OCCURRENCE AND IMPACT OF THE ASIAN FISH TAPEWORM

BOTHRIOCEPHALUS ACHEILOGNATHI IN THE RIO GRANDE

(RÍO BRAVO DEL NORTE)

THESIS

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by

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San Marcos, Texas
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ACKNOWLEDGEMENTS

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SUPERVISING PROFESSOR: TIMOTHY H. BONNER

Bothriocephalus acheilognathi is native to Asia and was introduced worldwide as an incidental hitchhiker of its native host, *Ctenopharyngodon idella* (Andrews et al. 1981; Hoffman 1999). It is established in ten states in USA with the most recent discovery in the Rio Grande in Texas (Bean et al. 2007). Occurrence of this exotic tapeworm in the Rio Grande potentially is detrimental to native fishes, causing intestinal blockage and perforation, hemorrhaging, reduced growth, significantly decreased survivorship, and

mortality (Scott and Grizzle 1979; Hoffman 1980; Granath and Esch 1983; Hoole and Nisan 1994; Hansen et al. 2006). Endemic fishes of conservation concern in the Rio Grande include *Hybognathus amarus, Dionda diaboli, Notropis jemezanus*, and *Notropis Chihuahua* and likely are affected by the tapeworm.

The purpose of the first part of this study was to examine the presence and mean intensity of *B. acheilognathi* in *Cyprinella lutrensis* to determine spatial and temporal patterns in several reaches of the Rio Grande. The study also examined seasonal trends in tapeworm maturation and intraspecific competition. The second study assessed impacts of *B. acheilognathi* infection on condition and reproduction in *C. lutrensis* to make inferences about potential impacts on other endemic cyprinid species.

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CHAPTER I

SPATIAL AND TEMPORAL PATTERNS OF
BOTHRIOCEPHALUS ACHEILOGNATHI IN THE RIO GRANDE BASIN

ABSTRACT

Bothriocephalus acheilognathi (Cestoda: Pseudophyllidea), the Asian fish

tapeworm, was reported recently in fishes from the Rio Grande in the vicinity of Big

Bend National Park (NP). Objectives of this study were to determine distribution,

prevalence, mean intensity, and definitive host species of *B. acheilognathi* throughout the

Rio Grande drainage. From surveys in 2006 and 2007, occurrence of *B. acheilognathi* in

fishes ranged from Presidio, Texas to San Ygnacio, Texas in the Rio Grande mainstem,

from Fort Sumner, New Mexico to Artesia at Highway 82, New Mexico in the Pecos

River, near Highway 90 on Pinto Creek, and near Dolan Falls in the Devils River. New

host species include *Cyprinella proserpina*, *Dionda argentosa*, and *Cycleptus elongatus*.

In *C. lutensis*, highest prevalence (27%) was observed in Big Bend NP in the winter.

Within Big Bend NP, prevalences were higher at mainstem sites across months ($P <$

0.01) than the tributary site (Terlingua Creek). Mean intensities across Big Bend NP sites

ranged from 1.4 to 1.7 tapeworms per fish. Mean biomass of the tapeworm was $1.3\% \pm$

0.2% for all infected fish and did not differ ($P = 0.09$) among different intensities.

Information from this study provides information on potential impacts of *B. acheilognathi* infections on imperiled Rio Grande fish species.

INTRODUCTION

Introduction of exotic and invasive host taxa into new drainages facilitates the spread of potentially detrimental exotic parasites and pathogens (Hoffman and Schubert 1984) and contributes to the global spread of exotic parasites (Hoffman and Schubert 1984; Font 2003; Taraschewski 2006). Successful invaders often exhibit similar characteristics, such as wide ecological tolerance to physiological conditions, adaptation to harsh environments, and short generation times (Brown 1989, Elrich 1989). One such invader is *Bothriocephalus acheilognathi*, the Asian fish tapeworm, which has been introduced worldwide with one of the most recent findings in the Rio Grande (Río Bravo del Norte) of North America (Bean et al. 2007). This tapeworm is a successful invader and colonizer in part because of low definitive and intermediate host specificity (Körting 1975; Dove and Fletcher 2000).

Bothriocephalus acheilognathi (Cestoda: Pseudophyllidea) has several synonyms including *B. opsariichthydis*, *B. gowkongensis*, *B. kivuensis*, *B. phoxini*, *B. aegyptiacus*, and *Schyzocotyle fluviatilis* (Scholz 1997). The most distinguishing morphological characteristic is the heart-shaped scolex with a pair of dorsally and ventrally positioned bothria (Scholz 1997). *Bothriocephalus acheilognathi* lacks a neck with proglottids longitudinally compressed, campanulate in shape, and narrower than the scolex (Scholz 1997; Hoffman 1999). Natural geographic range of *B. acheilognathi* is Japan (original description – Yamaguti 1934), China, and the Amur River basin in Russia (Bauer and

Hoffman 1976; Pool and Chubb 1985; Pool 1987; Scholz 1997). Grass carp, *Ctenopharyngodon idella*, is one of the tapeworm's native hosts (Choudhury et al. 2006) and worldwide shipments of grass carp for macrophyte control subsequently led to the global introduction of *B. acheilognathi* (Hoffman 1980; Andrews et al. 1981). *Bothriocephalus acheilognathi* is established on six continents and currently infects over 100 species of fish (Salgado-Maldonado and Pineda-López 2003). In USA, *B. acheilognathi* occurs in the Colorado River drainage in Arizona, the Virgin River in Nevada, Arizona, and Utah, Belews Lake in North Carolina, the Yampa River in Colorado, Peter Lake in Wisconsin, and the South Platte River in Nebraska (Granath and Esch 1983b; Heckmann and Deacon 1987; Brouder and Hoffnagle 1997; Ward 2005; Choudhury et al. 2006). It is reported also in Kentucky, Arkansas, New Mexico, and Texas (Choudhury et al. 2006; Bean et al. 2007). Transfer into new drainages within the USA likely is attributable to bait fish introductions instead of grass carp introductions (Heckmann et al. 1993; Choudhury et al. 2004).

Ease of colonization of *B. acheilognathi* is, in part, a result of a short and direct life cycle. The tapeworm requires as little as two weeks to complete its life cycle in the intermediate host (Körting 1975). Eggs are passed with the feces of the fish and mobile coracidia emerge from the eggs after embryonation. Coracidia are consumed by the intermediate host, cyclopoid copepods (e.g., genera *Acantocyclops*, *Macrocyclops*, *Mesocyclops*, *Tropocyclops*, *and Diacyclops)* (Körting 1975; Marcogliese and Esch 1989; Díaz-Castaneda et al. 1995). The life cycle is completed when fish ingest infected copepods. Water temperature has a discernable influence on the successful completion of each stage of the life cycle (Granath and Esch 1983a; Brouder and Hoffnagle 1997) with

optimal temperatures for development and growth ranging between 25 – 30°C (Granath and Esch 1983a).

 Bothriocephalus acheilognathi is recognized as a causative agent of detrimental infection in aquaculture operations in Asia and Europe where it is reported to cause up to 100% mortality in hatchery ponds (Liao and Shih 1956; Körting 1975). Pathogenic effects of this exotic helminth include intestinal blockage and perforation, distended abdomen, necrosis, inflammation, hemorrhaging, loss of intestinal microvilli, and loss of enterocytes, which can reduce growth and decrease survivorship (Scott and Grizzle 1979; Hoffman 1980; Granath and Esch 1983a; Hoole and Nisan 1994; Hansen et al. 2006). Consequently, occurrence of *B. acheilognathi* might have negative ecological impacts on the Rio Grande native fishes, which includes a number of threatened and endemic taxa (Hubbs et al. 1991).

 Objectives of this study were to determine distribution and definitive hosts of *Bothriocephalus acheilognathi* within the Rio Grande drainage and to quantify occurrences, abundances, and reproduction of *B. acheilognathi* in one of its host, a non-threatened or endangered red shiner *Cyprinella lutrensis* taken from multiple sites on the Rio Grande. Mean intensity, and mean biomass were used as measures of tapeworm abundance. Information from this study will be used to assess the spread and extent of *B. acheilognathi*, possible source of introduction, and potential threat to the Rio Grande ichthyofauna.

