Georgia Journal of Science

Volume 73 No. 2 *Scholarly Contributions from the Membership and Others*

Article 8

2015

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Sydney M. Anderson

Riccardo A. Fiorillo
Georgia Gwinnett College, rfiorill@ggc.edu

Tamara J. Cook

William I. Lutterschmidt
Sam Houston State University, lutterschmidt@shsu.edu

Recommended Citation

Anderson, Sydney M.; Fiorillo, Riccardo A.; Cook, Tamara J.; and Lutterschmidt, William I. (2015) "Helminth Parasites of Two Species of Lepomis (Osteichthyes: Centrarchidae) from an Urban Watershed and their Potential Use in Environmental Monitoring," *Georgia Journal of Science*, Vol. 73, No. 2, Article 8.
Available at: https://digitalcommons.gaacademy.org/gjs/vol73/iss2/8

HELMINTH PARASITES OF TWO SPECIES OF *LEPOMIS* (OSTEICHTHYES: CENTRARCHIDAE) FROM AN URBAN WATERSHED AND THEIR POTENTIAL USE IN ENVIRONMENTAL MONITORING

Sidney M. Anderson[1], Riccardo A. Fiorillo[2*], Tamara J. Cook[3]
and William I. Lutterschmidt[3,4*]

[1]Texas Veterinary Medical Diagnostic Laboratory
Texas A&M University, College Station, Texas 77843
[2]School of Science and Technology
Georgia Gwinnett College, Lawrenceville, Georgia 30043
[3]Department of Biological Sciences
Sam Houston State University, Huntsville, Texas 77341
[4]Texas Research Institute for Environmental Sciences
Sam Houston State University, Huntsville, Texas 77341

*Corresponding Authors: rfiorill@ggc.edu, lutterschmidt@shsu.edu

ABSTRACT

We provide a checklist of the common parasites of bluegill (*Lepomis macrochirus*) and redbreast sunfish (*Lepomis auritus*) collected from eight creeks within an urban watershed located in Columbus, Georgia. A total of 12 parasite species were observed from 427 fish dissected. Bluegill (n = 222) were infected with 11 species, which included five species of larval helminths (*Proteocephalus* sp., *Bothriocephalus* sp., *Posthodiplostomum minimum, Diplostomulum* sp. and *Clinostomum complanatum*), one adult trematode (*Pisciamphistoma stunkardi*), four adult nematodes (*Philometra* sp., *Philometra intraoculus, Spinetectus carolini* and *Camallanus oxycephalus*) and one adult acanthocephalan (*Neochinorhynchus cylindratus*). Redbreast sunfish (n = 205) were infected with the same parasite species, except for the absence of larval *Bothriocephalus* sp. and the presence of an adult trematode (*Crepidostomum cooperi*). Metacercariae of the trematode *P. minimum* were the most common parasite in both fish species. Similar parasite communities in these sunfishes suggest that these species share similar microhabitats within the watershed, food resources and foraging sites. Seasonal comparison between the number of summer and winter parasites indicate a greater mean number of parasites per host in summer. We discuss the utility of such baseline parasite data in the potential environmental monitoring of the Bull and Upatoi Creeks watershed.

Key Words: Checklist, trematodes, nematodes, cestodes, acanthocephalans, sunfish, Centrarchidae, *Lepomis macrochirus, Lepomis auritus*, Muskogee County.

INTRODUCTION

Parasite assemblages of sunfishes (*Lepomis*) have received much interest and have been extensively surveyed (McDaniel 1963, McGraw and Allison 1967, Harley and Keefe 1970, Meade and Bedinger 1972, McDaniel and Bailey 1974, Cloutman 1975, Deselle *et al.* 1978, Jilek and Crites 1980, Fiorillo and Font 1996, Wilson *et al.* 1996, Muzzall and Peebles 1998, Fiorillo and Font 1999) thus making them a good investigative model. However, parasite community structure in fishes can vary greatly with environment and geographic region (Holmes and Price 1986). While regional variation in parasite communities of *Lepomis* is well documented in the literature, environmental influences on temporal variation of parasites within a region or locality may be of specific interest for environmental monitoring (*e.g.*, Lafferty 1997, Kennedy 1997, Sures 2004). However, such monitoring is only possible with a baseline assessment of typically occurring parasites and their abundance. By documenting what parasites infect specific host fishes and their abundance in a natural system, investigators may be able to monitor ecosystem health and identify environmentally-induced disease (Overstreet 1997).

