Experimental infection of the endangered bonytail chub (*Gila elegans*) with the Asian fish tapeworm (*Bothriocephalus acheilognathi*): impacts on survival, growth, and condition

S.P. Hansen, A. Choudhury, D.M. Heisey, J.A. Ahumada, T.L. Hoffnagle, and R.A. Cole

Abstract: *Bothriocephalus acheilognathi* Yamaguti, 1934, a tapeworm known to be pathogenic to some fish species, has become established in the endangered humpback chub (*Gila cypha* Miller, 1964) in Grand Canyon, USA, following the tapeworm's introduction into the Colorado River system. The potential impact of this tapeworm on humpback chub was studied by exposing the closely related bonytail chub (*Gila elegans* Baird and Girard, 1853) to the parasite under a range of conditions that included potential stressors of humpback chub in their natal waters, such as abrupt temperature change and a limited food base. Survival of infected fish under low food rations was considerably lower than that of control fish, and mortality of infected fish began 20 days earlier. Growth of infected fish was significantly reduced, and negative changes in health condition indices were found. No significant negative impacts were revealed from the synergistic effects between temperature shock and infection. *Bothriocephalus acheilognathi* does present a potential threat to humpback chub in Grand Canyon and should be considered, along with conventional concerns involving altered flow regimes and predation, when management decisions are made concerning conservation of this endangered species.

Résumé : *Bothriocephalus acheilognathi* Yamaguti, 1934, un ver plat connu comme pathogène pour certaines espèces de poissons, s'est associé au « méné bossu » (*Gila cypha* Miller, 1964), une espèce menacée du Grand Canyon, É.-U., après l'introduction du ver dans le réseau hydrographique du Colorado. Nous avons étudié l'impact potentiel de ce ver plat sur le méné bossu en exposant l'espèce proche *Gila elegans* Baird et Girard, 1853 au parasite sous une gamme de conditions qui incluent les facteurs potentiels de stress des ménés bossus dans leurs cours d'eau d'origine, tels que les changements abrupts de température et des ressources alimentaires limitées. La survie des poissons infectés dans des conditions de nourriture limitée est substantiellement réduite par comparaison aux poissons témoins et la mortalité des poissons infectés débute 20 jours plus tôt. La croissance des poissons infectés est significativement réduite et il y a des changements négatifs dans les indicateurs des conditions de santé. Il n'y a pas d'impact négatif significatif apparent des effets de synergie du choc thermique et de l'infection. *Bothriocephalus acheilognathi* est donc une menace potentielle pour le méné bossu dans le Grand Canyon et il devra être pris en considération, au même titre que les préoccupations habituelles au sujet de la modification du régime des eaux et de la prédation, lorsque des décisions seront prises au sujet de la conservation de cette espèce menacée.

[Traduit par la Rédaction]

Introduction

The ecology of the Colorado River in Grand Canyon, Arizona, has changed dramatically since the closure of the Glen Canyon Dam (GCD) in 1963 (Valdez and Ryel 1995). Once a turbid river with seasonally influenced temperatures, water downstream of the dam now rarely exceeds 16 °C in its 400 km run to Lake Mead (Cole and Kubly 1976; Arizona Game and Fish Department 1996; US Bureau of Reclamation 1999). Changes in water flow and temperature, as well

Received 13 April 2006. Accepted 3 August 2006. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 22 November 2006.

S.P. Hansen,[1] D.M. Heisey, and R.A. Cole.[4] US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711, USA.
A. Choudhury. Division of Natural Sciences, St. Norbert College, 100 Grant Street, DePere, WI 54115, USA.
J.A. Ahumada.[2] Pacific Cooperative Studies Unit, University of Hawaii, Manoa, HI 96822, USA.
T.L. Hoffnagle.[3] Arizona Game and Fish Department, 2221 West Greenway Road, Phoenix, AZ 85023, USA.

[1]Present address: Wisconsin Department of Natural Resources, 101 South Webster Street, Madison, WI 53706, USA.
[2]Present address: Center for Applied Biodiversity Science, Conservation International, 1919 M Street, NW Suite 600, Washington, DC 20036, USA.
[3]Present address: Oregon Department of Fish and Wildlife, 203 Badgely Hall, Eastern Oregon University, La Grande, OR 97850, USA.
[4]Corresponding author (e-mail: rebecca_cole@usgs.gov).

 doi:10.1139/Z06-126 © 2006 NRC Canada

as the presence of introduced fishes, are believed to have contributed significantly to the loss or reduction of some native fishes, including the endangered humpback chub (*Gila cypha* Miller, 1964) (Minckley 1991; Valdez and Ryel 1995; US Bureau of Reclamation 1999).

Humpback chub persist in Grand Canyon largely because of the population in the Little Colorado River (LCR), the largest tributary below GCD (Valdez and Ryel 1995). Higher water temperatures and natural flow in this tributary provide conditions suitable for humpback chub recruitment (Hamman 1982; Kaeding and Zimmerman 1983). However, the streamflow pulses that occur in the LCR during the monsoon rain periods of September–October often wash young-of-the-year chub from the warm LCR into the cold Colorado River, where they are subjected to thermal stress and predators (Valdez and Ryel 1995; Robinson et al. 1998).

Unfortunately, conditions in the LCR have also proved suitable for the establishment and continued propagation of several introduced parasites (Choudhury et al. 2004), of which the Asian fish tapeworm (*Bothriocephalus acheilognathi* Yamaguti, 1934) remains the most worrisome because of its known pathogenic effects on some fish species (see below). *Bothriocephalus acheilognathi* was first described from Japan and has spread to parts of Europe, Africa, Australia, and North America (Körting 1975; Boomker et al. 1980; Andrews et al. 1981; Font and Tate 1994; Dove et al. 1997; Choudhury et al. 2006). First introduced into the USA in the 1970s with its native host, the grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) (Hoffman 1976), *B. acheilognathi* has since spread to portions of the Colorado River drainage (Heckman et al. 1986; Brouder and Hoffnagle 1997) and now exploits humpback chub as its main host in the LCR (Choudhury et al. 2004).

Effects of this parasite range from mortality in carp, golden shiners (*Notemigonus crysoleucas* Mitchill, 1814), and mosquitofish (*Gambusia affinis* Baird and Girard, 1853) (Liao and Shih 1956; Hoffman 1980; Granath and Esch 1983) to a decrease in body mass of carp (Bauer et al. 1973) and may include growth retardation in roundtail chub (*Gila robusta* Baird and Girard, 1853) (Brouder 1999). Pathogenic changes can include necrosis, inflammation, and hemorrhagic enteritis of the gut, causing destruction and ultimately dysfunction of the intestinal mucosa (Scott and Grizzle 1979; Schäperclaus 1991). In addition, hematological parameters such as hematocrit and erythrocyte counts are also reduced (Liao and Shih 1956; Sopinska 1985), while suppression of proteolytic gut enzymes by the tapeworm may interfere with absorption of nutrients of ingested food in carp (Matskási 1984). Most evidence, however, remains circumstantial, and controlled experiments using high levels of infection to demonstrate the tapeworm's effect on host growth and survival are lacking.

We infected hatchery-propagated bonytail chub (*Gila elegans* Baird and Girard, 1853) (Endangered Species Permit No. TE676811-8) with *B. acheilognathi* to examine the tapeworm's impact on fish growth, survival, and health. The humpback chub was unavailable from a hatchery source. The bonytail chub is an ideal surrogate for the humpback chub because of the similar biology and close relationship of the two species, including the ability to hybridize (Holden and Stalnaker 1970; Hamman 1981; Valdez and Clemmer 1982). A pilot study of similar design was conducted in our laboratory using older (nearly 1 year old) bonytail chub. Results of that study indicated no significant adverse effects of *B. acheilognathi* infection except for reduced hematocrit levels in infected male fish. Consequently, the present study focused on younger fish and different experimental conditions to reflect some of the natural conditions and potential stressors experienced by young-of-the-year chub in their native waters, such as the LCR and Colorado River. Stressors included varying levels of exposure to the parasite, decreased food rations to reflect periods of low food resources, and cold shock, since juvenile chub also suffer adverse effects from exposure to the low water temperature of the Colorado River (Clarkson and Childs 2000). The overarching goal of each experiment was to examine for potential adverse effects of *B. acheilognathi* infections on survival, growth, and general health of bonytail chub. However, stressors were applied to a portion of fish from each experiment: fish from experiment I were exposed to an extreme reduction in water temperature, while fish from experiment II were subjected to reduced food levels. Each stressor was introduced to investigate the potential of tapeworms to exacerbate reduced survival due to known stressful conditions. Parasites have been considered a factor in the life history of chub in the Colorado River drainage in Grand Canyon (Hoffnagle et al. 2006), but information from controlled studies is lacking. Experimental results reported here will provide data that will assist in making management decisions regarding imperilled fish species such as the humpback chub in the Lower Colorado River Basin.