MATERIALS AND METHODS

Seventeen Rio Grande mainstem or tributary sites were sampled from 2006

through 2007. Upper Rio Grande sites were located at Las Lunas, New Mexico (Site 1;

13S E 342902, N 3852484) and Highway 380, New Mexico (Site 2; 13S E 328446, N

3754566). Middle Rio Grande sites were located at Presidio, Texas (Site 3; 13R E

558488, N 3270614), Santa Elena Canyon, Big Bend NP (Site 4; 13R E 635271, N

3226912), Terlingua Creek, Big Bend NP (Site 5; 13R E 635173, N 3226953), and

Boquillas, Big Bend NP (Site 6; 13R E 702576, N 3231651). Lower Rio Grande sites

were located at Pinto Creek, Texas (Site 7; 14R E334378, N 3229972), Quemado, Texas

(Site 8; 14R E 339851, N 3201952), Laredo, Texas (Site 9; 14R E 449947, N 3041685),

and San Ygnacio, Texas (Site 10; 14R E 455409, N 2993677). Pecos River sites were

located at Hwy 84, New Mexico (Site 11; 13S E 494307, N 3894107), Fort Sumner, New

Mexico (Site 12; 13S E 568090, N 3814643), Highway 70E, New Mexico (Site 13; 13S E

558124, N 3714600), Hwy 507, New Mexico (Site 14; 13S E 562547, N 3651050),

Artesia at Hwy 82, New Mexico (Site 15; 13S E 563294, N 3633150), and Independence

Creek, Texas (Site 16; 14R E 238573, N 3371658). The Devils River was also sampled

near Dolan Falls (Site 17; 14R E 307503, N 3307544). Site GPS coordinates were

recorded in the UTM NAD 27 coordinate system.

Sites were sampled monthly or seasonally for assessment of tapeworm prevalence

and abundance. Other sites were sampled once for determination of tapeworm

distribution and occurrence within the Rio Grande. Fish were taken monthly from

mainstem Site 4 (Santa Elena Canyon) and Site 6 (Boquillas) as well as tributary Site 5

(Terlingua Creek) within Big Bend NP. Mainstem Big Bend NP sites were sampled from

February through December 2006 and the tributary was sampled March through December 2006. Site 8 (Quemado, TX) was sampled seasonally from September 2006 through October 2007. Other sites were sampled opportunistically: upper Rio Grande (sites 1, 2) and Pecos River (sites 11, 12, 13, 14, and 15) were sampled in March 2007. Site 7 (Pinto Creek) was sampled in March 2007, and site 17 (Devils River) was sampled in December 2007. At each site, fish were captured from available geomorphic units with a 3-m x 1.8-m seine (mesh size: 3.1 mm). Up to 40 *Cyprinella lutrensis*, or *C. venusta* when *C. lutrensis* were unavailable, were randomly retained from each site. Other fishes with distended abdomen were also retained. Fish were anesthetized with a lethal dose of tricaine methanesulfonate (MS-222), and preserved in 10% formalin solution. Mean monthly temperatures (°C) were calculated from TCEQ gage CAMS 721 at Rio Grande Village in Big Bend NP.

In the laboratory, total length (mm) and weight (to the nearest 0.1mg) were obtained from each fish, and gastrointestinal tracts were removed. Tapeworms were teased from the intestinal lining and identified by their heart-shaped scolex with a pair a deep bothria (Scholz 1997). Tapeworms were enumerated in each fish to determine prevalence and mean intensity (terminology from Bush et al. 1997). Each tapeworm was weighed (to the nearest 0.1mg) to determine tapeworm biomass relative to the total weight of the fish as well as estimating stage of maturation. Stage of maturation for tapeworms was divided into two categories based on weight of the tapeworms; immature tapeworms weighed 0.1 - 4.0 mg and gravid tapeworms weighed 4.1 mg or more.

Prevalence [(number of fish infected / total number fish collected)*100] was compared among monthly Big Bend NP mainstem (sites 4, 6) and tributary sites (site 5)

using a heterogeneity Chi-square test. Difference in mean intensity (total number of

tapeworms collected from a sample of hosts / number of infected hosts) between monthly

Big Bend NP mainstem and tributary sites was assessed using a bootstrap t-test (Rózsa et

al. 2000). Bootstrapped 95% confidence intervals were constructed for mean intensity

following Rózsa et al. (2000). Biomass [(total mass of tapeworms / total mass of

fish)*100] was estimated by combining all monthly Big Bend NP sites and was analyzed

between differing intensities using a single factor ANOVA. Proportional data were

transformed ($p' = \arcsin \sqrt{p}$) to meet the assumptions of normality of the linear model

because proportional data typically follow a binomial distribution (Zar 1999).

Seasonality of tapeworm maturation was constructed using the monthly Big Bend NP

sites.

RESULTS

A total of 1,993 fishes spanning 11 families and 32 species were collected from

17 sites within the Rio Grande drainage (Table 1). Four families (Cyprinidae,

Catostomidae, Fundulidae, and Poeciliidae) and 10 species (25% of total number of taxa

collected) were definitive hosts for *B. acheilognathi*. Definitive hosts included four new

host records, *C. venusta*, *C. proserpina, Dionda argentosa*, and *Cycleptus elongatus*, two

previously reported hosts from the Rio Grande, *C. lutrensis* and *N. braytoni* (Bean et al.

2007), and four species previously reported as definitive hosts, *N. stramineus, Carpiodes

carpio, Fundulus zebrinus,* and *Gambusia affinis* (summarized in Dove and Fletcher

2000). Among all definitive hosts, infected fish were taken from the mainstem Rio

Grande at Presidio, Texas, to the farthest downstream site at San Ygnacio, Texas (Figure

1). *Bothriocephalus acheilognathi* were not found in fish taken from the mainstem Rio

Grande in New Mexico. Among tributaries, infected fish were taken at Fort Sumner, New Mexico, to Artesia, New Mexico, on the Pecos River, at Big Bend NP and Terlingua Creek, near Dolan Falls on the Devils River, and at Hwy 90 Crossing on Pinto Creek.

Among sites with *B. acheilognathi*, sufficient numbers of *Cyprinella* were obtained to calculate prevalence for the Big Bend reach of the Rio Grande (3 sites), middle Pecos River in New Mexico (2 sites), and the lower Rio Grande (1 site at Quemado, Texas). Prevalence was 11.3% in *C. lutrensis* (*N*= 1,096) taken from the Big Bend reach across monthly collections in 2006, and 7.9% (*N*= 38) taken from the middle Pecos River at Fort Sumner and Highway 507 in March 2007. Prevalence was 1.7% in *C. venusta* (*N*= 177) taken from lower Rio Grande seasonally in 2007.

Within the Big Bend reach of the Rio Grande, 790 *C. lutrensis* were taken across monthly collections from two mainstem sites, and 306 *C. lutrensis* were taken across monthly collections from one tributary site. Among all sites, total lengths of *C. lutrensis* ranged from 14 to 62 mm. Total lengths of infected *C. lutrensis* ranged from 16 to 54 mm. Monthly prevalence differed ($\chi^2 = 32.88$, df = 8, $P < 0.01$) between mainstem sites and the tributary sites. Mean prevalence of mainstem sites was 13.8% across months, ranging from 7.2% in April to 27% in January (from Bean et al. 2007). Among months, prevalence was highest (>15%) in January through March and again in December, and was lowest (<10%) in April through June and again in October. Mean prevalence in the tributary site was 8.7% across months, ranging from 0% in April and September to 27.5% in June (Figure 2).

Mean annual tapeworm intensities did not differ ($t = -0.91$, $N= 112$, $P = 0.37$) between mainstem and tributary sites. Therefore, mean mainstem and tributary

intensities were combined for an annual estimate. Mean annual intensity was 2.2

tapeworms per fish (±0.38 SE), ranging from 1.5 in April to 5.4 in May (Figure 3).

Highest intensity was 37 in a 43 mm fish. Mean percent biomass was 1.3% (±0.2% SE)

ranging from 0.6% (±0.14% SE) in fish with five or more tapeworms to 2.8% (±1.08%

SE) in fish with four tapeworms (Figure 4). Percent biomass did not differ (ANOVA, F

= 2.05, df = 4, P = 0.09) among densities. Greatest percent biomass was two tapeworms

at 19.6% found in a 16 mm fish.

Tapeworm reproduction, based on the occurrence of gravid tapeworms, was from

December through July in the Big Bend reach of the Rio Grande. Proportion of gravid

tapeworms (≥4.1 mg) within the population generally increased from December through

May with increasing water temperatures. When water temperatures exceeded 20°C,

monthly proportions of gravid tapeworms decreased and were absent by August.

Immature tapeworms (0.1 to 4.0 mg) were found year round, and exclusively from

August through November (Figure 5).

DISCUSSION

Spatial differences were found in occurrences and prevalences among reaches of

the Rio Grande drainage with higher prevalences in the Big Bend reach than in the lower

Rio Grande and upper Pecos River. Differences among reaches might be attributed to

interactions with biotic and abiotic factors including species abundance fluctuations in

intermediate host communities composed of potential hosts with differing susceptibilities

to infection (Marcogliese and Esch 1989) and sensitivity of the different tapeworm

lifecycle stages to abiotic parameters. Temperature is an important abiotic parameter

effecting the survivability of *Bothriocephalus acheilognathi* as temperatures below 12°C

inhibit eggs from hatching (Chubb 1981) and nearly half of procercoids in intermediate

hosts will not mature at 14°C (Granath and Esch 1983a). The infectivity and survival of

free-living stages of tapeworms can be reduced as increases in salinity can affect

coracidia, depleted oxygen concentrations can affect eggs, and increased depth as well

pressure can affect eggs (summarized in Pietrock and Marcogliese 2003). Exposure of

fish to pollutants can increase hosts susceptibility to parasitism by impairing

immunological responses (Lafferty and Kuris 1999). These abiotic parameters could

contribute to the observed distribution and abundance patterns observed in the Rio

Grande drainage if any combination of the parameters were outside of the tapeworms'

critical maxima or minima. Large hypolimnetic release reservoirs alter stream habitats

by lowering temperatures, decrease available nutrients, and alter water chemistry

(Edwards 1978) and the effects of Amistad reservoir on the Rio Grande might reduce

parasite survival and result in lower prevalence in the lower Rio Grande. Another

potential influence is the difference in suitable copepod populations exploiting creeks.

