

**Parasites of native and non-native fishes of the Little Colorado River, Grand Canyon, Arizona.**

Anindo Choudhury<sup>1</sup>, Timothy L. Hoffnagle<sup>2</sup>, and Rebecca A. Cole  
USGS-National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711,  
U.S.A.

<sup>2</sup>Oregon Department of Fish and Wildlife, 211 Inlow Hall, Eastern Oregon University, La Grande, OR 97850

Corresponding author:

Rebecca Cole, USGS – National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, U.S.A. Phone: (608) 270 2468. E-mail: Rebecca\_Cole@usgs.gov.

<sup>1</sup>Current address: Division of Natural Sciences, St. Norbert College, 100 Grant Street, DePere, Wisconsin 54115, U.S.A.

Running Head: Fish parasites in the Little Colorado River.

**ABSTRACT:** A 2-year, seasonal, parasitological study of 1435 fish, belonging to 4 species of native fishes and 7 species of non-native fishes from the lower Little Colorado River (LCR) and tributary creeks, Grand Canyon, Arizona, yielded 17 (possibly 18) species of parasites. These comprised 1 myxozoan (*Henneguya exilis*), 2 copepods (*Ergasilus arthrosis* and *Lernaea cyprinacea*), 1 acarine (Oribatida gen. sp.), 1 piscicolid leech (*Myzobdella lugubris*), 3 (possibly 4) monogeneans (*Gyrodactylus hoffmani*, *Gyrodactylus* sp., *Dactylogyrus extensus*, and *Ligictaluridus floridanus*), 4 nematodes (*Contracacecum* sp., *Eustrongylides* sp., *Rhabdochona* sp., *Truttaedacnitis truttae*), 3 cestodes (*Bothriocephalus acheilognathi*, *Corallobothrium fimbriatum* and *Megathylacoides giganteum*), and 2 trematodes (*Ornithodiplostomum* sp., *Posthodiplostomum* sp.). Of these, *Rhabdochona* sp. is the only adult parasite native to the LCR. Infection intensities (worm burden) of *Ornithodiplostomum* sp and *B. acheilognathi* were positively correlated with length of humpback chub, *Gila cypha*. Adult helminths showed a high degree of host specificity, the exception being *B. acheilognathi*, which was recovered from all fish species examined but was most abundant in cyprinids. Significantly higher abundance of *B. acheilognathi* in speckled dace, *Rhinichthys osculus* in the larger of the two creeks, Big Canyon Creek (BCC), may be related to higher population densities of dace. Abundance of *B. acheilognathi* in the humpback chub was highest in the fall and lowest in the summer, in both reaches of the LCR. This pattern was mirrored only partially by the tapeworms in speckled dace, and not by dace in the creeks, indicating some difference in transmission dynamics in the two fish hosts in different habitats. There were no major taxonomic differences in parasite assemblages between the two different reaches of the river (LC1 and LC2), mainly due to their connectivity, similar physical properties and, fish assemblages. Parasite community diversity was very similar in humpback chub, regardless of sampling site or time. Differences among sites (BCC, Salt Creek (SAC), LC1 and LC2) can be related to the fish hosts present (or absent), and a result of the host specificity shown by the adult parasites. The parasite fauna of the LCR is numerically dominated by *B. acheilognathi*, and by the metacercariae of *Ornithodiplostomum* sp. The richest and most diverse component community occurred in a non-native species, the channel catfish, *Ictalurus punctatus*, but infracommunity species richness was highest in a native host, humpback chub. The generally species-poor native parasite fauna is due to the physical and biological characteristics of the LCR, namely the high travertine component and seasonal flooding. This in turn results in an invertebrate community which is low in species richness and diversity, as well as a species-poor native fish fauna.

Key words: Parasites, fish, Little Colorado River, Grand Canyon, *Bothriocephalus acheilognathi*, *Gila cypha*.

## INTRODUCTION

The closure of Glen Canyon Dam (Figure 1) in 1963 had a profound affect on the physical nature and ecology of the Colorado River in Grand Canyon, transforming a once seasonally warm, turbulent, muddy river, to one that is perennially cold and relatively clear. The flow of the river is now strictly regulated by the operation of the dam (National Academy of Sciences, 1991). There is also concern that introductions of non-native fishes into the system may be affecting native fish populations (Minckley, 1991; Marsh &

Douglas, 1997; Fuller *et al.*, 1999). At least 24 species of non-native fishes have been introduced into the Colorado River and its tributaries in Grand Canyon, and 13 have reproducing populations (Valdez *et al.*, in press). In contrast, the native fish fauna in Grand Canyon today is comprised of only four species: two catostomids, bluehead sucker (*Catostomus discobolus* Cope) and flannelmouth sucker (*C. latipinnis* Baird and Girard), and two cyprinids, speckled dace (*Rhinichthys osculus* Girard) and endangered humpback chub (*Gila cyph* Miller).

Non-native fishes have also introduced potentially pathogenic fish parasites into the system. Two such parasites, the Asian fish tapeworm, *Bothriocephalus acheilognathi*, and the anchor worm, *Lernaea cyprinacea*, have been found in native and non-native fishes of the Colorado River and its tributaries in this region (Carothers *et al.*, 1981; Brouder & Hoffnagle, 1997; Clarkson *et al.*, 1997; Hoffnagle & Cole, 1999). These studies also indicate that the two parasites were more abundant in the Little Colorado River (LCR), the major, relatively unaltered, tributary of the Colorado River in Grand Canyon. Since the closure of Glen Canyon dam and the alteration of the Colorado River, the LCR has become increasingly significant in the biology of the native fishes in the canyon. The LCR is an important spawning and nursery site for all four native fish species, and the most important (and perhaps exclusive) spawning site for the endangered humpback chub (Robinson *et al.*, 1996; Valdez & Ryel, 1997; Stone, 1999). In addition, seven non-native fish species also inhabit the LCR; This assemblage includes three cyprinids; common carp (*Cyprinus carpio* L.), fathead minnow (*Pimephales promelas* Rafinesque), and red shiner (*Cyprinella lutrensis*, Baird and Girard); two ictalurid catfishes including channel catfish (*Ictalurus punctatus* Rafinesque) and yellow bullhead (*Ameirus natalis* Lesueur); and one cyprinodontid *Fundulus zebrinus* Jordan and Gilbert have reproducing populations. Stocked rainbow trout (*Oncorhynchus mykiss* Walbaum) from Lees Ferry reach, a 26 km tailwater immediately below Glen Canyon Dam, are not uncommon in the LCR.

Although previous studies have reported the distribution and host-associations of *B. acheilognathi* and *L. cyprinacea*, the seasonal patterns of these pathogenic parasites as well as the parasite fauna of native and introduced fishes in Grand Canyon remain largely unknown. The importance of the LCR in sustaining the endangered humpback chub and at least two introduced pathogenic parasites known to parasitize it, and the presence of non-native fishes, made this river a natural site for a 2-year seasonal study on the parasite fauna. The study addresses the characteristics of the parasite fauna, host-parasite associations, and seasonal patterns of potentially pathogenic parasites in this ecosystem.

## **MATERIALS AND METHODS**

### **Study Area**

The study area comprises the lower 18 km of the Little Colorado River to its confluence with the Colorado River at river kilometre (Rk) 98.6, within Grand Canyon, Arizona (Figure 1). The Little Colorado River, with headwaters in the White Mountains of northeastern Arizona, is the largest tributary of the Colorado River in the Grand Canyon

area. It has a length of 536.3 km and a drainage area of 69,790 km<sup>2</sup> (Oberlin *et al.*, 1999). The major source of its perennial lower 22km stretch is Blue Springs and a series of smaller springs, which together discharge approximately 6.3m<sup>3</sup>/s of 20°C water that is supersaturated with calcium carbonate and charged with free CO<sub>2</sub>. (Johnson & Sanderson, 1968; Cole, 1975). These carbonates give the LCR its characteristic aqua-blue colour during base flow periods. Deposits of carbonates (mainly CaCO<sub>3</sub>), known as travertine, form on the stream bottom, along stream banks, and on rocks, in turn encrusting vegetation and smothering the benthos. Travertine formations along the edges of riffles and rapids, result in low travertine ‘dams’, and impede flow. During periods of flooding, usually in the monsoon season (July-September) and in the Spring (February-April), the flow in the LCR can reach 3400m<sup>3</sup>/sec (USGS [www.usgs.gov/nwis/](http://www.usgs.gov/nwis/)). Vegetation along the stream bank is generally sparse and consists of stands of *Phragmites australis*, *Salix exigua* and *Tamarix chinensis*.

