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Bothriocephalus acheilognathi and Other Intestinal Helminths of *Cyprinella lutrensis* in Deep Creek, Kansas

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ABSTRACT: We investigated the intestinal parasites of a wild fish population in a Kansas stream to determine the prevalence and abundance of potentially harmful parasites. In total, 180 red shiners (*Cyprinella lutrensis*) were collected from 6 sites in October–November 2007. Fifteen Asian tapeworms (*Bothriocephalus acheilognathi*) were recovered from 13 fish (prevalence of 7.2%). Prevalence did not differ among sites; however, *B. acheilognathi* abundance was greatest at the site of a public fishing area. A total of 39 roundworms (*Rhabdochona canadensis*) were recovered from 28 fish (prevalence of 15.6%). Prevalence did not differ among sites, nor did abundance. However, mean abundance tended to be about 50% greater at the site of a public fishing area compared to all other sites. This paper documents the presence of both *B. acheilognathi* and *R. canadensis* in Kansas and offers a compilation of the known potential impacts these parasites may have on the native, federally endangered Topeka shiner (*Notropis topeka*).

Parasitic infections in native fishes are common, and although negative effects of these infections are generally minimal, certain parasites can cause severe pathology, and even mortality, in their target host (Bauer et al., 1973; Scott and Grizzle, 1979; Hoole and Nisan, 1994; Brouder, 1999; Salgado-Maldonado and Pineda-Lopez, 2003). An example of such a pathogenic parasite, the Asian tapeworm (*Bothriocephalus acheilognathi* Yamaguti, 1934), has recently been isolated from cyprinid fishes in Kansas, although to date, no publication documents its presence (Hoffman, 1999; Choudhury et al., 2006). Its first appearance coincided with its chance discovery in late 2004 in a captive population of federally endangered Topeka shiners (*Notropis topeka*) at the University of Kansas Field Station and Ecological Reserves. Later, in November 2006, staff scientists representing the Kansas Biological Survey, the Kansas Department of Wildlife and Parks, and the U.S. Fish and Wildlife Service isolated *B. acheilognathi* from several red shiners (*Cyprinella lutrensis*) and 1 redfin shiner (*Lythrurus umbratilis*) in Deep Creek, a Kansas stream known to contain Topeka shiners. Most recently, *B. acheilognathi* was isolated from a sample of golden shiners (*Notemigonus crysoleucas*) collected from a bait shop in Lawrence, Kansas in February 2007.

While *B. acheilognathi* is considered to be most lethal in cultivated fish populations (where increased infection intensities occur), the concern is that pathogenicity associated with low infection intensities has also been observed in several wild species, including the Topeka shiner (Scott and Grizzle, 1979; Granath and Esch, 1983; Hoole and Nisan, 1994; Brouder, 1999; Heckmann, 2000; Salgado-Maldonado and Pineda-Lopez, 2003; Koehle and Adelman, 2007). In addition, *B. acheilognathi* exhibits low definitive host specificity (Hoffman, 1999; Salgado-Maldonado and Pineda-Lopez, 2003; Hansen, 2006) and post-cyclic transmission (Hansen et al., 2007), the combination of which contribute to rapid dissemination of this invasive tapeworm.

The worldwide distribution of *B. acheilognathi* is largely unknown, and awareness of its potential to have a negative impact on native fish species is highly limited. In the United States, it has been observed in several states, with recent documentation extending its range northward into Canada (Choudhury et al., 2006). Currently, however, management and control of *B. acheilognathi* in native fish populations is minimal. The objective of this study was to identify the cyprinid-parasitizing intestinal helminths in a prairie stream inhabited by the endangered Topeka shiner, while confirming and officially documenting the presence of *B. acheilognathi* in Kansas. Sampling revealed the presence of a second parasite not previously recorded in Kansas, *Rhabdochona canadensis* (*Rhabdochona* Railliet, 1916), so this publication serves to document its presence as well.

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Deep Creek, in northeast Kansas, is a 4th order tributary to the Kansas River and was selected because the stream contains both the Topeka shiner, a federally endangered cyprinid, and the red shiner, a non-endangered cyprinid. Six sample sites were chosen based on location and accessibility, ranging from the upper portion of the watershed (site A, 26.5 km upstream from the confluence with the Kansas River), with sites (B–E) located approximately every 5 km downstream to the Kansas River, and 1 site located in the Kansas River (site F, 9.5 km upstream of the confluence with Deep Creek).