Methods

Animal culture

Fish

Young-of-the-year bonytail chub were hatched at the Dexter National Fish Hatchery and Technology Center in Dexter, New Mexico, and sent to the National Wildlife Health Center (NWHC), Madison, Wisconsin. Upon arrival, fish were housed in a recirculating water system using a municipal water source (general hardness 268.5 ppm) that was filtered through a 5 μm prefilter, an activated carbon filter, and a 1 μm finishing filter before use. Water in the system was circulated with 0.04-horsepower pumps circulating 1900 L/h. The pump was a component of a System Pak® (Aqua Logic Inc., San Diego, California, USA) that also included vessels that filtered water physically and chemically prior to distribution among 76 L glass aquaria. Outflow from all aquaria was collected in a 361 L sump tank filled with biofilter media cultured with *Nitrosomas* sp. and *Nitrobacter* sp. bacteria for ammonia and nitrite removal. Fish were fed Biokyowa® (Kyowa Hakko, New York, New York, USA) commercial fish food and *Artemia* sp. nauplii, which were cultured in-house. All vertebrates were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care (1993).

Copepods

Cyclopoid copepods (*Acanthocyclops robustus* G.O. Sars, 1863) were collected using a 153 μm plankton tow net from the Colorado River and a pool in Big Canyon Creek (BCC),

© 2006 NRC Canada

a tributary to the LCR, and returned to the laboratory. There they were separated by collection source, housed in 9.5 and 19 L tanks containing carbon-filtered water, and fed *Spirulina*, zooplankton meal, and *Paramecium* sp. Pilot studies indicated that copepods collected from each location were suitable hosts for larval tapeworms; however, infection levels in copepods from BCC were considerably higher (S. Hansen, personal observation 2001). Therefore, copepods from BCC were cultured and used as the intermediate host for each experiment.

Bothriocephalus acheilognathi

Eggs of *B. acheilognathi* were initially harvested from tapeworm-infected fish collected from the LCR in June 2001, stored in vials filled with sterile spring water, and kept at 14–19 °C for several days until transport to the laboratory. Supernatant with emergent coracidia (first larval stage of the tapeworm) was removed from the vials and introduced to copepod cultures. Cultured bonytail chub were fed infected copepods, became infected, and served as a source of eggs for a pilot study and subsequent experiments.

Experiment I

Infection of copepods

Infected pilot-study fish were euthanized with an overdose of tricaine methanesulfonate (MS 222), and tapeworms were removed from the stomach and placed in deionized water to expel eggs. Eggs not expelled were dissected free from tapeworm segments using fine needles. Eggs were incubated in carbon-filtered hard water at 24–26 °C.

Emergent coracidia were transferred to a 50 mL test tube, and numbers of coracidia were estimated by mixing test tube contents, removing a 10 μL sample, and enumerating coracidia using a microscope. The mean of three samples was calculated and multiplied by the volume of sample in the test tube. Coracidia were poured into bowls containing approximately 75 copepods at an average of 4807 coracidia (SD 1260) per bowl. At 13–15 days after exposure to coracidia, a minimum of five copepods were removed from each bowl and examined using a compound microscope to estimate the total number of infective procercoids (second larval stage of tapeworm). Procercoids were considered mature and infective if developed cercomers (constriction of body bearing embryonic hooks) could be observed (Hanzelová and Žitňan 1986). The mean number of procercoids per copepod was determined and multiplied by the total number of copepods in the bowl.

Fish exposure

Bonytail chub (n = 144) 60–61 days old and with a mean fork length of 22.28 mm (SD 2.14; range 17–28 mm) were randomly allocated to twelve 76 L tanks on the same recirculation system at 12 fish per tank. Randomization was accomplished using the combination of a die and a random numbers table. Four of the tanks with fish were subsequently assigned as controls. Fish were fasted for a minimum of 19 h and then exposed to enough copepods to yield approximately 100 infective procercoids per fish. In an attempt to increase the prevalence and levels of tapeworm infection, fish were exposed in two stages over the course of 3 days. On day 0, water levels were reduced to approximately one third and fish were allowed to feed for 3 h on half of the infected copepods; thereafter, the water level was increased to one half for 16 h. The tanks were then filled and the process was repeated on day 3 with the remainder of the copepods. Each tank from this treatment process was then replaced with a new one to remove any uneaten copepods. This was done to prevent establishment of copepod populations in the recirculation system and thus the potential for infection of control fish and recurring infections in exposed fish. Control fish were treated in the same manner, but no copepods were added to control tanks.

Fish husbandry

During the first 4 weeks postexposure, all fish were fed a pinch of Biokyowa commercial fish food per tank and an *Artemia* sp. nauplii suspension twice daily. (Observations during pilot studies suggested that tapeworm numbers and development were positively affected in fish fed a diet supplemented with *Artemia* nauplii versus a purely artificial diet.) After this 4-week period, commercial food was rationed to each tank at 2% of fish biomass for 2 weeks and then at 1.5% of fish biomass for the remainder of the experiment. Rations were divided between a morning and an afternoon feeding and were adjusted after each 2-week weighing date. *Artemia* rations were not fed as a proportion of fish biomass and were kept relatively consistent between tanks. *Artemia* nauplii are not considered a highly nutritious food source because of low lipid levels (Léger et al. 1986), and since we considered our rations to be relatively low, the amounts were not apportioned as rigorously as the Biokyowa. Wastes were siphoned daily from tanks along with an approximately 20% water change. Submersible heaters were placed in the sump tank and temperature was recorded daily from random tanks. Mean water temperature in this system was 23.40 °C (SD 0.33). Water temperature was set to simulate upper-level water temperatures recorded in the LCR, as reported in Choudhury et al. (2004). System ammonia and nitrite levels were measured weekly and the dissolved oxygen level was measured for each tank at approximately the beginning, middle, and end of the experiment to monitor water quality and consistency among tanks.

Cold-shock treatment

A recirculating water system was fitted with a 0.5-horsepower "Turbo-Fin Plus"® fluid chiller (Aqua Logic, Inc., San Diego, California, USA), which reduced the water temperature to 9.5 °C, to simulate the low water temperatures of the Colorado River at its confluence with the LCR (Arizona Game and Fish Department 1996). At 160 days postexposure, exposed fish from four tanks and control fish from two tanks were placed into six tanks with 9.5 °C water and examined for mortality, morbidity, or behavioral changes. Three-minute video recordings were made of the group of fish in each tank immediately upon placement into the chilled water and subsequently at 0.5, 1, 2, and 3 h. Fish were not fed during the course of the cold-shock treatment. Individual fish were observed on the videotape for abnormal behaviors including equilibrium loss, stationary positioning, hyperactivity, fanning slowly in place, surface swimming,

© 2006 NRC Canada

and inability to maintain position in the current below the tank inlet. These categories of aberrant behavior were determined from preliminary viewing of recordings and comparison with "normal" behaviors of fish that were not subjected to cold shock, as well as descriptions from the literature (Griffith 1978; Clarkson and Childs 2000). Each fish was timed for occurrence of these abnormal behaviors during a 2 min period following initial exposure and for 1 min periods at each of the subsequent recording times. During each recording time after initial exposure (i.e., 0.5, 1, 2 and 3 h), a quick "knocking" on the tank was applied in an attempt to stimulate a response. A movement of one or more body lengths was recorded as a strong response, while a movement of less than one body length or no movement was considered a weak response. Categorization of the stimulus responses was derived from observations by Griffith (1978) of threadfin shad (*Dorosoma petenense* Günther, 1867) exposed to low temperatures.

Growth monitoring and terminal sampling

On day –1, fish were individually anaesthetized with MS 222 (1:10 000), and fork length (mm) and batch mass (0.01 g) were recorded. Every 14 days thereafter, individual lengths and masses were determined. Excess water was removed from each fish after anaesthetization and prior to weighing by blotting with a moist Kimwipe® towel. At 161 days postexposure, fish were euthanized with an overdose of MS 222 and necropsied. Termination of the experiment on this day allowed ample time for development and maturation of tapeworms, as tapeworm eggs were found in fish fecal samples as early as 44 days postexposure in a pilot study (S. Hansen, personal observation 2002). Final length and mass were recorded and tapeworms were enumerated for each fish. Intensity of infection was reported as number of worms per infected fish (Bush et al. 1997). Recovered tapeworms were fixed in 10% hot-buffered formalin and transferred to 70% ethanol for subsequent biomass analysis. At necropsy, a general health assessment was conducted based on selected parameters used in the health index developed by Goede and Barton (1990). Specific anatomical structures suggested by the literature, such as spleen, liver, and kidney, were examined for abnormalities. Sex was determined using an acetocarmine squash method (Guerrero and Shelton 1974). Using heparinized microhematocrit tubes, blood was collected from the severed caudal peduncle and centrifuged for 4 min, after which hematocrit was measured. The liver was removed and weighed to the nearest milligram to obtain a liver–somatic index (LSI; liver mass/fish mass × 100) (Heidinger and Crawford 1977). As a reflection of reduced liver glycogen storage, LSI values have been used as an indicator of nutritional state (Heidinger and Crawford 1977; Bulow et al. 1978; Adams and McLean 1985). The amount of mesenteric fat was quantified using a modified version of Goede and Barton's (1990) mesenteric fat index that was used by Hoffnagle et al. (2006) for humpback chub. The following values were assigned based on visceral fat coverage: 1, 0%–25% coverage; 2, 26%–50% coverage; 3, 51%–75% coverage; and 4, 75%–100% coverage. Gastrointestinal tract tissues were fixed for histological analysis from select fish with high worm burdens or in cases where possible mechanical damage to the gut was suspected.