Three sampling reaches were initially chosen on the lower 18 km of the LCR, designated LC1, LC2 and LC3. The most upstream reach (LC3) is above the Atomizer/Chute Falls Complex which is 13.6 river kilometres (RK) upstream of the confluence with the Colorado River. This reach usually contains only speckled dace and non-native common carp and fathead minnow (Robinson *et al.* 1996; D. Stone, USFWS, personal communication), due to the waterfall barrier. This reach was only sampled once (Sept. 1999) because river conditions did not permit helicopter landing on a regular basis. The middle reach (LC2, RK 10.6-11.5) is in the area of Salt Trail Canyon (RK 10.8) and contains all fish species that complete their life cycles in the LCR as well as occasional rainbow trout (Robinson & Clarkson 1992; U.S. Fish and Wildlife Service 1994). Two clear, saline creeks, Big Canyon Creek (BCC) in Big Canyon, Salt Creek (SAC) in Salt Canyon, and a saline spring (Big Canyon Springs) drain into the LCR in this reach. These tributaries were also sampled. The most downstream reach (LC1) is in the vicinity of Boulder Camp (RK 2) and ranged from RK 2.5 to the mouth (RK 0). This reach contains all species present in the LCR, including those that move into the LCR from the Colorado River, such as rainbow trout and brown trout, *Salmo trutta* (Robinson & Clarkson 1992; AZGF, 1996; Brouder & Hoffnagle 1997). Nets and other sampling gear (see below) were set within a 1 km stretch at each reach.

## Sampling

**Fish:** Eleven fish species were sampled including all 4 native fishes and 7 non-native species (Tables I, II). The term ‘non-native’ refers to fish species that are not native to the Colorado River system, and consequently includes fishes that are native to other parts of the U.S. (channel catfish, red shiners etc.), as well as fishes that were originally introduced into the U.S. from Europe (e.g., common carp). Most species were caught using medium (45 cm) and large (91cm) hoop nets (each with 10cm mouths and 6 mm mesh), and minnow traps (each with 25 mm mouth and 3 mm mesh). Six hoop nets of each size were deployed in each reach. Minnow-traps were deployed in pods of 5 traps, comprising a total of 4 or 5 pods in each reach (LC1 and LC2). One or 2 pods of minnow traps were set in the tributary creeks (BCC, BCS and SAC) and only minnow traps were used at these sites. In addition, baited trot-lines were used to catch channel catfish and

rainbow trout. These two species were also taken by angling. Gill nets yielded few or no fish and were not used after September 1999. Seines were used less frequently to catch very small fish and mainly when the yield from other sampling gear was low. Hand held sling spears and spear guns were used to capture carp trapped in shallow pools. Nets and traps were checked once a day and fish were brought back live to camp for necropsy work. In total, 1435 fish were necropsied for parasites. The relatively low numbers of the endangered humpback chub examined were due restrictions on both sample size and fish size (U.S. Fish and Wildlife Service Permit No. TE008513-0). During each trip (sampling period), a maximum of 10 individuals could be taken for necropsy work from each of the two reaches, LC1 and LC2, of the river, and sampled fish had to be less than 150 mm (i.e. juveniles). Two larger fish (190 mm and 232 mm) that had died during the sampling were also examined and included in the analysis. This quota of 20 fish (10 per reach) per trip was met in most cases (Table II).

Fish were processed shortly after capture, or were held in 19 litre buckets with aerated water, or in live cars in SAC or in the LCR, before processing. Fish were weighed and measured (total lengths), and necropsied using a binocular dissecting microscope.

**Parasites:** Parasites were collected and first briefly washed in saline or clear LCR water (naturally saline). Procedures for killing and preserving parasites were conducted as outlined by Van Cleave and Mueller (1932) and Pritchard and Kruse (1982). Briefly, parasites were killed and simultaneously fixed in steaming 10% buffered (or non-buffered) formalin (for platyhelminths and occasionally nematodes) or hot 70% ethanol (for nematodes only). Hot fixatives were used to fix and collect enough material for taxonomic work, but once the fauna was known, hot fixation was discontinued and parasites were fixed in unheated fixatives. When monogeneans were numerous, the gill-arches were separated and placed in dilute (1%) formalin for 6-12 hours, causing dissociation of the epithelia along with the monogeneans. The monogeneans, mostly free, were collected from the dissociated epithelial debris. Copepods and myxozoan cysts were collected in 70% ethanol. Platyhelminths were stained in acetocarmine or Ehrlich's haematoxylin, dehydrated, cleared in xylene or methyl salicylate, and permanently mounted on slides, in balsam. Nematodes were cleared in a solution of 5% glycerine in 70% ethanol by evaporation of ethanol and water. Copepods were initially cleared in hot lactophenol and temporarily mounted on slides and later washed in 70% ethanol, stained in acetocarmine, and permanently mounted in balsam, on slides. Parasites were identified using Hoffman (1999) and museum specimens for comparison. The following species (accession numbers are in parentheses) are being deposited in the U.S. National Parasite Collection (USNPC), Beltsville, Maryland and Harold W. Manter Laboratory (HWML) collection, University of Nebraska, Lincoln, Nebraska: *Henneguya exilis* (USNPC XXXX), *Ergasilus arthrosis* (USNPC XXXX), *Lernaea cyprinacea* (USNPC XXXX), Oribatida gen. sp. (USNPC XXXX, HWML XXXX), *Myzobdella lugubris* (USNPC XXXX), *Gyrodactylus hoffmani* (USNPC XXXX, HWML XXXX), *Gyrodactylus* sp. (from SPD) (USNPC XXXX), *Dactylogyrus extensus* (USNPC XXXX), *Ligictaluridus floridanus* (USNPC XXXX, HWML XXXX), *Contracacecum* sp. (USNPC XXXX, HWML XXXX), *Eustrongylides* sp. (USNPC XXXX, HWML XXXX), *Rhabdochona* sp. (USNPC XXXX, HWML XXXX), *Truttaedacnitis truttae* (USNPC

XXXX, HWML XXXX), *Bothriocephalus acheilognathi* (USNPC XXXX, HWML XXXX), *Corallobothrium fimbriatum* (USNPC XXXX, HWML XXXX), *Megathylacoides giganteum* (USNPC XXXX, HWML XXXX), *Ornithodiplostomum* sp. (USNPC XXXX, HWML XXXX) and *Posthodiplostomum* sp. (USNPC XXXX).

### ***Temperature recording***

Water temperature was recorded with programmable stowaway Hobo® Temp temperature data loggers (Onset Computer Corporation, Bourne, Massachusetts) in submersible waterproof, polycarbonate cases. Rocks were used as anchors to ensure the encased data loggers remained submerged at all times. Data loggers were programmed to record temperatures every 6 hours or every 12 hours (shorter intervals for longer recording duration, since this affects the memory capacity of the loggers), and deployed in SAC and BCC to record temperatures continuously between September 27, 2000 and June 6, 2001. Data loggers were retrieved during each sampling trip and new ones were deployed in their place. The BoxCar Pro 4.0 Programme (for Windows) (Onset Computer Corporation) was used to programme and activate the Hobo Temp data loggers and to download data from them. Raw data were subsequently exported to a Microsoft Excel spreadsheet. Temperature data loggers were not deployed in the LCR between sampling trips since periodic flooding events would have them washed away. Water temperature in the LCR from 1998 and 2001 was recorded by the USGS temperature gauge approximately 1 km upstream from the confluence with the Colorado River (William Vernieu, USGS Grand Canyon Monitoring Research Center, Flagstaff, Arizona, unpublished data).

***Analyses:*** The terms abundance, intensity, and prevalence follow that of Margolis *et al.*, (1982). Species richness refers to the number of species in an assemblage or community. Infracommunity refers to the community of parasite species in an individual host, while component community refers to the assemblage of parasites recovered from a sample of a host species. A compound community is the assemblage of parasites in all host species at a locality (Sousa, 1994). Species richness of gut helminth communities have also been calculated separately because it provides a basis for comparison of parasite community patterns as they relate to trophic-web dynamics, and because parasite assemblages in the gut are due to a common process (ingestion). The terms richness and diversity follow usage in Magurran (1988) and Peet (1974). Regression analyses and Pearson's correlation coefficient were employed to examine relationships between fish length, parasite burden (total number of parasites in an individual fish), and infracommunity parasite species richness. Parasite community parameters examined include total component community richness, component community diversity (Shannon-Wiener's index) and mean (infracommunity) richness. Comparisons of richness were made using the Mann-Whitney U-Test and correlations were examined using Pearson's correlation and Spearman's rank correlation (Zar, 1996). Results of all tests were considered significant at  $P < 0.05$ .

