PARASITES OF THE REDBREAST SUNFISH (*LEPOMIS AURITUS*) IN THE SOUTH

CONCHO RIVER OF WEST CENTRAL TEXAS

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DEDICATION

I dedicate this thesis to my parents for their unwavering support throughout the years and Lance Alot Johnson.

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ABSTRACT

Redbreast sunfish (*Lepomis arturis*) were caught from Head of the River Ranch, Texas, and their parasite communities were documented. Fish were caught in the spring (n= 8), summer (n= 12) and fall (n= 6) so that seasonal differences in the parasite community could be investigated. A total of 9 parasite species were recovered from these fish. An NMDS ordination plot and a permutational MANOVA (n= 10,000 iterations) revealed that the fish have a seasonally distinct parasite community in the fall. A series of ANOVAs were used as post-hoc tests to better understand which parasites may be contributing to this difference. Intensity significantly differed between spring and fall for *Posthodiplostomum minimum*. Intensity is likely influenced by the biotic and abiotic factors. This report serves as the first from this region, necessitating further exploration of the presence of parasites and their community dynamics.

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INTRODUCTION

Helminth community assemblages in freshwater fishes reflect complex and dynamic processes that are influenced by many biotic and abiotic factors (Fellis and Esch, 2004; Violante-Gonzalez et al., 2008). Holmes and Price (1986) described helminth communities as either isolationist or interactive. Isolationist communities are composed of species with low colonizing ability, small infrapopulations, and low interspecific interactions so that community composition is the result of stochastic colonization processes. Interactive communities are composed of individuals with high colonizing ability, large infrapopulations, and high competition among species. As such, parasite-parasite interactions are the primary factors generating and structuring the parasite community (Holmes and Price, 1986). Because of the complexity and variability among helminth communities, studies have documented parasite communities that do not match either classification such as those in both grebes and salamanders (Goater et al., 1987; Stock and Holmes, 1987; Stock and Holmes, 1988). In an investigation of differences between helminth communities of fish and birds it was concluded that fish helminth communities had fewer taxa and lower abundances than communities found in avian hosts (Kennedy et al., 1986). These differences in fish were proposed to be due to the simplicity of the alimentary canal, the ectothermic nature of the host, and selectivity of host diet (Kennedy et al., 1986).

Diet characteristics, demography, and host identity shape the composition of helminth communities within fish. These hosts display indeterminate growth in most cases, so host length and age are inter-dependent variables (Devries and Frie, 1996). However, both fish length and age can influence parasite communities in fish. Cloutman (1975) and Fiorillo and

Font (1999) both documented that parasite communities in larger centrarchids were more diverse than those found in smaller centrarchids. This positive relationship between parasite diversity and length or age of fish may be explained by increased exposure time in the system and the larger quantity of food required for bigger hosts. Specific differences in parasite composition found within the different size classes of fish is thought to reflect shifts in diet, habitat use, or behavior that can mediate parasite exposure (Fiorillo and Font, 1996). The changes in diet and habitat with age are common characteristics of many freshwater fishes, which can lead to significantly different helminth communities between age or size classes (Garvey and Chipps, 2012). These shifts in diet and habitat can also occur seasonally due to changes in the abundance of prey, and altered habitat use due to spawning behavior (Pflieger, 1997). Although extensive research has focused on the determination of factors driving helminth parasite structure and composition in freshwater fish in lentic systems, few studies have investigated the effects of these determinants on parasite communities in permanently flowing systems (Barger and Esch, 2001).

Across North America, *Lepomis* sp. exhibit a large diversity of parasites. Mizelle and McDougal (1970), in their key of the Monogenea genus D*actylogyrus*, reported 1 species on *Lepomis auritus*. Arnold (1967) examined 3 species of *Lepomis* across Louisiana. In *Lepomis cyanellus*, he found 1 Digenea, 3 Nematoda, and 2 Acanthocephala species. In *Lepomis macrochirus*, he found 9 Digenea, 4 Cestoda, 7 Nematoda, and 6 Acanthocephala species. In *Lepomis megalotis*, he recovered 3 Digenea, 1 Cestoda, 2 Nematoda, and 2 Acanthocephaln species. Lewis and Nickum (1964) found 1 Digenea species in both bluegill and largemouth bass in 10 lakes across Illinois. Rawson et al. (1973) studied *Lepomis* in Walter F. George

Reservoir on the Chattahoochee River, Alabama, and found 1 new species of Monogenea on *L. macrochirus*.

