Parasites of Native and Non-native Fishes of the Lower Little Colorado River, Arizona

2001 Annual Report

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Submitted to:

Arizona Game and Fish Department
Phoenix, Arizona
Permit # SP621145

Navajo Fish and Wildlife Branch
The Navajo Nation
Window Rock, Arizona
Permit # 030301-025

Grand Canyon National Park
U. S. Park Service
Grand Canyon, Arizona
Permit # 9902-04-002

U.S. Fish and Wildlife Service
Albuquerque, New Mexico
Permit # TE008513-0

January 2002

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Introduction

The Colorado River in Grand Canyon and its biota have been dramatically changed by the closure of Glen Canyon Dam in 1963 (National Academy of Sciences 1991). Today, among approximately twenty non-native fishes only four of the original eight native species remain (bluehead sucker *Catostomus discobolus*, flannelmouth sucker *C. latipinnis*, humpback chub *Gila cypha*, speckled dace *Rhinichthys osculus*); and one of these (humpback chub) is federally endangered (http://endangered.fws.gov/i/E0B.html). Due to perennially cold, clear water, native fish in Grand Canyon now reproduce only in tributaries of the Colorado River (Valdez and Ryel 1995; Arizona Game and Fish Department 1996), of which the Little Colorado River (LCR) is the largest in Grand Canyon and the main spawning area for all native species (Arizona Game and Fish Department 1996).

Non-native fishes introduced into the Little Colorado and Colorado river systems have brought various parasites with them. At least four exotic parasites are known to infect fishes of the LCR. Two of these parasites, Asian fish tapeworm *Bothriocephalus acheilognathi* (Cestoda) and anchor worm *Lernaea cyprinacea* (Copepoda) are particularly worrisome, since they infect humpback chub at a higher rate than any other species in the system (Brouder and Hoffnagle 1997; Hoffnagle and Cole 1999; Hoffnagle et al 2000). Both *B. acheilognathi* and *L. cyprinacea* have been reported as pathogenic and potentially fatal (directly or indirectly) to fish of various age classes (Schäpperclaus 1986). *Bothriocephalus acheilognathi*, in particular, has caused high mortality in native fishes that it has infected outside of its native range (Hoffman and Schubert 1984). These parasites cannot complete their life cycles in the mainstem Colorado River under present, cold water conditions. However, they can be transported by infected individuals to other suitable tributaries, such as Kanab Creek.

Information on parasite distribution, host affiliation and impact of water temperature regimes has been requested by managers for making decisions regarding the future operation of Glen Canyon Dam and the proposed multi-level intake structure. This study is the first survey of the parasites of all fishes of the Little Colorado River and will provide information on prevalence of parasites and diseases present in the Little Colorado River drainage, with emphasis on native fishes and those species likely to be injurious to native fishes. Herein we report the results of our second year of sampling.

Study Area

The Little Colorado River is the major tributary to the Colorado River in Grand Canyon. Its perennial flow comes from Blue Spring and a series of lesser springs which discharge approximately 6.3 m^3/s of 20° C water which is supersaturated with calcium carbonate and charged with free CO_2 (Johnson and Sanderson 1968; Cole 1975). This study is conducted in the lower 21 km of the Little Colorado River, Grand Canyon, Arizona. These lands are administered by the Navajo Natural Heritage Program (Navajo Reservation) and U. S. National Park Service (Grand Canyon National Park).

The study area was originally divided into three reaches. Reach 3 is above the Atomizer/Chute Falls Complex (RK 13.6; RK = distance upstream in the Little Colorado River from its confluence with the Colorado River). This reach was not sampled after the first trip in June 1999, because river conditions did not permit us to land a helicopter there. Reach 2 is in the area of Salt Trail Canyon (RK 10.8) and is a middle reach of river containing all fish that complete their life cycles in the LCR and rare migrant rainbow trout *Oncorhynchus mykiss* (Robinson and Clarkson 1992; U.S. Fish and Wildlife Service 1994). This reach also contains three clear water, saline springs (Big Canyon Creek, Big Canyon Spring and Salt Creek), which were sampled, as well. Reach 1 is in the vicinity of Boulder Camp (RK 2) and ranged from RK 2.6 to the mouth (RK 0). This area 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 and Clarkson 1992; Hoffnagle et al 1997; Brouder and Hoffnagle 1998).

Methods

We conducted one main sampling trip in 2001. We entered the Little Colorado River Canyon by helicopter from the Salt Trail helicopter pad on the Navajo Reservation. Reaches 1 and 2 were sampled during this trip.

Fish were collected by commonly used gears such as seines, minnow traps, hoop nets, gill nets and trot lines.