## RESULTS

### Fish

In total, 1435 fish belonging to 11 species were examined in this study (Table I). Of these, the 4 native fishes made up 67.4% of the total sample. Speckled dace comprised 43.9% of the total sample. All but one rainbow trout were caught from the LCR (Table II). Similarly, humpback chub were rarely caught from sites other than the LCR (LC1 and LC2). Big Canyon Creek and Salt Creek provided mainly speckled dace and occasionally a few other smaller species. Big Canyon Springs yielded mainly plains killifish and speckled dace. In general, the two reaches of the LCR (LC1 and LC2) consistently yielded a major proportion of the samples (Figure 2) as well as the richest assemblage of fish species throughout the study (Figure 3). High turbidity during the September 1999 sampling made collection of fish difficult. A sudden flooding event while sampling LC1 forced an early termination of sampling. This is reflected in the poor returns from sampling in the LCR during that time.

Most species were sampled over a considerable size range (Table I). However, most (> 90%) of the flannelmouth suckers examined were < 100 mm long. Similarly, most of the bluehead suckers were also immature individuals. The majority of carp samples were concentrated in one sampling period (June 2000) (Table II) and consisted primarily of small immature individuals. Other fish species, including most of the channel catfish examined, were taken as older juveniles and adults (see lengths and weights in Table II).

Some fish were not caught in each reach but rather were collected predominately at specific reaches. Rainbow trout were caught mostly at the downstream reach, LC1 (Table II). Species such as the red shiner, yellow bullhead, and common carp were caught more sporadically.

### Parasites

A total of 17 species of parasites was recovered from fishes in this study (Tables III, IV). The species of monogenean found in speckled dace resembles closely the species found in fathead minnow, and may be conspecific with it, but has been treated separately due to condition of samples. Eleven of the 16 metazoan parasites were adults. Of these, *Rhabdochona* sp. was the only adult parasite native to the LCR. All other adult parasite species were most likely introduced with their fish hosts. Parasites found as larval or juvenile stages mature in fish-eating birds (i.e., 2 nematodes, *Contracaecum* sp., *Eustrongylides* sp, and 2 trematodes, *Ornithodiplostomum* sp., *Posthodiplostomum* sp.), in channel catfish (i.e., plerocercoids of Corallobothriinae), or in the aquatic environment as free living adults (i.e. juvenile mites on gills).

The degree of host specificity varied but was highest in most of the adult parasites (Tables III, IV). The monogeneans were specific for their fish hosts. The nematodes, *Truttaedacnitis truttae* and *Rhabdochona* sp were specific for rainbow trout and speckled dace respectively. The cestodes *Corallobothrium fimbriatum* and *Megathylacoides giganteum*, the myxozoan *Henneguya exilis*, and the leech *Myzobdella lugubris*, were

found only in channel catfish. *Bothriocephalus acheilognathi* was recovered in all fish species examined, but was rare in the catostomids, rainbow trout and ictalurids (channel catfish and yellow bullhead). The species of *Rhabdochona* from speckled dace appears to be previously unknown and is being described elsewhere. Gravid females of this nematode were not found in any other fish host in the LCR.

Component community richness was highest in channel catfish (7 species) but infracommunity richness was highest in humpback chub (1.86) and channel catfish (1.23) (Figure 4). There was no common pattern of seasonal change in infra-community richness in the different fish species examined (Figure 5), except that both humpback chub and speckled dace showed lowest infra-community richness values in the summer (June) sampling periods in both 1999 and 2000. The component and infra-communities of the two native sucker species were consistently species poor (Table III, Figures 4, 5). Diversity of parasite component communities was remarkably uniform in humpback chub over the study period (Table V), compared to assemblages in speckled dace and channel catfish. Furthermore, the overall diversity of chub parasite communities in LC1 and LC2 were nearly identical (Table VI).

*Bothriocephalus acheilognathi* reached its highest abundance in humpback chub (Table III, Figure 6). Abundance of *B. acheilognathi* showed a clear seasonality in humpback chub, and was significantly lower in the summers than in any other time in this study (Figure 7). Abundance values of the tapeworm were also consistently (and in most sampling periods significantly) higher at LC2 than at LC1 (Figure 7). Except for abundance values of *B. acheilognathi* in chub from LC1 during April 2001, the two reaches of the river (LC2 and LC1) showed similar seasonal patterns (Figure 7). This pattern was not mirrored by the infections of *B. acheilognathi* in speckled dace from any of the sampling sites (Figure 8). Abundance of *B. acheilognathi* infections in speckled dace was significantly higher in Big Canyon Creek throughout the study than in any other location (Figure 9). Of the two creeks, a seasonal pattern of tapeworm abundance was only observed in Big Canyon Creek, where abundance values of the tapeworm were significantly higher in the spring and lower in the fall and summer (Figure 9). In total, 3930 individuals of *B. acheilognathi* were recovered in this study. Of these, 2130 (or 54.1%) were found in humpback chub. Regression analyses showed that, over all, there was a positive correlation between length of humpback chub and tapeworm burden ( $R^2 = 0.12$ ), but  $R^2$  values varied from as low as 0 (Sept. 1999) to 0.19 (April 2000).

*Ornithodiplostomum* sp. occurred consistently, and also followed trends in humpback chub similar to those seen with *B. acheilognathi* (Figure 10). *Ornithodiplostomum* sp. also shows significantly higher abundance values in Big Canyon Creek than elsewhere (Figure 11). Regression analyses indicated that infections (worm burden) of *Ornithodiplostomum* sp. were positively and significantly correlated with body size of humpback chub ( $R^2 = 0.28$ ). The  $R^2$  values varied between a low of 0.03 (Sept. 1999) and a maximum of 0.75 (Sept 2000) indicating this relationship fluctuates through time.



## Temperature

Temperature profiles were generated from the temperature data recorded by the Hobo Temp data loggers for BCC and SAC (Figure 12), and for the LCR from the data recorded by the USGS temperature data logger (Figure 13). The sharp decrease in temperatures in both SAC and BCC near the end of October (Figure 12) was possibly due to a flooding event in Salt Canyon and Big Canyon.

## DISCUSSION

Unlike most other investigations of fish parasite communities, this study provided us with a dataset of the entire fish parasite community from one river, and allowed us to address questions relating to parasite community structure, as well as historical and contemporary processes affecting parasite community richness and diversity.

Fifteen of the 17 species of parasites identified in this study (Tables III, IV) are first published records for the LCR and Colorado River in Grand Canyon. Only *Bothriocephalus acheilognathi* and *L. cyprinacea* have been previously reported from the LCR and other sites in the Grand Canyon (Carothers *et al.*, 1981; Brouder & Hoffnagle, 1997; Clarkson *et al.*, 1997; Hoffnagle & Cole, 1999). The parasite assemblage is species poor and unique in having only one species of native adult helminth (i.e., the ‘new’ species of *Rhabdochona* in speckled dace). The parasite assemblage is numerically dominated by parasites (*B. acheilognathi*) that have been presumably introduced with non-native fish, or by larval parasites (*Ornithodiplostomum* sp.) of fish eating birds. The host specificity and preferences of the parasite species observed in this study follow patterns of host-associations elsewhere (Hoffman, 1999). Although *B. acheilognathi* was found in all fish species examined, it clearly has a predilection for cyprinids. This is in keeping with its host preferences (Bauer, 1991; Kennedy, 1991; Scholz, 1997, 1999; Hoffman, 1999), and is not surprising since it is originally a parasite of cyprinids in northeast and far-east Asia (Yeh, 1955; Bauer, 1991; Scholz, 1997; Hoffman, 1999). More recently, the parasite has also been reported from the roundtail chub (*G. robusta* Baird and Girard) in a hatchery in Arizona (Brouder, 1999) and in wild populations of the arroyo chub (*G. orcutti* Eigenmann and Eigenmann) (Kuperman and Matthey, pers. comm.). In the U.S., the tapeworm also infects the more distantly related cyprinodontiforms such as the mosquito fish (Granath and Esch, 1983a), and the plains killifish demonstrated in this study and by Brouder and Hoffnagle (1997).

The parasite fauna in the LCR is also unusual in lacking adult trematodes. This absence, and the presence of larval strigeids only, can be related to the presence of only one species of mollusc, a species of *Physa*, in the LCR and its tributaries (unpublished observations). *Physa* spp. are known to be first intermediate hosts of strigeids such as *Ornithodiplostomum* spp. and *Posthodiplostomum* spp. In contrast, *Physa* spp. are not known to be intermediate hosts of the typical digenean fauna of North American freshwater fish parasites (e.g. Allocreadiidae, Gorgoderidae, Lissorchiidae in catostomids, Macroderoididae etc.). Furthermore, the benthic macroinvertebrate fauna of the LCR is severely affected by the unstable nature of their habitat. Periodic flooding

causes periodic reduction of some or most of this fauna from the river. Recolonization is made difficult by extensive travertine deposition which smothers the benthos and encrusts vegetation and rocks.