Parasites of *Lepomis* sp. have been studied in east Texas, but few if any studies have been conducted in west central Texas. From San Angelo, TX, the closest study is over 300 km away. Underwood and Dronen (1984) studied *Lepomis* sp. in the San Marcos River, Hays County, Texas. They found 8 Digenea, 1 Cestoda, 12 Nematoda, and 9 Acanthocephala species in *L. aurtius*; 6 Digeanea, 12 Nematoda, and 5 Acanthocephala species in *L. cyanellus;* and 11 Digenea, 4 Cestoda, 11 Nematoda, and 9 Acanthocephala species in *L. macrochirus*. Allison (1967) examined fish on the Navasota River and the Little River of east central Texas and found 3 Monogenea species: 2 *Cleidodiscus* sp. on *L. cyanellus* and 1 *Macrohaptor* sp. on *L. megalotis*. McGraw and Allison (1967) also studied the Little River system of Texas and found 8 Digenea and 2 Nematoda species in *L. megalotis;* 7 Digenea, 3 Nematoda, and 1 Acanthocephala species in *L. cyanellus;* and 7 Digenea and 2 Nematoda species in *L. macrochirus*. Meade and Bedinger (1972) found 1 Monogenea species on *L. cyanellus* and 2 Monogenea species on *L. macrochirus* in Madison and Walker counties in eastern Texas. Even within Texas, the different species of *Lepomis* exhibit variation in their parasite community. Notably, the parasites of *L. auritus* have only been reported for a single location in Texas: the San Marcos River (Underwood and Dronen, 1984).

Compared to these studies, fish from the Concho Valley are expected to have parasite communities similar to those recovered from other locations in Texas. The primary objective is to report and document the parasites found in and on *L. auritus* from a single location in the Concho Valley in west central Texas. The second objective is to examine the seasonal

variation of the parasite community in this fish host. This information will serve as a baseline for studies on the parasite community of *Lepomis* collected from other locations in the Concho Valley.

METHODS

Study Site

The Concho Valley is a 50000 km^2 region consisting of 13 counties including Coke, Concho, Crockett, Irion, Kimble, Mason, McCulloch, Menard, Reagan, Schleicher, Sterling, Sutton, and Tom Green Counties. The river system in the Concho Valley is the Concho River, which has three primary tributaries: the North Concho River, the South Concho River, and the Middle Concho River. The Concho River is known for its abundance of freshwater bivalves. This study took place on the South Concho River on the Head of the River Ranch, 7 km south of Christoval, TX (Fig. 1).

Figure 1. Map of fishing location at Head of the River Ranch in Christoval, TX.
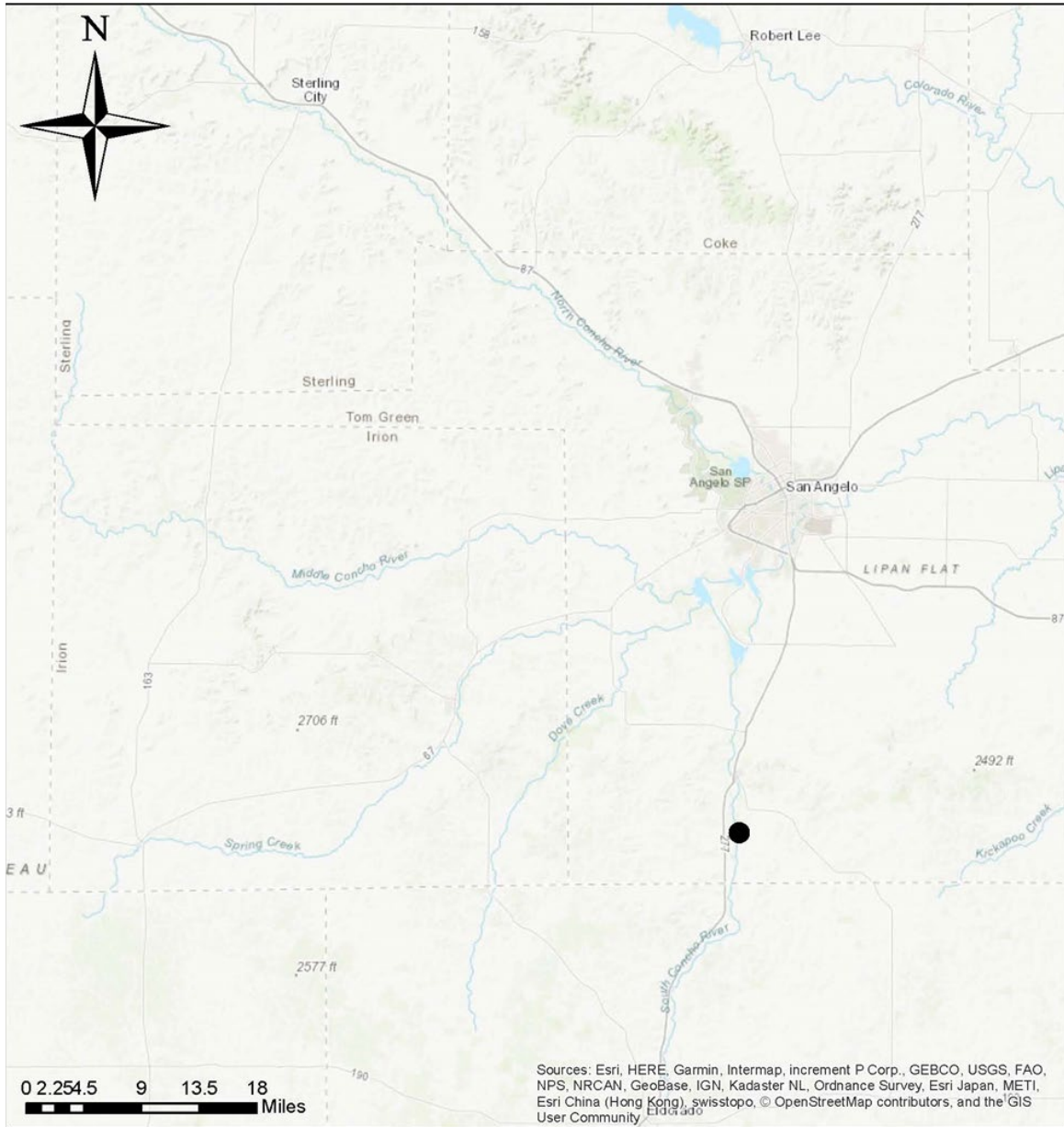
Fish Collection Techniques