Tapeworm biomass was assessed on a dry mass basis. Recovered worms were transferred onto alcohol-cleaned glass slides and placed in a drying oven at 60 °C for a minimum of 24 h, whereafter worms were weighed to the nearest 0.1 mg. Due to the 0.1 mg lower limit of the balance, only worm masses ≥ 1.0 mg were recorded. Worm masses below 1.0 mg or non-detectable were pooled and the calculated mean mass was assigned to each sample.

Experiment II

Procedures and conditions are the same as in experiment I except where noted.

Fish exposure

Young-of-the-year bonytail chub, age 60–61 days, were randomly (computer-generated random numbers) allocated to twenty-four 76 L tanks at 8 fish per tank. Mean fork length was 22.04 mm (SD 1.41; range 18–25 mm). Exposed and control fish were maintained in separate recirculating water systems in separate rooms, each containing 12 tanks. Estimations of procercoid numbers in copepods were not conducted. Approximately 225 exposed copepods were released into each tank. To promote the establishment of a copepod population within the system and thereby repeated exposure to tapeworm infections, no attempt was made to remove uneaten copepods after initial exposure. Control fish were treated identically except they were given approximately the same number of *Artemia* sp. nauplii in place of copepods. Uninfected copepods were released into each treatment system periodically throughout this experiment to facilitate establishment of the parasite life cycle within the exposed-fish system.

Fish husbandry

Half of the fish were fed once daily with Biokyowa at a rate of 1.5% of body mass per tank, and the remaining fish were fed at a rate of 0.5%. As deaths occurred, these rations were adjusted by taking the mean amount of Biokyowa per fish and subtracting it from the total tank ration as necessary. Fish diets were again supplemented with an *Artemia* nauplii suspension, with lower ration fish receiving 0.25 mL and higher ration fish receiving 0.5 mL per day per tank. Mean water temperatures in control and infected systems were 24.10 °C (SD 0.17) and 24.10 °C (SD 0.28), respectively.

Growth monitoring and terminal sampling

Fish were batch weighed to the nearest 0.001 g and individual fork lengths (mm) were measured on day –1. Individual masses and lengths were measured every 14 days thereafter. At day 170, fish were euthanized and mass and length were determined. Small fish size precluded blood sampling and determination of sex and liver mass. Final mean lengths of high-ration control and exposed fish in experiment II were less than half the respective lengths of fish in experiment I. Other health index tissues (e.g., spleen, kidney, and liver) were not examined for this experimental group with the exception of mesenteric fat coverage.

© 2006 NRC Canada

Data analyses

Experiment I

All analyses were conducted using the SAS® 8.01 statistical software package (SAS Institute Inc. 1999–2000), and results for all statistical tests were considered significant at $\alpha = 0.05$. Because the treatment design consisted of introducing parasites at the tank level, the data from exposed and control groups were analyzed using tank as the experimental unit. Owing to variation among tanks, fish within a tank are likely to be correlated. Therefore, for single time endpoint variables such as length, mass, hematocrit, and LSI, an intraclass correlation model was used to allow fish within a tank to be correlated. Specifically, this was implemented in SAS PROC MIXED using a repeated measures model with an error structure of compound symmetry. Unlike approaches based on first collapsing the data to tank means, this approach allowed individual-level covariates, such as sex, to be included without difficulty. Exposed and control fish data were analyzed further to include a third group consisting of fish that were exposed but not infected (hereafter referred to as uninfected) because not all exposed fish in each tank were found to be infected at the end of the study. Significant differences between groups from multiple comparison analyses were determined using the protected least significant difference method on population marginal means. Unrestricted maximum likelihood was used for all analyses. Because no significant sex effect was observed, sex was not included as a covariate in these multiple comparison analyses. Since individual fish identities were not maintained over time, longitudinal length and mass growth curve data were analyzed using the tank mean values. These data were transformed as needed to best approximate linearity. Mass was successfully linearized using a square root transform (1/2 power), while 3/2 power was used to linearize length. A within-tank repeated measures analysis-of-covariance model was then used to estimate the daily growth rate and test for differences between exposed and control tanks. The error structure for this model was assumed to be first-order autoregressive, or AR(1). Because randomization ensures that the expected differences between tanks are initially 0, the model was constrained to have the same origin regardless of treatment. Tests to examine effects of worm biomass and number of worms on fish length used similar model statements. Because a portion of fish from the first experiment was exposed to a cold-shock treatment, chilled and non-chilled fish along with treatment interactions were examined using the same model statement. Logistic regression was used for binary variables such as the presence or absence of parasites. For binary data, the consequence of intra-tank correlation is overdispersion, which was tested for using tank as the grouping factor. Differences in cold shock response and strength of response to the "knocking" stimulus between control and exposed fish were analyzed using the same intraclass correlation model approach used for endpoint analysis.

Experiment II

Growth curve analyses were performed as in experiment I. The same transformations were used to help linearize length and mass data over time. While these transformations improved the linearity, the data could not be perfectly linearized because of several inflections. Final length and mass were analyzed with a compound symmetry repeated measures analysis of variance with parasite exposure and ration levels expressed as a one-way design with four levels. Pairwise comparisons were performed with population marginal means comparisons. Uninfected fish were not used as a group in these analyses because of the low proportion of uninfected fish in this experiment. Endpoint comparisons between control and exposed low-ration fish were not conducted because of the high mortality rates in these groups. Visceral fat coverage indices of exposed and control fish within the high-ration group were analyzed with a compound symmetry repeated measures analysis of variance.

Kaplan–Meier survival estimates (Kaplan and Meier 1958) were calculated for each of the treatment factors (control high ration, control low ration, exposed high ration, and exposed low ration). Survival curves were constructed as the proportion of fish from each of these cohorts surviving to day *t*. Fish that survived until the endpoint of 170 days were treated as right censored. Three fish apparently removed during cleaning on days 38 and 39 were treated as censored losses on the day of disappearance. Omnibus differences between the survival curves were tested for with the log-rank test, and the pairwise hazard ratios and *P* values were obtained with a Cox proportional hazards (PH) model (Cox 1972). The log-rank and Cox PH likelihood ratio tests assume each fish is independent and do not allow within-tank correlations in mortality. To examine whether this assumption resulted in "overly significant" results, an analysis of survival at day 170 was performed with logistic regression, which allows for an explicit examination of within-tank correlation (overdispersion). The above analysis also assumes the three accidental losses during tank cleaning were independent of those fishes' predisposition to die. However, it seems reasonable to postulate that they were more "frail" and more vulnerable, hence the assumption of independence might be inappropriate. Therefore, to examine the sensitivity of the results to this potential independence violation, we examined the "worst case", where the removals correspond to deaths.

Results

Experiment I

Forty-four of 92 (48%) exposed fish were infected at the end of this experiment. No fish died as a result of treatment. Intensity of infection ranged from 1 to 151 worms. The distribution was heavily skewed to low levels of infection, with 54% of the fish having five or fewer worms. Mean intensity of infection was 17.2 worms (SD 30.56). Mean worm biomass was 7.8 mg (SD 7.6). Exposed fish grew at a significantly slower rate throughout the course of the experiment (Fig. 1), with the result that these fish were, on average, 3.48 mm smaller than control fish by the end of the experiment (Table 1). Separation of infected fish and uninfected fish revealed significant differences in final length ($F_{[2,6]}$ = 8.22, P = 0.019) and suggestive significant differences in final mass ($F_{[2,6]}$ = 4.51, P = 0.064) within the exposed group. Infected fish were, on average, 5.8 and 5.9 mm shorter and

© 2006 NRC Canada

Fig. 1. Experiment I. Mean growth, by tank, of bonytail chub (*Gila elegans*) in fork length and mass over 161 days for control fish (△; n = 4) and those exposed to *Bothriocephalus acheilognathi* (○; n = 8). Growth of control fish was significantly greater than that of exposed fish in both length ($F_{[1,142]}$ = 14.85, P < 0.001) and mass ($F_{[1,142]}$ = 15.53, P < 0.001).

