	$Y = 40.14 - 0.75(T)$	0.05	4.03	*
<i>Micropterus dolomieu</i>	$Y = 51.96 - 5.28(S) - 5.56(W)$	0.33	8.52	**
Leukocrit				
<i>Catostomus insignis</i>	$Y = 1.09 - 0.27(M)$	0.08	6.26	*
<i>Gila robusta</i>	$Y = 2.01 - 0.36(M)$	0.35	19.85	***
<i>Cyprinus carpio</i>	$Y = 1.45 - 0.36(M)$	0.10	8.98	**

TABLE 8. Means (numerator) and sample size (denominator) of water physicochemical parameters measured over one 24 h period during two months at two sites on the Verde River, 1996. Significant (sig.;  $p < 0.05$  ANOVA) differences between months (a) and sites (b) also are indicated.

Parameter	February		June		Sig.
	Childs	Perkinsville	Childs	Perkinsville	
Water temperature ( $^{\circ}\text{C}$ )	13.3/7	11.7/7	23.3/6	21.2/7	a,b
Dissolved oxygen (mg/L)	8.22/7	7.87/7	6.80/6	5.68/7	a,b
pH	8.13/7	8.23/7	8.84/6	8.96/7	a,b
Conductivity ( $\mu\text{S}/\text{cm}$ )	578/7	522/7	836/6	535/7	a,b
Nitrite-Nitrate (mg/L)	0.10/2	0.19/1	0.11/3	0.10/3	--
Orthophosphate (mg/L)	0.03/3	0.10/2	0.04/3	0.04/3	--

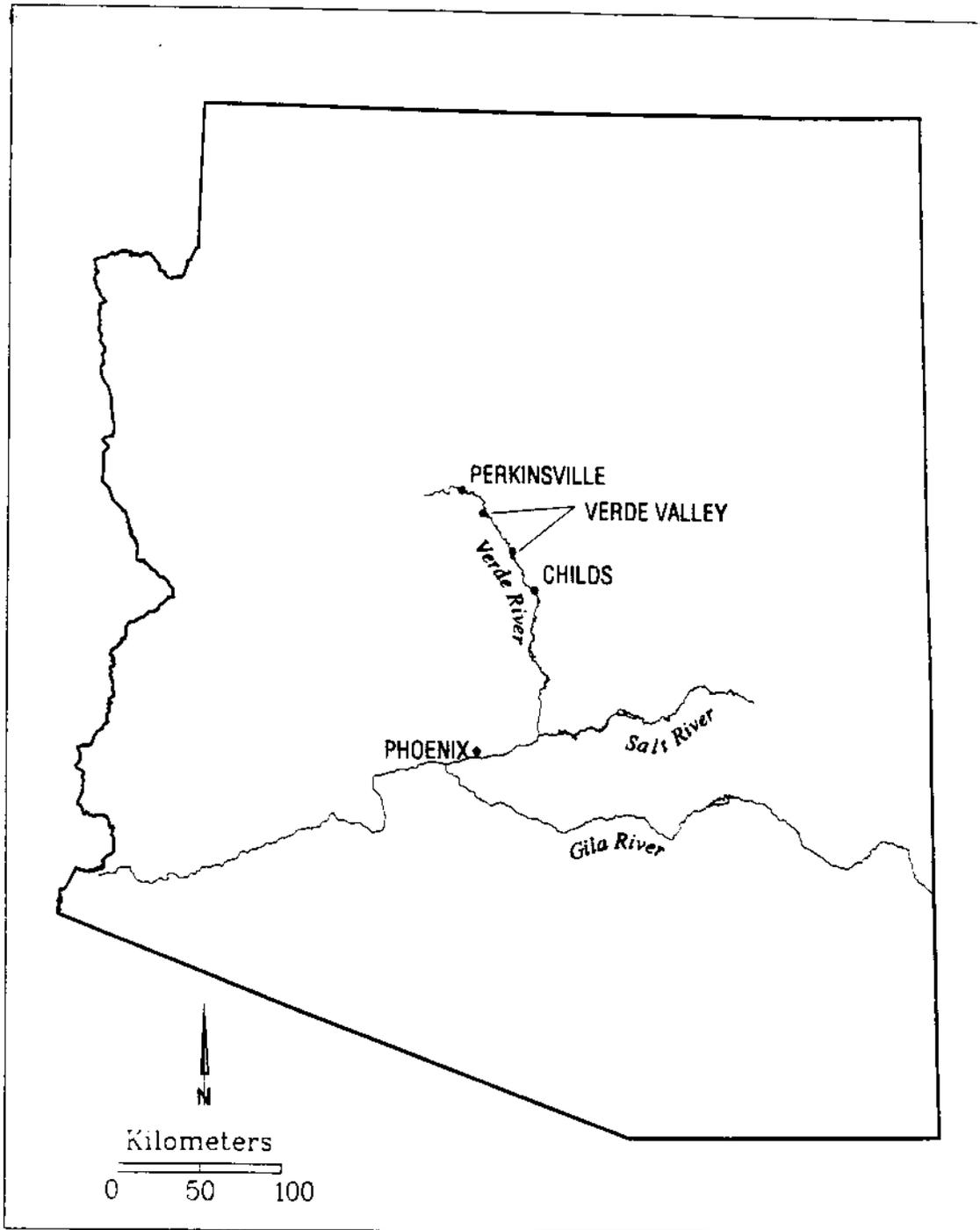


FIGURE 1. Map of the study area, showing the two study sites, Childs and Perkinsville.