Red shiners were sampled in this study because of their known ability to function as a definitive host for *B. acheilognathi* (Hoffman, 1999, Choudhury et al. 2004), their known abundance in Kansas, and their possession of life history characteristics similar to the Topeka shiner. Thirty red shiners were collected at each site and transported to Kansas State University, where they were kept in fiberglass holding tanks until necropsy. Individuals were identified, measured, and killed via decapitation. The intestinal tract was cut loose at the anus and esophagus and dissected in phosphate-buffered saline (pH 7.0) using a binocular dissecting microscope. All intestinal parasites were collected and identified.

Cestodes were carefully removed from the intestinal epithelium using fine-bristled paint brushes and placed in room temperature distilled water, where they were allowed to relax. Once the tapeworm's movements slowed, they were placed in 5% ethanol for 48 hr after which they were transferred into 70% ethanol where they were fixed and stored. Specimen whole mounts were stained using Semichon's acetocarmine, dehydrated through a graded alcohol series, cleared in toluene, and mounted in Canada balsam. All tapeworms were identified as *B. acheilognathi* using keys from Hoffman (1999) and Scholz (1997). A specimen fixed in 70% ethanol was deposited in the Harold W. Manter Laboratory (HWML) parasite collection at the University of Nebraska–Lincoln State Museum, Lincoln, Nebraska, accession no. 48816.

Nematodes were removed from the intestine and straightened in ice-cold, phosphate-buffered saline for 60 sec and then suspended in 70% ethanol for fixation and storage. Nematodes were cleared and mounted using Grey and Wess media and identified as *R. canadensis* (Moravec and Arai, 1971; Moravec and Huffman, 1988; Hoffman, 1999). A male specimen was deposited in the HWML parasite collection at the University of Nebraska–Lincoln State Museum, Lincoln, Nebraska, accession no. 48815.

We use the terms abundance, mean intensity, and prevalence in accordance with Margolis et al. (1982). Frequency was defined as the number of fish infected. To determine if the mean length of infected and non-infected fish differed for each parasite, *t*-tests were used. An analysis of variance (ANOVA) was used to examine the relationship between parasite abundance and site proximity to the Kansas River. Wald's chi-square analysis was used to test for differences in parasite prevalence among sites. Results were considered significant at $\alpha = 0.05$.

In total, 180 red shiners, ranging from 29–65 mm total length, were collected from all sites. Two different intestinal parasites were isolated; both were helminths. Fifteen Asian tapeworms were recovered from 13 fish (prevalence of 7.2%; Table I). *Bothriocephalus acheilognathi* was found at 4 of 6 sample sites (B–E); it was absent from the farthest upstream site (site A) and the site in the Kansas River (site F). All tapeworms were located within the anterior portion of the digestive tract, with only 1 isolate being gravid. Overall, infection intensity ranged from 0–2 tapeworms per fish examined, with a mean intensity of 1.2 tapeworms per infected fish. Prevalence of *B. acheilognathi* ranged from 0–26.7%, but did not differ among sites ($\chi^2 = 8.65$, df = 5, $P = 0.124$). However, *B. acheilognathi* abundance was greatest at the site of a public fishing area (site B) compared to all other sites ($F = 4.21$, df = 5, 174, $P = 0.0012$). Mean length (mean = 44 mm) of fish infected with *B. acheilognathi* did

TABLE I. Parasites isolated from red shiners collected from Deep Creek, Kansas, 2008 (headwater site A downstream to the confluence with the Kansas River site E, and the mainstem Kansas River, site F) and statistical computations by sample site.

Species Recovered	Site	Parasites isolated (n)	Infected fish (n)	Mean intensity (parasites / infected fish)	Prevalence (% infected)	Abundance (no. of parasites / no. of fish)
Bothriocephalus acheilognathi	A	0	0	–	–	–
	B*	9	8	1.125	26.7%	0.30
	C	1	1	1.0	3%	0.03
	D	3	2	1.5	6.7%	0.10
	E	2	2	1.0	6.7%	0.067
	F	0	0	–	–	–
	Total	15	13	1.15	7.2%	0.083
Rhabdochona canadensis	A	9	7	1.286	23.3%	0.30
	B*	11	5	2.2	16.7%	0.367
	C	7	3	2.333	10%	0.233
	D	7	6	1.167	20%	0.233
	E	2	2	1.0	6.7%	0.067
	F	2	2	1.0	6.7%	0.067
	Total	38	25	1.52	13.9%	0.211

* Site B is the location of a public fishing area.

not differ from non-infected fish (mean = 45.5 mm; $t = 0.61$, df = 148, $P = 0.541$).

