Journal of Aquatic Animal Health 18:184–193, 2006
© Copyright by the American Fisheries Society 2006
DOI: 10.1577/H05-046.1

[Article]

Parasitism and Body Condition in Humpback Chub from the Colorado and Little Colorado Rivers, Grand Canyon, Arizona

TIMOTHY L. HOFFNAGLE*[1]
Arizona Game and Fish Department, 2221 West Greenway Road, Phoenix, Arizona 85023, USA

ANINDO CHOUDHURY[2] AND REBECCA A. COLE
U.S. Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53701, USA

Abstract.—Glen Canyon Dam has greatly altered the Colorado River in Grand Canyon. The Little Colorado River (LCR) provides a small refuge of seasonally warm and turbid water that is thought to be more suitable than the Colorado River for endangered humpback chub *Gila cypha*. However, the LCR has low productivity and contains nonnative fishes and parasites, which pose a threat to humpback chub. The Colorado River hosts a different suite of nonnative fishes and is cold and clear but more productive. We compared condition factor (*K*), abdominal fat index (AFI), and presence and number of two introduced pathogenic parasites (*Lernaea cyprinacea* and *Bothriocephalus acheilognathi*) between juvenile (<150 mm total length) humpback chub from the LCR and those from the Colorado River during 1996–1999. Both *K* and AFI were lower and *L. cyprinacea* prevalence and *B. acheilognathi* prevalence were higher in LCR fish than in Colorado River fish for all years. Mean *K* and AFI were 0.622 and 0.48, respectively, in the LCR and 0.735 and 2.02, respectively, in the Colorado River, indicating that fish in the Colorado River were more robust. Mean prevalence of *L. cyprinacea* was 23.9% and mean intensity was 1.73 *L. cyprinacea*/infected fish in the LCR, whereas prevalence was 3.2% and intensity was 1.0 *L. cyprinacea*/infected fish in the Colorado River. Mean prevalence of *B. acheilognathi* was 51.0% and mean intensity was 25.0 *B. acheilognathi*/infected fish in the LCR, whereas prevalence was 15.8% and intensity was 12.0 *B. acheilognathi*/infected fish in the Colorado River. Increased parasitism and poorer body condition in humpback chub from the LCR challenge the paradigm that warmer LCR waters are more suitable for humpback chub than the colder Colorado River and indicate the need to consider the importance and benefits of all available habitats, as well as biotic and abiotic factors, when managing endangered species and their environment.

The humpback chub *Gila cypha* is an endangered fish that is native to the Colorado River basin in the western United States; the largest extant population is found in the area of the Colorado River–Little Colorado River (LCR) confluence in Grand Canyon, Arizona (Minckley 1991; Valdez and Ryel 1997). It has been hypothesized that the LCR, a seasonally warm and turbid stream, contains better habitat for juvenile humpback chub because it has not been greatly altered by human activity, whereas the Colorado River has been greatly altered by Glen Canyon Dam, located immediately upstream of Grand Canyon. Hypolimnetic releases from the dam turned a turbid and seasonally warm stream into a usually clear and constantly cold one (Stanford and Ward 1991). Therefore, juvenile humpback chub are thought to be better off if they remain in the warmer LCR for as long as possible (Gorman 1994; Douglas and Marsh 1996). Management actions that would warm the Colorado River to make it more hospitable for native fishes are being considered.

Juvenile humpback chub in Grand Canyon are exposed to a variety of stressors in their habitat that may affect their health and condition. Reproduction occurs nearly exclusively in the LCR, a stream with poor productivity (Haury 1981; Oberlin et al. 1999), where juveniles must compete for food with young native bluehead suckers *Catostomus discobolus,* flannelmouth suckers *C. latipinnis*, and speckled dace *Rhinichthys osculus* and with nonnative fathead minnow *Pimephales promelas*, red shiners *Cyprinella lutrensis*, and plains killifish *Fundulus zebrinus* (AGFD 1996). Juveniles also must avoid predation by larger conspecifics and by nonnative rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, channel catfish *Ictalurus punctatus*, black bullheads *Ameiurus melas*, yellow bullheads *A. natalis*, and red shiners (Ruppert et al. 1993; Marsh and Douglas 1997;

* Corresponding author: tim.hoffnagle@eou.edu
[1] Present address: Northeast Oregon Fish Research, Oregon Department of Fish and Wildlife, 203 Badgley Hall, Eastern Oregon University, La Grande, Oregon 97850, USA.
[2] Present address: Division of Natural Sciences, St. Norbert College, 100 Grant Street, De Pere, Wisconsin 54115, USA.

Received October 3, 2005; accepted February 22, 2006
Published online August 14, 2006

Valdez and Ryel 1997; Stone 1999). Additionally, juvenile humpback chub may be infected by a number of parasites that can affect their growth, behavior, and ultimately, survival (Choudhury et al. 2004).