During this survey, 5 *Lepomis* sp. were collected each mo from the South Concho

River at the Head of the River Ranch from the months of May to October of 2018. The site

was chosen for presence and abundance of *Lepomis* sp. Fish were collected using hook and

line (rod and reel) to ensure the capture of larger fish to obtain a representation of parasites of

adult *Lepomis* sp., which should have a greater diversity of parasites compared to yearling or

juvenile fish. Sampling occurred a minimum of once per month.

Laboratory Holding Facilities

Fish were captured and transported to the laboratory at Angelo State University in

aerated 19 L buckets where they were transferred into a 100 L aquarium. Fish were fed

crickets daily until examined. When processed, the fish were identified to species using the

Peterson Field Guide to Freshwater Fishes of North America North of Mexico, Second

Edition (Page and Burr, 2011); then length (cm) and weight (g) were recorded. Fish were

measured from the tip of the lower jaw to the end of the tail. Fish were necropsied by

standard procedures as outlined by *Necropsy Techniques for Fish* (Yanong, 2003). All

Lepomis sp. were sexed, and their age determined by counting the light and dark ring patterns

on their scales (Hoxmeier et al., 2001). All methods were approved by the Angelo State

University's Institutional Animal Care and Use Committee (Protocol #15-16).

Parasite Collection

The external anatomy of the fish was examined under a dissection microscope to

identify ectoparasites. Locations included the fins, inside the mouth and gill chamber, on the

gills, and the scales. After the external anatomy was completely examined, fish were

immediately examined for endoparasites. Locations examined include the eyes, muscle, stomach, pyloric caeca, intestines, visceral organs, mesentery, and reproductive tracts. The stomach, pyloric caeca, and intestines were opened longitudinally, and all contents were washed into a petri dish using Ringer's solution. Mucosal surfaces of the gastrointestinal tract were examined for attached helminths. Visceral organs (heart, kidney, liver, and spleen) were removed from the body cavity, compressed between two slides (larger organs were compressed between two petri dishes) and examined for encysted helminths under a dissection or compound light microscope. The location of all endoparasites was recorded prior to their removal from the fish. Endoparasites were preserved in 70% ethanol except for nematodes and encysted parasites. Nematodes were first placed in glacial acetic acid before being transferred into 70% ethanol. A sample of the encysted parasites were removed from their cysts and stored in 70% ethanol until stained. For *Huffmanela* sp., the number of eggs in the swim bladder was quantified using a scale from 0 to 4, with 0 being no eggs detected and 4 meaning that more than 50% of the swimbladder contained eggs of *Huffmanela* sp. This method was outlined by Moravec (1987) and summarized in Table I.

