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An Initial Survey on Black-Spot Disease (Digenea: Strigeoidea: Diplostomidae) in Select Arkansas Fishes

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Running Title: Black-Spot Disease (Digenea: Strigeoidea: Diplostomidae) in Select Arkansas Fishes

Much has been published on black-spot disease caused by infection with neascus-type metacercariae or "black grub" of digene trematodes (e. g., *Neascus* sp., *Uvulifer ambloplitis*, *Diplostomum* sp., and others) of North American fishes (see Hoffman 1999); however, little is known about these parasites in Arkansas fishes, and most of that information is on game fishes (Cloutman 1974, Becker and Cloutman 1975). Hlass et al. (1998), in a study on biotic integrity of streams of the lower Ouachita River, examined 15 species of fishes for black-spot disease but did not delineate those which were infected. Here, we provide some new host records for these parasites from select fishes of the state.

As the primary purpose of our study was an initial survey to report only what fish species were infected, we did not study the number of hosts infected and examined (prevalence of infection), abundance and/or intensity of infection per Bush et al. (1997). Using an American Optical stereomicroscope (Model 568, Buffalo, NY), we examined the external anatomy of both game and non-game fishes deposited in the museum collection at Henderson State University (HSU), Arkadelphia. Fishes had been previously fixed in 10% formalin and stored in 60% isopropanol or ethanol. We removed the caudal fin containing embedded metacercariae from a single fish and placed the tissue into a vial of 70% ethanol; this was followed by decalcification of the tissue mass in a 1% HCL solution for 24 hr. After decalcification, we used standard histological techniques to prepare the tissue for light microscopy following Presnell and Schreibman (1997). The tissue was dehydrated in a graded series of increasing ethanol solutions (70-100%), cleared with xylene, and infiltrated and embedded in paraffin. A single paraffin tissue block was trimmed and sagittally sectioned into ribbons 10 µm in thickness using a rotary microtome. The ribbons were affixed to microscope slides using Haupt's adhesive on a slider warmer while floating them on a 2% neutral buffered formalin solution. We stained the slides using Harris hematoxylin followed by counterstaining with eosin (H & E) and then applied coverslips using Permount® (Fisher Scientific) mounting medium.

For photomicroscopy, we used a Nikon Eclipse 600 epifluorescent light microscope with a Nikon DXM 1200C digital camera (Nikon Instruments Inc, Melville, NY). Macrophotography was accomplished by using the above camera on a Nikon SM2800 stereomicroscope. Common names of fishes are capitalized and scientific names follow Page et al. (2013).

A total of 47 fish species (within four families) collected between 1976 and 2012 were found to harbor the digene parasite that causes black-spot disease (Table 1). These included 2 species of catostomids, 22 species of cyprinids, 2 species of centrarchids, and 21 species of percids (Table 1). Interestingly, several members of the Percidae were commonly infected (41% of all Arkansas percid species) and this may be related to their habit of lying on or burying in the substrate (Robison and Buchanan 1988) and perhaps coming more often into closer contact with the first intermediate snail hosts. Not surprisingly, nearly a third of these counties (Fig. 1) where positive fishes were collected was centered around the Arkadelphia area (Ouachitas) where collections were made by one of us (RT) and other personnel at HSU.

The life cycle of the parasite is quite complex. It involves 2 intermediate hosts, a planorbid snail (*Helisoma* spp.), which is infected with miracidial, daughter sporocyst, and cercarial stages in the digestive gland and liver, and second intermediate host, a fish, infected with the metacercarial stage in the dermis, muscle, and fins (Fig. 2). When cercariae

leave the snail, they enter a fish where it remains in the metacercarial stage. The host fish surrounds the cyst with a response by depositing black pigment (melanin, Fig. 2) that gives the disease its name while the parasite secretes a hyaline cyst around itself (Fig. 3).

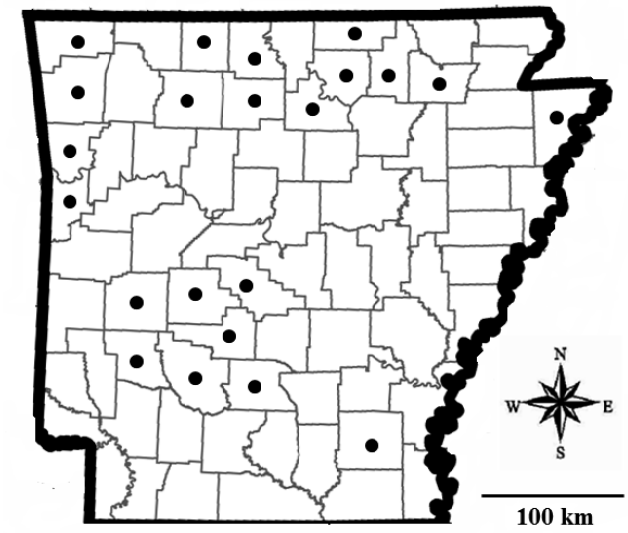

Figure 1. Twenty-two Arkansas counties where fishes were collected with black-spot disease.