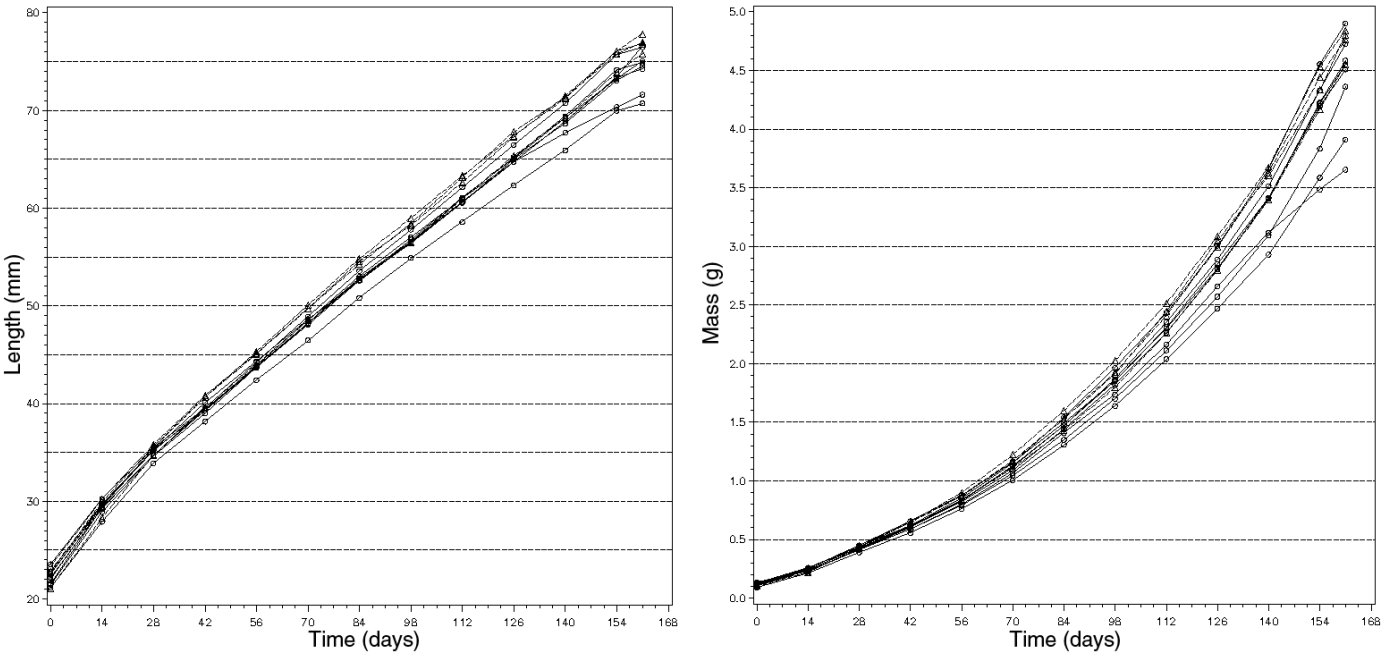


Table 1. Experiment I: final mean (1 SE) length (mm), mass (g), hematocrit, and liver–somatic index (LSI) for control and parasite-exposed bonytail chub (*Gila elegans*).

	Population marginal means				
	Control	Exposed	Difference	P	df
Length	77.57 (0.99)	74.09 (0.70)	3.48 (1.22)	0.017	1, 10
Mass	4.86 (0.19)	4.36 (0.14)	0.50 (0.24)	0.061	1, 10
Hematocrit	37.1 (0.69)	36.2 (0.49)	0.9 (0.84)	0.297	1, 10
LSI	1.5 (0.07)	1.4 (0.05)	0.1 (0.1)	0.498	1, 10

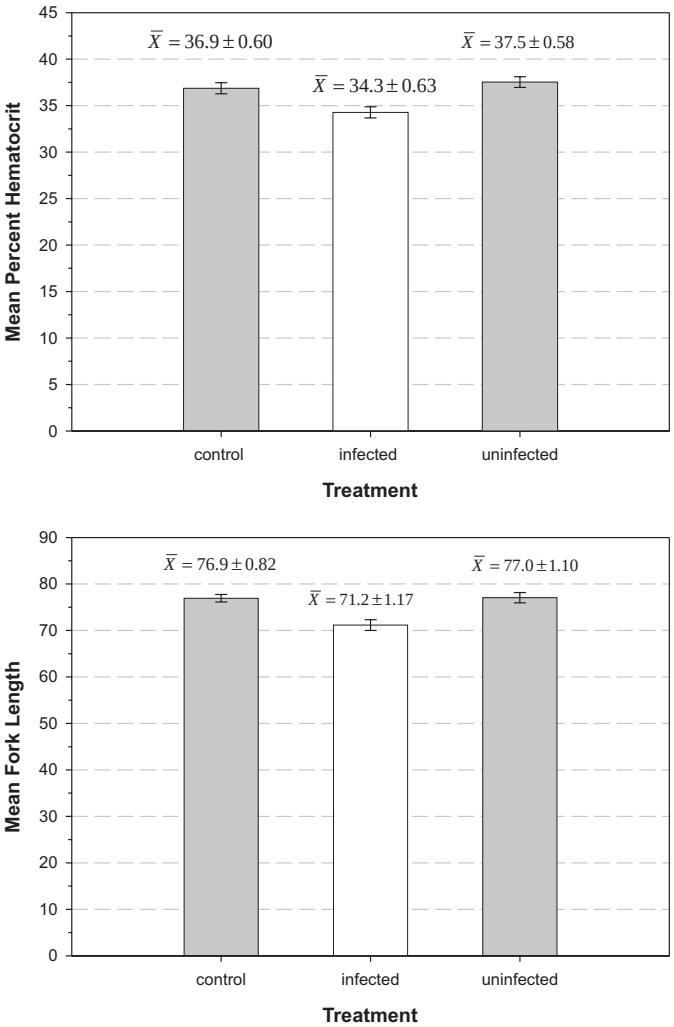
0.83 and 0.96 g lighter than control and uninfected fish, respectively (Fig. 2). Mean hematocrit values for exposed fish were 0.9 lower than those for controls, but the difference was not significant (Table 1). Within the exposed group, infected fish had significantly lower mean hematocrit values than uninfected fish ($F_{[2,6]}$ = 7.97, P = 0.020) (Fig. 2). A suggestive significant sex effect was found in the hematocrit analysis ($F_{[1,10]}$ = 4.17, P = 0 0.068), with mean hematocrit values of 35.8 for females and 37.4 for males. No significant differences in LSI were found when comparing control and exposed fish (Table 1) or when separating infected and uninfected fish ($F_{[2,6]}$ = 0.40, P = 0.688), although chilled fish had a significantly higher LSI ($F_{[1,10]}$ = 11.82, P = 0.006) than non-chilled fish. Fat coverage of the viscera was generally in the higher index range for both control and exposed fish. A significant positive relationship between worm biomass and fish length was revealed ($F_{[1,77]}$ = 12.52, P < 0.001), while the relationship between worm numbers and length was negative but not significant ($F_{[1,77]}$ = 1.91, P = 0.171). Abnormalities in selected health index tissues examined during necropsy were not outwardly apparent. Histological analysis of gut tissue from two infected fish revealed heavy eosinophilic and lymphocytic responses when compared with controls (histology not shown). No fish died as a result of the cold-shock treatment. No significant differences in thermal stress responses between control and exposed fish were revealed ($F_{[1,4]}$ = 3.13, P = 0.152), although there was a significant time by treatment interaction ($F_{[4,16]}$ = 4.62, P = 0.011), which was likely due to the influence of a strong time effect ($F_{[4,16]}$ = 20.84, P < 0.001) (Table 2). In general, there appeared to be a decline in the time spent performing abnormal behaviors with increasing cold shock exposure time. However, the maximum time spent performing abnormal behaviors occurred at the 0 h observation time for control fish and at the 0.5 h observation time for parasite-exposed fish. There was no significant difference in stimulus response between exposed and control fish due to cold-shock treatment ($F_{[1,4]}$ = 0.18, P = 0.694), and there was no treatment by time interaction ($F_{[3,11]}$ = 2.01, P = 0.172).

Experiment II

Seventy-seven of the 96 (80%) exposed fish were infected. Of the fish initially allocated to each of the four treatment groups, 29 (62%) exposed low-ration fish, 4 (8%)

© 2006 NRC Canada

Fig. 2. Experiment I. Least squares mean (±1 SE) final fork length and hematocrit values for control, infected, and exposed but uninfected bonytail chub (*G. elegans*). Comparison of lengths of infected fish with those of control and uninfected fish produced $P = 0.007$ and $P = 0.024$, respectively. Comparison of mean hematocrit values for infected fish with those for control and uninfected fish produced $P = 0.024$ and $P = 0.009$, respectively.