The physical nature of the LCR has likely had a long-standing influence on the ability of invertebrates to periodically colonise the river, and on the development of trophic webs in the system. The relatively short links in this trophic web are reflected in the parasite fauna. Four species (*Contracaecum* sp., *B. acheilognathi*, *C. fimbriatum*, and *M. giganteum*) are transmitted to their fish hosts by copepods. Ten species (*H. exilis*, *L. cyprinacea*, *Ergasilus arthrosis*, *M. lugubris*, *Ornithodiplostomum* sp., *Posthodiplostomum* sp., the oribatid mite, and the 3 species of monogeneans) infect or infest their hosts by direct attachment of larvae or by penetration. Seven of these do not utilise any prior intermediate host. Two species, *Rhabdochona* sp. and *Eustrongylides* sp. are transmitted by macroinvertebrates in the diet. *Eustrongylides* spp. utilise tubificid oligochaetes as intermediate hosts as do myxozoans, while *Rhabdochona* spp. commonly have some insect larvae (e.g., ephemeropterans) as intermediate hosts (Anderson, 2000). The physical nature of the aquatic environment may explain why the ectoparasites were so sporadic in occurrence and relatively rare in the system, and why there are so few parasites with a riverine benthic life cycle.

The physical, and hence biotic, characteristics of the LCR also explain why the two native catostomids (bluehead sucker and flannelmouth sucker) have unusually depauperate parasite faunas. Catostomids generally possess a rich and taxonomically diverse parasite fauna comprising of monogeneans, digeneans, acanthocephalans and nematodes (Margolis & Arthur, 1979; McDonald & Margolis, 1995; Hoffman, 1999). The characteristic digenean fauna of catostomids utilise sphaeriid bivalves or ancyliid snails as first intermediate hosts (Hoffman, 1999), neither of which are known from the LCR. The humpback chub also has no parasite that could be considered native to the LCR. Higher infracommunity richness in humpback chub was a result of the high prevalences of *B. acheilognathi* and *Ornithodiplostomum* sp. The lack of information on the parasite fauna of these fishes in the Colorado River main stem makes it impossible to determine whether these native LCR fishes possess more diverse parasite faunas. If they do, then the parasites possess the potential to serve as natural markers in understanding the movement of these fishes between the LCR and the Colorado River.

The parasite fauna of the non-native fishes is also species poor and reduced in taxonomic diversity compared to the fauna in their native range. At first glance, this is in keeping with the generalisations by Dogiel (1964) and Dogiel *et al.* (1961) that host species that are introduced into areas outside their native ranges undergo a reduction in or loss of their parasite fauna. Rainbow trout have only one parasite specific to them (and other salmonids) viz. *Truttaedacnitis truttae* (Hoffman, 1999) in the LCR. Since this trout is an occasional migrant and temporary resident in the LCR, and is mainly confined to the lower reaches of the river (Tables III, IV), it is likely that its parasite fauna in the Colorado River is equally species poor. It is most probable that rainbow trout were stocked from one or several hatcheries, in which case it is not a true case of translocation and Dogiel's generalisations, or those of Kennedy and Bush (1994), cannot be easily

applied. *T. truttae* has been found in trout in hatcheries, and could have survived the translocation process. The finding of *T. truttae* of various sizes in rainbow trout in the Grand Canyon has bearing on the biology and taxonomy of this parasite. Brook lampreys are considered to be obligate intermediate hosts of this parasite in Europe, and brown trout in Europe become infected by ingesting lampreys (Moravec, 1994). As there are no lampreys in the Colorado River in Grand Canyon, some other intermediate host is involved. Choudhury and Dick (1996) showed that there was a morphological difference between North American and continental European *T. truttae*. This study highlights the fact that there are biological differences as well.

Channel catfish, which possess a characteristic parasite fauna within their native range (Hoffman, 1999; Pérez-Ponce de León & Choudhury, 2002), are infected in the LCR by 4 species that are specific to them and other *Ictalurus* spp.: (i.e., *H. exilis*, *C. fimbriatum*, *M. giganteum*, and *L. floridanus*). In addition, the leech *M. lugubris* and the parasitic copepod, *E. arthrosis*, also appear to be typical of channel catfish (Roberts, 1970; Hoffman, 1999). Channel catfish were introduced from an unknown source into the lower Colorado River basin around 1890 (Miller and Alcorn, 1943, U.S. Fish and Wildlife Service, 1980). As result it is impossible to conclude with certainty whether their parasite fauna in the LCR indicates a significant reduction or retention of parasites that were introduced with them. However, in native waters, channel catfish are parasitised by several digeneans that are specific to ictalurids: the urinary bladder fluke (*Phyllodistomum lacustri*), the intestinal flukes (*Crepidostomum ictaluri* and *Polylekithum ictaluri*) and two macroderoidids (*Alloglossidium corti* and *A. geminum*). None of these species are present in the LCR. At least part of the reason for this may lie in the abiotic conditions, and the resulting biotic composition, of the LCR. Most of these trematodes utilise intermediate mollusc hosts that are not present in the LCR. Although the yellow bullhead is also an ictalurid, it did not share any parasites with channel catfish. This is likely due to the fact that the typical cestode fauna of *Ameiurus* spp. comprises corallobothrines such as *Corallotaenia minutia*, and *C. parafimbriatum*, not *C. fimbriatum* or *M. giganteum*. Likewise, *Ameiurus* spp. have their own specific species of *Ligictaluridus* (Hoffman, 1999), while the myxozoan, *H. exilis* appears to be specific to the channel catfish.

The abundance of *B. acheilognathi* in non-native cyprinids, such as fathead minnows, red shiners, and small carp, as well as in the plains killifish, implicates any of these fish species as hosts that introduced the tapeworm into the LCR. It is possible that bait bucket transfers into the upper reaches of the LCR or into the Colorado River may have been responsible for the introductions. The data on *B. acheilognathi* infections in this study (Tables III, IV, Figures 6, 7, 8) demonstrate the high colonising potential of this parasite. This is in keeping with its establishment in non-native areas worldwide (Boomker et al., 1980; Bauer, 1991; Kennedy, 1991; Esch & Fernández, 1993; Heckman & Furtak, 1993; Font & Tate, 1994; Pérez-Ponce de León et al., 1996; Dove et al., 1997; Hoffman, 1999). Comparisons of *B. acheilognathi* infections with past studies (Brouder & Hoffnagle, 1997; Clarkson et al., 1997) show that the parasite is well established in native cyprinids (i.e., humpback chub and speckled dace). Humpback chub appears to be the most important host in the LCR (Figure 7). Although chub comprised only 8% of the

total number of fish examined in this study, it harboured 54.1% of the tapeworms recovered. These findings are in contrast to those of Dove (1998), who found that non-native carp were the main hosts of *B. acheilognathi* in Australia, both in terms of numbers and biomass of the tapeworm. Dove predicted that *B. acheilognathi* required carp as a reservoir host for the infections to be maintained in native fishes in Australia. This is possibly due to the fact that Australia has a unique and isolated freshwater fish fauna, devoid of native cyprinids (Nelson, 1994). Our study suggests that *B. acheilognathi* would persist and maintain its presence in the LCR even in the absence of carp or other non-native hosts.

A combination of behavioural and physiological traits may make humpback chub suitable hosts. First, chub are highly omnivorous (Valdez & Hoffnagle, 1999), and zooplankton comprises a large percentage of their diet (AZGF, 1996). They commonly feed on copepods even as larger juveniles. Second, as a native species, the humpback chub is well adapted to exploit habitat and food in the LCR. Speckled dace are also native but they are primarily benthivores (AZGF, 1996), as reflected by the presence of *Rhabdochona* sp. Although dace are suitable hosts of *B. acheilognathi*, their foraging behaviour may prevent heavier infections. Finally, the humpback chub is a much larger species than the speckled dace. While this in itself should not necessarily mean higher infections in humpback chub, ingestion of large concentrated amounts of zooplankton indirectly through predation on small planktivorous fish, and its omnivorous feeding in the water column may expose it to infected zooplankton at all stages in its life history. However, the restriction of our sampling to chub less than 150 mm prevents firm conclusions about the nature of chub-tapeworm interactions or about the transmission dynamics of *B. acheilognathi* in larger fish.