Figure 2. Black-spot on Creek Chub, *Semotilus atromaculatus* (Pike Co., HSU 1928).

When another fish or fish-eating bird (typically a great blue heron or belted kingfisher) eats an infected fish, the fluke develops into an adult in about one mo in the intestinal tract of this definitive host. Unembryonated eggs are released in feces of the definitive host where free-swimming miracidia emerge and penetrate a snail; thus, the cycle repeats itself (Olsen 1974, Schell 1985, Roberts et al. 2012).

Although this disease is usually not pathogenic to fish, studies do show that infected juvenile fish can experience heavy blood losses, physiological stress, and perhaps, even death (Hunter and Hunter 1934, Krull 1934, Lemly and Esch 1983). Some species of fish have been shown to lose weight when infected with the black spot as well. To prevent or help control the incidence of black-spot, research has focused on decreasing the snail population.

In conclusion, we document 30 new host records for fishes with black-spot disease. With further surveys, we expect additional Arkansas fish species to be added to the list of hosts and additional data included on prevalence, intensity of infection and abundance.

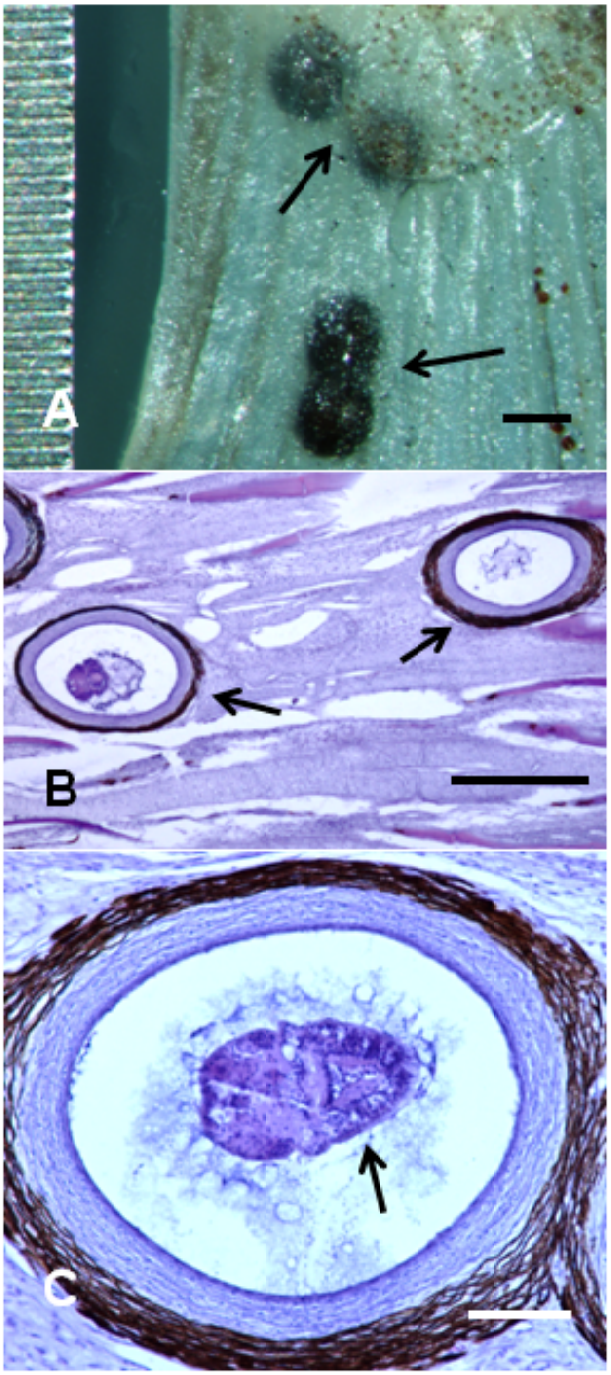

Figure 3. Black-spot on Hornyhead Chub, *Nocomis biguttatus* (Stone Co., HSU 2386). (A) Caudal fin with black-spot (arrows); scale bar = 500 µm. (B) Microscopic view of two black-spot cysts (arrows); scale bar = 500 µm. (C) Closer view of black-spot showing melanin deposition and hyaline cyst with metacercaria (arrow); scale bar = 100 µm.

Table 1. Arkansas fishes from the HSU collection infected with black-spot disease.

