

Infection of American Bullfrog Tadpoles, *Lithobates catesbeianus* (Shaw), by Anchor Worm, *Lernaea cyprinacea* L., in Streams of Northeastern Ohio

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Abstract: In some years, the copepod parasite *Lernaea cyprinacea* L. (Anchor Worm) parasitizes fishes in the Grand River, Conneaut Creek, and the Ashtabula River of northeastern Ohio. Here, I report on the occurrence and prevalence of this parasite on *Lithobates catesbeianus* (Shaw) (American Bullfrog) tadpoles at sites in reaches of these streams.

Keywords: *Lernaea cyprinacea*, Anchor Worm, *Lithobates catesbeianus*, American Bullfrog, amphibian parasite

Introduction

The copepod *Lernaea cyprinacea* L. (Anchor Worm) is primarily an opportunistic ectoparasite of freshwater fishes (Demaree 1967, Eisen 1977, Enders and Rifenburgh 1928, Wilson 1914). It also infects amphibians, primarily tadpoles (Baldauf 1961; Hoffman 1976; Kupferberg et al. 2009; Ming 2001; Tidd 1934, 1962), caudates including *Dicamptodon ensatus* (Eschscholtz) (Kupferberg et al. 2009) and the axolotl *Amyxstoma mexicanum* (Shaw and Nodder) (Carnevia and Speranza 2003), and several orders of insects (McAllister et al. 2011). *Lernaea cyprinacea* is a Eurasian species that has become widespread globally through the introduction of fishes, often via the aquarium trade (Tidd 1934) or fish culture (Enders and Rifenburgh 1928, Hoffman 1976). The infection is usually first recognized through observation of barbel-like filaments extending from various locations on the body of the host. In fish, the copepods typically anchor on or at a fin base (Whitaker and Schlueter 1975, Wilson 1914; pers. obs.). In amphibians, *L. cyprinacea* may embed posterior to the papillae of the mouth (Baldauf 1961); in or near the groove of the body and tail juncture, especially near the vent and the origin of the hind legs (Alcalde and Batistoni 2005, Kupferberg et al. 2009, Tidd and Shields 1963); or near the spiracle (Bird 1968; Shields and Tidd 1968.).

The life cycle of *L. cyprinacea* includes three naupliar free-swimming larval stages (two nauplii and a metanauplii), followed by five parasitic copepodid stages. Larval entry into the tadpole host is through the spiracle (Bird 1968, Shariff and Somerville 1986); the parasitic larvae attach inside the mouth or to gill filaments within the branchial chamber (Shields and Tidd 1963) or to the integument (Tidd 1970). Sexual differentiation and copulation occur during the fifth copepodid stage. Following copulation, the males die within 24 h, whereas the female moves to the exterior surface integument, attaches, and feeds on the mucosa of the host (Shariff and Somerville 1986, Shields and Tidd 1963, Tidd 1970). She then penetrates the integument and undergoes metamorphosis, during which the body segments elongate and the cephalothorax transforms and develops four horns, two of which are branched (Enders and Rifenburgh 1928). The horns embed and anchor the female in the integument, in muscle, and often in organs within the coelom (Tidd and Shields 1963). Two egg sacs develop on the distal end of the elongated parasite; they may each contain 50–250 eggs (Shields and Goode 1978, Streckler and Yanong 2017) and are replaced multiple times (Fig. 1). The entire life cycle requires only 18–25 days at water temperatures of 25–30 °C and is temperature dependent (Grabda 1963, Streckler and Yanong 2017). Development is slowed in all stages at lower temperatures (<20 °C; Shields and Tidd 1968) and the overwintering population consists of transformed females (Nakai and Kokai 1931, Shields and Tidd 1968). Infections of native species *Lithobates catesbeianus* (American Bullfrog) and *Lithobates clamitans* (Latreille) (Green Frog) tadpoles by *L. cyprinacea* in streams of central Ohio were first described by Tidd (1962, 1970) and Tidd and Shields (1963). Fishes infected with *L. cyprinacea* were netted at numerous sites over the years in northeastern Ohio stream drainage systems; consequently, the copepod is widely distributed in these streams and is environmentally available to infect amphibians. Here, based upon long-term observations, I report on infections of amphibian tadpoles by *L. cyprinacea* in reaches of three streams in northeastern Ohio.

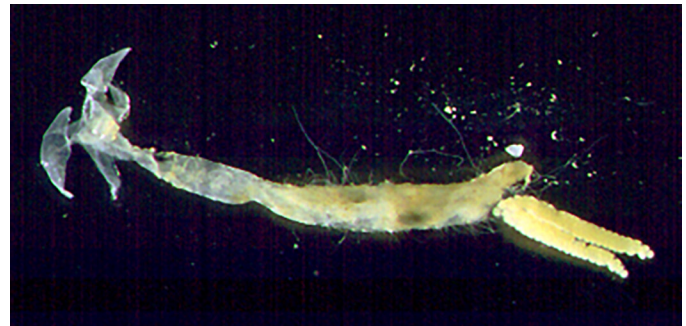


Figure 1. Adult female *Lernaea cyprinacea*, illustrating the anterior anchor (left) and two posterior egg sacs (right).