The use of parasites as a bioindicator of ecosystem health is not novel and has been reviewed extensively (Adams 1990, Marcogliese 2005). Parasites may be particularly useful in monitoring anthropogenic related pollution (Khan and Billiard 2007), as increased parasitism is often observed as host defense mechanisms are compromised by certain pollutants. For example, toxic chemicals impair mucus production in fishes, making them more susceptible to gill ciliates (*e.g.* Khan *et al.* 1994). In addition, most helminth parasites of fish rely on multiple hosts, connected through the food web or other ecological interactions, for completing their complex life cycles and thus their presence or absence may provide useful information about the ecological integrity of an aquatic system. By establishing baseline data, potential increases (or decreases, see Marcogliese 2004) in parasite richness and abundance could be used by future investigators as a mechanism for environmental monitoring (Lafferty 1997, Kennedy 1997, Sures 2004). Furthermore, both the presence of parasites and their abundance within a host individual (or population) may be costly and can be characterized specifically as disease. Such epidemiological information regarding disease and parasitism is becoming more common in monitoring the health of biological and aquatic systems (*e.g.*, Kennedy 1997, Hoffman 1999, Sures 2004).

A specific aquatic system of interest, the Bull and Upatoi creeks watershed, consists of eight creeks located within and just east of Columbus, Georgia (Figure 1). Both urban development and rapid changes in land-use are predicted to influence the environmental quality of this aquatic system. Additionally, potential land-use change along the northern boundary of Fort Benning has been of great interest and a stimulus for this funded study (ERDC-CERL Contract #DACA 42-00-C-0047). This study and another investigation of fish diversity (Martin and Lutterschmidt 2013) were aimed at investigating how urbanization and land-use type may influence biological diversity. Biodiversity and identification of potential indicator species could be used as ecological drivers in the application of the Land use Evolution and impact Assessment Model (LEAM) at Fort Benning.

LEAM was developed with funding from the National Science Foundation and simulates changes in land use across a landscape that may result from spatial and dynamic interactions among economic, ecological, and social systems (Deal 2001). Insight gained from LEAM simulations would inform environmental policy and planning and has been used with other models (Wang *et al*. 2005) to help in land-use management decisions.

Here we surveyed the parasites of bluegill (*Lepomis macrochirus*) and redbreast sunfish (*Lepomis auritus*) to: (1) inventory and provide a checklist of the parasites found in the Bull and Upatoi Creeks watershed, Muskogee County, Georgia, (2) compare seasonal variation in parasite richness and abundance, within a host species, (3) compare variation in parasite richness and abundance between host species, and (4) calculate indices of parasite biodiversity to potentially serve as an indicator (Marcogliese 2005) and ecological driver in future land-use models. These data may ultimately provide investigators with information on the parasites of two common fishes and needed baseline data to evaluate potential changes in environmental quality due to land-use change, urbanization, or pollution within an urban watershed of Columbus, Georgia.

MATERIALS AND METHODS

A total of 222 bluegill (*Lepomis macrochirus*) and 205 redbreast sunfish (*Lepomis auritus*) were collected from eight small creeks (Lindsey, Cooper, Flatrock, Bull, Dozier, Randall, Kendall and Baker) within the Bull and Upatoi Creeks watershed, Muskogee County, Georgia. Creeks are located perpendicular to Macon Road (U.S. Highway 22) east of Columbus, Georgia, and just north of the northern boundary of Fort Benning (Figure 1). We accessed each creek from Macon Road and sampled fish from stream pools and runs both north and south of where each creek intersects Macon Road. A backpack electro-fisher (Smith-Root® Inc.) was used to collect fishes which were transported live to our field laboratory and euthanized prior to dissection. Individuals that could not be freshly dissected, were frozen for transport and later dissection at Sam Houston State University, Huntsville, Texas. Fish were collected in February (winter) and August (summer) 2002, six months apart, to investigate potential temporal variation in host parasite communities. All field research, specimen collection, and dissections were conducted in accordance with the methods and protocols outlined by the American Society of Icthyologists and Herpetologists (1987) Guidelines for the Use of Fishes in Field Research. All fish were collected under a permit (29-WMB-01-147) issued to William I. Lutterschmidt by the Georgia Department of Natural Recourses.