Family/Species	County and Watershed	Date Collected	HSU #
Catostomidae			
*Erimyzon claviformis**	Dallas Co., E Tulip Creek	21 Apr. 1999	2373
*Moxostoma duquesnei**	Pike Co., Caddo River	2 Apr. 1994	342
Cyprinidae			
Campostoma anomalum	Boone Co., Long Creek	22 Mar. 1999	2634
*C. oligolepis**	Fulton Co., Spring River	15 Apr. 2005	3043
*C. spadiceum**	Clark Co., Caddo River	19 Mar. 2005	3326
*Chrosomus erythrogaster**	Carroll Co., S Fork Dry Creek	22 Mar. 1999	2609
*Cyprinella venusta**	Clark Co., Terre Noir Creek	2 Mar. 2005	3016
Cyprinella whipplei	Clark Co., L'Eau Frais Creek	19 Feb. 1994	669
*Erimystax harryi**	Fulton Co., Spring River	11 Mar. 2012	3484
*E. x-punctatus**	Pike Co., Caddo River	2 Apr. 1994	335
*Luxilus cardinalis**	Benton Co., Sugar Creek	5 May 1994	1228
*L. chrysocephalus**	Hot Spring Co., Holly Creek	17 Jan. 1999	2483
*L. pilsbryi**	Searcy Co., Holder Creek	5 Apr. 1997	1820
	Stone Co., N Sylamore Creek	20 Mar. 1999	2377
*L. zonatus**	Fulton Co., Spring River	18 Apr. 1997	1729
*Lythrurus umbratilus**	Hot Spring Co., L'Eau Frais Creek	22 Mar. 1997	1491
Nocomis biguttatus	Izard Co., Piney Creek	20 Feb. 1999	2417
	Stone Co., W Livingstone Creek	21 Mar. 1999	2386
Notemigonus chrysoleucus	Clark Co., Mill Creek	15 Apr. 1991	8
*Notropis boops**	Hot Spring Co., Curl Creek	22 Jul. 1995	1103
*N. greenei**	Crawford Co., Frog Bayou	9 Jul. 1991	3223
N. ortenburgeri	Clark Co., Hollywood Creek	25 Jun. 1978	3205
*N. telescopus**	Sharp Co., Mill Creek	18 Mar. 1984	949
Opsopoeodus emiliae	Clark Co., Tupelo Creek	25 Feb. 1999	2342
Pimephales notatus	Montgomery Co., Little Missouri River	26 Feb. 1994	666
	Pike Co., Blocker's Creek	29 Apr. 1994	623
Semotilus atromaculatus	Montgomery Co., Lick Creek	2 Apr. 1994	370
	Pike Co., Bear Creek	20 Apr. 1997	1928
Centrarchidae			
*Ambloplites ariommus**	Montgomery Co., Caddo River	30 Jul. 1993	230
*A. constellatus**	Stone Co., Sylamore Creek	19 Nov. 1976	3296
Percidae			
*Ammocrypta clara**	Lawrence Co., Strawberry River	24 Sept. 1976	3235
*Crystallaria asprella**	Drew Co., Saline River	23 Jun. 1972	3232
Etheostoma blennioides	Fulton Co., S Fork Spring River	24 Mar. 1997	2032
E. caeruleum	Marion Co., Crooked Creek	25 Mar. 1997	2012
*E. chlorosoma**	Hot Spring Co., Holly Creek	17 Jan. 1999	2477
*E. collettei**	Hot Spring Co., Ouachita River	6 Mar. 1994	932
*E. euzonum**	Newton Co., Buffalo River	25 Mar. 1997	1919
E. flabellare	Crawford Co., Lee Creek	27 Apr. 1984	3272
*E, gracile**	Mississippi Co., Little River	15 Mar. 1997	1905
*E. juliae**	Newton Co., Buffalo River	25 Mar. 1997	1922
*E. pallididorsum**	Montgomery Co., Collier Creek	1 May 1997	1734
*E. proeliare**	Clark Co., Little Brushy Creek	18 Feb. 1999	2407
	Hot Spring Co., Caney Creek	22 Mar. 1994	1004
*E. radiosum**	Montgomery Co., Caddo River	2 Apr. 1994	363
	Pike Co., Wolf Creek	16 Feb. 1997	1443
E. spectabile	Washington Co., W Fork White River	19 Aug. 2010	3370
*E. whipplei**	Clark Co., Caddo River	2 Feb. 1975	1335
	Pike Co., Wolf Creek	26 Mar. 1994	2678
E. zonale	Fulton Co., S Fork Spring River	24 Mar. 1997	2034
Percina caprodes	Garland Co., Cooper Creek	20 Aug. 1993	96
	Hot Spring Co., Lake Catherine	17 Apr. 1994	393
P. copelandi	Sebastian Co., Vache Grasse Creek	24 May 1997	2164
P. maculata	Hot Spring Co., DeLisle Creek	25 Feb. 1997	1480
*P. sciera**	Sebastian Co., Vache Grasse Creek	24 May 1997	2163
Sander vitreus	Hot Spring Co., Lake Catherine	24 Mar. 1997	2109

*New host record

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