We attempted to capture at least ten individuals of each species in each reach. Fishes collected for necropsy were limited by species: no humpback chub >150 mm could be necropsied and no more than five bluehead or flannelmouth suckers >150 mm could be sacrificed from each reach / trip. As many as 200 speckled dace or non-native species could be collected on each trip. The utility of these common fish as surrogates for monitoring parasite abundance in humpback chub will be examined.

All fish captured were measured for total length (mm), weighed (g) and examined for external parasites (*Lernaea cyprinacea*). Fish not collected for necropsy were released alive at the site of capture. Necropsied fish were examined for parasites in the gastrointestinal (GI) tract, eyes, brain, kidney, liver, gall bladder, musculature and gills. Parasites were identified, counted, preserved and their maturity and attachment location in/on the fish recorded. Blood smears were also made, air-dried and preserved in 100% methanol and later stained with Giemsa to examine for blood parasites.

Results

A total of 495 fish were captured and 278 fish were necropsied for internal parasites during the trip (Table 1). Eleven species of fish were examined, including all four native species and seven non-native species (channel catfish, common carp, fathead minnow, plains killifish *Fundulus zebrinus*, rainbow trout, red shiner *Cyprinella lutrensis* and yellow bullhead *Ameiurus natalis*). Ten species of parasites were recovered (Table 2): four Nematoda, three Cestoda, three Monogenea, one Trematoda, two Myxosporea, one Copepoda, and one Acarina (the Acarina are most likely ectocommensal, not obligatory parasites). Humpback chub and speckled dace were the most highly infected fishes. Parasite species richness in humpback chub was 5 species (*Ornithodiplostomum* sp., *Posthodiplostomum* sp., *Lernaea cyprinacea*, *Bothriocephalus acheilognathi,* and *Rhabdochona* sp.), as did speckled dace (Oribatida gen. sp., *Ornithodiplostomum* sp., *Posthodiplostomum* sp., *B. acheilognathi*, *Rhabdochona* sp.) (Table 2). The remaining fishes contained three species or less and at low rates of infection.

Of the 10 species of parasites recovered, only one adult parasite, *Rhabdochona* sp., appears to be native to the Little Colorado River in Grand Canyon. This nematode was found

only in speckled dace and humpback chub but appears to mature only in speckled dace. It was found in 13 of 124 speckled dace and in 5 of 18 humpback chub examined and its intensity of infection was also low (1-9 worms in infected dace). Comparisons with other species of *Rhabdochona* are under way to determine if it represents a previously undescribed species.

All other adult parasites found were probably introduced with non-native fishes and some of these are known to be or are potentially pathogenic. Some of these species may be particularly problematic due to their life cycle and/or the native fishes that they infect. The introduced Asian fish tapeworm (*Bothriocephalus acheilognathi*) was most abundant (prevalence and intensity) in humpback chub (100% prevalence and up to 115 worms in one fish). Heavy infections of *B. acheilognathi* may decrease growth of humpback chub, leaving them susceptible to predation for a longer period (Hoffman and Schubert 1984; Schäpperclaus 1986). *Lernaea cyprinacea* was relatively rare and was found on 2 species of fish (humpback chub, speckled dace). This ectoparasitic crustacean has been shown to be very common on humpback chub in the LCR in previous years (Hoffnagle and Cole 1999). *L. cyprinacea* has been show to affect fish health, causing changes in blood leucocyte density, loss of blood, secondary infection by *Chondrococcus*, *Costia* and *Saprolegnia* and decreased growth (McNeil 1961; James 1968; Bauer 1970).

Three species (*Ornithodiplostomum* sp., *Posthodiplostomum* sp., and *Contracaecum* sp.) were recovered as larval stages. These parasites mature in fish-eating birds that may be resident or transient in Grand Canyon. The identification of the strigeid larvae as *Ornithodiplostomum* sp was confirmed and we continued to find them in the viscera of fish (humpback chub, speckled dace) during this trip.

Changing the behavior of intermediate hosts is a well-documented strategy by parasites to increase their likelihood of being passed on to the next host (Beckage 1997). The catfish tapeworms (*C. fimbriatum* and *M. giganteum*) use small fish (possibly humpback chub) as an intermediate host. *Ornithodiplostomum* sp. was found in 88.9% of the humpback chub examined. This parasite uses fish-eating birds as its definitive host, but uses small fishes as intermediate hosts. Additionally, *Ornithodipostomum ptychocheilus* (Radabaugh 1980) and other related trematodes (Crowden and Broom 1980; Brassard et al. 1982; Lemly and Esch 1984; Lafferty and Morris 1996; Shirakashi and Goater, 2001) have been shown to alter host behavior in such a way as to increase the risk of predation and, thus, the likelihood of continuing the life

cycle of the parasite. Cestodes may also be able to increase the likelihood of their intermediate host to be consumed by the next host by decreasing growth (Pennycuick 1971) or increasing oxygen demand (Smith and Kramer 1987), which expose the fish to an increased risk of predation (Milinski 1985). It may be that parasites that use humpback chub as an intermediate host areas or more important to the survival of this endangered species than the parasites that use them as definitive hosts.