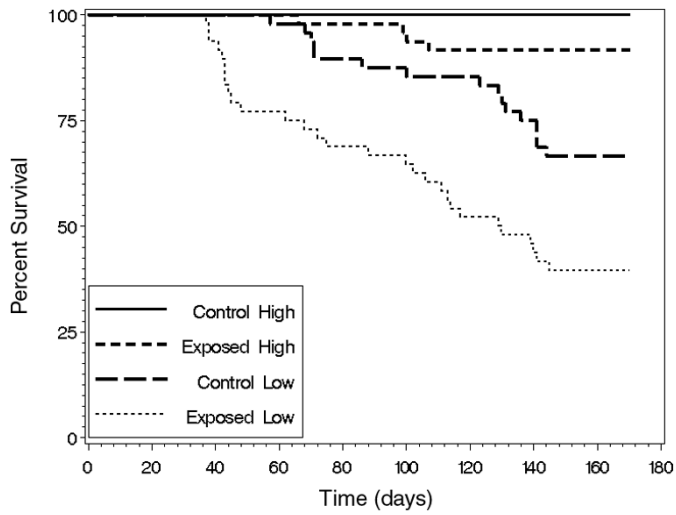
exposed high-ration fish, and 16 (35%) control low-ration fish died or were determined as moribund and removed during the course of the experiment (Fig. 3). None of the control high-ration fish died. All but one of the exposed fish that died were infected. The log-rank test revealed significant differences among survival curves ($P < 0.001$, df = 3). Significant or nearly significant differences were revealed for all pairwise hazard ratios between treatment groups (Table 3). Analysis of the data treating the three accidental fish losses as deaths caused little quantitative change in the results and had no effect on the qualitative patterns or interpretation. Logistic regression results for survival at day 170 were similar to results of the hazard-based analyses (log-rank and PH models); therefore, no evidence of within-tank correlation (overdispersion) was revealed. Overall mean worm intensity for this experiment was 24.30 (SD 26.56; range 1–143), with high-ration fish having a mean of 28.76 worms (SD 30.16; range 1–143) and low-ration fish having

Table 2. Experiment I: population marginal means (1 SE) of total time (in seconds) spent performing abnormal behaviors for control ($n = 2$) and parasite-exposed ($n = 4$) bonytail chub (*G. elegans*).

Observation time (h)	Control	Exposed
0	62.57 (4.81)	45.88 (3.36)
0.5	43.18 (4.81)	50.19 (3.36)
1	49.48 (4.81)	35.14 (3.36)
2	33.70 (4.81)	25.81 (3.36)
3	37.53 (4.81)	30.27 (3.36)

Note: Observation time is time after cold-shock treatment began.

Fig. 3. Experiment II. Kaplan–Meier survival curves for four treatment groups of bonytail chub (*G. elegans*), showing a distinct survival curve for each group.



a mean of 20.18 worms (SD 22.34; range 1–131). Mean worm biomass for fish on the high food ration was 1.0 mg (SD 0.5). Worm biomass measurements from surviving low-ration infected fish are not reported because small worm size (<0.1 mg) precluded accurate mass measurements.

Exposed fish on a high-ration diet grew at a significantly slower rate than control fish, while exposed fish on a low-ration diet ultimately grew at an overall faster rate than controls in both length ($F_{[3,284]} = 317.18$, $P < 0.001$) and mass ($F_{[3,284]} = 287.95$, $P < 0.001$) (Fig. 4). Significant differences were revealed among the four groups for both final mean length ($F_{[3,20]} = 177.32$, $P < 0.001$) and mass ($F_{[3,20]} = 128.90$, $P < 0.001$). Pairwise comparisons indicated that within the high-ration group, exposed fish were significantly smaller than controls in final mean length (33.22 mm versus 35.40 mm, $P < 0.001$) and mass (0.30 g versus 0.35 g, $P = 0.002$). For high-ration fish, a significant negative relationship between worm numbers and length was revealed ($F_{[1,35]} = 20.26$, $P < 0.001$), while the relationship between worm biomass and length was positive, although nonsignificant ($F_{[1,35]} = 2.45$, $P = 0.126$). Significant differences in mean fat index values were found among the four groups ($F_{[3,20]} = 30.59$, $P < 0.001$). Pairwise comparisons between fish within the high-ration group revealed that fat indices were significantly lower in the exposed fish (1.69) than in controls (2.50) ($P < 0.001$).

Figure 4 shows a curious acceleration in mass and length

© 2006 NRC Canada

Table 3. Experiment II: pairwise hazard ratios and *P* values (in parentheses) for differences between bonytail chub (*G. elegans*) survival curves for each ration and exposure treatment combination; rows are the reference for each comparison.

	Control high ration	Control low ration	Exposed high ration
Control low ration	0.0 (<0.001)		
Exposed high ration	0.0 (0.018)	4.17 (0.004)	
Exposed low ration	0.0 (<0.001)	0.564 (0.060)	0.135 (<0.001)

near the end of the study. Additional analyses demonstrated conclusively that fish that died before the end of the study were both shorter ($P < 0.001$) and lighter ($P < 0.001$) than the survivors, providing a likely explanation for the apparent acceleration.

Discussion

Our studies demonstrate that infection with *B. acheilognathi*, in addition to reducing growth in bonytail chub, has the potential to accelerate mortality rates under conditions of reduced food availability. Mortality of low-ration exposed fish from experiment II began 20 days earlier and occurred at nearly twice the rate of low-ration controls, indicating a strong physiological disruption by the parasite. While we were not able to determine the cause of increased mortality, diversion of vital nutrients to the parasite is the most likely explanation. Cestodes acquire their nutrition through absorption across the tegument, which allows them to compete effectively for host resources (Pappas 1983) and can ultimately inhibit host development and survival. In addition to reduced growth from nutrient loss to competitive absorption by tapeworms, adverse effects due to adult tapeworm infection may include mechanical obstruction, hematological changes, lesions and inflammation, reduced feeding, and behavioral changes (Bauer et al. 1973; Boyce 1979; Hoffman 1980; Arme et al. 1983; Crompton 1984; Williams and Jones 1994). Adult cestodes can alter the host's gut enzymatic activity and intestinal pH and also produce their own hydrolases, providing a competitive advantage over host mucosa in absorption of nutritive compounds such as glucose (Matskási 1984; Halton 1997). Fish from our experiments, however, appeared to feed well until shortly before death, and we observed no evidence of intestinal blockage in our experiments.

Stressors such as restricted food rations or high doses of a particular chemical (e.g., zinc, cadmium) often induce mortality of infected fish (Walkey and Meakins 1970; Boyce and Yamada 1977; Pascoe and Cram 1977; Pascoe and Mattey 1977). Our results supported and highlighted the effect of such synergism between parasitism and a decreased nutrient and energy base.

