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Chris T. McAllister

Eastern Oklahoma St. College, cmcallister@se.edu

Henry W. Robison

retired, hwrobison@yahoo.com

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Cover Page Footnote

The Arkansas Game and Fish Commission (AG&F) provided a Scientific Collecting Permit to CTM. We thank Dr. Ronald M. Bonett (University of Tulsa) for technical assistance and several AG&F staff from the Mountain Home office who provided assistance in the collection of specimens, especially Kenneth Shirley.

Clinostomum marginatum (Digenea: Clinostomidae) from Fishes of Crooked Creek, Boone and Marion Counties, Arkansas

C.T. McAllister^{1*}, and H.W. Robison²

¹Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

²9717 Wild Mountain Drive, Sherwood, AR 72120

*Correspondence: cmcallister@se.edu

Running Title: *Clinostomum marginatum* from Crooked Creek Fishes

Abstract

Crooked Creek is a renowned trophy (blue ribbon) Smallmouth Bass (*Micropterus dolomieu*) fishing stream. This fish, however, has been previously reported to commonly harbor some of the highest population densities of the digenean trematode parasite, *Clinostomum marginatum*, otherwise known as “yellow grub”. The parasite infects the orobranchial cavity, gills, and peritoneal cavity of Smallmouth Bass. Historical studies on this fish over the last 3 decades or more from various sites on the creek have shown that *M. dolomieu* also have high mean abundances but fish from upstream sites had lower prevalence than those collected from downstream sites. Here, we survey several fishes from the creek for *C. marginatum* as well as compare our data on *M. dolomieu* to some of the previous studies conducted on the same species from the watershed. In addition, a new host record for *C. marginatum* is documented in the Ozark Bass, *Ambloplites constellatus*.

Introduction

Crooked Creek is a 129 km (80 mi.) long Ozark highland White River tributary in Boone, Marion, and Newton counties of northern Arkansas (Fig. 1). The stream's headwaters (36°06'47"N, 93°02'19"W) begins in Newton County at Sulphur Spring just south of Harrison on the north side of Sulphur Mountain and east of Marble Falls and flows north passing under State Highway (St. Hwy.) 206 just west of Elmwood (Boone County). The stream continues north traveling parallel (eastward) to St. Hwy. 7 passing through the southeastern part of Harrison and under US 65. It immediately turns eastward, beginning a long series of meanders and then turns southeast passing the communities of Pyatt and Summit (Marion County) and under US 62. It continues to flow eastward roughly paralleling US 62 (south) and crossing under St. Hwy. 14

Figure 1. Crooked Creek and 5 accesses (•). 1 = Harmon; 2 = Pyatt; 3 = Snow; 4 = Kelly's Slab; 5 = Doe Bambi. Abbreviation: WR (White River).

south of Yellville. The stream continues further eastward and goes under St. Hwy. 101 near Rea Valley until entering the confluence of the White River just south of Buford Station below the town of Cotter in Baxter County (36°10'15"N, 93°06'55"W).

The yellow grub, *Clinostomum marginatum* (Digenea: Clinostomidae) has a long published history of scientific documentation from basses (*Micropterus* spp.) from Crooked Creek. Studies on this trematode in mostly Smallmouth Bass (*M. dolomieu*) have originated from seminal research done by Daly and associates (Daly *et al.* 1987, 1991, 2002, 2007; Daly 2013, 2014). In addition, McAllister *et al.* (2016) reported *C. marginatum* from Northern Studfish, *Fundulus catenatus* from Crooked Creek at Kelly's Slab, Yellville, Marion County.

Clinostomum marginatum has also been found in Channel Catfish (*Ictalurus punctatus*) from a commercial pond in northwestern Arkansas (Daly and Singleton 1994). Tumilson *et al.* (2019) reported a hyperinfection of *C. marginatum* in a Black Bullhead (*Ameiurus melas*) from the upper White River, Arkansas. Other records of *C. marginatum* in Arkansas fishes include: **FUNDULIDAE**: Golden Topminnow, *Fundulus chrysotus* (McAllister *et al.* 2020); **ICTALURIDAE**: Ozark Madtom, *Noturus albater* and Caddo Madtom, *Noturus taylori* (McAllister *et al.* 2015); **CENTRARCHIDAE**: Bluegill, *Lepomis macrochirus*, Warmouth, *Lepomis gulosus*

(Becker and Cloutman 1975; Cloutman 1975); Spotted Bass, *Micropterus punctulatus*, Largemouth Bass, *Micropterus salmoides* (Becker and Cloutman 1975; Daly *et al.* 1999, 2002; Daly 2013).

The larval (metacercarial) stage of this trematode is commonly found in a variety of fish and Hoffman (1999) reported that it is likely capable of infecting any species of freshwater fish. The first intermediate host is a planorbid snail which has been infected from ova deposited in watersheds by definitive host fish-eating ardeid birds (herons, egrets and bitterns) (Olsen 1967). Larval forms are found in tissues of fish, amphibians, and reptiles (Bonett *et al.* 2012; Calhoun *et al.* 2019).