Seasonal patterns of infection with *B. acheilognathi* were observed in humpback chub in both reaches (LC1 and LC2) of the river (Figure 7). The general trend was lowest abundance in the summer months with significantly higher abundance values in the following fall (September). Temperature related dynamics of *B. acheilognathi* have been demonstrated in the past (Granath & Esch, 1983a, b; Marcogliese & Esch, 1989a, b). The LCR provides the tapeworm with the temperatures necessary for its development and maturation, mainly during the summer months (Figure 12), but the low abundance of worms in the summer (Figure 7) may be related to seasonal changes in copepod abundance (see Marcogliese and Esch, 1989a). In addition to seasonal patterns, the data also revealed spatial differences in abundance of *B. acheilognathi* infections in humpback chub (Figure 7). The higher abundance values of tapeworm infections in the upper reach (LC2) as compared to the LC1 may be due to the presence of both creeks (BCC and SAC) and Big Canyon Springs at LC2. The creeks and Big Canyon Springs provide landscape diversity and ideal habitat for copepods. Of these habitats, Big Canyon Creek may be particularly important. Although humpback chub were rarely caught in Big Canyon Creek (2 of 116 fish, with 243 and 45 tapeworms), access to such creeks may expose fish to functional reservoirs of infection, maintained by the dace in the creeks. Furthermore, the creeks may serve as refugia for copepods, intermediate hosts of *B. acheilognathi*, during flooding episodes in the LCR and as such serve as sources for the rapid colonisation by copepods of the near shore backwater environments in the LCR

after base flows return to normal levels. The absence of such creeks in the lower reaches of the LCR precludes such opportunities.

Significantly higher abundance of *B. acheilognathi* in speckled dace in the larger of the two creeks, BCC (Figure 9), is possibly related to the greater population size of dace (mean CPUE: 180.1 dace / 24 hrs in BCC vs. 52.2 dace / 24 hrs in SAC) and potentially larger populations of copepods. Salt Creek apparently provides year-round temperatures suitable for transmission of the tapeworm, while water temperatures in Big Canyon Creek show greater seasonal fluctuation (Figure 12). In Big Canyon Creek, the pattern clearly indicates low points in the summer and fall, which contrasts with the pattern shown by the parasite in dace in the LCR (Figure 8), where high fall (September 2000) values are flanked by low summer and spring values. A temperature-dependent increase in spring infection values (abundance) from those of the previous respective fall lows (Figure 8) is supported by the temperature profile of Big Canyon Creek (Figure 12).

Metacercariae of the bird fluke, *Ornithodiplostomum* sp., comprised the other abundant parasite in this system. An increase in abundance in the fall from preceding summer levels and a decrease in the subsequent spring is evident in both seasonal cycles of this study (Figure 10). Significantly higher abundances of *Ornithodiplostomum* sp in dace were also found in the larger of the two tributary creeks (BCC) (Figure 11). Whether these seasonal and habitat patterns correlate with habitat use by nesting or feeding piscivorous birds and (or) the abundance of the mollusc intermediate host (*Physa* sp.), is open to further investigation. The metacercariae have been shown to alter visually mediated behaviour in fish, increasing risk to predation and impacting foraging success (Sho & Goater, 2001). Its presence in chub and dace indicates that this parasite needs to be monitored as well.

This study demonstrates the intricate interplay between hosts, parasites and habitat in a species poor, yet clearly complex system. The high colonising potential of *B. acheilognathi* is reflected in its ability to infect a variety of fishes and habitats in the LCR and in its abundance in two native cyprinids. The study also demonstrates the importance of perennial tributary creeks as reservoir areas for copepods and fish, and consequently for *B. acheilognathi*. These facts make monitoring of this parasite an important issue in maintaining the biotic integrity of the Grand Canyon ecosystem. Sustained and continued studies on *B. acheilognathi* and other potentially pathogenic parasites (*Ornithodiplostomum* sp., *L. cyprinacea*) would allow the impact of these parasites on native fish species to be better understood as management and remedial activities are planned. Finally, the scope of such studies must be broadened to include the Colorado River main stem, its tributaries, and warmer feeder creeks that may be critical to success of these parasites. Monitoring fishes of the main stem would provide information on the entire distribution of these parasites within the Grand Canyon and document if the parasites spread, as alternate flow regimes from Glen Canyon Dam and other management strategies are executed.

## ACKNOWLEDGEMENTS

Numerous people contributed to the success of this project. We gratefully acknowledge the following: Dave Baker, Karl Hayden, L.B. Myers, Gary Burton, Lara Myers, Bruce Michael, Wes Shoop, Scott Hale, Scott Hansen, Anson Koehler, Jonathan C. Cambron, Ben Galuardi, and Bobbi Hervin, for volunteering their hard work and assistance in the field, the field personnel and biologists of the U.S. Fish and Wildlife Service (USFWS), Flagstaff, Randy Van Haverbeke, Dennis Stone, Lew Coggins, and Ben Galuardi for their help and advice, Scott Reger and Jodi Niccum, Arizona Game and Fish Department (AGFD), Flagstaff, and Bill Persons, AGFD, Phoenix, for logistic support, and Carol Fitzinger, Grand Canyon Monitoring Research Center (USGS-GCMRC) for loaning the satellite phone. AC is deeply indebted to Dave Baker for his help during the June 1999 sampling trip, and to Scott Hansen for his outstanding technical assistance on this project. We also thank Bill Vernieu, USGS-GCMRC, Flagstaff, Arizona, for the unpublished LCR temperature data, and the USFWS (#TE008513-0), NPS –Grand Canyon (#9902-04-002 ), AGFD (#SP920526) and the Navajo Nation ( #030301-025) for sampling permits. Finally, we also acknowledge the staff of the USGS-National Wildlife Health Center for logistic support. The project was funded by the U.S. Geological Survey (USGS) Southwestern Initiative.

## References

- Anderson, R.C. (2000). *Nematode parasites of vertebrates. Their development and transmission*. CABI Publishing, Wallingford, U.K., 650pp.
- Arizona Game and Fish Department. (1996). The ecology of Grand Canyon backwaters. Final Report. Submitted to Glen Canyon Environmental Studies, U. S. Bureau of Reclamation, Flagstaff, AZ. Arizona Game and Fish Department, Phoenix.
- Bauer, O.N. (1991) Spread of parasites and diseases of aquatic organisms by acclimatization: a short review. *Journal of Fish Biology* **39**, 679-686.
- Boomker, J., Huchzermeyer, F.W. & Naude, T.W. (1980). Bothriocephalosis in the common carp in the eastern Transvaal. *Journal of the South African Veterinary Association*, **51**, 263-264.
- Brouder, M.J. & Hoffnagle, T.L. (1997). Distribution and prevalence of the Asian fish tapeworm, *Bothriocephalus acheilognathi*, in the Colorado River and tributaries, Grand Canyon, Arizona, including two new host records. *Journal of the Helminthological Society of Washington* **64**, 219-226.
- Brouder, M.J. (1999). Relationship between length of roundtail chub and infection intensity of Asian fish tapeworm *Bothriocephalus acheilognathi*. *Journal of Aquatic Animal Health* **11**, 302-304.
- Carothers, S. W., Jordan, J.W., Minckley, C.O. & Usher, H.D. (1981). Infestations of the copepod parasite, *Lernaea cyprinacaeca*, in native fishes of the Grand Canyon. *National Park Service Transactions and Proceedings Series* **8**, 452-460.
- Clarkson, R.W., Robinson, A.T. & Hoffnagle, T.L. (1997). Asian tapeworm (*Bothriocephalus acheilognathi*) in native fishes from the Little Colorado River, Grand Canyon, Arizona. *Great Basin Naturalist* **57**, 66-69.
- Choudhury, A. & Dick, T.A. (1996). Observations on the systematics and biogeography of the genus *Truttaedacnitis* (Nematoda: Cucullanidae). *Journal of Parasitology*, **82**, 965-976
- Cole, G. A. (1975). Calcite saturation in Arizona waters. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **19**, 1675-1685.
- Dogiel, V.A. (1964). *General Parasitology*. English Translation. Oliver and Boyd, Edinburgh. 516 p.
- Dogiel, V.A. , Petrushevski, G.K.& Polyanski, Y.I. (1961). *Parasitology of Fishes*. English translation. Oliver and Boyd. Edinburgh. 384 p.