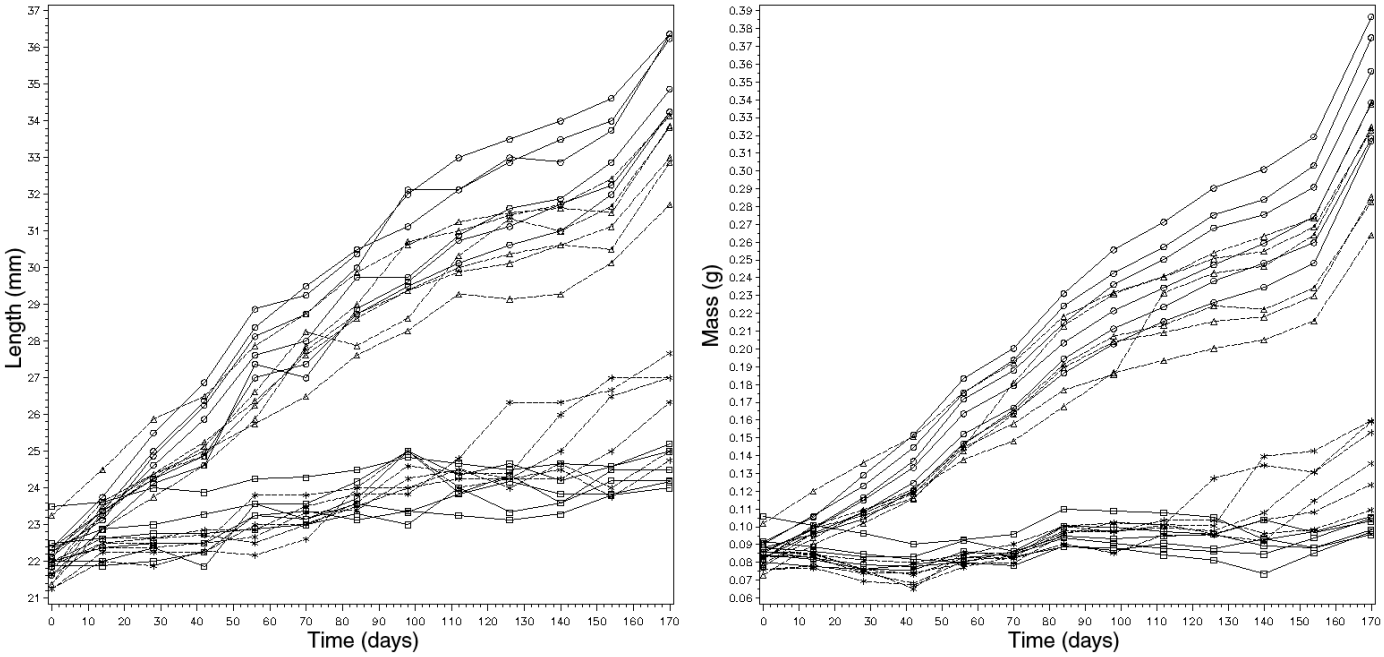
Where mortality occurs, such as under conditions of extreme virulence or where other stressors act synergistically, the effects of parasitism are relatively easy to document; in other cases, the effects of parasitism may be subtle and insidious (Minchella and Scott 1991; Ballabeni and Ward 1993). Differences in the growth curves among exposed fish in experiment I and among high-ration fish from experiment II appeared to be small even though these data included exposed but non-infected fish. The differences in length were most apparent at the termination of the experiments, when uninfected fish could be distinguished from infected fish in the exposed group. This was especially true in experiment I, where the mean final length of infected fish was approximately 6 mm (8%) less than that of both control and uninfected fish. The difference between the infected fish and the other two groups in experiment I was clear evidence of growth depression. Exposed high-ration fish in experiment II were, on average, over 2 mm smaller than high-ration controls. Though this difference is small, the mean final lengths of control fish and infected fish were only 35.40 and 33.22 mm, respectively, so the 2 mm difference amounts to a reduction in length of more than 6%. These results again suggest tapeworm competition for nutrients within the host gut or a reduction in food intake. That the exposed fish in the low-ration group ultimately grew larger than controls by the end of experiment II seems likely to be an artefact of high losses of fish within the groups and the influence of the few uninfected fish in the exposed group. This explanation is supported by analysis indicating that smaller fish within the groups were indeed censored and by the greater mortality rate in the infected group.

Residual effects of small changes in fish growth should not be underestimated. Even modest variation in time spent in the larval stage because of a decline in growth rate due to various factors, including disease, can influence cumulative mortality and lead to sizable population changes at later stages (Houde 1987; Rice et al. 1993). Timing is of critical importance in the ontogeny of fishes, since transformation of fish and abundance of important prey items must overlap spatially and temporally. For example, a critical transition may occur when fish switch from zooplankton to macroinvertebrates such as chironomid or simuliid larvae, two food items important to humpback chub in the Colorado and Little Colorado rivers (Kaeding and Zimmerman 1983; Arizona Game and Fish Department 1996). Moreover, retarded growth may prolong feeding on copepods as a primary food item, thereby resulting in an increased window of time in which fish are exposed to parasites. The extended predation risk from larger fish that is associated with growth inhibition must also be considered.

Chub larvae measured approximately 22 mm (fork length) at initiation of each experiment; humpback chub of this size in the LCR would probably be 4 to 6 weeks of age (Hamman 1982; Kaeding and Zimmerman 1983). As copepods are an important food source throughout the larval period, infections by *B. acheilognathi* are likely to occur much earlier than the age at which we exposed the fish in our experiments. Infections in these more sensitive early developmental stages may result in even greater mortality or growth depression than recorded in our experiments. Furthermore, fish in the wild are constantly exposed to infected copepods during the warmer seasons. Although we attempted to promote recurring

© 2006 NRC Canada

Fig. 4. Experiment II. Mean growth, by tank, of bonytail chub (*G. elegans*) in fork length and mass over 170 days for control high-ration fish (○; n = 6), exposed high-ration fish (△; n = 6), control low-ration fish (□; n = 6), and exposed low-ration fish (*; n = 6). Within the high-ration group, growth of control fish was significantly greater than that of exposed fish in length ($P < 0.001$) and mass ($P < 0.001$), whereas in the low-ration group, growth of exposed fish was significantly greater than that of control fish in length ($P = 0.007$) and mass ($P < 0.001$). A significant bias toward death was revealed for lighter ($P < 0.001$) and shorter fish ($P < 0.001$), explaining a late increase in length and mass of exposed low-ration fish.



infections by providing hosts necessary for completion of the parasite life cycle in the fish husbandry systems during experiment II, it is doubtful that we were successful given that few tapeworms obtained at necropsy were gravid.

Other physiological and anatomical parameters (hematocrit, visceral fat amounts, LSI, gut histopathology) provided less conclusive results. Although the hematocrit values in infected fish (experiment I) were 7% and 9% lower than those in control and uninfected fish, respectively (Fig. 2), these values need to be interpreted cautiously (Houston 1997). Berry (1984) reported a mean hematocrit value of 34.50 (SD 5.80) for similarly sized hatchery bonytail chub, which is comparable to the mean level of 34.28 (SD 4.40) observed in our infected fish. In some cases, hematological values have been correlated with sex and gonadal development (reviewed by Blaxhall 1972). This may have been the case in experiment I, where a marginally significant sex effect was demonstrated and, as noted in the pilot study, where infected males had lower hematocrit values than control males (unpublished data). In general, visceral fat coverage was quite high in experiment I, reflecting the higher feeding ration, and tapeworm infections appeared to have no influence on fat storage. Restricted food in experiment II greatly reduced fat storage, and our results indicate the effect was exacerbated by the tapeworm. However, glycogen storage does not appear to have been affected by the parasite, as we did not detect any significant differences in LSI measurements among treatment groups. Interestingly, chilled fish had significantly higher LSI measurements than non-chilled fish, suggesting increased liver glycogen storage, as noted in other studies (Dean and Goodnight 1964; Heidinger and Crawford 1977). Upon necropsy, no gross lesions were observed in the gut epithelium. The heavy leucocyte (eosinophil and lymphocyte) infiltration throughout the submucosa and lamina propria and into the intestinal epithelium, as seen in histological sections, is in keeping with other studies (Liao and Shih 1956; Bauer et al. 1973; Hoole and Nisan 1994). However, we were unable to confirm any severe mechanical damage to the gut from two infected fish that were examined histologically.

Cold-shock treatment did not result in any significant increase in the performance of abnormal behaviors or reduction in the stimulus response in exposed fish compared with controls. As expected, all shocked fish were lethargic, and the general effect of thermal shock likely overwhelmed any additive effects of tapeworm infection. Abrupt reaction to cold shock is more notable at much earlier life stages (Houston 1972; Berry 1988); therefore, fish in the experiment (8 months old) may have been more capable of acclimation than fish washed into the Colorado River, which are typically 3 months younger. Furthermore, our inability to visually distinguish between uninfected and infected fish within the exposed group may have tempered any possible signals from infected fish. Similar studies with younger fish with ubiquitous infections may be valuable.

Our study corroborates the suggestion that the biomass of tapeworms, including *B. acheilognathi*, reflects food quantity and quality and, by extension, food intake by the host (Davydov 1978; Kennedy 1983; Smyth and McManus 1989). The mean biomass of worms recovered from fish in experiment I was over 7 times that of worms recovered from the high-ration group in experiment II, despite the comparable time span for each trial. Although the ration for each of these two groups was 1.5% of body mass throughout

© 2006 NRC Canada

most of each trial, fish from experiment I had an overall greater food intake because they were fed approximately 2%–4% body mass for the first 6 weeks. Further evidence is provided from experiment II, where low worm biomass from low-ration fish precluded making accurate measurements. These worm biomass data also address life-history strategies adopted by *B. acheilognathi* for survival, growth, and recruitment. Such strategies balance the needs of the parasite (nutrition for growth and maturity) with the potentially conflicting need to maintain an optimal habitat (their host). Results from our study demonstrate that under certain conditions, the balance between conflict and coexistence (Toft and Aeschlimann 1991) can be tipped in either direction. In experiment I, where food supply was ample, the cost of infection to the host was reflected in reduced somatic growth and interruption of physiological processes (e.g., red blood cell production). In experiment II, where food was restricted, competition between host and parasite for limited resources resulted in growth reduction, loss of fat stores, and ultimately death in a portion of the fish. This hypothesis of host–parasite competition was supported by the resulting significant negative relationship between worm intensity and fish length in experiment II. In experiment I, where food was not limiting, the absence of such a relationship suggests that competition for food resources between the host and the parasite did not occur to such an extent. Furthermore, the significant positive relationship between fish length and tapeworm biomass in experiment I suggests that larger fish competed effectively with other fish for food within the tank, thereby providing ample resources to the parasites they harbored.