Choudhury et al. (2004) noted that higher abundances in upstream reaches might result

from copepods utilizing creek habitats and serve as source populations that repopulate

mainstem habitats after disturbance events. A similar scenario is likely for the Big Bend

reach where Terlingua creek and other large tributaries could serve as source populations

sustaining infection as these tributaries are often warmer and more lentic favoring the

production of copepod intermediate hosts. Possible sources of infection include spread of

infection from the Río Conchos, as the occurrence of the tapeworm has been confirmed

in many Mexican systems (Salgado-Maldonado et al. 2001a; Salgado-Maldonado et al.

2001b), bait bucket transfers, stockings of grass carp, or introduction in the Pecos River from Dextar Federal Hatchery in New Mexico, where *B. acheilognathi* has been documented in the hatchery (Choudhury et al. 2006).

Tapeworm prevalence exhibited seasonality in *C. lutrensis* collected from Rio Grande mainstem sites in Big Bend NP. Prevalence trends observed from Big Bend NP were similar to those observed in North Carolina (Marcogliese and Esch 1989). Both systems exhibited decreases in prevalence at high seasonal temperatures. Marcogliese and Esch (1989) attribute the fluctuations in seasonal trends to abundance and availability of suitable copepod intermediate hosts. The decrease and subsequently low prevalences observed in Big Bend NP from March through October could also be temperature related and result from increased host mortality. Granath and Esch (1983a) observed that infected *Gambusia affinis* had significantly decreased survivorship in water temperatures >20°C. Our results are consistent with this observation and decreases in prevalence at temperatures >20°C might indicate mortality associated with the interacting stressors of temperature and parasitism. At the monthly Big Bend NP tributary site, prevalence remained relatively low throughout the year except in June. During May, the tributary was completely cut off from the mainstem and existed as a series of long pools near the confluence. The sharp increase in prevalence from May to June might be a consequence of the tributary becoming detached from the mainstem lending to copepod intermediate hosts and fish isolated and in close confinement increasing the opportunity for more fish to become infected.

Immature and gravid tapeworms exhibited seasonality in Big Bend NP. The proportion of immature tapeworms increased after water temperatures warmed in the

summer and fall and proportions of gravid tapeworms increased in the winter and spring.

Bothriocephalus acheilognathi also exhibits size seasonality in other regions. Liao

(2002) recognized a winter generation of gravid tapeworms from October through April

and immature tapeworms from May through August. In North Carolina, the majority of

segmented and gravid worms were collected in the spring and summer and non

segmented worms were collected in the fall and winter (Granath and Esch 1983a).

Collectively, these results suggest that tapeworm reproduction is regulated by

temperature (Chubb 1981) but might also be regulated based on seasonality of copepod

hosts (Marcogliese and Esch 1989).

Mean intensity was less than five worms per fish for the majority of the year at

Big Bend NP mainstem sites. Levels of mean intensity for Big Bend NP tributary site

was comparable to mainstem sites, however levels were greater than five in September,

October, and December. Intensity levels observed in the *C. lutrensis* in Big Bend NP are

similar to those observed in *Gambusia affinis* from North Carolina (Marcogliese and

Esch 1989). Biomass was not significantly different among intensities possibly resulting

from a crowding effect (Morand et al. 1995; Read 2000; Roberts 2000). Roberts (2000)

postulated that the crowding effect could be a result of exploitative competition of limited

host resources potentially explaining the similarities in biomass among different

intensities observed in this study.

Existing reports of tapeworms in Texas range from tapeworm intensities of 1 to

7.5 in a central Texas river (Underwood and Dronen 1984) and tapeworm biomass can

exceed 40% or total stomach weight in East Texas (Williams and Bonner 2006). There

are no known reports of fish tapeworms from the Rio Grande and no other species were

collected during this study. This could indicate that the Rio Grande tapeworm population

was depauperate before the introduction of *B. acheilognathi* or that *B. acheilognathi* has

replaced the native tapeworm assemblage. Pathogenic effects might be exacerbated if

host populations in the Rio Grande historically experienced low native tapeworm

biomass. If the tapeworm assemblage was previously nonexistent, the introduction of

any tapeworm species could negatively impact fish populations not adapted to heavy

parasite loads presented by *B. acheilognathi*. *Bothriocephalus acheilognathi* potentially

impacts a host by modifying host behavior (Bush et al. 1997) by impairing swimming

ability (personal observation), increasing susceptibility to pollution (Brown and Pascoe

1989), increasing susceptibility to predation (Combs 1991), changing host competitive

interactions (Price 1980), impacting host reproduction (Kuris 1974), and inducing

mortality (Lemly and Esch 1984).

Table 1. List of species surveyed in the Rio Grande drainage in 2006 and 2007. Species documented as definitive hosts are indicated by an X.