Humpback chub generally rear for at least their first summer in the LCR. Fishes in the LCR are infected by several exotic parasites, the most common of which are the ectoparasite *Lernaea cyprinacea* (Copepoda) and the gastrointestinal tapeworm *Bothriocephalus acheilognathi* (Cestoda) (Hoffnagle and Landye 1998; Choudhury et al. 2004). Both of these parasites have been shown to infect humpback chub with greater prevalence and intensity than other fishes in Grand Canyon (Brouder and Hoffnagle 1997; Clarkson et al. 1997; Choudhury et al. 2004), and infection by *B. acheilognathi* probably reduces growth and survival of humpback chub (Hansen 2004). During late summer, when the LCR floods due to monsoon storms, a portion of the juvenile humpback chub population disperses into the Colorado River. If these juveniles are able to find suitable shoreline habitats in the Colorado River downstream from the LCR, they will grow to adulthood (Valdez and Ryel 1997). After entering the Colorado River, the humpback chub must first withstand the quick transition from warm (20–25°C) to cold (10–12°C) water (Lupher and Clarkson 1994; Clarkson and Childs 2002) as well as continue to find food and avoid predation. However, the threat of most or all parasites found in the Grand Canyon is greatly reduced by cold temperature, and food is more abundant in the Colorado River than in the LCR (Blinn and Cole 1991; AGFD 1996; Oberlin et al. 1999).

In light of these field and laboratory studies, it may be that the colder Colorado River provides benefits in the form of increased food availability and the prohibitive effects of cold temperature on parasitic infections. We compare two health indices (condition factor [$K = 100{,}000 \times$ body weight/length^3] and abdominal fat index [AFI]) and the prevalence and intensity of *B. acheilognathi* and *L. cyprinacea* in juvenile humpback chub from the Colorado River and LCR in the Grand Canyon to examine the paradigm that the LCR is better suited for growth of juvenile humpback chub than is the Colorado River.

Study Site

The LCR is the largest tributary of the Colorado River in Grand Canyon, Arizona, joining the Colorado River 99 km downstream from Lees Ferry and 124 km downstream from Glen Canyon Dam (Figure 1). This survey was conducted in the lower 3 km of the LCR and in the Colorado River from its confluence with the LCR to approximately 6.5 km downstream. The LCR is the spawning area for the largest extant population of humpback chub, and the vast majority of the humpback chub in Grand Canyon inhabit the LCR and this reach of the Colorado River (AGFD 1996; Valdez and Ryel 1997).

Methods

We captured 152 juvenile humpback chub (young of the year and age 1; <150 mm total length) in the Colorado River and LCR during September 1996–1999. Sample sizes, limited by our collection permit due to the endangered status of humpback chub and by our ability to capture them, ranged from 8 to 34 fish in each stream and year. We sampled the fish in September to ensure that the parasites would have time to infect and mature in young humpback chub. We measured total length and weight and later calculated K for each fish (Anderson and Neuman 1996). We assigned an AFI modified from Goede (1993) to characterize the amount of abdominal fat in each specimen by visually estimating the percentage of the internal organs covered by fat (0 = 0%; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%). We also examined each fish for the presence of two parasite species, *L. cyprinacea* and *B. acheilognathi*. The fish were not examined for any other parasites or diseases. We recorded numbers of *L. cyprinacea* for all years and numbers of *B. acheilognathi* in 1999. We conducted Mann–Whitney U-tests to compare length, weight, K, AFI, and parasite intensity (number/infected fish) between the two rivers (Sokal and Rohlf 1981). To further examine the effect of parasites on fish health, we used Mann–Whitney U-tests to compare length, weight, K, AFI, and parasite intensity in the presence or absence of one or both parasite species in each river. We calculated Pearson's product-moment correlation coefficients (Sokal and Rohlf 1981) between *L. cyprinacea* and *B. acheilognathi* intensity versus weight, length, K, and AFI. The significance level (α) for all statistical tests was set at 0.05.

Results

Fish Size

Juvenile humpback chub ranged in length from 35 to 147 mm and in weight from 0.2 to 19.0 g (Table 1). The mean length of all fish did not vary between rivers ($P = 0.0850$), but mean weight was greater ($P = 0.0018$) in the Colorado River (3.93 g) than in the LCR (2.79 g; Table 2). Both the mean length and weight of uninfected juvenile humpback chub were greater ($P \leq 0.0066$) in the Colorado River than in the LCR. However, the size of juvenile humpback chub infected by one or both parasites did not differ between rivers ($P \geq 0.0744$).

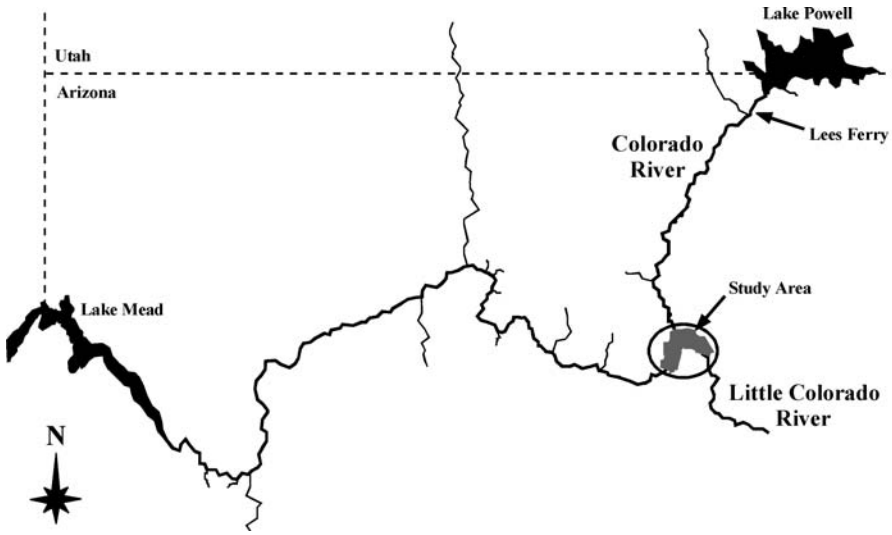

FIGURE 1.—The Colorado and Little Colorado rivers in the Grand Canyon, Arizona. The study area (circle) encompasses the lower 3 km of the Little Colorado River and the 6.5 km of the Colorado River immediately downstream from its confluence with the Little Colorado River.