Rhabdochona canadensis was isolated at every site, with 15.6% of fish being *R. canadensis*-infected (Table I). Thirty-nine roundworms were recovered from 28 red shiners. Infection intensity ranged from 0–5 roundworms per fish, with a mean intensity of 1.39 roundworms per infected fish. No female isolates were gravid. Prevalence of *R. canadensis* ranged from 6.7–23.3%. Prevalence did not differ among sites ($\chi^2 = 5.86$, df = 5, $P = 0.320$), nor did abundance ($F = 1.0$, df = 5, 174, $P = 0.4165$). However, mean abundance tended to be about 50% greater at the site of a public fishing area compared to other sites. The mean length (mean = 49 mm) of red shiners infected with *R. canadensis* was greater than that of non-infected fish (mean = 45 mm; $t = -2.53$, df = 148, $P = 0.013$).

Although the Asian tapeworm has been associated with retarded growth at low infection intensities in some fish species, including the Topeka shiner (Koehle and Adelman, 2007), we observed no difference in length between *B. acheilognathi*-infected and non-infected red shiners ($P = 0.029$). *Bothriocephalus acheilognathi* abundance was different between sample sites, an observation that may be attributed to a number of factors. For example, *B. acheilognathi* is transmitted directly to its definitive host upon consumption of infected copepods (Chubb, 1981; Hansen et al., 2007), and it is possible that morphological characteristics of the river are not ideal for copepods at every location. Community structure and, therefore, predator-prey interactions may differ, causing variability in host consumption of infected copepods. Seasonal population dynamics of copepod communities may play a role (Riggs and Esch, 1987a; Marcogliese and Esch, 1989; Vincent and Font, 2003), as could variations in water temperature between sites (Chubb, 1981; Granath and Esch, 1983). Piscivorous birds have been demonstrated to passively transfer Asian tapeworms (Prigli, 1975; Chubb, 1981), adding local avian habitat to the list of potential contributors. It is possible that infected baitfish released by anglers could be a source for *B. acheilognathi* introduction, or that higher nutrient loads at fishing areas accessible to the public create a more productive habitat. Also, in the Deep Creek watershed, there are approximately 150 privately owned small impoundments, and it is feasible that landowners near some sites have introduced infected grass carp into their impoundments in an effort to control macrophyte production. Obviously, additional research would be required to determine the precise cause of this observed variability in abundance, as this study was designed to investigate the presence of potentially harmful parasites to stream fishes in Kansas, and no data regarding copepod variability–habitat or stream morphology and site temperatures were collected.

Only a single gravid tapeworm was found in this study. The scarcity of gravid females suggests that our fall sampling occurred near the end of optimal growth conditions for *B. acheilognathi*, and this coincides with previous observations that *B. acheilognathi* growth and development is restricted during winter months (Bauer et al., 1973; Chubb, 1981; Granath and Esch, 1983; Riggs and Esch, 1987b). Because *B. acheilognathi* is prevalent year-round (Chubb, 1981; Granath and Esch, 1983; Marcogliese and Esch, 1989), we believe that our isolates are representative of the population in Deep Creek, although summer sampling may yield a slight increase in prevalence.

Limited information is available regarding the distribution, in nature, of our other isolated parasite, *R. canadensis*. Within the United States, records indicate its prevalence in Texas, Michigan, Nebraska, and now Kansas (Moravec and Huffman, 1988; Muzzall et al., 1992; Barger and Janovy, 1994). The *R. canadensis* species uses mayfly nymphs as intermediate hosts (Barger and Janovy, 1994) and, unlike the Asian tapeworm, it exhibits a high level of specificity for its definitive host (Hoffman, 1999). It is known to infect very few fish species throughout the world and only *C. lutrensis* in the locality of this study (Barger and Janovy, 1994). Our results indicate that mean body size of red shiners infected with *R. canadensis* was slightly larger than that of uninfected fish, and pathogenicity has not been associated with *R. canadensis* infection. No concerns regarding fish growth or survival have been expressed in other literature and, as no studies examining histopathology have been conducted, no information is available implicating *R. canadensis* as a threat to the endangered Topeka shiner or to any other fish species. Differences in abundance and prevalence of *R. canadensis* were not observed among sites, although mean abundance tended to be highest at the site that functions as a public fishing area.

The present study documents the presence of *B. acheilognathi* and *R. canadensis* in Kansas' streams that are also known to contain the federally endangered Topeka shiner. Although *B. acheilognathi* is considered to be most lethal in cultivated fish populations, it remains a threat to native species for 3 reasons: (1) *B. acheilognathi* exhibits a high capacity for transmission, (2) the definitive host range for this tapeworm is very broad, and (3) pathogenicity has been demonstrated in wild fish species, several of which are endangered. While little is known about the effects of *R. canadensis* infection on native fish, the documentation of its isolation in Kansas will provide regional managers with a basis for future research. More intensive sampling is needed for a precise estimate of the distribution of *B. acheilognathi* and *R. canadensis* in Kansas, and additional research is required to determine the threat these parasites pose to native fish species in the Great Plains.

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