	Species	Common Name	N	Definitive host
Cyprinidae	*Cyprinella lutrensis*	red shiner	1411	X
	Cyprinella venusta	black tail shiner	257	X
	Cyprinella proserpina	proserpine shiner	1	X
	Cyprinus carpio	common carp	1	-
	Dionda argentosa	Manantial roundnose minnow	1	X
	Dionda episcopa	roundnose minnow	3	-
	Hybognathus placitus	plains minnow	4	-
	Macrhybopsis aestivalis	speckled chub	31	-
	Notropis amabilis	Texas shiner	3	-
	Notropis braytoni	Tamaulipas shiner	91	X
	Notropis girardi	Arkansas River shiner	25	-
	Notropis simus pecosensis	Pecos bluntnose shiner	1	-
	Notropis stramineus	sand shiner	72	X
	Pimephales promelas	fathead minnow	3	-
	Pimephales vigilax	bullhead minnow	1	-
Catostomidae	*Carpiodes carpio*	river carpsucker	3	X
	Catastomous commersonii	white sucker	4	-
	Cycleptus elongatus	blue sucker	22	X
	Moxostoma congestum	gray redhorse	1	-
Characidae	*Astyanax mexicanus*	Mexican tetra	4	-
Ictaluridae	*Ictalurus furcatus*	blue catfish	1	-
	Ictalurus punctatus	channel catfish	3	-
Atherinopsidae	*Menidia beryllina*	inland silverside	26	-
Fundulidae	*Fundulus grandis*	Gulf killifish	3	-
	Fundulus zebrinus	plains killifish	3	X
Poeciliidae	*Gambusia affinis*	western mosquitofish	9	X
	Gambusia geiseri	largespring gambusia	1	-
Cyprinodontidae	*Cyprinodon variegatus*	sheepshead minnow	1	-
Centrarchidae	*Lepomis megalotis*	longear sunfish	1	-
	Micropterus salmoides	largemouth bass	1	-
Percidae	*Etheostoma grahami*	Rio Grande darter	4	-
Cichlidae	*Cichlasoma cyanoguttatum*	Rio Grande cichlid	1	-
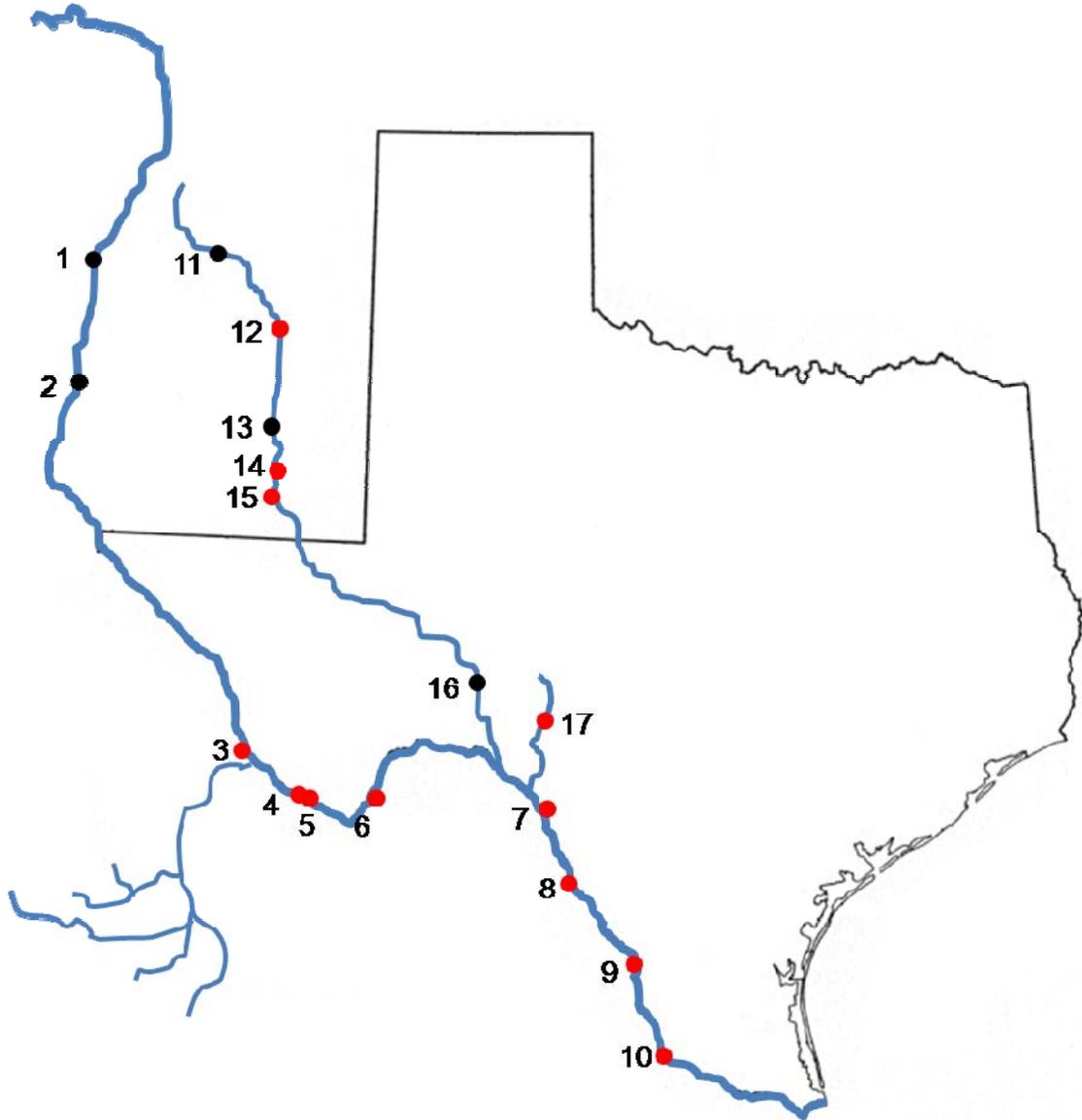
Figure 1. Locations of fish collections from the Rio Grande, Pecos, and Devils River in 2006 and 2007. Red dots indicate sites of confirmed *Bothriocephalus acheilognathi* presence and black dots indicate tapeworm's occurrence not detected.
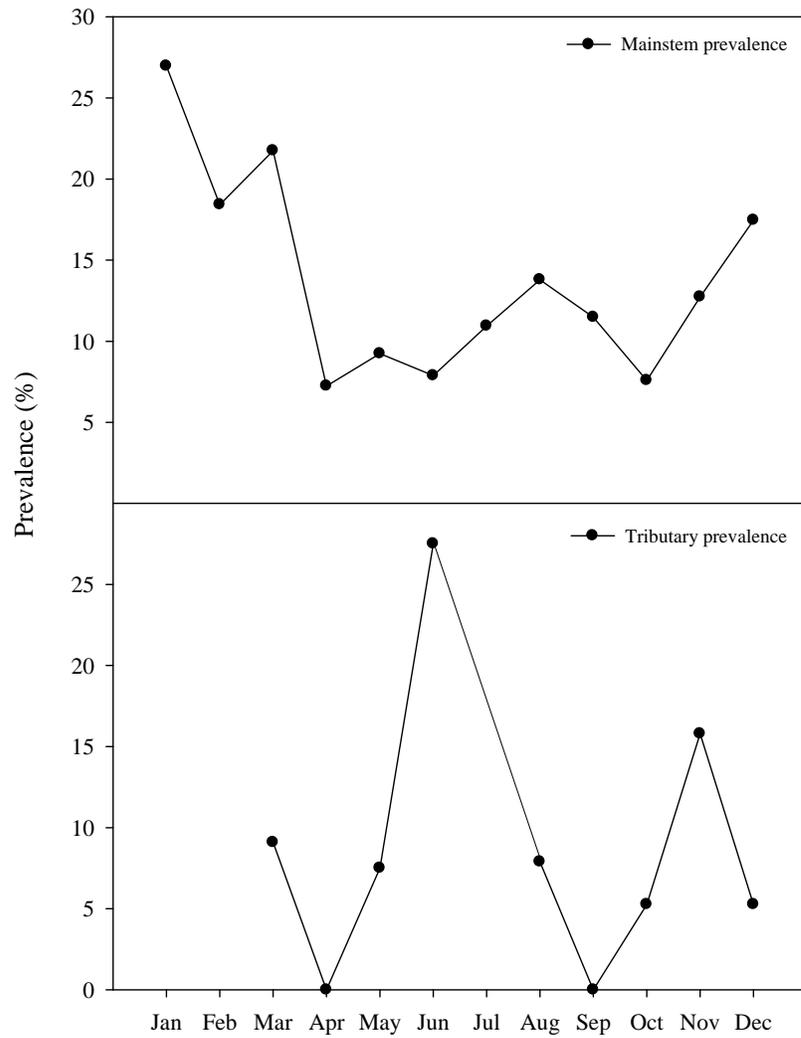
*January data point from Bean et al. 2007.