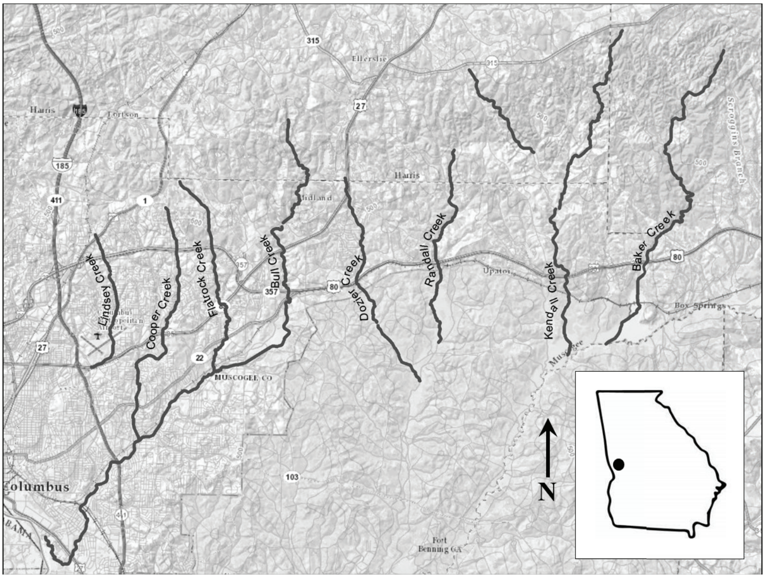

Figure 1. Location of the Middle Chattahoochee watershed in west Georgia. The eight sampled creeks within the smaller Bull and Upatoi creeks watershed are highlighted and are located within and east of Columbus, Georgia. The northern boundary of Fort Benning is shown and is located just south of Macon Road (US Highway 22). Map modified from Martin and Lutterschmidt (2013).

Body mass (g) and standard length (mm) of hosts were measured prior to dissection. The eyes, eye cavities, viscera (heart, spleen, liver, kidney and gonads), and intestinal tract (intestinal mesentery, intestinal lumen, pyloric ceca and stomach) were examined for helminth parasites. Gills were not examined for parasites (*e.g.*, Monogenes). All parasites were identified to the lowest possible taxon (Hoffman 1999). Trematodes were fixed in Berland's solution (9 parts acetic acid, 1 part 37% formaldehyde) and stored in alcohol-formalin-acetic acid (AFA). Nematodes were fixed in Berland's solution and placed in glycerine alcohol. Acanthocephalans were refrigerated in distilled water overnight to extrude the proboscis, and several small holes were made in the body wall with fine dissecting pins prior to fixation in AFA. Trematodes and acanthocephalans were stained with Semichon's Carmine procedure (Meyer and Olsen 1975), dehydrated in a graded alcohol series, cleared in xylene, and using gum damar, mounted on slides for positive identification. Nematodes were cleared and examined as temporary mounts in glycerine jelly.

Nematodes and acanthocephalans were observed only for presence or absence due to their low occurrence. Trematodes and cestodes were most abundant and therefore the seven species in these two groups were used in our community analyses to summarize species richness, total parasites, and the mean number of parasites per host. We used Shannon-Weiner indices of diversity (Shannon

1948 as cited in Zar 1999) to calculate species diversity (H') and evenness (J' = H'/H'max). Trematode and cestode counts were used to calculate percent similarity index (PSI) between fish species and all parasites species (n = 12) were used to calculate the Jaccard community coefficient (Table I). We used SigmaPlot© Version 11.0 and SPSS© Version 22 to run a 2 x 2 factorial, two-way analysis of variance with both species (bluegill and redbreast sunfish) and season (winter and summer) as independent factors and total parasite load per host fish as the dependent factor. Total parasite load data were corrected for normality and equal variance using the square-root transformation. All results are considered significant at $P \leq 0.05$.