Table I. Descriptions of the categories used to estimate the intensity of infection with eggs of *Huffmanela huffmani* (Moravec, 1987) in the ventral wall of the swim bladders of wild-caught centrarchids.

Infection-intensity category	Description
0	no eggs detected
1	trace infection; only a few eggs observed
2	up to 25% of the swim bladder infected with eggs
3	Greater than 25% of the swim bladder infected
4	Greater than 50% of the swim bladder infected

Morphological Identification of Parasites

Helminth mounts were prepared by isolating and staining the worms with Harris' hematoxylin or Semichon's acid carmine and mounting in gum damar (Sepulveda and Kinsella, 2013). Monogeneans were identified while they were alive. Parasites were identified using the textbook *Parasites of North American Freshwater Fishes* (Hoffman, 1999).

Statistical Analysis

Statistical analysis was performed in R (R Core Team, 2018). Mean length of *L. auritus* between the spring, summer, and fall collecting periods was analyzed with an ANOVA after confirming that all assumptions of the test were met. To determine if the parasite communities differed between spring, summer, and fall, a non-metric multidimensional scaling analysis (NMDS) with Bray-Curtis dissimilarities was performed (Oksanen et al., 2019). A statistical assessment of differences between the seasons was

performed with a permutational MANOVA of the Bray-Curtis distance matrix with 10,000 iterations using the adonis function in the vegan package (Oksanen et al., 2019). Prevalence and mean intensity for each parasite were also analyzed to determine if differences existed between the seasons. Prevalence was analyzed using logistic regression. ANOVAs were used to analyze differences in mean intensity.

RESULTS

A total of 30 fish were examined from May through October 2018. Twenty-six fish were *L. auratus*. There were also 4 other *Lepomis* sp. caught in this study: 2 were longear sunfish *(L. megalotis)*, 1 was a bluegill sunfish (*L. macrochirus*), and 1 was an orangespotted sunfish (*Lepomis humilis*; Table II). From these 30 fish, a total of 3535 helminths were recovered. This included 1 species of Monogenea, 2 species of Digenea, 1 species of Cestoda, 1 species of Acanthocephala, and 4 species of Nematoda.

Table II. Parasite intensity (prevalence in parentheses) of parasites from *Lepomis humilis* (n= 1), *Lepomis macrochirus* (n= 1), and *Lepomis megalotis* (n= 2) collected at the Head of the River Ranch.

Parasite	*Lepomis humilis*	*Lepomis macrochirus*	*Lepomis megalotis*
Posthodiplostomum minimum	407.00	37.00	4.50 (50%)
Capillaria cantenata	-	-	3.00 (50%)
Contracecum sp.	-	-	2.50 (100%)
Huffmanela sp.	4.00	-	1.50 (50%)
Cleidodiscus sp.	-	19.00	17.50 (100%)
Leptorhynchoides thecatus	-	22.00	9.00 (100%)

Host demographics

Lepomis auritus (n= 26) was the most frequently encountered *Lepomis* sp., so seasonal analysis was performed only on this species. Eight fish were caught in the spring (May 10th to June 21st), 12 were caught in the summer (June 22nd to September 21st), and 6 were caught in the fall (September 22nd through October). The mean length of *L. auritus* was 15.78 cm (SE= 0.52 cm) in spring, 16.43 cm (SE= 0.64 cm) in summer, and 18.16 cm

(SE=1.4 cm) in the fall. Quantile-quantile plots suggest that length was normally distributed, and variances were equal (Brown-Forsyth Test: $F_{2,23}=0.74$, $P=0.49$). The mean length of *L. auritus* did not differ between the three seasons (ANOVA: $F_{2,23}=1.82$, $P=0.18$).

Parasite Community

From *L. auritus*, the most common and abundant helminth was metacercariae of *Posthodiplostomum minimum*, which were found to infect 100% of all fish. Metacercariae of *Clinostomum marginatum* (prevalence = 11%) were encysted in various tissues. The gills were infected with *Cleidodiscus* sp. with a prevalence of 66%. Swim bladders were infected with the eggs of *Huffmanela* sp. (prevalence = 53%). Adults of *Leptorhynchoides thecatus* (prevalence = 53%), *Bothriocephalus* sp. (prevalence = 7%), and *Camallanus oxycephalus* (prevalence = 19%) were found in the intestine. Juveniles of *Capillaria contenata* (prevalence = 96%) and *Contracecum* sp. (prevalence = 15%) were encysted in tissue of the gastrointestinal tract (Table III).