Methods

Field observations of *L. catesbeianus* tadpoles were made in the Grand River, Ashtabula River, and Conneaut Creek of Lake and Ashtabula counties of northeast Ohio while conducting vertebrate and crayfish surveys from 1993–2017 (Fig. 2). During an amphibian population study in the Grand River in 1993, numerous second-year *L. catesbeianus* tadpoles were netted, examined, and found to be infected with mature female *L. cyprinacea*. Thereafter, netted tadpoles in reaches of all northeast Ohio streams were examined for lernaids from July through early September. Sampling effort to net and examine tadpoles was not evenly distributed between sites. Site 1 in both the Grand and Ashtabula rivers were sites visited repeatedly during population studies of *Necturus*. Other sites were sampled once annually on various dates or irregularly over the years, the presence of *L. catesbeianus* and *Lernaea* having been detected during earlier fish surveys. Beginning in 1999, the number of *L. cyprinacea* and their attachment positions were recorded. Parasite prevalence (number of infected tadpoles/total number of tadpoles inspected) was calculated for sites in years when tadpoles were encountered. Morphological abnormalities of host tadpoles were noted in the field, and eight parasitized tadpoles or metamorphosing individuals with morphological abnormalities were transported to the lab and reared to determine whether metamorphosis would proceed normally. Tadpoles were reared in dechlorinated tap water in a 20-gallon (85 L) aquarium at room temperature ($21\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) under 8 h of fluorescent lighting supplemented with plant grow lights; they were fed tetra fish food and algae pellets twice weekly until metamorphosis was complete.

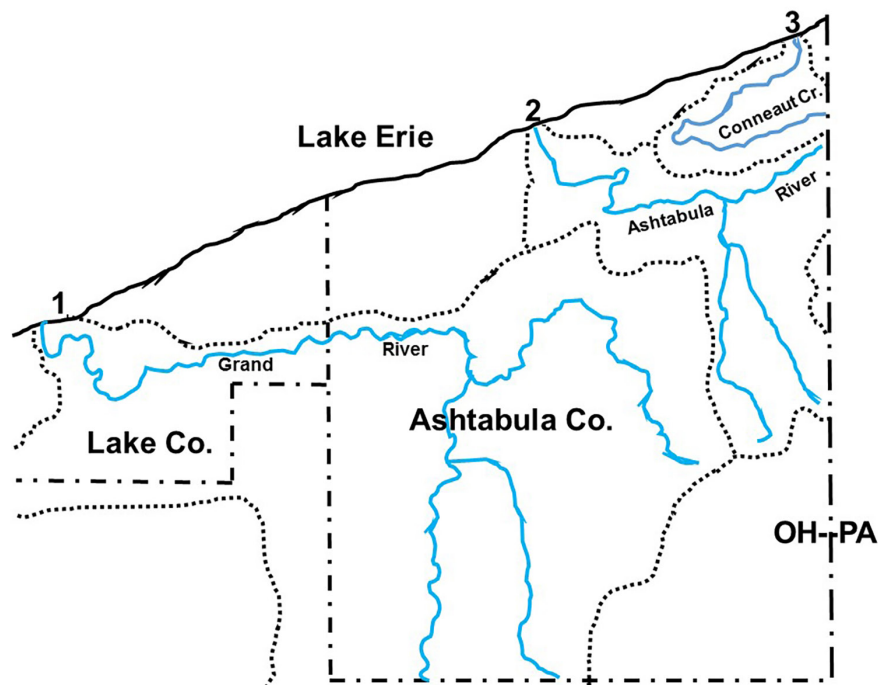


Figure 2. Map of Ashtabula County and eastern Lake County, showing the three drainage systems studied and their relative position in northeast Ohio: Grand River (1), Ashtabula River (2), Conneaut Creek (3). Blue lines represent the streams, dotted lines show the drainage demarcations, and alternating dot-dash lines represent county and state lines.

Results

First- or second-year tadpoles of *L. catesbeianus* were not present or were present in low numbers in the reaches of streams during some years when stream studies were conducted. At sites where only first-year tadpoles were captured, no tadpoles were found infected with adult female *L. cyprinacea*. However, it is probable that some first-year tadpoles were infected with earlier, microscopic stages of the parasite's life cycle on the gills or in the oral chamber, which would not be observable in the field. All mature *L. cyprinacea* were found on second- or, in a few cases (one of three), third-year tadpoles. The intensity of the infections and the prevalence of parasites are presented for sites within the reaches of the streams studied in Table 1. Most parasitized tadpoles were infected with one copepod; however, tadpoles with 2–7 copepods in various stages of development were not uncommon. Female *L. cyprinacea* were embedded posterior to the papillae of the mouth, in or near the groove of the body–tail junction, or near the vent or the origin of the hind legs (Fig. 3). They also were attached near the spiracle, on the tail near its base, on the thigh, and on the distal end of the shank. Infected tadpoles frequently showed edema of the thigh and subdermal hemorrhaging around the site of anchor penetration; connective (scar) tissue encircling the external base of the mature female was present (Fig. 3). Tadpoles and metamorphosing individuals with parasites embedded near or at the tail base