Robison *et al.* (2011) reported a total of 65 species of fishes distributed among 14 families in Crooked Creek. The purpose of our study was 2-fold: (1) to survey some of those fishes from the Crooked Creek watershed within Boone and Marion counties for *C. marginatum*, and (2) specifically, compare some of our data gathered from *M. dolomieu* to the historical studies involving this fish species previously done on specimens from the various parts of the creek.

Materials and Methods

During 2010 and 2011, 11 species of fish ($n = 203$) were collected with a boat electrofisher or traditional hook and line from 5 access sites (see Fig. 1, upstream to downstream) along Crooked Creek as follows: (1) Harmon (36°15'16.182"N, -92°57' 3.4092"W), (2) Pyatt (36°14'46.8456"N, -92°50' 4.722"W), (3) Snow (36°14'13.0164"N, -92°48' 16.7292"W), (4) Kelly's Slab (36°13'50.5596"N, -92°42'34.2216"W), and (5) Doe Bambi near White River (36°14'09.7836"N, -92°32'09.7332"W) (Fig. 1). Fish species and numbers collected and examined included: **CENTRARCHIDAE**: 99 *M. dolomieu*, 11 *M. salmoides*, 1 *M. punctulatus*, 13 Ozark Bass (*Ambloplites constellatus*), 9 Green Sunfish (*Lepomis cyanellus*), 30 Longear Sunfish (*L. megalotis*); **CATOSTOMIDAE**: 11 Golden Redhorse (*Moxostoma erythrurum*), 22 Northern Hogsucker (*Hypentelium nigricans*); **LEUCISIDAE**: 3 Southern Redbelly Dace (*Phoxinus erythrogaster*); 3 Central Stoneroller (*Camptostoma oligolepis*), 1 Striped Shiner (*Luxilus chrysocephalus*). Fish were measured for total length (TL, mm) and killed by severing their cervical vertebrae. The gills and orobranchial cavity were immediately examined macroscopically for metacercariae; the peritoneal cavity and visceral organs were not examined invasively. The location of parasites was noted and individual worms were counted. Several specimens were retained as vouchers

and fixed in 70–95% DNA grade ethanol. A voucher specimen of *C. marginatum* from *M. dolomieu* was accessioned into GenBank as MF38189. Population parameters follow Bush *et al.* (1997).

Results and Discussion

Of the 203 fish examined, 43 (21%) were infected with *C. marginatum* (Fig. 2, Table 1). Hosts included: 31 of 99 (31%) *M. dolomieu*, 1 of 1 (100%) *M. punctulatus*, 4 of 11 (36%) *M. salmoides*, 2 of 13 (15%) *A. constellatus*, 3 of 9 (33%) *L. cyanellus*, and 2 of 22 (9%) *H. nigricans* (Table 1); none of the other 48 fish, including 30 *L. megalotis*, 11 *M. erythrurum*, 3 *P. erythrogaster*, 3 *C. pullum*, or 1 *L. chrysocephalus* was infected. A new host record is documented for *C. marginatum* in *A. constellatus*.

Figure 2. *Clinostomum marginatum* from *Micropterus dolomieu* from Crooked Creek. (A) Two metacercariae within capsule on gills. Scale bar = 1 mm. (B) Single stained metacercaria. Scale bar = 250 μ m.

Smallmouth Bass from Crooked Creek (collected between Pyatt and Yellville) have been reported with a very high prevalence (78%) of *C. marginatum*, containing an average of 23.2 metacercariae per fish with a range from 1 to 227 worms (Daly *et al.* 1987). In a follow-up study, Daly *et al.* (2002) reported a prevalence of 72% at Pyatt, 84% at Clear Creek, 91% at Turkey, 86% at Yellville, and 70% near the White River; however, orobranchial as well as invasive soft tissue counts were included. In the present study, only 37% of *M. dolomieu* from Kelly's Slab (Access 4,

Yellville) were infected containing an average of 2 metacercariae per fish with a range from 1 to 6 worms (Table 1). When other comparative sites on the creek for *C. marginatum* in *M. dolomieu* are compared to Daly *et al.* (2007), who only used non-invasive orobranchial counts (same as present study), prevalence at Harmon (Access 1) was higher in our study (24% vs. 16%) but at the far downstream site near the confluence with the White River at Doe Bambi, prevalence was lower in our study (Access 5, 40% vs. 59%). However, samples sizes were unequal. Also, we did not find any *C. marginatum* in Smallmouth Bass at our Pyatt (Access 2) and Snow stations (Access 3), which may also be the result of smaller sample sizes ($n = 2$ and 6 fish, respectively).

In conclusion, Smallmouth Bass from all reaches of Crooked Creek harbor a high prevalence of *C. marginatum*. When our data is compared to previous studies of this trematode infecting *M. dolomieu* at 3 of the same stations on the creek (Daly *et al.* 2007), prevalence was lower at each station, (except Harmon site) in the present study (Table 2). In addition, however, we did discover that *M. dolomieu* from the upper reaches of the creek possessed lower prevalence compared to those of lower reaches; this is in agreement with previous historical studies. We suggest additional surveys of *M. dolomieu* for yellow grub that include 3 parameters: (1) the density of intermediate hosts, (2) the density of host fishes, and (3) the river flow at different reaches.