Table I. A checklist of the parasites of the bluegill (*Lepomis macrochirus*) and redbreast sunfish (*L. auritus*) sunfishes within the Bull and Upatoi creeks watershed. Parasites are listed in order of greatest to least abundance. Either counts, presence (X), or absence (–) are indicated and followed by (prevalence) for each parasite species. Due to low occurrence, nematodes and acanthocephalans counts are not included. Trematode and cestode counts were used to calculate percent similarity index (PSI = 99.49%) between fish species, and all parasites species (n = 12) were used to calculate Jaccard community coefficient (J = 0.833). Asterisk (*) indicates larval form of the parasite.

Parasite Species (n)	***Bluegill L. macrochirus***	**Redbreast Sunfish L. auritus**
Trematodes		
*Posthodiplostomum minimum** (26742)	15810 (94.5%)	10932 (94.6%)
Diplostomulum sp.* (84)	18 (4.9%)	66 (8.2%)
Pisciamphistoma stunkardi (31)	12 (3.6%)	19 (6.8%)
*Clinostomum complanatum** (7)	3 (0.4%)	4 (1.9%)
Crepidostomum cooperi (3)	–	3 (0.9%)
Cestodes		
Proteocephalus sp.* (73)	50 (12.1%)	23 (6.8%)
Bothriocephalus sp.* (1)	1 (0.4%)	–
Nematodes		
Philometra intraoculis	X	X
Philometra sp.	X	X
Spinetectus carolini	X	X
Camallanus oxycephalus	X	X
Acanthocephalans		
Neochinorhynchus cylindratus	X	X

RESULTS AND DISCUSSION

Parasites (Table I) collected from bluegill (*Lepomis macrochirus*) and redbreast sunfish (*L. auritus*) from eight creeks of the Bull and Upatoi creeks watershed (Figure 1) have been previously documented in these hosts (see Hoffman 1999). Bluegill (n = 222, mean body mass (± SE) = 11.0 ± 0.54 g, mean standard length = 84.6 ± 1.35 mm) were infected with 11 parasites species including five larval helminths (*Proteocephalus* sp., *Bothriocephalus* sp., *Posthodiplostomum minimum, Diplostomulum* sp. and *Clinostomum complanatum*), one adult trematode (*Pisciamphistoma stunkardi*), four adult nematodes (*Philometra* sp., *Philometra intraoculus, Spinetectus carolini* and *Camallanus oxycephalus*) and an adult acanthocephalan (*Neochinorhynchus cylindratus*). Redbreast sunfish (n = 205, mean body mass = 14.6 ± 0.85 g, mean standard length = 88.4 ± 1.64 mm) were infected with the same parasites, except for the absence of larval *Bothriocephalus* sp. and the presence of an adult trematode (*Crepidostomum cooperi*).

Bluegill prey mostly on aquatic insect larvae, microcrustaceans, fallen terrestrial insects and occasionally plant matter/algae, which they likely consume accidentally while foraging for aquatic insects (Etnier 1971, Sadzikowski and Wallace 1976), and redbreast sunfish feed on surface dwelling invertebrates, insect larvae, crayfish, gastropods and fishes (Goldstein 2000, Murphy *et al*. 2005). From stomach contents of hosts, we found primarily finger-nail clams, freshwater snails, insect larvae, and algae in bluegill. We observed fewer of these prey in redbreast sunfish and noted partially digested fishes (Cyprinidae) suggesting that redbreast sunfish in this system are feeding on larger prey, including vertebrates. We might expect the parasites of these two *Lepomis* species to differ due to their differences in trophic strategies. While bluegill are generalists, other sunfishes (*e.g.*, redbreast sunfish and pumpkinseed) are considered specialists in their feeding ecology (*e.g.*, Goldstein 2000, Robinson *et al*. 1993). Regardless of these apparent differences in feeding strategies, these hosts shared 10 of the 12 helminth species observed in this study (Table I) and their parasite communities were similar (percent similarity index = 99.49%, Jaccard community coefficient = 0.833, Table I) which suggests that bluegill and redbreast sunfish may share foraging sites and food resources, as nine of the 12 helminths infect fish through the food web. However, the high degree of similarity between these two parasite communities is likely due to the high abundance of *P. minimum* representing 99% of all helminths. This parasite infects fish by penetrating through a host’s skin and encysting in its visceral organs. It is likely that both bluegill and redbreast sunfish co-occur in areas where physid snails, first intermediate host in the life cycle of this trematode, are actively shedding the parasite.