Both length and weight varied with the presence or absence of parasites (Table 2). In both rivers combined, mean length did not differ between infected and uninfected fish ($P = 0.1439$), but mean weight was greater in uninfected than infected fish ($P = 0.0466$). In the Colorado River, infected fish were smaller in both length and weight ($P \leq 0.0075$) than were uninfected fish, while in the LCR there was no difference between the two groups ($P \geq 0.0766$). Mean length and weight did not differ in fish infected by *L. cyprinacea* ($P \geq 0.0535$) except in the LCR, where infected fish were longer than uninfected fish ($P = 0.0357$). In both rivers combined, humpback chub infected by *B. acheilognathi* were neither longer nor heavier than uninfected fish ($P \geq 0.1502$). *Bothriocephalus acheilognathi*-infected fish from the Colorado River were both smaller and lighter than the uninfected humpback chub ($P \leq 0.0485$), while the mean size of infected humpback chub from the LCR was not different from that of uninfected fish ($P \geq 0.1988$).

Both the length and weight of juvenile humpback chub were positively correlated with AFI in both rivers combined and in the LCR ($P \leq 0.0219$) but not in the Colorado River ($P \geq 0.0886$) (Table 3). Fish size was not correlated with numbers of *L. cyprinacea* or *B. acheilognathi* ($P \geq 0.0815$).

Condition Factor

Overall, mean K was higher ($P < 0.0001$) in the Colorado River (0.735) than in the LCR (0.622) (Table 1). Mean K was also higher in the Colorado River than in the LCR during each year ($P \leq 0.0040$) except 1997 ($P = 0.6680$). Uninfected humpback chub exhibited higher K in the Colorado River than in the LCR ($P < 0.0001$), but there was no difference between rivers for humpback chub infected by one or both parasites ($P = 0.1631$).

Condition factor also varied with the presence or absence of parasites (Table 2). Mean K was higher in uninfected than infected humpback chub for both rivers combined and in the Colorado River ($P \leq 0.0252$), but in the LCR there was no difference in mean K between infected and uninfected humpback chub ($P \geq 0.2274$).

Condition factor was correlated with AFI and *L. cyprinacea* intensity (Table 3). Condition factor was positively correlated with AFI ($P \leq 0.0003$) and negatively correlated with number of *L. cyprinacea* ($P \leq 0.0072$) in both rivers combined and in the Colorado River. There were no significant correlations with K in the LCR or with *B. acheilognathi* intensity ($P \geq 0.0721$).

Abdominal Fat Index

Mean AFI was higher ($P < 0.0001$) overall in Colorado River fish (2.02) than in LCR fish (0.48) (Table 1). The annual mean AFI in Colorado River fish ranged from 1.62 in 1996 to 2.85 in 1999 and was higher than that of LCR fish in every year ($P \leq 0.0001$). The annual mean AFI in LCR fish ranged from 0.19 in 1996 to 1.00 in 1999.

The mean AFI in juvenile humpback chub infected by one or both parasites did not vary between rivers (P

TABLE 1.—Number sampled (N) and mean, minimum, and maximum length (mm), weight (g), abdominal fat index (AFI), condition factor (K), and number and percent of humpback chub from the Colorado River (CR) and Little Colorado River (LCR), Arizona, infected by *Lernaea cyprinacea* and *Bothriocephalus acheilognathi,* 1996–1999. Significant differences between annual and total means are indicated (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$).

	1996		1997		1998		1999		Total	
Variable	CR	LCR	CR	LCR	CR	LCR	CR	LCR	CR	LCR
N	34	21	15	8	16	19	20	19	85	67
Length										
Mean	69.0	59.1*	72.8	66.8	61.8	56.2	79.2	89.1	70.7	67.7
Minimum	42.0	42.0	56.0	48.0	41.0	51.0	40.0	35.0	40.0	35.0
Maximum	103.0	92.0	100.0	100.0	129.0	63.0	121.0	147.0	129.0	147.0
Weight										
Mean	2.85	1.45**	3.28	2.21	6.12	1.09	5.72	6.20	3.93	2.79
Minimum	0.5	0.4	1.2	1.0	1.7	0.8	0.5	0.2	0.5	0.2
Maximum	8.5	4.4	7.3	6.0	18.0	1.5	15.5	19.0	18.0	19.0
AFI										
Mean	1.62	0.19***	2.13	0.38**	1.75	0.32****	2.85	1.00***	2.02	0.48***
Minimum					1.00			1.00		
Maximum	4.0	1.0	4.0	1.0	3.0	1.0	4.0	1.0	4.0	1.0
K										
Mean	0.724	0.629**	0.709	0.688	0.800	0.610****	0.759	0.599***	0.735	0.622***
Minimum	0.456	0.496	0.572	0.572	0.752	0.540	0.605	0.466	0.456	0.466
Maximum	1.082	0.905	0.916	0.904	0.839	0.678	0.896	0.694	1.082	0.905
Lernaea cyprinacea										
Intensity[a]										
Mean	1.0	1.9**	1.00	1.67		1.0*		1.0*	1.00	1.73**
Minimum	1.0	1.0	1.00	1.0		1.0		1.0	1.00	1.0
Maximum	1.0	7.0	1.00	2.0		1.0		1.0	1.00	7.0
Number of fish infected	2.0	10.0	1.00	3.0		1.0		1.0	3.00	15.0
% infected	5.9	47.6	6.7	37.5		5.3		5.3	3.5	22.4
Bothriocephalus acheilognathi										
Intensity[a]										
Mean							12.0	25.0****	12.00	25.0****
Minimum							4.0	1.0	4.00	1.0
Maximum							26.0	178.0	26.00	178.0
Number of fish infected	3.0	8.0	4.00	4.0	2.0	6.0	3.0	16.0	12.00	34.0
% infected	8.8	38.1	26.7	50.0	12.5	31.6	15.0	84.2	14.1	50.7

[a] Number of parasites per infected host.