Beyond the consistent stressors to native fishes of the Colorado River Basin, such as competition for resources and predation from other fishes, stochastic events may cause stressful situations such as reduction in the food supply or low dissolved oxygen levels that can exacerbate the tapeworm's influence on chub development and survival. Additionally, the cost of tapeworm infections may divert energy needed for gonadal development and thus reduce fecundity, which may warrant investigation. Our results demonstrate the negative impacts of *B. acheilognathi* infections on young fish and that a low food ration exacerbates this negative pressure. Native fish management strategies that could ensure a robust food supply may help offset some of the negative impacts of tapeworm infections. Optimal temperatures for recruitment and development of *B. acheilognathi* are between 22 and 25 °C (Bauer et al. 1973; Hanzelová and Žitňan 1986; Oškinis 1994); drought conditions of the recent past and proposed multilevel intake structures at GCD could raise temperatures in the Colorado River and facilitate the spread of *B. acheilognathi* throughout the Grand Canyon system. The potential for *B. acheilognathi* to colonize other areas as a result of its wide host specificity (Dove et al. 1997; Hoffman 1999) and occurrence in extensively transplanted fish such as carp, mosquitofish, and baitfish species, combined with the negative impacts we report in the present study, serves as a warning of the potential threat this parasite poses to other fish populations.

Acknowledgements

We are grateful to Jennifer Phillips, Skip Sterner, and Connie Roderick for their excellent animal care and technical assistance throughout these experiments. We thank Andrew Kotecki for his fish husbandry assistance. We thank Chad Linder for reviewing the fish behavior video recordings. We also wish to express our appreciation to Roger Hamman for providing the bonytail chub and for his expertise in fish husbandry. We thank Dr. Janet Reid and Dr. Stanley Dodson for identifying the copepods from field sites. We are also grateful to Dr. Victoria Matey and the late Dr. Boris Kuperman for their intellectual support and for providing tapeworm eggs during the early stages of this study. Funding for this research was provided by the US Geological Survey.

References

Adams, S.M., and McLean, R.B. 1985. Estimation of largemouth bass, *Micropterus salmoides* Lacépède, growth using liver somatic index and physiological variables. J. Fish Biol. **26**: 111–126. doi:10.1111/j.1095-8649.1985.tb04248.x.

Andrews, C., Chubb, J.C., Coles, T., and Dearsley, A. 1981. The occurrence of *Bothriocephalus acheilognathi* Yamaguti, 1934 (*B. gowkongensis*) (Cestoda: Pseudophyllidea) in the British Isles. J. Fish Dis. **4**: 89–93. doi:10.1111/j.1365-2761.1981.tb01113.x.

Arizona Game and Fish Department. 1996. Ecology of Grand Canyon backwaters. Final report submitted to Glen Canyon Environmental Studies, US Bureau of Reclamation, Flagstaff, Arizona. Arizona Fish and Game Department, Phoenix.

Arme, C., Bridges, J.F., and Hoole, D. 1983. Pathology of cestode infections in the vertebrate host. *In* Biology of the Eucestoda. Vol. II. *Edited* by C. Arme and P.W. Pappas. Academic Press, New York. pp. 499–538.

Ballabeni, P., and Ward, P.I. 1993. Local adaptation of the trematode *Diplostomum phoxini* to the European minnow *Phoxinus phoxinus*, its second intermediate host. Funct. Ecol. **7**: 84–90. doi:10.2307/2389870.

Bauer, O.N., Musselius, V.A., and Strelkov, Y.A. 1973. Diseases of pond fishes. Israel Program for Scientific Translations, Jerusalem. pp. 115–122.

Berry, C.R., Jr. 1984. Hematology of four rare Colorado River fishes. Copeia, 1984: 790–793. doi:10.2307/1445172.

Berry, C.R., Jr. 1988. Effects of cold shock on Colorado squawfish larvae. Southw. Nat. **33**: 193–197.

Blaxhall, P.C. 1972. The haematological assessment of the health of freshwater fish. J. Fish Biol. **4**: 593–604. doi:10.1111/j.1095-8649.1972.tb05704.x.

Boomker, J., Huchzermeyer, F.W., and Naude, T.W. 1980. Bothriocephalosis in the common carp in the eastern Transvaal. J. S. Afr. Vet. Assoc. **51**: 263–265. PMID:7241498.

Boyce, N.P. 1979. Effects of *Eubothrium salvelini* (Cestoda: Pseudophyllidea) on the growth and vitality of sockeye salmon *Oncorhynchus nerka*. Can. J. Zool. **57**: 597–602.

Boyce, N.P., and Yamada, S.B. 1977. Effects of a parasite, *Eubothrium salvelini* (Cestoda: Pseudophyllidea), on the resistance of juvenile sockeye salmon, *Oncorhynchus nerka*, to zinc. J. Fish. Res. Board Can. **34**: 706–709.

Brouder, M.J. 1999. Relationship between length of roundtail chub and infection intensity of Asian fish tapeworm *Bothriocephalus acheilognathi*. J. Aquat. Anim. Health, **11**: 302–304. doi:10.1577/1548-8667(1999)011<0302:RBLORC>2.0.CO;2.

Brouder, M.J., and 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. J. Helminthol. Soc. Wash. **64**: 219–226.

© 2006 NRC Canada

Bulow, F.J., Coburn, C.B., Jr., and Cobb, C.S. 1978. Comparisons of two bluegill populations by means of the RNA-DNA ratio and liver-somatic index. Trans. Am. Fish. Soc. **107**: 799–803. doi:10.1577/1548-8659(1978)107<799:COTBPB>2.0.CO;2.

Bush, A.O., Lafferty, K.D., Lotz, J.M., and Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. **83**: 575–583.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Vol. 1. 2nd ed. Canadian Council on Animal Care, Ottawa, Ont.

Choudhury, A., Hoffnagle, T.L., and Cole, R.A. 2004. Parasites of native and non-native fishes of the Little Colorado River, Grand Canyon, Arizona. J. Parasitol. **90**: 1042–1053. doi:10.1645/GE-3244. PMID:15562604.

Choudhury, A., Charipar, E., Nelson, P., Hodgson, J., Bonar, S., and Cole, R.A. 2006. Update on the distribution of the invasive Asian fish tapeworm, *Bothriocephalus acheilognathi*, in the U.S. and Canada. Comp. Parasitol. **73**: 269–273.

Clarkson, R.W., and Childs, M.R. 2000. Temperature effects of hypolimnial-release dams on early life stages of Colorado River basin big-river fishes. Copeia, 2000: 402–412. doi:10.1643/0045-8511(2000)000[0402:TEOHRD]2.0.CO;2.

Cole, G.A., and Kubly, D.M. 1976. Limnological studies on the Colorado River from Lee's Ferry to Diamond Creek. Colorado River Research Series Contribution No. 37. Grand Canyon National Park, Ariz.

Cox, D.R. 1972. Regression models and life tables (with discussion). J. R. Stat. Soc. Ser. B Stat. Methodol. **34**: 187–220.

Crompton, D.W.T. 1984. Influence of parasitic infection on food intake. Fed. Proc. **43**: 239–245. PMID:6363136.

Davydov, O.N. 1978. Growth, development and fecundity of *Bothriocephalus gowkongensis* (Jen, 1955), a cyprinid parasite. Hydrobiol. J. **14**: 60–66.

Dean, J.M., and Goodnight, C.J. 1964. A comparative study of carbohydrate metabolism in fish as affected by temperature and exercise. Physiol. Zool. **37**: 280–299.

Dove, A.D.M., Cribb, T.H., Mockler, S.P., and Lintermans, M. 1997. The Asian fish tapeworm, *Bothriocephalus acheilognathi*, in Australian freshwater fishes. Mar. Freshw. Res. **48**: 181–183. doi:10.1071/MF96069.

Font, W.F., and Tate, D.C. 1994. Helminth parasites of native Hawaiian freshwater fishes: an example of extreme ecological isolation. J. Parasitol. **80**: 682–688. doi:10.2307/3283246. PMID:7931902.

Goede, R.W., and Barton, B.A. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. Am. Fish. Soc. Symp. **8**: 93–108.

Granath, W.O., Jr., and Esch, G.W. 1983. Survivorship and parasite-induced host mortality among mosquitofish in a predator-free, North Carolina cooling reservoir. Am. Midl. Nat. **110**: 314–323. doi:10.2307/2425272.

Griffith, J.S. 1978. Effects of low temperature on survival and behavior of threadfin shad, *Dorosoma petenense*. Trans. Am. Fish. Soc. **107**: 63–70. doi:10.1577/1548-8659(1978)107<63:EOLTOT>2.0.CO;2.

Guerrero, R.D., III, and Shelton, W.L. 1974. An aceto-carmine squash method for sexing juvenile fishes. Prog. Fish-Cult. **36**: 56.