Figure 2. Monthly prevalences and mean temperature of the Big Bend National Park
mainstem and tributary sites for 2006.
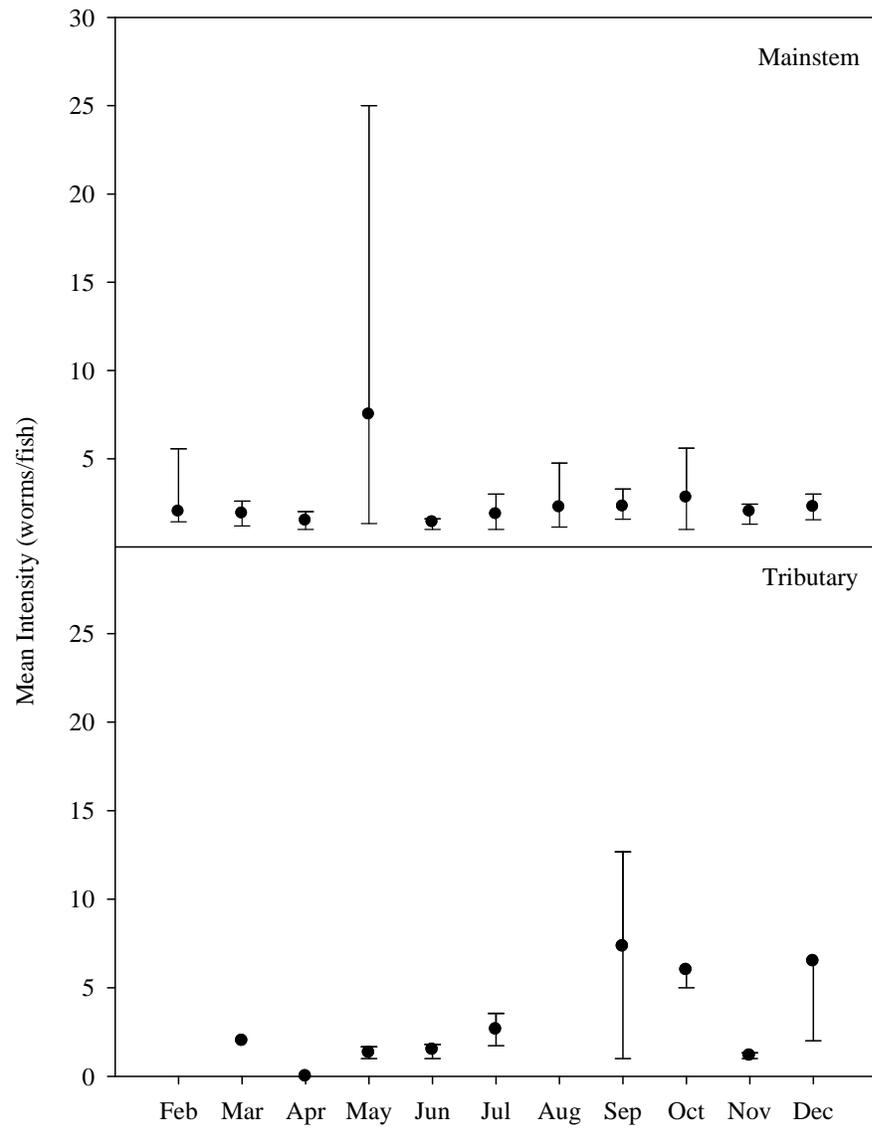
Figure 3. Monthly mean intensities for the Big Bend National Park mainstem and tributary sites. Estimates include bootstrapped 95% confidence intervals.
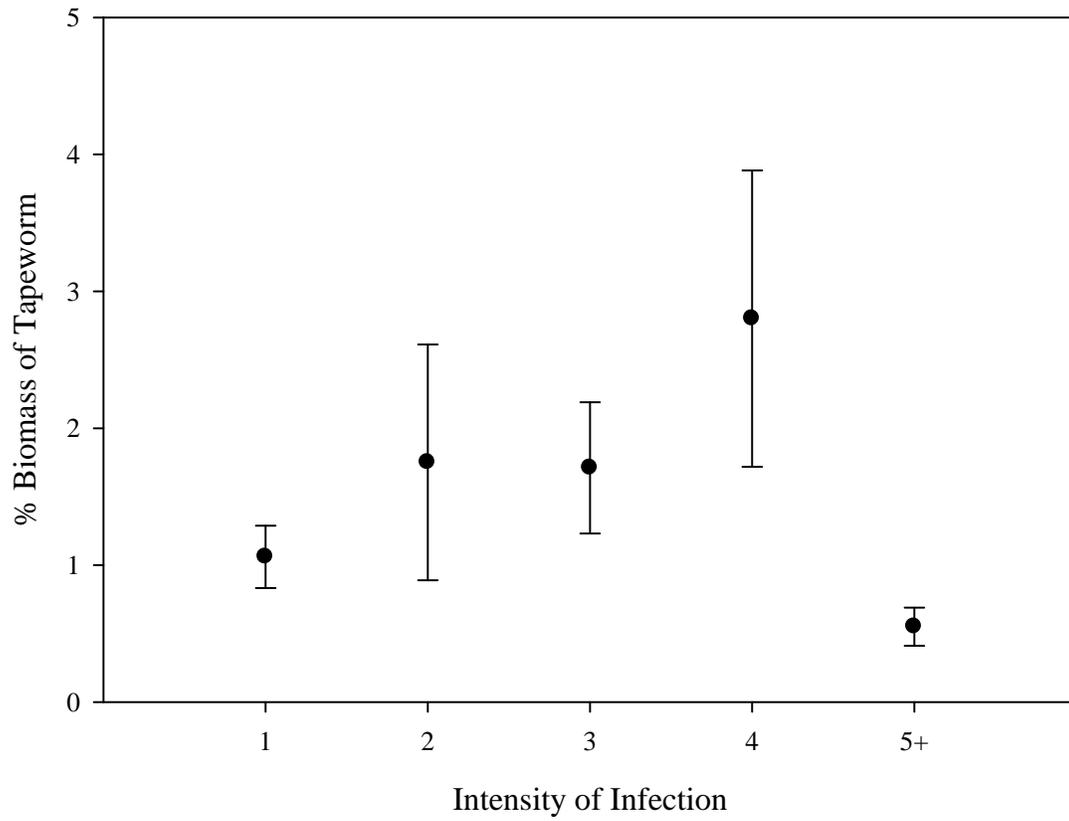
Figure 4. Tapeworm biomass (±SE) among infection intensities in *Cyprinella lutrensis* from the Rio Grande in Big Bend National Park in 2006.
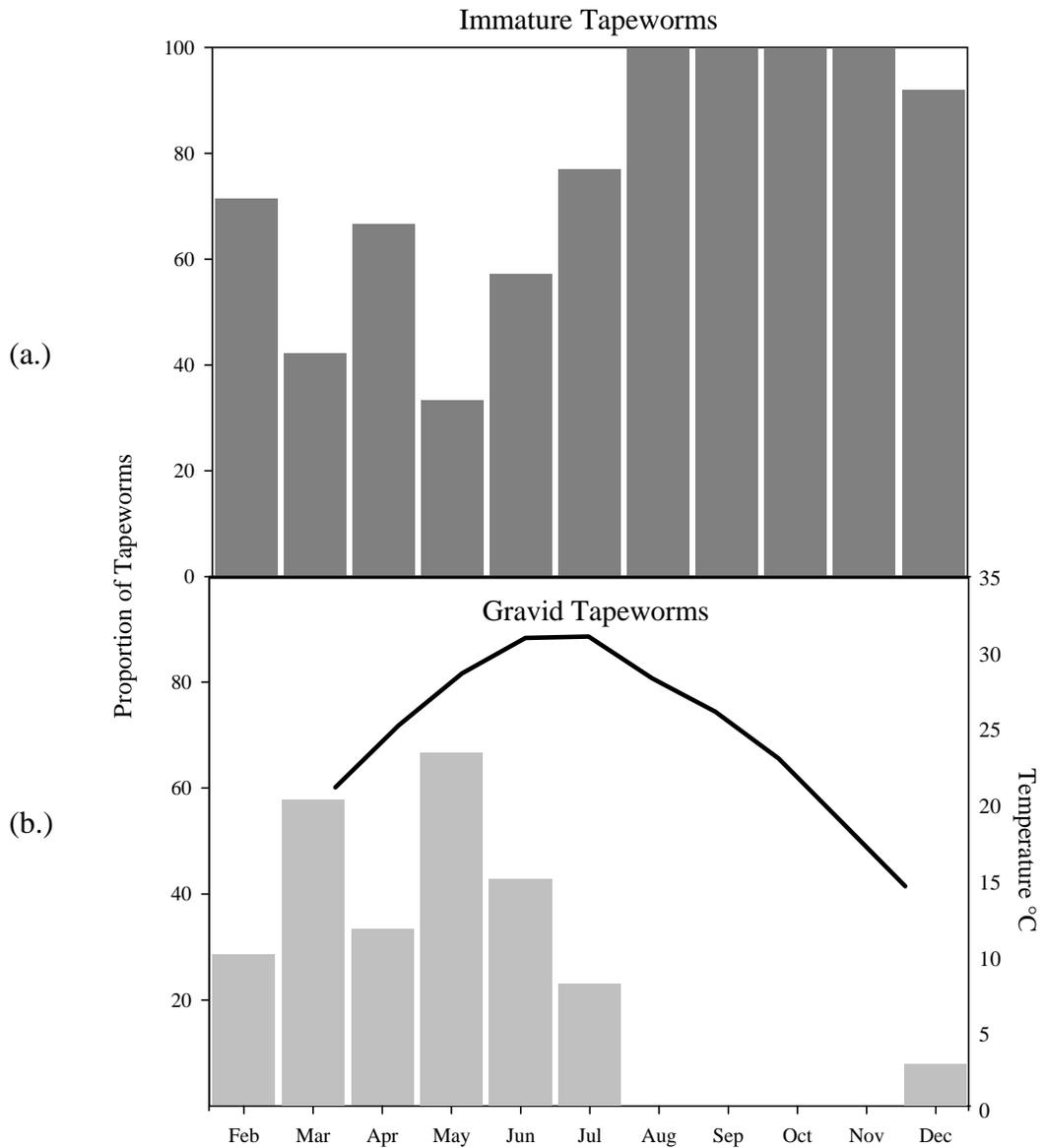
Figure 5. Seasonal trends for immature (a.) and gravid (b.) *Bothriocephalus acheilognathi* from Big Bend National Park 2006. Solid line shown in graph (b.) depicts mean monthly temperature recorded at Rio Grande Village Big Bend NP, gage station CAMS 721.

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CHAPTER II

IMPACT OF *BOTHRIOCEPHALUS ACHEILOGNATHI*
ON FISH CONDITION AND REPRODUCTION

ABSTRACT

Over 50% of imperiled fish species in Texas are endemic to the Rio Grande drainage. *Bothriocephalus acheilognathi* was recently collected from the Rio Grande and might pose further risk for these species. The objectives of this study were to examine the impact of this exotic tapeworm on fish condition and reproduction using a surrogate species, *Cyprinella lutrensis*. Fish were collected from two mainstem sites and one tributary site within Big Bend National Park from February through December 2006. Adjusted mean fulton condition was higher for uninfected fish than infected fish ($P = 0.04$). No differences were detected between uninfected fish for gonadosomatic indices ($P = 0.13$), number of vitellogenic oocytes ($P = 0.88$), number of late vitellogenic oocytes $P = 0.36$), late vitellogenic occyte maximum diameters *($P = 0.96$)*, or proportion of late vitellogenic oocytes to total number of oocytes ($P = 0.88$). These findings suggest that *C. lutrensis* is investing energy in reproduction at the expense of somatic growth, but energy invested in reproduction was not affected by tapeworm occurrence.

INTRODUCTION

The Asian fish tapeworm *Bothriocephalus acheilognathi* has rapidly disseminated worldwide from Asia. The tapeworm is a generalist currently infecting over 100 species of fish spanning 7 orders and 14 families (Salgado-Maldonado and Pineda-López 2003). The tapeworm has a short, direct lifecycle (Körting 1975) contributing to its ease of colonization of new drainages (Dove and Fletcher 2000). Much of the current research has focused on determining pathogenic effects for fishes. Host pathogenic effects can be as serious as intestinal disintegration (Hoole and Nisan 1994), decreased survivorship (Granath and Esch 1983), and mortality (Körting 1975). These pathogenic effects would be detrimental to endemic fish populations, especially to those already listed as of conservations concern.