Future Studies

This concludes the survey phase of the study. Evaluation of all sampled parameters (parasites, tissues, fish diet, benthos, plankton, temperature, etc.) has begun. Phase 2 has also started (work is already in progress) and will focus on the impact of *B. acheilognathi* on the growth rate of its host and acute pathogenicity in very young fish. We are concerned that field data may be skewed as to impacts of this parasite on the very young fish (i.e., young fish that are heavily infected may not survive long enough to be sampled). Experimental work will help us fill this information gap. Parasite impact on growth is relevant because of the potential to increase the window of vulnerability to predation for these fish. Additionally, vulnerability to thermal stress is greater in smaller fish so infected fish that disperse into the mainstem Colorado River may also suffer increased mortality.

At least two introduced parasites (*B. acheilognathi* and *L. cyprinacea*) infecting humpback chub are restricted to the warmer LCR by the colder Colorado River (Carothers et al. 1981; Brouder and Hoffnagle 1997; Clarkson et al. 1997; Hoffnagle and Cole 1999). *T. truttae* is not restricted by cold temperatures, since it is found in rainbow trout in the Colorado River at Lees Ferry and in the LCR (McKinney et al. 1999; Hoffnagle et al. 2000). We do not know the temperature requirements of the other parasites. Increasing the water temperature in the Colorado River below Glen Canyon Dam has been proposed. Implementing this strategy may allow these parasites to increase their range and, thus, their effect on native fishes. We strongly recommend that a survey of fish parasites of the Colorado River and other tributaries in Grand Canyon be conducted as part of the studies concerning the temperature control device.

Acknowledgments

This work was funded by a grant to the National Wildlife Health Center (U.S. Geological

Survey - Biological Resources Division). We appreciate Grand Canyon National Park, Navajo Natural Heritage Program and the U.S. Fish and Wildlife Service for granting permits to conduct this work. Jon Cambron, Ben Galuardi, U.S.F.S., Flagstaff, Scott Hansen, Bobbi Hervin, and Anson Koehler provided skillful and enthusiastic field assistance.

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Table 1. Number of fish of each species necropsied on each trip and in each sampled reach during AGFD/USGS-BRD Little Colorado River fish parasite research, 2001. Note: Big Canyon Creek, Big Canyon Springs and Salt Creek were considered part of LCR Reach 2 for permitted humpback chub collections.

Table 2 continued.

	Native Fishes*				Non-native Fishes*							
Trip/Reach	BHS	FMS	HBC	SPD	CCF	CRP	FHM	PKF	RBT	RSH	YBH	Total
April 2001 (1 April – 12 April)												
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

*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 2. Frequency of occurrence* and range of the intensities of infection (in parentheses) of each parasite found in each species of fish sampled in the Little Colorado River during AGFD/USGS-BRD Little Colorado River fish parasite research, 2001.

	Native Fishes**				Non-native Fishes**						
Parasites	BHS	FMS	HBC	SPD	CCF	CRP	FHM	PKF	RBT	RSH	YBH
Acari (Mites)	0	0	0	4/124 (1)	0	0	0	1/40	0	0	0
Monogenea											
Dactylogyrus sp.	0	0	0	0	0	1/5 (2)	0	0	0	0	0
Gyrodactylus sp.	0	0	0	0	0	0	4/53 (1-2)	0	0	0	0
Copepoda											
Lernaea cyprinacea	0	0	1/18 (1)	0	0	0	0	0	0	0	0
Cestoda											
Bothriocephalus acheilognathi	0	0	18/18 (1-115)	55/124 (53)	0	0	7/53 (1-6)	1/40 (1)	0	2/2 (1-3)	0
Trematoda											
Ornithodiplostomum sp. (larva)	0	0	16/18 (1-27)	30/124 (1-32)	0	0	0	0	0	0	0
Posthodiplostomum sp. (larva)	0	0	1/18 (1)	1/124 (1)	0	0	0	0	0	0	0

Unidentified metacercaria	0	0	0	0	0	0	0	0	0	0	0
Nematoda											
Rhabdochona sp.	0	0	5/18 (1-2)	13/124 (1-9)	0	0	0	0	0	0	0
Truttaedacnitis truttae	0	0	0	0	0	0	0	0	1/1 (2)	0	0
Contracaecum sp.(larva)	0	0	0	0	0	0	0	0	0	1 / 2 (1)	0
Unidentified cysts	0	0	0	1/124 (1)	0	0	0	1/40 (1)	0	0	0

* Number of fish infected / number of fish examined

**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.