- Dove, A.D.M., Cribb, T.H., Mockler, S.P. & Lintermans, M. (1997). The Asian fish tapeworm, *Bothriocephalus acheilognathi*, in Australian freshwater fishes. *Marine and Freshwater Research*, **48**, 181-183.
- Dove, A.D.M (1998). A silent tragedy: Parasites and the exotic fishes of Australia. *Proceedings of the Royal Society of Queensland* **107**, 109-113.
- Esch, G.W & Fernandez, J. (1993). *A functional biology of parasitism*. Academic Press, New York.
- Font, W.F. & Tate, D.C. (1994). Helminth parasites of native Hawaiian freshwater fishes: An example of extreme ecological isolation. *Journal of Parasitology*, **80**, 682-688.
- Fuller, P.L., Nico, L.G. & Williams, J.D. (1999). Nonindigenous fishes introduced into inland waters of the United States. American Fisheries Society, Special Publication 27, Bethesda, Maryland. 613 p.
- Granath, W.O. Jr. & Esch, G.W. (1983a). Seasonal dynamics of *Bothriocephalus acheilognathi* in ambient and thermally altered areas of a North Carolina cooling reservoir. *Proceedings of the Helminthological Society of Washington*, **50**, 205-218.
- Granath, W.O. Jr. & Esch, G.W. (1983b). Temperature and other factors that regulate the composition and infrapopulation densities of *Bothriocephalus acheilognathi* (Cestoda) in *Gambusia affinis* (Pisces). *Journal of Parasitology*, **69**, 1116-1124.
- Heckmann, R.A., Greger, P.D., & Furtek, R.C. (1993). The Asian fish tapeworm, *Bothriocephalus acheilognathi*, in fishes from Nevada. *Journal of the Helminthological Society of Washington*, **60**, 127-128.
- Hoffman, G. (1999). *Parasites of North American Freshwater Fishes*. Ithaca: Cornell University Press.
- Hoffnagle, T. L. & Cole, R.A. (1999). Distribution and prevalence of *Lernaea cyprinacea* in fishes of the Colorado River and tributaries in Grand Canyon, Arizona. *Proceedings of the Desert Fishes Council*, **29**, 45-46.
- Johnson, P. W. and R. B. Sanderson. 1968. Spring flow into the Colorado River, Lees Ferry to Lake Mead, Arizona. *Arizona State Land Department, Water Resources Report* No. 34.
- Kennedy, C. & Bush, A.O. (1994). The relationship between pattern and scale in parasite communities: a stranger in a strange land. *Parasitology* **109**, 187-196.



- Kennedy, C.R. (1991). Introductions, spread and colonization of new localities by fish helminth and crustacean parasites in the British Isles: a perspective and appraisal. *Journal of Fish Biology* **43**, 287-301.
- Magurran, A.E. (1988). *Ecological diversity and its measurement*. Princeton University Press, New Jersey. 110 pp.
- Marcogliese, D. & Esch, G.W. (1989a). Experimental and natural infection of planktonic and benthic copepods by the Asian tapeworm, *Bothriocephalus acheilognathi*. *Proceedings of the Helminthological Society of Washington*, **56**, 151-155.
- Marcogliese, D. & Esch, G.W. (1989b). Alteration in seasonal dynamics of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir over a seven year period. *Journal of Parasitology*, **75**, 378-382.
- Margolis, L. & Arthur, J.R. (1979). Synopsis of the parasites of fishes of Canada. *Bulletin of the Fisheries Research Board of Canada*, 199. 269 p.
- Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M., & Schad, G.A. (1982). The use of ecological terms in parasitology. *Journal of Parasitology* **68**, 131-133.
- Marsh, P.C. & Douglas, M.E. 1997. Predation by introduced fishes on endangered humpback chub and other native species in the Little Colorado River, Arizona. *Transactions of the American Fisheries Society*, **126**, 343-346.
- McDonald, T. & Margolis, L. (1995). Synopsis of the parasites of fishes of Canada. *Canadian Special Publication of Fisheries and Aquatic Sciences*, 122. 265 pp.
- Milinski, M. (1985). Risk of predation of parasitized sticklebacks (*Gasterosteus aculeatus* L.) under competition for food. *Behaviour*, **93**, 203-216.
- Miller, R.R. and J.R. Alcorn. (1943). The introduced fishes of Nevada with a history of their introduction. *Transactions of the American Fisheries Society* **73**, 173-193.
- Minckley, W. L. (1991). Native fishes of the Grand Canyon region: an obituary? In *Colorado River Ecology and Dam Management* (National Academy of Sciences, ed.) National Academy of Sciences Press, Washington, DC. Pages 124-177.
- Moravec, F. (1994). *Parasitic nematodes of freshwater fishes of Europe*. Kluwer Academic Publishers, Boston. 473 pp.
- National Academy of Sciences. (1991). *Colorado River Ecology and Dam Management*. National Academy of Sciences Press, Washington, DC.
- Nelson, J.S. (1994). *Fishes of the World*. 3<sup>rd</sup> Edition. John Wiley and Sons Inc., New York. 600 p.

Oberlin, G.E., Shannon, J.P. & Blinn, D.W. (1999). Watershed influence on the macroinvertebrate fauna of ten major tributaries of the Colorado River through Grand Canyon, Arizona. *The Southwestern Naturalist*, **44**, 17-30.

Peet, R. (1974). The measurement of species diversity. *Annual Review of Ecology and Systematics*, **15**, 285-307.

Pérez-Ponce de León, G. & Choudhury, A. (2002). Adult endohelminth parasites of ictalurid fishes (Osteichthyes: Ictaluridae) in Mexico: Empirical evidence for biogeographical patterns. *Journal of the Helminthological Society of Washington*, **69**, 10-19.

Pérez-Ponce de León, G., García –Prieto, L., Osorio-Sarabia, D. & León-Règagnon, V. (1996). *Listados Faunísticos de México VI. Helmintos Parásitos de Peces de Aguas Continentales de México*. Instituto de Biología, UNAM, México. 100 pp.

Pritchard, M. H. and G. O.W. Kruse. 1982. The collection and preservation of animal parasites. University Nebraska Press, Lincoln. 141 pp.

Roberts, L.S. (1970). *Ergasilus* (Copepoda: Cyclopoida): Revision and key to species in North America. *Transaction of the American Microscopical Society*, **89**, 134-161.

Robinson, A. T., Kubly, D.M., Clarkson, R.W. & Creef, E.D. (1996). Factors limiting the distributions of native fishes in the Little Colorado River, Grand Canyon, Arizona. *The Southwestern Naturalist* **41**, 378-387.

Scholz, T. (1997) A revision of the species of *Bothriocephalus* Rudolphi, 1808 (Cestoda: Pseudophyllidea) parasitic in American freshwater fishes. *Systematic Parasitology* **36**, 85-107.

Scholz, T. (1999). Parasites in cultured and feral fish. *Veterinary Parasitology* **84**, 317-335.

Sho, S. & Goater, C.P. (2001). Brain-encysting parasites affect visually-mediated behaviours of fathead minnows. *Ecoscience*. **8**, 289-293.

Sousa, W.P. (1994). Patterns and processes in communities of helminth parasites. *Trends in Ecology and Evolution* **9**, 52-57.

Stone, D. (1999). Ecology of humpback chub, *Gila cypha*, in the Little Colorado River, near Grand Canyon, Arizona. M.Sc. thesis. Northern Arizona University, Flagstaff, Arizona, U.S.A.

U.S. Fish and Wildlife Service. (1980). Special report on distribution and abundance of fishes of the lower Colorado River. U.S. Fish and Wildlife Service, Albuquerque, NM. 157 pp.

U. S. Fish and Wildlife Service. (1994). Habitat use by humpback chub, *Gila cypha*, in the Little Colorado River and other tributaries of the Colorado River. Final report submitted to Glen Canyon Environmental Studies, U. S. Bureau of Reclamation, Flagstaff, Arizona. Arizona Fishery Resources Offices, U. S. Fish and Wildlife Service, Pinetop, Arizona.

Valdez, R. A. & Hoffnagle, T.L. (1999). Movement, habitat use, and diet of adult humpback chub. In *The controlled flood in Grand Canyon*. American Geophysical Union Monograph 110 (R. H. Webb, Schmidt, J.C., Marzolf, G.R. & Valdez, R.A. eds.) American Geophysical Union, Washington, DC., U.S.A., pp 297-308.

Valdez, R. A. & Ryel, R.J. (1997). Life history and ecology of the humpback chub in the Colorado River in Grand Canyon, Arizona. *Proceedings of the Biennial Conference of Research on the Colorado Plateau* **3**, 3-31.

Valdez, R. A., Persons, W.R. & Hoffnagle, T.L. (in press). A non-native fish control strategy for Grand Canyon, Arizona. In *Proceedings of a Symposium and Workshop on Restoring Native Fish to the Lower Colorado River: Interactions of Native and Non-native Fishes. 13-14 July 1999, Las Vegas, Nevada*. Region II, U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

Van Cleave, H.J. & Mueller, J.F. (1932). Parasites of Oneida Lake fishes, Part I. Descriptions of new genera and new species. *Roosevelt Wildlife Annals*, 3(1): 1-72.

Yeh, L.S. (1955). On a new tapeworm *Bothriocephalus gowkongensis* n. sp. (Cestoda: Bothriocephalidae) from freshwater fish in China. *Acta Zoologica Sinica*, 7: 69-72.

Zar, J. H. (1996). *Biostatistical Analysis*. Prentice Hall, Inc., Upper Saddle River, NJ.