In Texas, over 50% of imperiled fishes are endemic to the Rio Grande drainage (Hubbs et al. 1991). Fish in the Rio Grande are highly impacted because of anthropogenic impacts (Anderson et al. 1995). Habitat alterations associated with groundwater pumping, construction of dams, siltation, agricultural runoff, and the introduction of nonnative fish all threaten these endemic taxa (Contreras-Balderas 1974; Contreras-Balderas et al. 2002; Edwards et al. 2002; Contreras-Balderas et al. 2003). The added pathogenic effects of the Asian fish tapeworm in the system along with the anthropogenic impacts might have serious consequences for the continued persistence of Rio Grande taxa.

Several studies have examined the impact of *Bothriocephalus acheilognathi* on large-bodied and long-lived cyprinids. Brouder (1999) compared infected and uninfected *Gila robusta* (maximum length = 43.0 cm total length; Page and Burr 1991) and

determined that infected fish were significantly shorter than uninfected fish. Infected *Gila elegans* (maximum length = 62.0 cm total length; Page and Burr 1991) in a laboratory study grew at a significantly slower rate and weighed less than uninfected fish of the same age class (Hansen et al. 2006). The short-bodied and short-lived cyprinid *Notropis topeka* also exhibited reduced growth in infected fish (Koehle and Adelman 2007) in a laboratory experiment (maximum length = 7.6 cm TL; Page and Burr 1991). In the Rio Grande, many endemic cyprinids are small, growing to a maximum of around 7.0 cm TL (Thomas et al. 2007) with longevity ranging around two years for *Notropis* and three years for *Cyprinella*. As these species are short lived, many taxa will undergo one or two spawning seasons in their lifespan.

The purpose of this study was determining the potential impacts of *Bothriocpehalus acheilognathi* on small-bodied, short-lived cyprinid taxa. *Cyprinella lutrensis* was used as a surrogate to estimate potential impacts on condition and reproduction for other imperiled cyprinid taxa. Red shiners *Cyprinella lutrensis* were used because of a widespread distribution and high abundance in the Rio Grande drainage. The Big Bend National Park (NP) was selected for abundance of *B. acheilognathi* and prevalences observed by Bean et al. (2007). Fulton condition factors were compared between infected and uninfected *C. lutenesis* to determine impacts on growth. Several reproductive parameters were estimated to determine if there was an impact on infected *C. lutrensis*. Specifically, I examined monthly gonadosomatic indices, maximum late vitellogenic oocyte diameters, and proportion of oocytes in late vitellogenic stage to assess impact of *B. acheilognathi* infection on quality of mature oocytes produced, number of vitellogenic oocytes to assess impact of tapeworm infection

on number of mature oocytes produced, and number of late vitellogenic oocytes to assess impact of tapeworm infection on number of oocytes per clutch.

MATERIALS AND METHODS

Two mainstem sites were selected at Santa Elena Canyon (13R E 635271, N 3226912) and Boquillas (13R E 702576, N 3231651) and one tributary site at Terlingua Creek (13R E 635173, N 3226953) (Figure 6). Site GPS locations were recorded in the UTM NAD 27 coordinate system. Fish were collected monthly from February to December 2006. In October, flows inhibited the collection of a sufficient number of specimens from Boquillas, so an adjacent location was sampled (Hot Springs; 13R E 695091, N 3229250). Within each site, all available geomorphic units were sampled with a 3-m x 1.8-m seine (mesh size, 3.1mm). Approximately 40 red shiners were collected per site each month, anesthetized with a lethal dose of tricane methanesulfonate, and preserved in 10% formalin solution.

In the laboratory, fish were weighed and measured (total length in mm) before the gut tract was removed and examined for tapeworms. Tapeworms from each fish were enumerated and individually weighed. Fish gonads were removed and weighed. Eviscerated fish weight was obtained by removing the remaining internal organs from the gut cavity and reweighing the fish. Eviscerated fish weight was obtained to exclude tapeworm and gut content weight in calculations. All weights were recorded to the nearest 0.1mg.

To determine impact on growth, adjusted Fulton condition factors [(eviscerated fish weight / total length^3)*100,000)] were calculated from all fish collected. Monthly

adjusted gonadosomatic indices [GSI; (gonad weight / eviscerated weight of fish)*100] were calculated for fish at size of sexual maturity ($\geq$ 25 mm TL; Marsh-Matthews et al. 2002) collected from mainstem sites from February through December. Vitellogenic oocytes were counted from the left ovary of up to 13 uninfected and infected fish from February through September. Diameters of 100 oocytes were measured from all oocytes to the nearest 0.01 mm using a Nikon 1500 microscope with a Nikon Digital Sight DS-L1 digital camera. Differences in condition factors, number of vitellogenic oocytes, number of late vitellogenic oocytes ($\geq$ 0.74), maximum diameter of late vitellogenic oocytes, and proportion of late vitellogenic oocytes to total number of oocytes were tested with ANCOVA using length as a covariate. Covariates were natural log transformed ($X' = \ln(X +1)$) to improve linear model assumptions (Zar 1999). Among infected fish, different intensities of infection were compared with a single factor ANOVA after data were natural log transformed. Female adjusted GSIs and male adjusted GSIs between uninfected and infected fish were compared across months using a paired-sample t test.

RESULTS

A total of 969 red shiners (range in length: 14 – 62 mm in TL) were collected from the Big Bend NP mainstem sites. Fulton condition factors differed (ANCOVA, $F_{1,967} = 4.25$, $P = 0.04$) between uninfected fish (N = 858) and infected fish (N = 111; Figure 7). Mean ($\pm$SE) adjusted condition factors were slightly higher for uninfected fish (0.51$\pm$0.0042) than infected fish (0.49$\pm$0.0098). Among infected fish, Fulton condition factors were similar (ANOVA, $F_{4,107} = 0.52$, $P = 0.72$) across tapeworm intensities, ranging from 0.46$\pm$0.02 with one tapeworm to 0.48$\pm$0.04 with $\geq$5 tapeworms (Figure 8).

Gonadosomatic indices were calculated for 298 female and 158 male red shiners. Mean female GSIs were elevated in March and May through July for both uninfected and infected groups and was below 6% from August to February (Figure 9). Mean monthly GSIs did not differ (t-statistic; $P = 0.38$) between uninfected and infected females. Mean male GSIs were elevated in March and May through August (Figure 9) and between uninfected and infected males.

A total of 104 female red shiners (range in TL: 25 – 58 mm) were examined and 80 contained vitellogenic oocytes. Number of vitellogenic oocytes did not differ (ANCOVA, $F_{1,77} = 0.02$, $P = 0.88$) between infected and uninfected groups. In both groups, vitellogenic oocytes were first observed in February and found through July (Table 2 and Figure 10). Number of late vitellogenic oocytes (≥ 0.74 mm) did not differ (ANCOVA, $F_{1,77} = 0.84$, $P = 0.36$) between uninfected and infected groups. Late vitellogenic oocytes were present from February through July for uninfected fish with the largest number of mean late vitellogenic oocytes ($\pm$SE) occurring in June (9.55 ± 3.00). Late vitellogenic oocytes were present in March and May through July for infected fish with largest number of mean late vitellogenic oocytes occurring in June (9.00 ± 1.91). Likewise, late vitellogenic maximum diameters did not differ (ANCOVA, $F_{1,49} = 0.002$, $P = 0.96$) between uninfected and infected groups. Largest mean diameter ($\pm$SE) of vitellogenic oocytes was found in June for uninfected fish (0.88 ± 0.04) and in May for infected fish (0.93 ± 0.04). Proportion of late vitellogenic oocytes did not differ (ANCOVA, $F_{1,77} = 0.85$, $P = 0.88$) between uninfected and infected groups. Largest proportions of late vitellogenic oocytes ($\pm$SE) were found in June for both uninfected (0.1 ± 0.03) and infected (0.09 ± 0.02) groups.

DISCUSSION

Red shiners infected with *Bothriocephalus acheilognathi* in Big Bend NP had lower condition than uninfected red shiners but did not differ in GSI, number of vitellogenic oocytes, number of late vitellogenic oocytes, maximum diameter of late vitellogenic occytes, or proportion of late vitellogenic to total vitellogenic oocytes. Failure to detect differences in oocyte production or oocyte diameters is surprising given that a decrease in condition can indirectly indicate a deficit in available resources for reproduction (Bagamian et al. 2004). Wootton and Evans (1976) specify a strong relationship between both foraging ability and body weight with reproductive success in sticklebacks and observed a tradeoff between available resources invested in reproduction and somatic growth.