$= 0.3284$), but AFI was higher ($P < 0.0001$) for uninfected Colorado River fish than for uninfected LCR fish. Mean AFI was higher ($P \leq 0.0055$) in uninfected than infected humpback chub in both rivers combined and in the Colorado River but not in the LCR (Table 2).

The AFI was correlated ($P \leq 0.0219$) with length, weight, K, and *L. cyprinacea* intensity (Table 3). The AFI was positively correlated with length and weight in both rivers combined and in the LCR and was positively correlated with K in the Colorado River and in both rivers combined. There was a negative correlation between AFI and *L. cyprinacea* intensity in the Colorado River and in both rivers combined.

Lernaea cyprinacea Infection

The mean percentage of humpback chub infected with *L. cyprinacea* and the mean number of *L. cyprinacea* per infected humpback chub were higher ($P = 0.0003$) in the LCR than in the Colorado River (Table 1). The LCR humpback chub had an overall mean of 1.73 *L. cyprinacea*/infected fish, and 22.4% of the fish were infected. All infected Colorado River fish had only 1.0 *L. cyprinacea* each, and only 3.5% were infected. The mean number of *L. cyprinacea* per infected fish was higher for LCR humpback chub than for Colorado River fish in all years ($P \leq 0.0361$). Annual *L. cyprinacea* prevalence and intensity in LCR humpback chub ranged from 5.3% infected with a mean of 1.0 *L. cyprinacea*/infected fish (1998 and 1999) to 47.6% infected with 1.9 *L. cyprinacea*/infected fish (1996). The maximum number of *L. cyprinacea* found on a juvenile humpback chub in the LCR was seven in 1996. In the Colorado River, no fish were infected in 1998 and 1999, and only two (5.9%) and one (6.7%) infected fish were found in 1996 and 1997, respectively; only 1.0 *L. cyprinacea*/infected fish was found in Colorado River humpback chub in both 1996 and 1997.

In both rivers combined and in the Colorado River, *L. cyprinacea* intensity was negatively correlated ($P \leq 0.0219$) with K and AFI (Table 3). In the LCR, *L.*

TABLE 2.—Number of juvenile humpback chub from the Colorado River (CR), Little Colorado River (LCR), and both rivers combined that were infected by *Lernaea cyprinacea, Bothriocephalus acheilognathi,* or both parasites. Mean length, weight, condition factor (*K*), and abdominal fat index (AFI) of infected and uninfected fish are also shown. Significant differences between infected and uninfected fish are noted (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$).

	CR		LCR		Both rivers	
Variable	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
Both parasites						
N	82	3	62	5	144	8
Length	73.1	56.3**	59.9	71.8	69.9	68.4
Weight	4.29	1.64**	1.58	3.42	3.57	3.09*
K	0.746	0.665*	0.612	0.627	0.711	0.634****
AFI	2.18	1.08**	0.43	0.50	1.76	0.63****
Lernaea cyprinacea						
N	82	3	52	15	134	18
Length	71.0	61.7	66.1	73.1*	69.1	71.2
Weight	4.04	1.33	2.66	3.23*	3.46	2.91
K	0.742	0.574*	0.628	0.603	0.694	0.598***
AFI	2.09	0.33*	0.54	0.27	1.49	0.28****
Bothriocephalus acheilognathi						
N	73	12	33	34	106	46
Length	73.1	56.3**	62.5	72.7	69.8	68.4
Weight	4.29	1.64**	1.78	3.76	3.44	3.28*
K	0.746	0.665*	0.610	0.634	0.700	0.641**
AFI	2.18	1.08**	0.39	0.56	1.62	0.70****

cyprinacea intensity was not significantly correlated with any of the measured variables ($P \geq 0.0721$).

Bothriocephalus acheilognathi Infection

In the LCR, 50.7% of the humpback chub were infected with *B. acheilognathi*, a greater percentage ($P = 0.0209$) than the 14.1% that were infected in the Colorado River (Table 1). In 1999, there was a mean of 25.0 *B. acheilognathi*/infected fish (range = 1–178 *B. acheilognathi*/infected fish) in humpback chub from the LCR and 12.0 *B. acheilognathi*/infected fish (range = 4–26 *B. acheilognathi*/infected fish) in humpback chub from the Colorado River. The percentage of humpback chub infected in the LCR ranged from 31.6% (1998) to 84.2% (1999). In the Colorado River, the percentage of infected humpback chub ranged from 8.8% (1996) to 26.7% (1997). The intensity of *B. acheilognathi* was not significantly correlated ($P \geq 0.0731$) with any of the measured variables (Table 3), but the number of this parasite was only recorded in 1999.