Table III. Parasite mean intensity and prevalence (bootstrapped 95% CI in parenthesis) of parasites from *Lepomis auritus* (n= 26) collected at the Head of the River Ranch. *Posthodiplostomum minimum* does not have a bootstrapped 95% CI for prevalence because all fish were infected.

Parasite	Intensity	Prevalance
Clinostomum marginatum	13.67 (0.00 to 4.15)	11% (0% to 23%)
Posthodiplostomum minimum	83.77 (47.58 to 138.92)	100%
Camallanus oxycephalus	3.20 (0.11 to 1.23)	19% (4% to 35%)
Capillaria cantenata	15.88 (10.31 to 21.50)	96% (88% to 100%
Contracecum sp.	1 (0.04 to 0.31)	15% (4 to 31%)
Huffmanela sp.	2.64 (0.81 to 2.08)	53% (35% to 73%)
Bothriocephalus sp.	1.00 (0.00 to 0.12)	4% (0 to 19%)
Cleidodiscus sp.	12.47 (2.77 to 16.19)	66% (46% to 81%)
Leptorhynchoides thecatus	11.67 (2.00 to 13.73)	53% (35% to 73%)

To assess the differences in the parasite communities between the three seasons, NMDS and permutational MANOVA was performed. The ordination plot suggests that the spring, summer, and fall may constitute distinct groups based on their parasites (stress=0.177; Fig. 2). A permutational MANOVA supports this observation ($P = 0.018$). Furthermore, the permutational MANOVA supports an effect of fish length on the parasite community ($P = 0.028$). Intensity of each parasite species was not affected by the sex of the fish (Permutational MANOVA: $P = 0.624$). A series of ANOVAs were used as post-hoc tests to better understand which parasites may be contributing to these differences. For parasite intensities, a significant difference between the three seasons was observed only for *P. minimum* (ANOVA: $F_{2,23} = 3.45$, $P = 0.049$; Fig. 3). A Tukey's posthoc test suggests that parasite intensity differs only between spring and fall ($P = 0.049$). Length had a positive and

significant effect only on the intensity of *P. minimum* (ANOVA: $F_{1,24} = 22.56$, $P < 0.0001$;

Fig. 4). There was a significant effect of age of the host on intensity for *P. minimum* only

(ANOVA: $F_{1,24} = 7.18$, $P = 0.013$; Table IV). A significant positive effect of length on

prevalence of Contracecum sp. was also observed ($X^2 = 5.66$, df = 1, $P < 0.017$), but

prevalence did not differ across the seasons ($X^2 = 1.37$ df = 2, $P < 0.505$). Prevalence did not

differ for length or season for the remaining species (Logistic regression: *P*>0.05 for each

test).

Figure 2. Ordination plot from a non-metric multidimensional scaling procedure that examined the parasites of *Lepomis auritus* across the 3 seasons. The figure displays 95% confidence ellipses to illustrate the difference between the seasons, which were statistically significant (permutational MANOVA of dissimilarities: *P*<0.018).