Cyprinids examined in previous *B. acheilognathi* studies exhibiting impaired growth were large bodied, long lived fish hatched and reared in the laboratory (Brouder 1999; Hansen et al. 2006). This study examined the potential impacts of the tapeworm infections in fish from wild populations. Although there was a difference in condition between uninfected and infected groups, there was no significant difference in condition among infection intensities. These results suggest that an initial infection of one tapeworm impedes the absorption of nutrients by the host as higher intensities of infection. Reduced condition might reduce growth and cause delayed ontological shifts in diet increasing the time spent consuming copepods thereby increasing the likelihood of reinfection (Brouder 1999; Hansen et al. 2006). Hansen et al. (2006) also notes that impaired growth might increase risk of predation. Infections hindering the growth in small bodied cyprinids could also increase the time to sexual maturation. A delay in time

to maturation or even the presence of an infection could be sufficient to prevent fish from becoming reproductively active during the normal reproductive season (Heins and Baker 2001). This could further threaten many of the endemic *Notropis* and *Cyprinella* in the Rio Grande drainage as many species of *Notropis* have longevities of less than three years.

One plausible reason for failure to detect differences in reproduction between uninfected and infected fish in this study is reproductive strategy of red shiners. Red shiners continuously spawn over a reproductive season asynchronously releasing may ova clutches (Gale 1986). Asynchronous release of eggs has lead to high variability among individuals in timing of clutch release leading to different stages of ovarian development among individuals which might mask any effects of tapeworm infections. Other confounding factors might include time of infection, intensity of infection, biomass of tapeworms, and inability to determine the number of clutches per season per individual. However, it is likely that *B. acheilognathi*, an exotic tapeworm with documented detrimental pathology, is impacting fish reproduction. Tapeworm infections in sticklebacks did impact fish reproduction manifested as a decrease in ovum mass (Heins and Baker 2003). Carter et al. (2005) reported decreases in condition, gonadal development, and a reduction of almost 50% of gonadotrophin in roach, *Rutilus rutilus*. Laboratory experimentation is needed to fully assess the impact of *B. acheilognathi* on cyprinid reproduction in order to account for the above mentioned confounding variables.

Introduction of exotic parasites are detrimental to endemic fishes. Impacts include impeding blood flow through the gill lamella inhibiting efficient respiration in the endangered *Etheostoma fonticola* from *Centrocestus formosanus* infections (summarized

in McDonald et al. 2007) to extensive parasite induced mortality of *Gyrodactylus salaris* on native Norwegian salmon populations (Johnsen and Jensen 1986). Impacts of exotic parasites such as *Bothriocephalus acheilognathi* might be exacerbated by additional stresses on native fish. Stresses in the American Southwest and especially the Rio Grande include habitat alterations with excessive groundwater pumping (Contreras-Balderas 1974; Contreras-Balderas et al. 2002; Edwards et al. 2002; Contreras-Balderas et al. 2003). In addition to the direct effects of habitat degradation, decreased flows might expose fish to higher concentrations of infected copepods, increase rates and intensities of infection, and put native fish at risk for parasite induced mortalities. Considering the widespread distribution of *B. acheilognathi* in the Rio Grande, it is unlikely that the impact on native fish can be managed. Consequently, occurrence of *B. acheilognathi* in the Rio Grande emphasizes the importance of preventing incidental introductions and spread of exotic species in order to protect and conserve native species.

Table 2: Summary of reproductive characteristics and parameters (±SE) comparing uninfected and infected *Cyprinella lutrensis* by month collected in Big Bend NP 2006.

		N	Mean Number Late Vitellogenic Oocytes	Mean Number Vitellogenic Oocytes	Mean Proportion of Vitellogenic Oocytes
February	*Uninfected*	8	2.00 ± 1.00	28.75 ± 17.61	0.02 ± 0.01
	Infected	3	0.00	0.33 ± 0.33	0.00
March	*Uninfected*	9	3.11 ± 1.29	50.89 ± 20.52	0.03 ± 0.01
	Infected	7	6.71 ± 3.66	101.28 ± 27.11	0.08 ± 0.04
April	*Uninfected*	13	0.31 ± 0.31	36.77 ± 27.07	0.00 ± 0.003
	Infected	3	0.00	0.00	0.00
May	*Uninfected*	11	9.55 ± 3.00	85.18 ± 25.41	0.1 ± 0.03
	Infected	6	9.00 ± 1.91	107.17 ± 22.83	0.09 ± 0.02
June	*Uninfected*	11	9.36 ± 3.45	126.10 ± 14.07	0.09 ± 0.03
	Infected	5	2.40 ±0.81	87.00 ± 15.60	0.02 ± 0.01
July	*Uninfected*	11	6.82 ± 1.61	83.45 ± 16.19	0.07 ± 0.02
	Infected	5	0.60 ± 0.60	15.80 ± 15.80	0.01 ± 0.004
August	*Uninfected*	9	0.00	0.00	0.00