Discussion

These results suggest that juvenile humpback chub from the Colorado River are healthier than those from the LCR. Juvenile humpback chub from the Colorado River have higher *K*, more body fat, and a lower prevalence and fewer numbers of *L. cyprinacea* and *B.*

TABLE 3.—Pearson's product-moment correlation coefficients for length, weight, condition factor (*K*), abdominal fat index (AFI), and number of *Bothriocephalus acheilognathi* and *Lernaea cyprinacea* in juvenile humpback chub from the Colorado River, Little Colorado River, and both rivers combined during 1996–1999. The statistical significance of the coefficients is noted (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$).

River and variable[a]	Length	Weight	*K*	AFI	*B. acheilognathi* intensity
Little Colorado river					
AFI	0.366**	0.362**	−0.166		
B. acheilognathi intensity	0.223	0.186	−0.146		
L. cyprinacea intensity	0.090	0.016	−0.221	−0.069	0.072
Colorado river					
AFI	0.186	0.165	0.341**		
B. acheilognathi intensity	−0.399	−0.296	−0.038	0.038	
L. cyprinacea intensity	−0.082	−0.136	−0.310**	−0.248*	
Rivers combined					
AFI	0.204*	0.242**	0.462****		
B. acheilognathi intensity	0.175	0.112	−0.290	−0.233	
L. cyprinacea intensity	0.036	−0.041	−0.264**	−0.196*	0.121

[a] Parasite intensity is the number of parasites per infected host.

acheilognathi than similarly sized humpback chub from the LCR. These results challenge the paradigm that the LCR is better habitat for humpback chub than the Colorado River (Gorman 1994; Douglas and Marsh 1996). The cause of the disparity in these health indices between the two rivers is unclear but probably stems from the combined effect of these parasites and the differing productivities of the two rivers.

Since both *B. acheilognathi* and *L. cyprinacea* require warm water to mature, it seems clear that the differences in parasite intensity and abundance are caused by the temperature difference between the two rivers. Granath and Esch (1983) found that *B. acheilognathi* requires a temperature of 20°C or greater to mature, and Hoffman (1976) reported that *L. cyprinacea* requires a temperature of 17°C or greater. In the vicinity of the LCR, the temperature of the Colorado River is cold. Mean monthly temperature reaches only 12.2°C in the main channel and 14.9°C in backwaters (AGFD 1996) and does not approach the temperature necessary for either of these parasites to complete their life cycles. Therefore, all parasites found in this study must have been contracted during humpback chub residence in the LCR.

The infection parameters for *B. acheilognathi* in this study are similar to those reported previously. Brouder and Hoffnagle (1997) reported that 26.7% of the humpback chub caught in the Colorado River near the LCR were infected, and Clarkson et al. (1997) found that 47.6% of similarly sized humpback chub were infected in the LCR. Hoffnagle and Landye (1998) reported that *B. acheilognathi* infection rates in speckled dace were 27% in the LCR and 21% in Kanab Creek during 1998. The high prevalence of humpback chub infection by *B. acheilognathi* is of concern due to the endangered status of this species and because high-intensity infections can block the gastrointestinal tracts of young fish, perforate the intestines, and/or destroy the intestinal mucosa, thereby causing death (Hoffman 1980; Schäperclaus 1986). We do not know how *B. acheilognathi* has affected the humpback chub population in Grand Canyon. However, laboratory infection of the very similar and closely related bonytail *G. elegans* (22 mm fork length; approximately 2.5 months old) demonstrated that juvenile growth in length was reduced by up to 9% relative to that of uninfected controls; when food ration was restricted, infected fish began dying 20 d earlier and at nearly twice the rate of uninfected controls (Hansen 2004).

Humpback chub in and around the LCR comprise the largest remaining population of this species (Valdez and Ryel 1997); the population is estimated to include 4,508–10,444 humpback chub that are 150 mm or larger (Douglas and Marsh 1996). The potential for added mortality of juveniles reduces the potential for recruitment into the population, which does not bode well for this endangered fish. Native speckled dace, flannelmouth suckers, and bluehead suckers and non-native channel catfish, common carp, fathead minnow, plains killifish, rainbow trout, red shiners, and yellow bullheads are also infected by *B. acheilognathi* in the LCR, although the tapeworm matures mostly in cyprinids (Hoffnagle and Landye 1998; Choudhury et al. 2004).

The prevalence of *B. acheilognathi*, although high in 1996, showed no trends from 1996 to 1999. It is likely that *B. acheilognathi* is more resistant to the effects of flooding than is *L. cyprinacea*. Because of its life cycle, *B. acheilognathi* can overwinter in its host and can begin or resume shedding eggs in the spring, when the water temperature warms (Granath and Esch 1983; Riggs et al. 1987; Riggs and Esch 1987). In the LCR, water temperature remains cool during the spring runoff period, warming only when discharge returns to base flow. Therefore, eggs or infected copepod intermediate hosts, which are most commonly found on and among vegetation along stream margins, are less at risk of being flushed downstream by spring floods. By the time monsoon flooding arrives in late summer, young fish have already been infected.

The low numbers and prevalence of *L. cyprinacea*, particularly in the Colorado River, make comparisons difficult. However, the pattern of relationships between *L. cyprinacea* infection and *K* or AFI in infected and uninfected humpback chub is similar to that of *B. acheilognathi*. Infection parameters for *L. cyprinacea* in this study are lower than those reported from Grand Canyon in October 1978 (Carothers et al. 1981). At that time, 53.8% of 65 juvenile (58–189 mm) humpback chub were infected by *L. cyprinacea*, and the intensity of infection ranged from 1 to 5 *L. cyprinacea*/fish; only bluehead suckers from Kanab Creek had a higher prevalence of infection (55.1%). Hoffnagle and Landye (1998) reported that no *L. cyprinacea* were found on any of the fish (mostly speckled dace) sampled from Colorado River tributaries in Grand Canyon in 1998. This parasite has little host specificity and can infect nearly any fish, as well as frog tadpoles and salamanders (Hoffman 1999). Choudhury et al. (2004) found low infection rates in 1999 and 2000; only humpback chub, speckled dace, and plains killifish were infected with adult *L. cyprinacea*, whereas rainbow trout, red shiners, and yellow bullheads were infected with immature stages.