We found both low and similar parasite diversity (H’) and evenness (J’) for the two species of *Lepomis*, mostly due to the dominant abundance of *P. minimum* in both hosts (Tables I and II). Although more fish were collected in the winter (n = 241) than summer (n = 186) sample, the winter sample showed lower total parasites and greater difference in parasite diversity and evenness between host fishes (Table II). This might suggest that summer samples may be more repeatable and useful in the development of environmental monitoring regimes. With increased activity and frequent feeding during summer, fishes are more likely to acquire a parasite community that is more fully representative of the total helminth fauna available in the environment.

Table II. Summary of species richness, total parasites, mean (± standard error, SE) parasites per fish host, mean (± standard error, SE) *P. minimum* per fish host, species diversity (H') and species evenness (J') for bluegill (*L. macrochirus, Lm*) and redbreast sunfish (*L. auritus, La*) sunfishes collected in winter and summer 2002. Due to low occurrence, nematodes and acanthocephalans were included in the calculation of species richness only.

		Parasite Data					
Season	**Host (n)**	Species Richness	Total Parasites	Mean (SE) Parasites per Host	Mean (SE) *P. minimum* per Host	Species Diversity (H')	Species Evenness (J')
Winter	*Lm* (132)	5	6524	49.4 (7.9)	49.3 (7.9)	0.0068	0.0098
	La (109)	4	3591	32.9 (8.2)	32.7 (8.2)	0.0181	0.0300
Summer	*Lm* (90)	4	9370	104.1 (13.3)	103.3 (13.3)	0.0225	0.0374
	La (96)	5	7456	77.7 (8.9)	76.6 (8.9)	0.0343	0.0490

Although the parasite richness between these host fish species is similar, we found significant differences in the total parasites infecting bluegill and redbreast sunfish (F = 7.44; df = 1, 423; P = 0.007, Table III) with bluegill also harboring significantly more metacercariae of *P. minimum* (t = 4.038, df = 425, P = 0.042). However, both hosts appear to be equally exposed to snails shedding *P. minimum* cercariae, as prevalence of this parasite in both hosts is quite similar (Table I). *Posthodiplostomum minimum* shows preference, among centrarchid fishes, for *Lepomis* spp. suggesting this trematode may be less of a generalist than previously reported (Lane *et al.* 2015). Similarly, Fellis and Esch (2004) showed that green sunfish (*L. cyanellus*) are typically found in the littoral zone and presumably in closer proximity of snails shedding *P. minimum* cercariae than bluegill. However, green sunfish harbored significantly less worms than bluegill suggesting that green sunfish may be less susceptible to infection. Assuming both bluegill and redbreast sunfish have similar probability of encounter with this parasite, bluegill appear to be the preferred host in this watershed. Further laboratory studies are needed to critically examine a "differential susceptibility" versus a more parsimonious "differential exposure" hypothesis to explain these observed differences in how *P. minimum* parasitizes *Lepomis* species.

We also found significant differences between the winter and summer samples (F = 67.98; df = 1, 423; P = 0.001, Table III), suggesting that the use of parasites for environmental monitoring must control for seasonal effects. A non-significant interaction between fish species and season indicate that both host fishes experience similar changes in seasonally dependent parasite loads (Table III). Bluegill and redbreast sunfish were each more heavily parasitized in summer than in winter, with fish collected in winter harboring less than half the number of parasites infecting fish collected in summer (Table II). These data suggests greater winter mortality for fishes that are heavily parasitized. Lemly and Esch (1983, 1984a and 1984b) showed a similar pattern as bluegills infected with more than 50 metacercariae of *Uvulifer ambloplitis*, a close relative of *P. minimum*, suffered significantly higher winter mortality than less infected conspecifics. Like *U. ambloplitis, P. minimum* metacercariae are not lost once recruited, which leads to an accumulating worm burden and the potential for heavily infected individuals which may be more susceptible to environmental stressors (see Lutterschmidt *et al*. 2007). Previous studies (*e.g.* Spall and Summerfelt 1970, Amin 1984, Amin 1990, and Wilson and Camp 2003) have found substantial variation in the prevalence, abundance, and mean intensity of *P. minimum* over several geographic regions but *P. minimum* metacercariae are typically the dominant parasite. Because of the high abundance of *P. minimum* across creeks within this urban watershed, this parasite species may serve as good response variable for environmental monitoring. Metacercariae of this parasite in both fish species infected the pericardial sac of the heart, liver, spleen, kidney, gonads, and mesenteries of the peritoneal cavity.