Figure 6: Location of *Cyprinella lutrensis* collections from two Rio Grande mainstem sites (Santa Elena and Boquillas) and one tributary site (Terlingua Creek) in Big Bend National Park. Fish were collected monthly from February through December 2006.
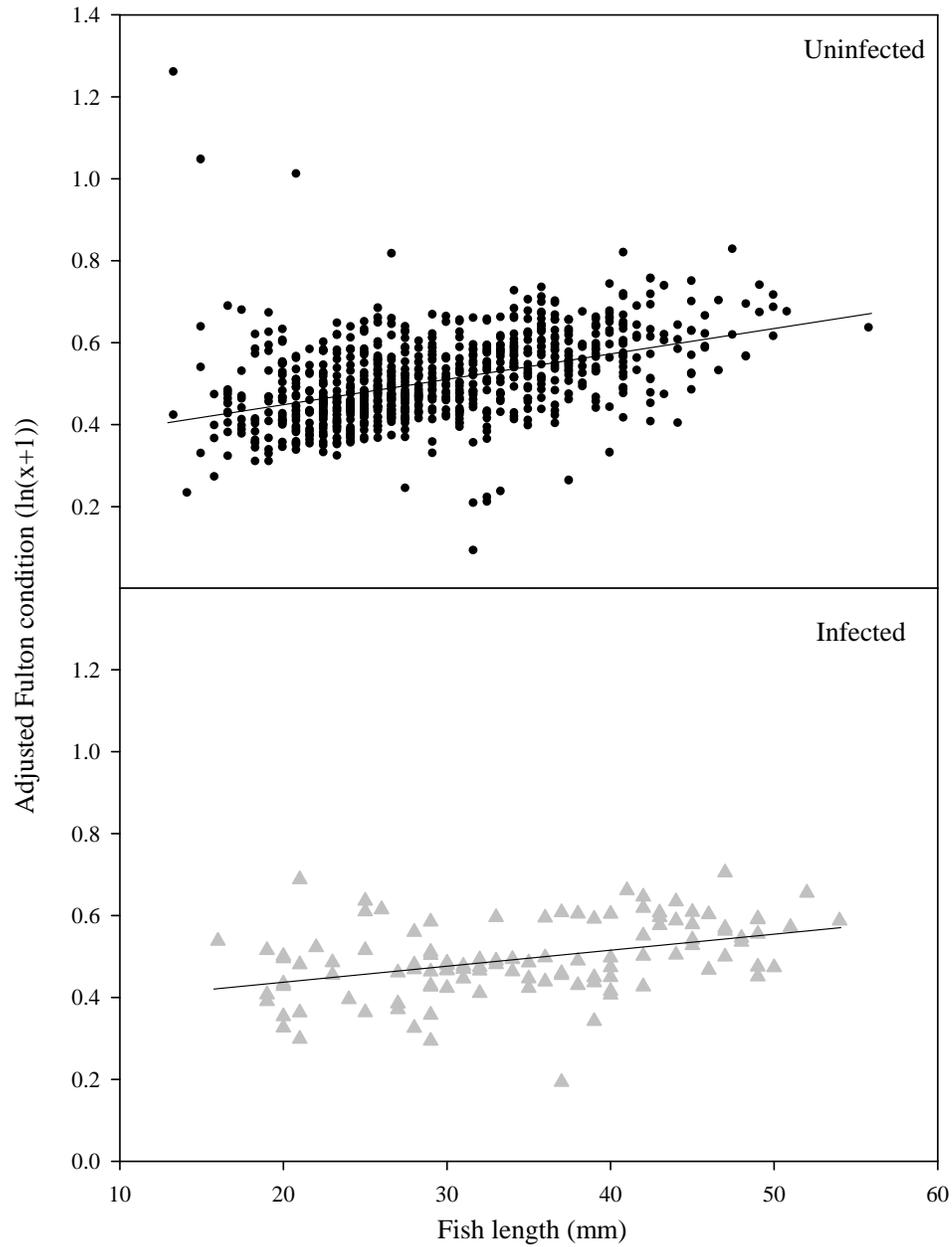
Figure 7: Adjusted Fulton condition factors for uninfected (N = 858) and infected (N = 111) *Cyprinella lutrensis* in Big Bend National Park taken monthly from three sites, February – December, 2006.
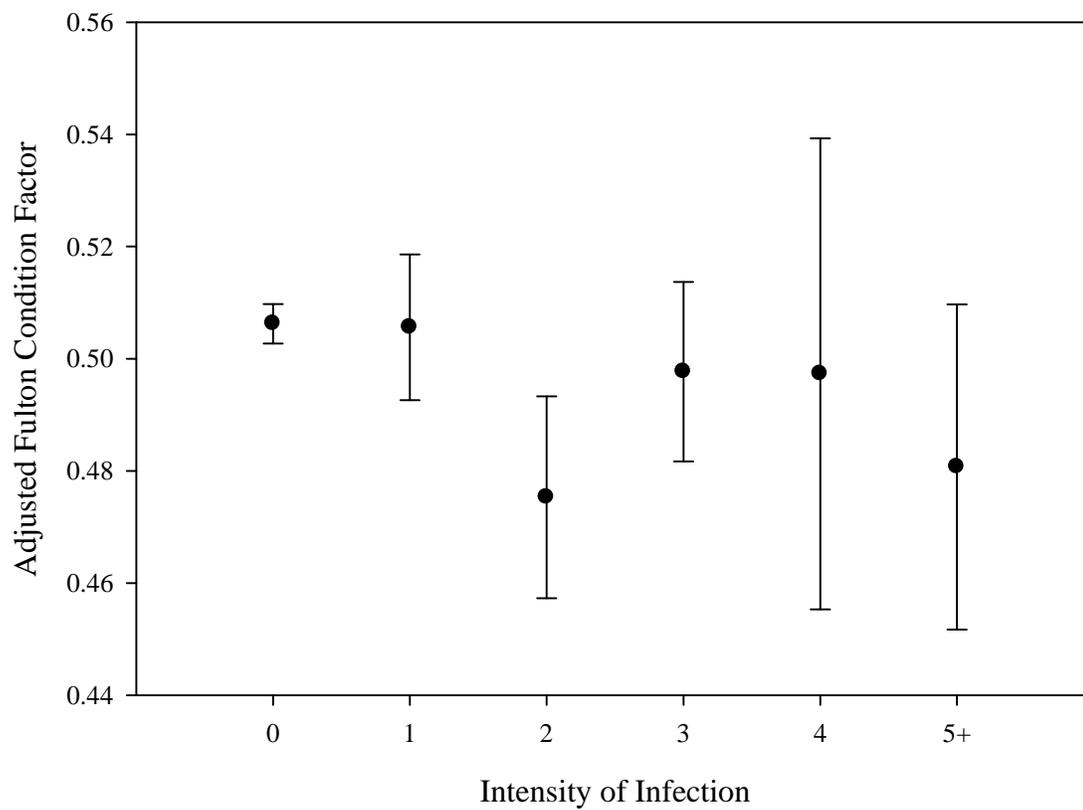
Figure 8: Adjusted Fulton condition factors comparing uninfected fish, infected *Cyprinella lutrensis* overall, and fish with different intensities of tapeworms from Big Bend National Park.
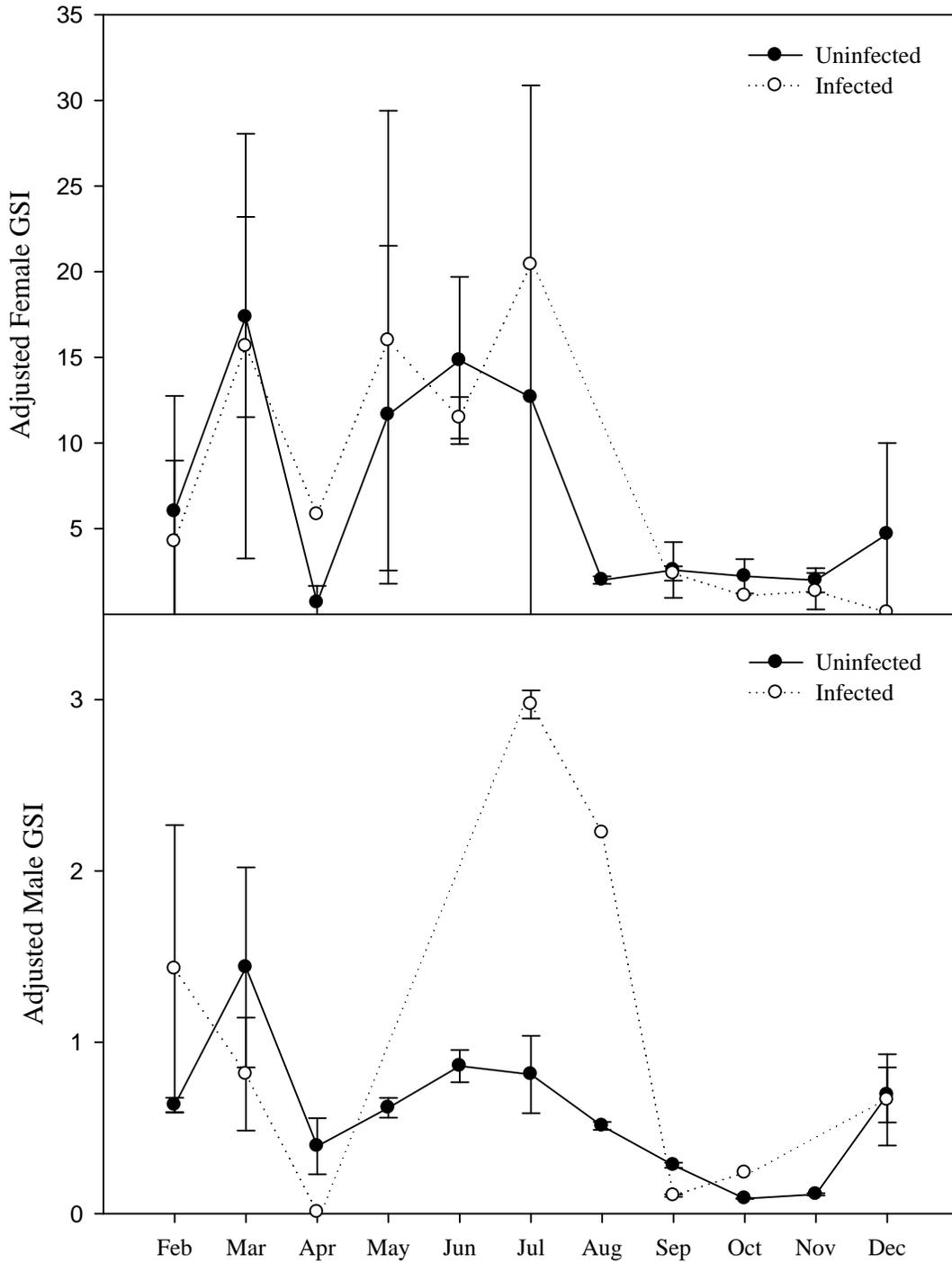
Figure 9: Monthly adjusted gonadosomatic indices ±SE for uninfected and infected female and male *Cyprinella lutrensis* collected from Big Bend National Park in 2006.
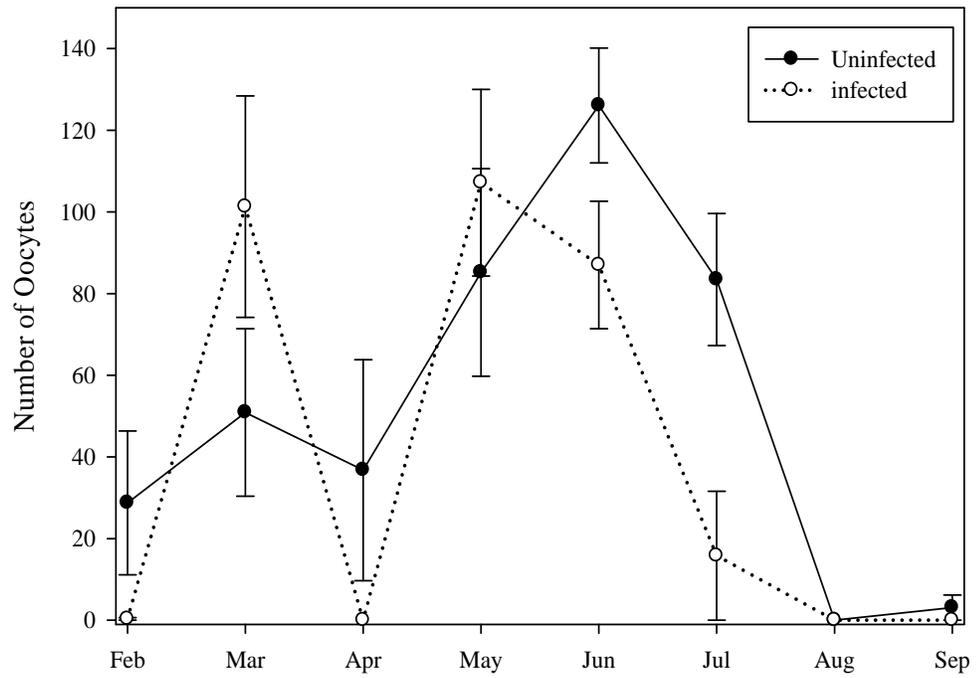
Figure 10: Average monthly counts ±SE of vitellogenic oocytes (left ovary) for uninfected and infected *Cyprinella lutrensis* from 2006.

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