The abundance of *L. cyprinacea* in humpback chub decreased over the 4 years of this study. It appears that annual *L. cyprinacea* abundance may be negatively

correlated with intensity of flooding in the LCR. The years 1998 and 1999 were characterized by extended springtime floods and early and strong monsoon seasons that resulted in extended periods of high discharge in the LCR. These floods may have flushed the free-swimming stages of *L. cyprinacea* out of the LCR. In contrast, dry winter and spring periods in 1996 and 1997 kept discharge low, thus flushing fewer of these copepods, and allowed the river to warm more quickly in the spring, which created a longer growing season for the parasite.

During the course of this study, we found adult *L. cyprinacea* embedded in the head and through the abdominal wall into the viscera of humpback chub. In all cases, gross pathology included inflammation associated with penetration lesions and fibrosis in the viscera. With respect to length–weight relationships, increased vulnerability to predation, and other factors, *L. cyprinacea* was found to have a sub-lethal impact on the fish sampled in this study. However, in experimental studies on fishes such as the fathead minnow (Vaughn and Coble 1975), *L. cyprinacea* is well documented as being potentially lethal or pathogenic for feral and cultured fishes alike (Paperna 1964; Lester and Roubal 1995; Hoffman 1999). Disease caused by this parasite is probably associated with the considerable pathology (lesions) created by the embedded anchor and neck (Khalifa and Post 1976; Lester and Roubal 1995), and disease severity is probably related to infection intensity, secondary microbial infections, and attachment site. The severity of infection to the humpback chub population in Grand Canyon is unknown, as we did not recover any fish that may have died from *L. cyprinacea* infection.

Given the intensity of infection and the effect that these parasites can have on their hosts, the question remains whether the observed differences in *K* and body fat were due to differences in the riverine environments (e.g., temperature or food availability); the influence of *B. acheilognathi*, *L. cyprinacea*, or other introduced parasites; or a combination of these or other factors. Our data suggest that a combination of these two factors, and perhaps others, is involved.

Length, weight, *K*, and AFI did not differ between infected and uninfected humpback chub in the LCR. However, we did find that unparasitized humpback chub from the LCR were smaller and had lower *K* and AFI than did unparasitized humpback chub from the Colorado River. The magnitude of the observed difference might have been affected by the sampling of some humpback chub that had only recently left the LCR and entered the Colorado River (thus retaining LCR influence while being counted as Colorado River fish). The true difference in *K* and AFI between the Colorado River and LCR may therefore be even larger. This suggests an environmental effect and is contrary to the paradigm that humpback chub are better off in the natural LCR than in the drastically altered Colorado River. The environmental effect may be explained by two factors: productivity and introduced species. Although the LCR is a warmer stream, it is much less productive than the Colorado River (Haury 1981; Oberlin et al. 1999). Since increased temperature increases the metabolism of poikilothermic animals, humpback chub metabolic demand in the LCR may be so high as to adversely affect *K*: that is, the fish may be using all of their energy for metabolism and growth in length at the expense of storing energy. However, for a stream in which environmental conditions are largely unaltered and humpback chub are native, this would seem unlikely—unless food is scarce. Food scarcity may be related to the number of nonnative fishes present in the LCR. Fathead minnow, plains killifish, and young common carp, which were introduced into this system as early as the late 1800s, are common. The humpback chub did not evolve with these exotic competitors, whose diets and habitat are similar to those of humpback chub. Ultimately, these new competitors and the poor productivity of the LCR mean that the humpback chub must spend more time and energy in foraging. The reduced growth rate caused by low forage abundance forces humpback chub to feed on copepods for a long period of time, which unfortunately means an increased exposure to and subsequently higher abundance of parasitic infections (Choudhury et al. 2004).

The added burden of parasites certainly exacerbates the environmental problems present in the LCR. As the number of parasites per infected host increased, *K* and AFI decreased in the Colorado River and overall, indicating that parasites may also affect these health indices. There was no difference in *K* or AFI between parasitized humpback chub in the two rivers or between infected or uninfected humpback chub in the LCR. Our finding that humpback chub in the LCR had little or no body fat indicates that they are already stressed by low food abundance. The added burden of parasitic infection may be visible only in the fish that we could not sample because they had already died. The data of Hansen (2004) indicate that heavily infected fish are likely to suffer high rates of mortality in the LCR, where food is limiting. When the fish reach the Colorado River, the effect of the parasites becomes apparent. In the Colorado River, diet restrictions are reduced as food becomes more abundant, and uninfected fish become healthier while infected fish maintain their low *K* and AFI. This may be similar to ecological release, which is observed when a competitor

is removed from an environment (Paine 1966; Dunham 1980). However, instead of competitor removal, in this case the humpback chub has moved into an environment where the suspected limiting factor (food) is more abundant and where environmental conditions (cold water temperature and high current velocity) are more debilitating to some competitors (e.g., fathead minnow and plains killifish) than to the species of interest (Hoffnagle et al. 1999).