Halton, D.W. 1997. Nutritional adaptations to parasitism within the platyhelminthes. Int. J. Parasitol. **27**: 693–704. PMID:9229252.

Hamman, R.L. 1981. Hybridization of three species of chub in a hatchery. Prog. Fish-Cult. **43**: 140–141.

Hamman, R.L. 1982. Spawning and culture of humpback chub. Prog. Fish-Cult. **44**: 213–216.

Hanzelová, V., and Žitňan, R. 1986. Embryogenesis and development of *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda) in the intermediate host under experimental conditions. Helminthologia, **23**: 145–155.

Heckman, R.A., Deacon, J.E., and Greger, P.D. 1986. Parasites of the woundfin minnow, *Plagopterus argentissimus*, and other endemic fishes from the Virgin River, Utah. Great Basin Nat. **46**: 662–676.

Heidinger, R.C., and Crawford, S.D. 1977. Effect of temperature and feeding rate on the liver-somatic index of largemouth bass, *Micropterus salmoides*. J. Fish. Res. Board Can. **34**: 633–638.

Hoffman, G.L. 1976. The Asian tapeworm, *Bothriocephalus gowkongensis*, in the United States, and research needs in fish parasitology. *In* Proceedings of the 1976 Fish Farming Conference and Annual Convention of the Catfish Farmers of Texas, College Station, Tex., 20–21 July 1976. Texas A&M University, College Station, Tex. pp. 84–90.

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

Hoffman, G. 1999. Parasites of North American freshwater fishes. Cornell University Press, Ithaca, N.Y.

Hoffnagle, T.L., Choudhury, A., and Cole, R.A. 2006. Parasitism and body condition in humpback chub from the Colorado and Little Colorado rivers, Grand Canyon, Arizona. J. Aquat. Anim. Health, **18**: 184–193. doi:10.1577/H05-046.1.

Holden, P.B., and Stalnaker, C.B. 1970. Systematic studies of the cyprinid genus *Gila*, in the Upper Colorado River Basin. Copeia, 1970: 409–429. doi:10.2307/1442267.

Hoole, D., and Nisan, H. 1994. Ultrastructural studies on the intestinal response of carp, *Cyprinus carpio* L., to the pseudophyllidean tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934. J. Fish Dis. **17**: 623–629. doi:10.1111/j.1365-2761.1994.tb00260.x.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. **2**: 17–29.

Houston, A.H. 1972. Environmental temperature and the body fluid system of the teleost. *In* Responses of fish to environmental changes. *Edited by* W. Chavin. Charles C. Thomas Publishers, Springfield, Ill. pp. 87–162.

Houston, A.H. 1997. Review: Are the classical hematological variables acceptable indicators of fish health? Trans. Am. Fish. Soc. **126**: 879–894. doi:10.1577/1548-8659(1997)126<0879:RATCHV>2.3.CO;2.

Kaeding, L.R., and Zimmerman, M.A. 1983. Life history and ecology of the humpback chub in the Little Colorado and Colorado Rivers of the Grand Canyon. Trans. Am. Fish. Soc. **112**: 577–594. doi:10.1577/1548-8659(1983)112<577:LHAEOT>2.0.CO;2.

Kaplan, E.L., and Meier, P. 1958. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. **53**: 457–481. doi:10.2307/2281868.

Kennedy, C.R. 1983. General ecology. *In* Biology of the Eucestoda. Vol. I. *Edited* by C. Arme and P.W. Pappas. Academic Press, New York. pp. 27–80.

Körting, W. 1975. Larval development of *Bothriocephalus* sp. (Cestoda: Pseudophyllidea) from carp (*Cyprinus carpio* L.) in Germany. J. Fish Biol. **7**: 725–733.

Léger, P., Bengston, D.A., Simpson, K.L., and Sorgeloos, P. 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Annu. Rev. **24**: 521–623.

Liao, H., and Shih, L. 1956. Contribution to the biology and control of *Bothriocephalus gowkongensis* Yeh, a tapeworm parasitic in the young grass carp (*Ctenopharyngodon idellus* C. and V.). Acta Hydrobiol. Sin. **2**: 129–185.

Matskási, I. 1984. The effect of *Bothriocephalus acheilognathi* Yamaguti, 1934 infection on the protease activity in the gut of carp fry. Parasitol. Hung. **11**: 51–56.

© 2006 NRC Canada

Minchella, D.J., and Scott, M.E. 1991. Parasitism: a cryptic determinant of animal community structure. Trends Ecol. Evol. **6**: 250–253. doi:10.1016/0169-5347(91)90071-5.

Minckley, W.M. 1991. Native fishes of the Grand Canyon Region: an obituary? *In* Colorado River Ecology and Dam Management: Proceedings of a Symposium, Santa Fe, N. Mex., 24–25 May 1990. National Academy Press, Washington, D.C. pp. 124–177.

Oškinis, V. 1994. Temperature and development of *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda) in laboratory conditions. Ekologia, **4**: 40–42.

Pappas, P.W. 1983. Host–parasite interface. *In* Biology of the Eucestoda. Vol. II. *Edited by* C. Arme and P.W. Pappas. Academic Press, New York. pp. 297–334.

Pascoe, D., and Cram, P. 1977. The effect of parasitism on the toxicity of cadmium to the three-spined stickleback, *Gasterosteus aculeatus* L. J. Fish Biol. **10**: 467–472. doi:10.1111/j.1095-8649.1977.tb04079.x.

Pascoe, D., and Mattey, D. 1977. Dietary stress in parasitized and non-parasitized *Gasterosteus aculeatus* L. Z. Parasitenkd. **51**: 179–186. doi:10.1007/BF00500957.

Rice, J.A., Crowder, L.B., and Rose, K.A. 1993. Interactions between size-structured predator and prey populations: experimental test and model comparison. Trans. Am. Fish. Soc. **122**: 481–491. doi:10.1577/1548-8659(1993)122<0481:IBSSPA>2.3.CO;2.

Robinson, A.T., Clarkson, R.W., and Forrest, R.E. 1998. Dispersal of larval fishes in a regulated river tributary. Trans. Am. Fish. Soc. **127**: 772–786. doi:10.1577/1548-8659(1998)127<0772:DOLFIA>2.0.CO;2.

SAS Institute Inc. 1999–2000. SAS®. Version 8.01 [computer program]. SAS Institute Inc., Cary, N.C.

Schäperclaus, W. 1991. Fish diseases. *Edited by* W. Schäperclaus, H. Kulow, and K. Schreckenbach. Amerind Publishing Co. Pvt. Ltd., New Delhi.

Scott, A.L., and Grizzle, J.M. 1979. Pathology of cyprinid fishes caused by *Bothriocephalus gowkongensis* Yea, 1955 (Cestoda: Pseudophyllidea). J. Fish Dis. **2**: 69–73. doi:10.1111/j.1365-2761.1979.tb00141.x.

Smyth, J.D., and McManus, D.P. 1989. The physiology and biochemistry of cestodes. Cambridge University Press, Cambridge.

Sopinska, A. 1985. Effects of physiological factors, stress, and disease on hematologic parameters of carp, with a particular reference to the leukocyte patterns. III. Changes in blood accompanying branchionecrosis and bothriocephalosis. Acta Ichthyol. Piscatoria, **15**: 141–168.

Toft, C.A., and Aeschlimann, A. 1991. Introduction: coexistence or conflict? *In* Parasite–host associations: coexistence or conflict? *Edited by* C.A. Toft, A. Aeschlimann, and L. Bolis. Oxford University Press, New York. pp. 1–12.

US Bureau of Reclamation. 1999. Glen Canyon Dam modifications to control downstream temperatures — plan and draft environmental assessment. US Bureau of Reclamation, Upper Colorado region, Salt Lake City, Utah.

Valdez, R.A., and Clemmer, G.C. 1982. Life history and prospects for recovery of the humpback and bonytail chub. *In* Fishes of the upper Colorado River system: present and future. *Edited by* W.H. Miller, H.M. Tyus, and C.A. Carlson. American Fisheries Society, Western Division, Bethesda, Md. pp. 109–119.

Valdez, R.A., and Ryel, R.J. 1995. Life history and ecology of the humpback chub (*Gila cypha*) in the Colorado River, Grand Canyon, Arizona: Final Report. Bureau of Reclamation, Salt Lake City, Utah.

Walkey, M., and Meakins, R.H. 1970. An attempt to balance the energy budget of a host–parasite system. J. Fish Biol. **2**: 361–372. doi:10.1111/j.1095-8649.1970.tb03294.x.

Williams, H., and Jones, A. 1994. Parasitic worms of fish. Taylor and Francis Ltd., Bristol, Pa.

© 2006 NRC Canada

Copyright of Canadian Journal of Zoology is the property of NRC Research Press and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.