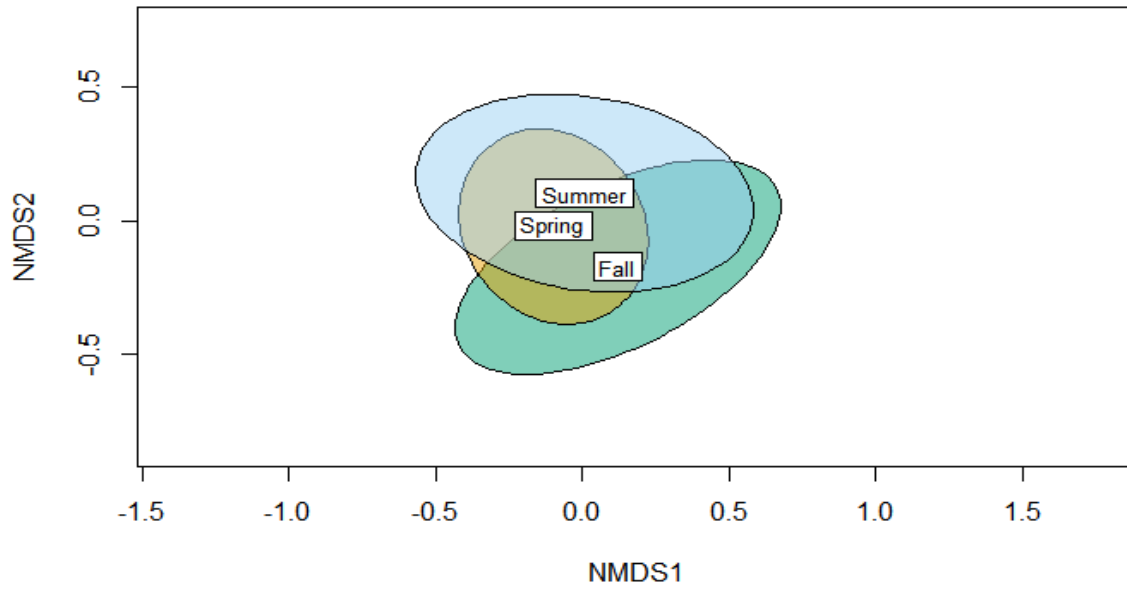Figure 3. Mean intensity of *Posthodiplostomum minimum* in *Lepomis auritus* for the spring, summer, and fall. The error bars are standard error. A difference was detected between the seasons, with mean intensity in the fall being higher than the values for both spring and summer.
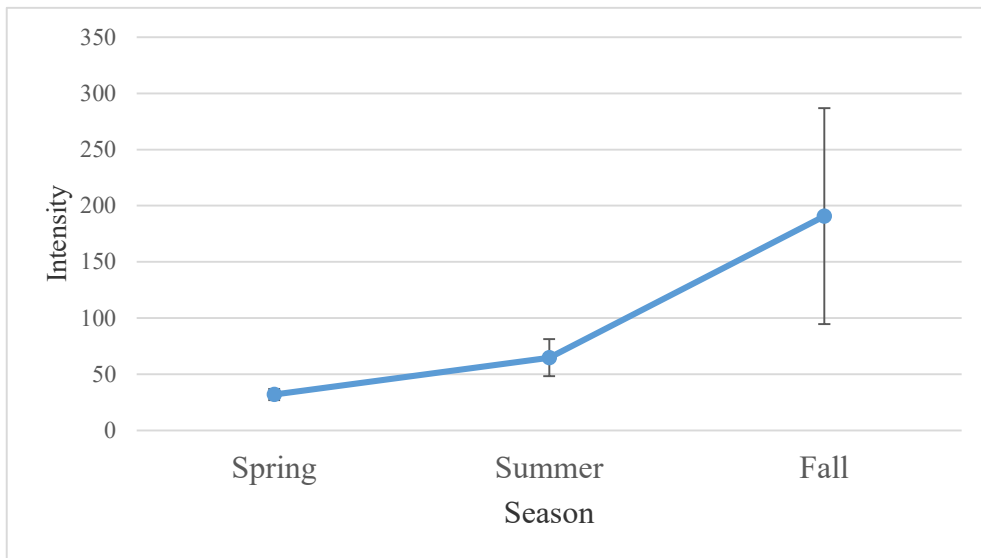
Figure 4. Standard length of *Lepomis auritus* is positively correlated to the mean intensity of

Posthodiplostomum minimum.
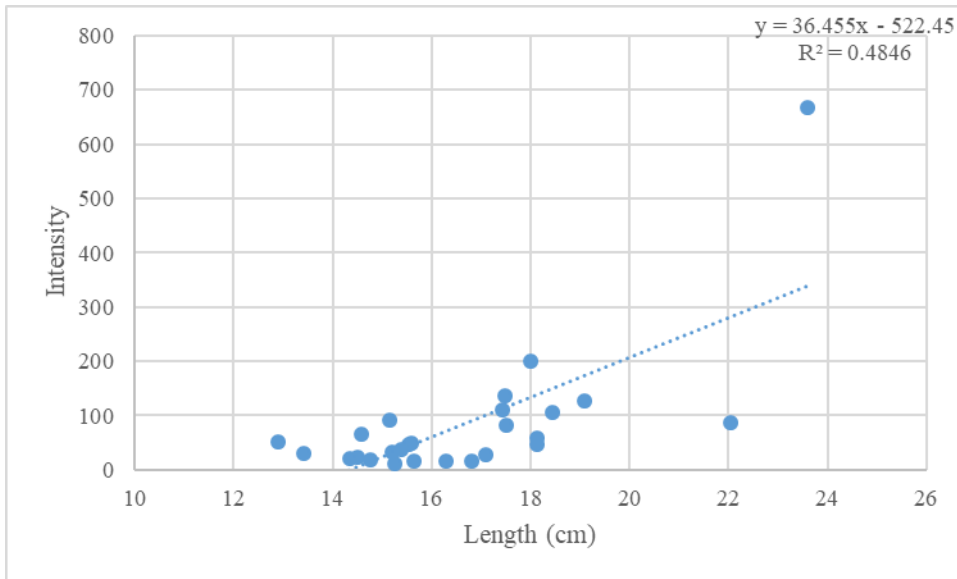
Table VI. Parasite mean intensity and prevalence from *Lepomis auritus* collected at the Head of the River Ranch for the three seasons. Bootstrapped 95% confidence intervals are included in parentheses. Values in bold indicate a significant difference was detected for that parasite species.