Parasite infection may be a major problem for humpback chub, as they appear to be particularly susceptible to infection by exotic parasites. Choudhury et al. (2004) found that among all fishes in the LCR, humpback chub had the highest abundances of three of the six parasites that infected this species. Immune reactions to foreign bodies come at a cost to fitness (Miller et al. 2002). It is highly likely that infection by internal and external parasites adds to the energy demands of humpback chub. The abundance of parasites in the LCR (in addition to *L. cyprinacea* and *B. acheilognathi*) probably further burdens the energy budget of this fish. In the Colorado River, food appears to be plentiful but the water is colder, which reduces the potential for growth (AGFD 1996). Cold water also reduces or eliminates the threat of these two and other pathogenic warmwater parasites, thus improving the energy budget of humpback chub and allowing them to store more fat. Valdez and Ryel (1997) reported that in 1990–1993, growth of adult humpback chub was greater in the Colorado River than in the LCR but that subadult growth was greater in the LCR. One would expect that juvenile humpback chub would also grow more slowly in the Colorado River; however, if food was limiting in the LCR during 1996–1999, possibly due to competition from nonnative fishes (Valdez et al. 2005), then even juvenile humpback chub growth may have been improved in the Colorado River.

The LCR is the only place in Grand Canyon where humpback chub regularly spawn and the only place from which new fish are recruited into the Grand Canyon population (AGFD 1996). Therefore, the LCR is still essential for humpback chub survival in Grand Canyon, and every effort must be made to protect it even though it may behoove the humpback chub to leave the LCR as soon as they are capable of withstanding the increased current speed and cold temperature of the Colorado River. Although it appears to be a more natural environment, the LCR has been compromised by exotic species. The Colorado River, while having completely unnatural physical characteristics, may be a refuge of sorts from these exotic parasites and some of the exotic fishes and may help to maintain humpback chub numbers. Warming the water of the Colorado River may reduce the effectiveness of this refuge, and management actions should be very cautious in this regard. Given the precarious status of the humpback chub population in Grand Canyon and elsewhere, we should take advantage of all habitats that are available for the humpback chub and be careful in our efforts to manage habitats to the benefit of native species. It is possible that the status quo in the Grand Canyon is the best situation that can be provided for humpback chub under the circumstances. In the final analysis, it appears that humpback chub face a trade-off between a warm stream with poor productivity and a higher parasite burden versus a cold but productive stream with a low risk of parasitism. The parasitism of humpback chub by *B. acheilognathi* and *L. cyprinacea* illustrates the unavoidable consequence of the introduction of nonnative species; the extreme success of these two introduced parasites as colonizers of new hosts and new environments; and the need to consider the importance and benefits of all available habitats as well as biotic and abiotic factors in the management of endangered species and their environment.

Acknowledgments

This work was conducted during Arizona Game and Fish Department (AGFD; 1996–1997) and U.S. Fish and Wildlife Service (USFWS; 1998–1999) Grand Canyon fish monitoring activities and U.S. Geological Survey (USGS) National Wildlife Health Center and AGFD fish parasite research (1999). Many people helped capture fishes for this work. R. Cordes of the South Dakota Department of Game, Fish, and Parks); P. Hines and D. Stone of the USFWS; and B. Michael, M. Brouder, and D. Brown of the AGFD assisted with necropsies and/or recording data. O. T. Gorman of the USFWS, W. R. Persons and M. Brouder of the AGFD, and A. Bersh of the USGS National Wildlife Health Center reviewed the manuscript.

References

Anderson, R. O., and R. M. Neuman. 1996. Length, weight, and associated structural indices. Pages 447–482 *in* B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

AGFD (Arizona Game and Fish Department). 1996. Ecology of Grand Canyon backwaters. Final report submitted to Glen Canyon Environmental Studies, U.S. Bureau of Reclamation, Flagstaff, Arizona.

Blinn, D. W., and G. A. Cole. 1991. Algal and invertebrate biota in the Colorado River: comparison of pre- and postdam conditions. Pages 103–123 *in* Committee to Review the Glen Canyon Environmental Studies, editors. Colorado River ecology and dam management. National Academy Press, Washington, D.C.

Brouder, M. J., and T. L. Hoffnagle. 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.

Carothers, S. W., J. W. Jordan, C. O. Minckley, and H. D. Usher. 1981. Infections of the copepod parasite, *Lernaea cyprinacea*, in native fishes of Grand Canyon. National Park Service Transactions and Proceedings Series 8:452–460.

Choudhury, A., T. L. Hoffnagle, and A. R. A. Cole. 2004. Parasites of native and nonnative fishes of the Little Colorado River, Grand Canyon, Arizona. Journal of Parasitology 90:1042–1053.

Clarkson, R. W., A. T. Robinson, and T. L. Hoffnagle. 1997. Asian tapeworm, *Bothriocephalus acheilognathi*, in native fishes from the Little Colorado River, Grand Canyon, Arizona. Great Basin Naturalist 57:66–69.

Clarkson, R. W., and M. R. Childs. 2002. Temperature effects of hypolimnial release dams on early life stages of Colorado River basin big-river fishes. Copeia 2002:402–412.

Douglas, M. E., and P. C. Marsh. 1996. Population estimates/population movements of *Gila cypha*, an endangered cyprinid fish in the Grand Canyon region of Arizona. Copeia 1996:15–28.

Dunham, A. E. 1980. An experimental study of interspecific competition between the iguanid lizards *Sceloprus merriami* and *Urosaurus ornatus*. Ecological Monographs 50:309–330.