Table III. Summary statistics of a 2 x 2 factorial, two-way analysis of variance showing significance differences between host fish species (bluegill and redbreast sunfish) and season (winter and summer) for total parasite load.

Source of Variation	**df**	**SS**	**MS**	**F**	**P**
Species	1	144.84	144.84	7.44	0.007
Season	1	1323.04	1323.04	67.98	0.001
Interaction (Species x Season)	1	7.57	7.57	0.39	0.533
Residual	423	8232.79	19.46		
Corrected Total	426	9659.97	22.67		

Both field and laboratory studies suggest that there are 2 subspecies of *P. minimum* which infect North American freshwater fishes. *Posthodiplostomum minimum minimum* infect minnows (Cyprinidae), while *P. minimum centrarchi* infects fishes in the family Centrarchidae (Hoffman 1999). However because *P. minimum* has such a wide distribution and can infect so many different fish hosts, absolute identification to species can be obtained only from molecular techniques or from adult parasites recovered from experimental infection with the metacercariae.

Pisciamphistoma stunkardi was the second most common parasite in both fish species and was observed in the intestinal lumen and pyloric ceca. *Proteocephalus* sp. was observed in the liver and associated connective tissues of both fish species, *Diplostomulum* sp. was found in the aqueous humor and lens of the eye in both fish species, while *Crepidostomum cooperi* was observed in the digestive tract and *Bothriocephalus* sp. was recovered from the liver in only the redbreast sunfish.

There may be numerous abiotic and biotic considerations for the temporal changes in parasite diversity and abundance, and the assumption that infection increases/decreases with environmental pollution and stress (Schwaiger 2001) must be carefully evaluated (Sures 2004, Marcogliese 2005). However, there is overwhelming evidence that parasites abundance may serve as an excellent metric for the monitoring of environmental change (*e.g.*, Mackenzie 1999, Lafferty 1997, Overstreet 1997, Sures *et al.* 1999, Sures 2001). Although parasites are not classically viewed as sentinel or indicator species, as are macroinvertebrates (Rosenburg and Resh 1993) and some fishes (Chovanec *et al.* 2003), the advantages of parasites are both numerous and great (Sures 2004, Marcogliese 2005). Additionally, both the presence and abundance of gill parasites (*e.g.*, monogenes) might serve as an additional indicator for environmental quality as they have direct interface with both the host and the environment. Monogenes were not investigated here due to our limited expertise with this group.

Here we establish a baseline for the parasites and their abundance in two common host fishes, the bluegill and redbreast sunfish, within eight creeks of the Bull and Upatoi Creeks watershed, Muskogee County, Georgia. Because this watershed is subject to future land-use change and anthropogenic disturbances associated with urbanization, we hope these data may serve future investigators and provide baseline data for the potential establishment or enhancement of environmental monitoring protocols and programs for the creeks of Columbus, Georgia.

ACKNOWLEDGEMENTS

We thank Laurieanne Dent, Daniel S. Millican, and Dennis K. Wasco for their dedicated field assistance as paid technicians on this project. We also acknowledge a research grant awarded to William I. Lutterschmidt by the Engineer Research and Development Center (ERDC) and the Civil Engineering Research Laboratory (CERL) of the US Army Corps of Engineers (ERDC-CERL Contract #DACA 42-00-C-0047). We acknowledge the assistance provided by Gordon A. Plishker and all personnel of the Texas Research Institute for Environmental Studies (TRIES) at Sam Houston State University. Special thanks to David R. Hoffpauir (Operations Manager of the TRIES GIS Laboratory) for his assistance in GIS mapping. We also thank Hugh Westbury of the Strategic Environmental Research and Development Program (SERDP), Ecosystem Management Project (SEMP), and Harold (Hal) Balbach of the US Army ERDC and CERL for their dedicated interest in and support of this project. Our sincere thanks to Julia C. Buck, William F. Font and Autumn Smith-Herron for their reviews of this manuscript.

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