Acknowledgments

The Arkansas Game and Fish Commission (AG&F) provided a Scientific Collecting Permit to CTM. We thank Dr. Ronald M. Bonett (University of Tulsa) for technical assistance and several AG&F staff from the Mountain Home office who provided assistance in the collection of specimens, especially Supervisor Kenneth Shirley (retired).

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Table 1. Prevalence, mean intensity, and mean abundance of *Clinostomum marginatum* in fishes from Crooked Creek.

Species	Access	Prevalence*	Mean intensity	Abundance
Centrarchidae				
<i>Amploplites constellatus</i> †	Kelly's Slab	2/9 (22%)	1.5	0.33
	Harmon	0/1 (0%)	—	—
	Pyatt	0/2 (0%)	—	—
	Doe Bambi	0/1 (0%)	—	—
<i>Lepomis cyanellus</i>	Kelly's Slab	2/4 (50%)	1.0	0.50
	Harmon	0/1 (0%)	—	—
	Pyatt	1/4 (25%)	1.0	0.25
<i>Lepomis megalotis</i> ‡	Kelly's Slab	0/21 (0%)	—	—
	Harmon	0/5 (0%)	—	—
	Pyatt	0/3 (0%)	—	—
	Doe Bambi	0/1 (0%)	—	—
<i>Micropterus dolomieu</i>	Kelly's Slab	17/46 (37%)	2.17	0.80
	Harmon	6/25 (24%)	1.67	0.40
	Pyatt	0/2 (0%)	—	—
	Snow	0/6 (0%)	—	—
	Doe Bambi	8/20 (40%)	3.75	1.50
<i>Micropterus punctulatus</i>	White River	1/1 (100%)	31	—
<i>Micropterus salmoides</i>	Kelly's Slab	1/6 (17%)	2.0	0.33
	Doe Bambi	3/5 (60%)	1.5	0.60
Catostomidae				
<i>Hypentelium nigricans</i>	Kelly's Slab	2/22 (9%)	1.0	0.09
<i>Moxostoma erythrurum</i>	Kelly's Slab	0/10 (0%)	—	—
	Pyatt	0/1 (0%)	—	—
Leucisidae				
<i>Campostoma oligolepis</i> ‡	Kelly's Slab	0/3 (0%)	—	—
<i>Luxilus chrysocephalus</i> ‡	Kelly's Slab	0/1 (0%)	—	—
<i>Phoxinus erythrogaster</i> ‡	Kelly's Slab	0/3 (0%)	—	—

*Prevalence = number infected/number examined (%).

†New host record.

‡Not previously reported as a host (Hoffman 1999).

||Previously reported as a host (Hoffman 1999).

***Clinostomum marginatum* from Crooked Creek Fishes**Table 2. Comparative prevalence, mean intensity, and mean abundance of *Clinostomum marginatum* in *Micropterus dolomieu* from upstream to downstream sites on Crooked Creek.

Access	Prevalence*	Abundance	Maximum Abundance	Reference
Huzzah Creek	3/10 (30%) 1/10 (10%)	2.10 0.10	10 1	Daly <i>et al.</i> (2002) *Daly <i>et al.</i> (2007)
Harmon	6/25 (24%) 83/120 (69%) 24/120 (16%)	0.40 3.0 0.33	3 27 3	This study Daly <i>et al.</i> (2002) *Daly <i>et al.</i> (2007)
Pyatt	0/2 (0%) 19/27 (72%) 16/27 (59%)	— 14.3 1.85	— 57 10	This study Daly <i>et al.</i> (2002) *Daly <i>et al.</i> (2007)
Snow	0/6 (0%)	—	—	This study
Clear Creek	36/43 (84%) 21/42 (49%)	19.0 1.84	92 17	Daly <i>et al.</i> (2002) *Daly <i>et al.</i> (2007)
Turkey	97/107 (91%) 67/105 (64%)	83.0 3.7	179 25	Daly <i>et al.</i> (2002) *Daly <i>et al.</i> (2007)
George's Creek	26/31 (84%)	22.5	144	Daly <i>et al.</i> (2002)
Kelly's Slab	17/46 (37%) 38/44 (86%) 22/44 (49%)	0.80 9.40 1.14	6 76 11	This study Daly <i>et al.</i> (2002) *Daly <i>et al.</i> (2007)
Doe Bambi†	8/20 (40%) 36/51 (70%) 31/53 (59%)	1.50 105.0 23.0	5 2,500 400	This study Daly <i>et al.</i> (2002) *Daly <i>et al.</i> (2007)

*Daly *et al.* (2007) used orobranchial counts only.†Our Doe Bambi access site equates to White River site of Daly *et al.* (2002, 2007).

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