Table I. Lengths, weights, and total number of fish examined in a study of the Lower Little Colorado River

Fish Species	Length <sup>1</sup> (mm)	Weight <sup>1</sup> (g)	N
Native			
<i>Catostomus discobolus</i>	84.48 ± 48.83 (35 – 288)	10.67 ± 23.32 (30 – 147)	148
<i>C. latipinnis</i>	103.64 ± 86.46 (36 – 492)	50.89 ± 173.17 (0.5 – 962)	73
<i>Gila cypha</i>	93.55 ± 36.43 (34 – 232)	7.76 ± 10.27 (0.2 – 78.3)	116
<i>Rhinichthys osculus</i>	69.1 ± 12.12 (29 – 115)	2.97 ± 4.47 (0.2 – 85)	630
Non-native			
<i>Ictalurus punctatus</i>	381.57 ± 212.71 (48 - 770)	1147.68 ± 469.3 (0.8 – 8030)	54
<i>Cyprinus carpio</i>	119.16 ± 133.83 (32 – 600)	139.64 ± 463.57 (0.5 – 2617.5)	63
<i>Pimephales promelas</i>	61.97 ± 12.12 (31 – 99)	2.42 ± 1.61 (0.3 - 10.3)	193
<i>Fundulus zebrinus</i>	55.48 ± 9.4 (28 – 81)	1.67 ± 0.9 (0.4 – 5.5)	113
<i>Oncorhynchus mykiss</i>	326.14 ± 44.34 (252 – 441)	298.69 ± 133.14 (122 – 441)	22
<i>Cyprinella lutrensis</i>	60.18 ± 10.31 (50 – 88)	1.97 ± 0.89 (1 – 3.9)	11
<i>Ameiurus natalis</i>	165.42 ± 63.15 (80 – 252)	76.77 ± 63.84 (5.7 – 185)	12

<sup>1</sup> Mean ± Standard deviation (minimum - maximum). N = sample size

Table II. Number of fish of each species necropsied on each trip and in each sampled reach during this study.

Trip/Reach	Native Fishes*				Non-native Fishes*							Total
	BHS	FMS	HBC	SPD	CCF	CRP	FHM	PKF	RBT	RSH	YBH	
<u>Summer 1999 (28 May – 9 June)</u>												
LCR Reach 3	0	0	0	20	1	0	4	0	0	0	0	25
Big Canyon Springs	0	0	0	0	0	0	0	0	0	0	0	0
Salt Creek	0	0	0	0	0	0	0	0	0	0	0	0
LCR Reach 2	10	13	10	33	4	1	3	0	1	0	0	75
LCR Reach 1	20	12	9	33	5	1	10	1	0	3	0	94
Summer 1999 Total	30	25	19	86	10	2	17	1	1	3	0	194
<u>Fall 1999 (Sept 4 - 13)</u>												
Big Canyon Creek	5	0	1	35	0	0	11	0	0	0	0	52
Big Canyon Springs	0	0	0	0	0	0	0	0	0	0	0	0
Salt Creek	0	0	1	64	0	0	2	0	0	0	0	67
LCR Reach 2	2	0	7	0	8	2	1	0	0	0	2	22
LCR Reach 1	1	0	10	0	3	1	0	0	0	0	0	15
Fall 1999 Total	8	0	19	99	11	3	14	0	0	0	2	156

Table II continued

Trip/Reach	Native Fishes*				Non-native Fishes*							Total
	BHS	FMS	HBC	SPD	CCF	CRP	FHM	PKF	RBT	RSH	YBH	
<u>Spring 2000 (9-21 April)</u>												
Big Canyon Creek	0	0	1	31	0	0	0	0	0	0	0	32
Big Canyon Springs	0	0	0	0	0	0	0	0	0	0	0	0
Salt Creek	0	0	3	19	0	0	0	0	0	0	0	22
LCR Reach 2	26	2	6	31	6	0	5	4	3	0	0	82
LCR Reach 1	6	3	10	16	6	1	14	1	7	1	1	67
Spring 2000 Total	32	5	20	97	12	1	19	5	10	1	1	203
<u>Summer 2000 (4 - 15 June)</u>												
Big Canyon Creek	0	0	0	22	0	0	0	0	0	0	0	22
Big Canyon Springs	0	0	0	31	0	0	0	6	0	0	0	37
Salt Creek	0	0	0	22	0	0	0	0	0	0	0	22
LCR Reach 2	26	0	10	17	3	27	9	4	0	0	3	99
LCR Reach 1	24	23	10	21	4	22	0	1	7	5	0	117
Summer 2000 Total	50	23	20	113	7	49	9	11	7	5	3	297

Table II continued.

Trip/Reach	Native Fishes*				Non-native Fishes*							Total
	BHS	FMS	HBC	SPD	CCF	CRP	FHM	PKF	RBT	RSH	YBH	
<u>Fall 2000 (25 September - 4 October)</u>												
Big Canyon Creek	0	0	0	25	0	0	0	0	0	0	0	25
Big Canyon Springs	0	0	0	20	0	0	0	21	0	0	0	41
Salt Creek	0	0	0	30	0	0	0	0	0	0	0	30
LCR Reach 2	6	4	10	26	3	0	47	19	3	0	2	120
LCR Reach 1	1	7	10	10	10	3	34	16	0	0	0	91
Fall 2000 Total	7	11	20	111	13	3	81	56	3	0	2	307
<u>April 2001 (1 – 12 April)</u>												
Big Canyon Creek	0	0	0	30	0	0	2	0	1	0	0	33
Big Canyon Springs	10	2	0	30	0	0	2	30	0	0	0	74
Salt Creek	0	0	0	25	0	0	2	7	0	0	0	34
LCR Reach 2	9	1	9	18	1	1	22	2	0	1	3	67
LCR Reach 1	2	6	9	21	0	4	25	1	0	1	1	70
April 2001 Total	21	9	18	124	1	5	53	40	1	2	4	278
Total	148	73	116	630	54	63	193	113	22	11	12	1435

\*BHS = bluehead sucker, FMS = flannelmouth sucker, HBC = humpback chub, SPD = speckled dace, CCF = channel catfish, CRP = common carp, FHM = fathead minnow, PKF = plains killifish, RBT = rainbow trout, RSH = red shiner, YBH = yellow bullhead.

Table III. Parasites of native fishes in the Little Colorado River<sup>1</sup>.

	BHS <sup>+</sup> (n = 148)	FMS (n = 73)	HBC (n = 116)	SPD (n = 630)
<b>Parasite species</b>				
<u>Monogenea</u>				
<i>Gyrodactylus</i> sp.	-	-	-	0.04 ± 0.44 (0 – 7) (0.01)
<u>Cestoda</u>				
<i>B. acheilognathi</i>	0.05 ± 0.58 (0 – 7) (0.01)	0.07 ± 0.48 (0 – 4) (0.03)	18.36 ± 34.55 (0 – 243) (0.84)	1.97 ± 6.06 (0 – 64) (0.43)
Corallobothriinae* (pl.)			0.009 ± 0.09 (0 – 1) (0.01)	-
<u>Trematoda</u>				
<i>Ornithodiplostomum</i> sp.* (viscera)	0.07 ± 0.59 (0 – 7) (0.03)	0.01 ± 0.12 (0 – 1) (0.01)	7.69 ± 20.67 (0 – 202) (0.67)	1.53 ± 3.36 (0 – 32) (0.4)
<i>Ornithodiplostomum</i> sp.* (brain)			0.15 ± 0.46 (0 – 3) (0.11)	0.03 ± 0.19 (0 – 2) (0.03)
<i>Ornithodiplostomum</i> sp.* (eye)			0.02 ± 0.13 (0 – 1) (0.01)	0.001 ± 0.04 (0 – 1) (0.001)
<i>Posthodiplostomum</i> sp. *	-	-	0.01 ± 0.09 (0 – 1) (0.01)	-
Unidentified metacercaria	0.006 ± 0.82 (0 – 1) (0.006)	-	0.009 ± 0.09 (0 – 1) (0.01)	0.003 ± 0.06 (0 – 1) (0.003)
<u>Nematoda</u>				
<i>Rhabdochona</i> sp.	-	-	0.11 ± 0.39 (0 – 2) (0.09)	0.37 ± 1.66 (0 – 23) (0.12)
<i>Eustrongylides</i> sp.*	-	-	-	0.001 ± 0.04 (0 – 1) ((0.001)
<i>Contracaecum</i> sp.*	-	-	-	0.003 ± 0.06 (0 – 1) (0.003)



Acari

Oribatida gen. sp.	-	-	-	0.02 ± 0.18 (0 – 3) (0.02)
--------------------	---	---	---	-------------------------------

Crustacea

<i>Lernaea cyprinacea</i> (adult female)	-	-	0.07 ± 0.25 (0 – 1) (0.01)	0.01 ± 0.11 (0 – 2) (0.01)
<i>L. cyprinacea</i> (copepodites)	-	-	0.03 ± 0.37 (0 – 2) (0.01)	0.008 ± 0.10 (0 – 2) (0.01)

---

<sup>1</sup>Mean abundance ± standard deviation (minimum – maximum) (prevalence)

Larval stages: pl = plerocercoid.