Goede, R. W. 1993. Fish health/condition assessment procedures. Utah Division of Wildlife Resources, Fisheries Experiment Station, Logan.

Gorman, O. T. 1994. Habitat use by humpback chub, *Gila cypha*, in the Little Colorado River and other tributaries of the Colorado River. Report submitted to Glen Canyon Environmental Studies, U.S. Bureau of Reclamation, Flagstaff, Arizona.

Granath, W. O., and G. W. Esch. 1983. 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.

Hansen, S. P. 2004. Experimental infection of bonytail chub (*Gila elegans*) with the Asian fish tapeworm (*Bothriocephalus acheilognathi*): impacts on survival, growth, and condition. Master's thesis. University of Wisconsin–La Crosse, La Crosse.

Haury, L. R. 1981. *Cladophora* drift and planktonic crustaceans in the Colorado River: Lee's Ferry to Diamond Creek. Report to Museum of Northern Arizona, Flagstaff.

Hoffman, G. L. 1976. The anchor parasite (*Lernaea elegans*) and related species. U.S. Fish and Wildlife Service Fish Disease Leaflet 46.

Hoffman, G. L. 1980. Asian tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934, in North America. Fisch und Umwelt 8:69–75.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes. Cornell University Press, Ithaca, New York.

Hoffnagle, T. L., and J. Landye. 1998. Summary of fish health and parasitology studies. Pages 27–30 *in* O. T. Gorman and R. G. Bramblett, editors. Monitoring and studies of native fishes of the Colorado River ecosystem in Grand Canyon, Arizona. Report submitted to Grand Canyon Monitoring and Research Center, Flagstaff, Arizona.

Hoffnagle, T. L., R. A. Valdez, and D. W. Speas. 1999. Fish abundance, distribution, and habitat use. Pages 273–288 *in* R. H. Webb, J. C. Schmidt, G. R. Marzolf, and R. A. Valdez, editors. The controlled flood in Grand Canyon. Geophysical Monograph 110.

Khalifa, K. A., and G. Post. 1976. Histopathological effect of *Lernaea cyprinacea* (a copepod parasite) on fish. Progressive Fish-Culturist 38:110–113.

Lester, R. J. G., and F. R. Roubal. 1995. Phylum Arthropoda. Pages 475–598 *in* P. T. K. Woo, editor. Fish diseases and disorders, volume 1. Protozoan and metazoan infections. CAB International, Wallingford, UK.

Lupher, M. L., and R. W. Clarkson. 1994. Temperature tolerance of humpback chub (*Gila cypha*) and Colorado squawfish (*Ptychocheilus lucius*), with a description of culture methods for humpback chub. Appendix 5.1 *in* Glen Canyon environmental studies, phase II: 1993 annual report. Submitted to the U.S. Bureau of Reclamation, Salt Lake City, Utah.

Marsh, P. C., and M. E. Douglas. 1997. Predation by introduced fishes on the endangered humpback chub and other native species in the Little Colorado River, Arizona. Transactions of the American Fisheries Society 126:343–346.

Miller, L. B., A. Scheuerlein, and M. Wikelski. 2002. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? Proceedings of the Royal Society of London B 270:153–158.

Minckley, W. L. 1991. Native fishes of the Grand Canyon region: an obituary? Pages 124–177 *in* National Academy of Sciences, editors. Colorado River ecology and dam management. National Academy Press, Washington, D.C.

Oberlin, G. E., J. P. Shannon, and D. W. Blinn. 1999. Watershed influences on the macroinvertebrate fauna of ten major tributaries of the Colorado River through Grand Canyon, Arizona. Southwestern Naturalist 44:17–30.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist 100:65–75.

Paperna, I. 1964. Parasitic helminths of inland fishes of Israel. Israel Journal of Zoology 13:1–20.

Riggs, M. R., A. D. Lemly, and G. W. Esch. 1987. The growth, biomass, and fecundity of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir. Journal of Parasitology 73:893–900.

Riggs, M. R., and G. W. Esch. 1987. The suprapopulation dynamics of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir: abundance, dispersion, and prevalence. Journal of Parasitology 73:877–892.

Ruppert, J. B., R. T. Muth, and T. P. Nesler. 1993. Predation on fish larvae by adult red shiner, Yampa and Green rivers, Colorado. Southwestern Naturalist 38:397–399.

Schäperclaus, W. 1986. Fish diseases, volume 2. Akademie-Verlag, Berlin. (German translation published for U.S. Department of the Interior and the National Science Foundation by Amerind Publishing Co., New Delhi).

Sokal, R. R., and F. J. Rohlf. 1981. Biometry, 2nd edition. Freeman, New York.

Stanford, J. A., and J. V. Ward. 1991. Limnology of Lake Powell and the chemistry of the Colorado River. Pages 75–102 *in* National Academy of Sciences, editors. Colorado River ecology and dam management. National Academy Press, Washington, D.C.

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

Valdez, R. A., and R. J. Ryel. 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., W. R. Persons, and T. L. Hoffnagle. 2005. A nonnative fish control strategy for Grand Canyon, Arizona. Pages 142–150 *in* M. J. Brouder, C. L. Springer, and S. C. Leon, editors. The lower Colorado River: restoring natural function and native fish within a modified riverine environment. U.S. Fish and Wildlife Service, Southwest Region, Albuquerque, New Mexico.

Vaughn, G. E., and P. W. Coble. 1975. Sublethal effects of three ectoparasites on fish. Journal of Fish Biology 7:283–294.