	Spring		Summer		Fall	
Parasite	Intensity	Prevalance	Intensity	Prevalance	Intensity	Prevalance
Clinostomum marginatum	-	-	27.00	8% (0% to 25%)	7.00	33% (0% to 66%)
Posthodiplostomum minimum	**32.12**	100%	64.75	100%	**190.7**	100%
Camallanus oxycephalus	-	-	3.00	25% (0% to 50%)	3.50	33% (0% to 66%)
Capillaria cantenata	14.00	100%	18.17	100%	11.17	83% (50% to 66%)
Contracecum sp.	-	-	1.00	25% (0% to 50%)	1.00	17% (0% to 50 %)
Huffmanela sp.	2.20	62% (25% to 87)	3.00	42% (16% to 66%)	2.75	67% (33% to 100%)
Bothriocephalus sp.	-	-	1.00	8% (0% to 25%)	-	-
Cleidodiscus sp.	16.62	50% (12% to 87%)	8.00	75% (50% to 100%)	1.75	67% (32% to 100%)
Leptorhynchoides thecatus	6.88	38% (12% to 75%)	13.86	58% (33% to 83%)	4.60	83% (50% to 100%)

DISCUSSION

All 9 parasite taxa documented in *L. auritus* in the South Concho River have been previously reported from other species of *Lepomis* (Hoffman, 1999). Additionally, numerous studies have also reported finding seasonal differences in the parasite community (Stromberg et al., 1975; Ingham et al., 1982; Fellis and Esch, 2004; Violante-Gonzalez et al., 2008). Parasite community structures often change because of seasonal variation in the abiotic and biotic environmental factors, which include changes in cercariae release from mollusks, temperature fluctuation, shifts in diet of both host and prey, and variation in the availability of intermediate hosts (Fellis and Esch, 2004; Violante-Gonzalez et al., 2008).

Total length of fish had a significant, positive effect on mean intensity for *P. minimum*. Similar trends have been found with other centrarchid species including largemouth bass (*Micropterus salmoides*), warmouth (*Lepomis gulosus*), and bluegill (Cloutman, 1975), four species of *Lepomis* (Fiorillo and Font, 1996), and *Lepomis miniatus* (Fiorillo and Font, 1999). This increase in mean intensity could be due to an increase in exposure because larger fish have lived longer allowing them to accumulate parasites.

Larger fish also have a higher energy demand that requires the consumption of more food or a change in diet as the fish grows. For example, *L. auritus* are primarily insectivores but can be opportunistic feeders (Flemer and Woolcott, 1966; Davis, 1972; Coomer et al., 1977; Cooner and Bayne, 1982). With increasing size, fish consume larger quantities of terrestrial insects (Cooner and Bayne, 1982) and other prey objects such as crustaceans. An increase in feeding on crustaceans could result in an increase in parasite diversity. An example of this phenomenon is *Contracaecum* sp. Larvae of *Contracaecum* sp. are long-lived

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in fish, which serve as intermediate hosts in the life cycle of the parasite (Szalai and Dick, 1990). Neves et al. (2013) suggest that host body size and diet composition are important factors influencing infections since *Contracaecum* sp. is acquired when the host ingests infected crustaceans (Koie, 1995). If larger fish supplement their diet with crustaceans, then the probability of infection with *Contracaecum* sp. is directly related to host size. As such, larger fish are more likely to be infected with and infected by more larvae of *Contracaecum* sp. Similarly, the presence of *C. oxycephalus* also suggests that the larger fish are eating copepods to supplement their diet since this parasite utilizes copepods as an intermediate host. However, infected copepods are only one possible transmission route to *L. auritus* for *Contracaecum* sp. and *C. oxycephalus*. Thomas (1937) observed that bass, *Micropterus* sp., also acquire *Contraceacum* sp. by feeding on fish, which suggests that they can be infected by consuming a parenetic fish host. Likewise, fish could acquire *C. oxycephalus* after consuming smaller, infected fish (Stromberg and Crites, 1974). Based on the presence of these nematodes, it is postulated that *L. auritus* are also consuming crustaceans and possibly smaller fish. Clearly, the lifecycle of the parasite, lifespan of the parasites, and multiple other biotic and abiotic factors can influence prevalence and intensity. Further research is needed to better understand if the pattern observed here is consistent with infection patterns of other fish in the region.