<sup>+</sup>BHS = bluehead sucker, FMS = flannelmouth sucker, HBC = humpback chub, SPD = speckled dace.

---

Table IV. Parasites of non-native fishes of the Little Colorado River<sup>1</sup>.

	CCF (N = 54)	CRP (N = 63)	FHM (N = 193)	PKF (N = 113)	RBT (N = 22)	RSH (N = 11)	YBH (N = 12)
<b>Parasites</b>							
<u>Myxozoa</u>							
<i>Henneguya exilis</i>	P (0.02)	-	-	-	-	-	-
<u>Monogenea</u>							
<i>Ligictaluridus floridanus</i>	1.13 ± 7.62 (0 – 56) (0.07)	-	-	-	-	-	-
<i>Dactylogyrus extensus</i>	-	0.27 ± 0.83 (0 – 4) (0.13)	-	-	-	-	-
<i>Gyrodactylus hoffmani</i>	-	-	0.24 ± 1.32 (0 – 15) (0.09)	-	-	-	-
<u>Cestoda</u>							
<i>Bothriocephalus acheilognathi</i>	0.04 ± 0.19 (0 – 1) (0.05)	3.5 ± 5.4 (0 – 28)(0.52)	0.84 ± 5.28 (0 – 72) (0.23)	1.26 ± 4.54 (0 – 26) (0.15)	0.04 ± 0.21 (0 – 3) (0.14)	1.2 ± 1.31 (0 – 3) (0.63)	0.08 ± 0.28 (0 – 1) (0.08)
<i>Corallobothrium fimbriatum</i>	1.97 ± 7.67 (0- 56) (0.35)	-	-	-	-	-	-
<i>Megathylacoides giganteum</i>	1.59 ± 2.51 (0 – 10) (0.4)	-	-	-	-	-	-
Corallobothriinae* (pl.)	0.18 ± 1.12 (0 – 8) (0.02)	-	-	-	-	-	-
<u>Trematoda</u>							
<i>Ornithodiplostomum</i> sp.* (v)	0.02 ± 0.14 (0 – 1) (0.02)	-	0.24 ± 1.56 (0 – 17) (0.06)	-	-	-	-
<i>Ornithodiplostomum</i> sp.* (b)	-	-	0.01 ± 0.11 (0 – 1) (0.01)	-	-	-	-
<i>Posthodiplostomum</i> sp.*	-	-	0.02 ± 0.36 (0 – 5) (0.005)	-	-	-	-

Nematoda

<i>Rhabdochona</i> sp.	-	-	-	0.01 ± 0.09 (0 – 1) (0.01)	-	-	-
<i>Truttaedacnitis truttae</i>	-	-	-	-	24.8 ± 25.57 (0 – 108) (1)	-	-
<i>Eustrongylides</i> sp.* (l)	0.24 ± 0.58 (0 – 3) (0.18)	-	-	-	-	-	-
<i>Contracaecum</i> sp.* (l)	0.18 ± 0.55 (0 – 3) (0.13)	-	-	-	-	0.09 ± 0.30 (0 – 1) (0.09)	0.08 ± 0.29 (0 – 1) (0.08)

Acari

Oribatida gen. sp.	-	-	-	0.18 ± 0.13 (0 – 1) (0.02)	-	-	-
--------------------	---	---	---	-------------------------------	---	---	---

Crustacea

<i>Ergasilus arthrosis</i> .	0.02 ± 0.14 (0 – 1) (0.02)	-	-	-	-	-	-
<i>Lernaea cyprinacea</i> (female)	-	-	-	0.01 ± 0.09 (0 – 1)(0.01)	-	-	-
<i>Lernaea cyprinacea</i> (copepodite)	-	-	0.005 ± 0.07 (0 – 1) (0.005)	0.12 ± 0.5 (0 – 3) (0.07)	0.04 ± 0.21 (0 – 1) (0.04)	-	-

Hirudinea

<i>Myzobdella lugubris</i>	0.02 ± 0.14 (0 – 1) (0.02)	-	-	-	-	-	-
----------------------------	-------------------------------	---	---	---	---	---	---

<sup>1</sup> Mean abundance ± standard deviation (minimum – maximum) (prevalence),

P = Present (prevalence)

\* = Larval stages: (l) = larva, pl = plerocercoid, v = visceral, b = brain.

Table V. Parasite component community diversity<sup>1</sup> in humpback chub, speckled dace, and channel catfish.

	<i>G. cypha</i>	<i>R. osculus</i>	<i>I. punctatus</i>
June 1999	0.22 (0.47) (N=19)	0.27 (0.57) (N=86)	0.36 (0.59) (N=10)
September 1999	0.28 (0.47) (N=18)	0.42 (0.89) (N=99)	0.50 (0.65) (N=11)
April 2000	0.25 (0.53) (N=20)	0.37 (0.48) (N=97)	0.58 (0.69) (N=12)
June 2000	0.26 (0.55) (N=20)	0.51 (0.72) (N=113)	0.50 (0.6) (N=7)
September 2000	0.31 (0.44) (N=20)	0.49 (0.63) (N=111)	0.35 (0.48) (N=13)
April 2001	0.29 (0.47) (N=20)	0.38 (0.55) (N=124)	

<sup>1</sup>Shannon-Wiener index (Shannon's H') followed by evenness (in parentheses), and the sample size (N) of hosts in parentheses.

Table VI. Parasite component community diversity<sup>1</sup> in humpback chub and speckled dace at different sampling sites.

	<i>G. cypha</i>	<i>R. osculus</i>
Sampling Site <sup>2</sup>		
LC1	0.27 (0.44) (N=58)	0.46 (0.59) (N=101)
LC2	0.29 (0.38) (N=52)	0.55 (0.81) (N=125)
BCC	-	0.35 (0.51) (N=143)
BCS	-	0.50 (0.65) (N=81)
SAC	-	0.43 (0.56) (N=160)

<sup>1</sup>Shannon-Wiener index (Shannon's H') followed by evenness (in parentheses), and the sample size (N) of hosts in parentheses

<sup>2</sup>See text for sampling sites



## Figure Legends

Figure 1. The Colorado River drainage and location of fish sampling sites in the Little Colorado River, Grand Canyon, Arizona. LC1 = RK 0 – 2.5; LC2 = RK 10.6-11.5; LC3 = RK 14.5-15.1; BCC = Big Canyon Creek; BCS = Big Canyon Springs; SAC= Salt Creek.

Figure 2. Contribution of the different sampling sites (%) to the total fish sample during the Lower Little Colorado River (LCR) Study. See Figure 1 and text for acronyms of sampling sites.

Figure 3. Number of species of fish recovered from the different sampling sites during the LCR study. See Figure 1 and text for acronyms of sampling sites.

Figure 4. Mean parasite species richness of the different species of fishes examined in LCR study. BHS: Bluehead sucker, FMS: Flannelmouth sucker, HBC: Humpback chub, SPD: Speckled dace, CCF: Channel catfish, CRP: Carp, FHM: Fathead minnow, PKF: Plains killifish, RBT: Rainbow trout, YBH: Yellow bullhead.

Figure 5. Mean parasite species richness in 5 of the more abundant fish species in the LCR at different sampling periods. Acronyms of fish species as in Figure 4.

Figure 6. Abundance of *B. acheilognathi* in 11 species of fishes in the LCR at different sampling periods. Acronyms of fish species as in Figure 4.

Figure 7. Abundance of *B. acheliognathi* in humpback chub in LC1 and LC2 at different sampling periods. Acronyms of sampling sites and fish species as in Figures 1 and 4.

Figure 8. Abundance of *B. acheilognathi* in speckled dace in different sampling sites at different periods. Big Canyon Creek and Salt Creek at different sampling times.

Figure 9. Abundance of *B. acheilognathi* in speckled dace in Big Canyon Creek (BCC) and Salt Creek (SAC) at different sampling times.

Figure 10. Abundance of *Ornithodiplostomum* sp. in humpback chub and speckled dace from different sampling sites. See text for acronyms.

Figure 11. Abundance values of visceral *Ornithodiplostomum* sp., in speckled dace from Big Canyon Creek (BCC) and Salt Creek (SAC) at different sampling periods. Corresponding abundance values of *B. acheilognathi* are provided for comparison.

Figure 12. Temperature profiles of Big Canyon Creek (BCC) and Salt Creek (SAC), 27 September 2000 - 6 June 2001.

Figure 13. Mean daily temperature of the Little Colorado River at RK 1.2. Blank section is due to instrument failure after a flood. Shaded bars are sampling trips.