Helminths that utilize contact transmission have different factors than trophically-transmitted parasites that affect their prevalence and intensity within the fish hosts. The trematodes *P. minimum* and *C. complanatum* both infect fish when their free-swimming cercariae attaches to and penetrates the skin. These two parasites exhibited a prevalence of

100% and 11.5%, respectively. The large difference in the prevalence of these two parasites is likely associated with abundance of and prevalence in the first intermediate host. The first intermediate host of *P. minimum* is a snail of the family Physidae (Hoffman, 1999), whereas the first intermediate host of *C. complanatum* is a snail of the family Planorbidae (Dias et al., 2003; Hunter and Hunter, 1934). Physid snails are ubiquitous throughout most aquatic systems in North America including in the South Concho River. In contrast, Planorbidae snails, such as *Helisoma antrosum* and *Helisoma campanulatum*, which are known intermediate hosts of *C. complanatum* in the USA (Hunter and Hunter, 1935), are less common in the South Concho River. Difference in abundance of the first intermediate hosts in this river suggests that fewer snails overall are infected by *C. complanatum* compared to the number of physids infected by *P. minimum*. Consequently, fewer infected snails shedding cercariae will lead to fewer second intermediate hosts.

    Proteocephalus ambloplitis did not occur in any of the fish examined in this study. The life cycle of *P. ambloplitis* is a three-host life cycle with an adult bass as the definitive host (Fischer and Freeman, 1969). The parasite is trophically transmitted with amphipods as the first intermediate host, smaller centrarchids as second intermediate hosts, and bass as the definitive host (Hunter III, 1928; Fischer and Freeman, 1969). In the South Concho River, *L. auritus* could possibly serve as a second intermediate host. The infection would be acquired through direct consumption of procercoid-infected amphipods or pleurocercoid-infected fish. Fish with pleurocercoids transmit the parasite to the definitive host, bass, when they are consumed. In aquatic systems with bass, the prevalence of *P. ambloplitis* in *Lepomis* sp. ranges between 22% and 38% (Amin, 1990; McDaniel and Bailey, 1974; Wilson, 2003).

Why this common parasite was not found in any of the sunfish is unknown, but one explanation is that there is an extremely small bass population at the Head of the River Ranch that cannot support the parasite in this system. However, this assumption is not in agreement with personal observations on the South Concho River, which suggests bass are abundant. Additional explanations are that the parasite might not have colonized this portion of the headwaters of the South Concho River, or if the bass were stocked in this portion of the river, then the stocked fish did not contain adults of *P. ambloplitis*. Finally, the amphipod intermediate host may be missing from the system and thus the life cycle cannot be completed. More research on the parasites of bass along the Concho River, especially in the South Concho River, is needed to better understand the distribution of *P. ambloplitis*.

CONCLUSION

The parasite community of *L. auritus* in this section of the South Concho River was low in both diversity and abundance, which better matches the characteristics of an isolationist community as described by Holmes and Price (1986). To date, there have been no published reports of parasites in aquatic systems in or near San Angelo, Texas. Further research should be conducted to better understand the variation and structuring factors of parasite communities in this unexplored region of Texas. Future directions should include surveying other freshwater fish to determine if their parasite communities exhibit a similar pattern of seasonal variation as that observed in *L. auritus*.

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APPENDIX I

Angelo State University's Institutional Animal Care and Use Committee (Protocol #15-16).

ANGELO STATE UNIVERSITY

College of Graduate Studies & Research

Institutional Animal Care & Use Committee

February 25, 2017

Dr. Nicholas Negovetich
Associate Professor
Biology Department
Angelo State University
ASU Station #10840
San Angelo, TX 76909

Your proposed project titled, "Parasite survey in west Texas" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective February 25, 2017 and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583iacuc-annual-review-progressreport) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 15-16. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Chase Runyan, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee

VITA

Keith Robert Johnson attended Indiana State University from August 2013 to May 2017, where he completed his Bachelor of Science with a major in Biology and minor in Chemistry. Following graduation, he moved to San Angelo, TX, to continue his education as a master's student at Angelo State University. He attended ASU from August 2017 to May 2019 and completed his Master of Science degree in Biology. His major field of specialization was parasitology, where he developed skills in animal necropsy procedures, and handling and preparing parasite specimens.

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