or along its length often developed various degrees of tail curvature. Eight metamorphosing tadpoles exhibiting moderate to severe tail curvature were maintained in captivity in the laboratory until the tail had been completely resorbed; metamorphosis resulted in juvenile frogs that appeared normal and moved without impediment. Only one out of 1489 tadpoles was observed in the field to have a developmental abnormality (a J-shaped tail); that tadpole was apparently not parasitized by *L. cyprinacea*.



Figure 3. Metamorphosing *Lithobates catesbeianus* with five embedded Anchor Worms, showing observed tail curvature.

Discussion

These results represent the first report of *L. cyprinacea* infecting *L. catesbeianus* in northeastern Ohio streams. *Lernaea cyprinacea* is a thermophilic species, and its rate of development varies greatly with water temperature (Grabda 1963, Kupferberg et al. 2009, Shields 1978.). Although water temperatures at stream sites we sampled were not routinely recorded, variation in the intensity of the infection and prevalence of *L. cyprinacea* may be attributed to yearly differences in water temperature caused by modifications of stream discharge, precipitation patterns, or alterations of riparian zone vegetation that modify stream shading. The number of generations per year is dependent upon environmental temperature and changes with latitude and climate (Grabda 1963). Geographic expansion is likely in areas experiencing variable weather patterns with elevated seasonal temperatures from year to year (Plaul et al. 2010) or with global warming (Kupferberg et al. 2009). Therefore, prevalence of amphibian infections with *L. cyprinacea* could predictably increase.

Lernaea cyprinacea has been found to cause mortality in fishes (Enders and Rifenburgh 1928, Goodwin 1999, Shariff and Sommerville 1986). In amphibians, *L. cyprinacea* caused limb abnormalities in field-caught populations of *Hylarana chalconota* (Schlegel) in Indonesia (67.3% prevalence; Ming 2001) and in *Rana boylei* Baird of Northern California (9.2–10.5% prevalence; Kupferberg et al. 2009); it was associated with high mortality (32%) in farm-raised *A. mexicanum* in Brazil (Carnevia and Speranza 2003) and in *L. catesbeianus* in Brazil (Martins and Souza 1995). Developmental abnormalities of limbs or digits and mortality from *L. cyprinacea* infection were not noted in field populations of *Hyla pulchella cordobae* Barrio in Argentina (Alcalde and Batistoni 2005), *L. catesbeianus* in Argentina (Salinas et al. 2016), or *L. catesbeianus* in central Ohio (Tidd 1962). However, swelling of the thigh was noted in this study when parasites were imbedded either in the thigh or near its base. Curvature of the tails of tadpoles with embedded *Lernaea* has previously not been described. It was beyond the scope of this study to examine tadpoles for other species of parasites, but tail curvature could result from parasitism by other species and requires additional study. If severe, tail curvature may affect swimming performance and impede predator avoidance.

Table 1. Parasitic female *Lernaea cyprinacea* found attached to second-year tadpoles of *Lithobates catesbeianus* in three streams of northeastern Ohio. N¹ = number of first-year tadpoles; N² = number of second-year tadpoles. When N² = 0, no second-year tadpoles were found that year. When N¹ is blank, the number of first-year tadpoles was either 0 or not recorded.

Locality	River mile	Year	N ¹	N ²	Intensity of parasitism (no. of attached parasites)								Prevalence	
					0	1	2	3	4	5	6	7		
Grand River														
Site 1	21.5	1993		106	87	19								17.9
		1997		106	103	3								2.8
		1999		3	2	1								33.3
		2002		1		1								100
		2008		0										0
		2009		0										0
		2010		114	7	16	35	33	17	5	1			93.9
		2011–2017		0										
Ashtabula River														
Site 1	6.5	2005		119	44	38	29	4	1	2	1			63
		2014–2016		0										0
		2017		75	75									0
Site 2	21.1	2016	89	173	0								0	
		2017		4	3	1								25
Conneaut Creek														
Site 1	22.3	2002		88	87	1								1.1
Site 2	17.7	1999		32	7	9	8	5	2	1				78.1
		2000		0										0
		2002		21	20	1								4.8
		2017		0										0
Site 3	5.4	2003		10	8	2							20	
Site 4	14.3	1999		3	2	1							33.3	
		2012		42	27	7	4	2	1	1			35.7	
Site 5	22.8	2002		32 ^a	4	8	5	3	10	2				87.5
		2003	69	0	69									0
		2005		42	5	3	15	6	9	3	0	1		88.1
		2006		175	151	21	3							13.7
		2007		197 ^b	145	42	10							26.4
		2008		18	17	1								5.6
		2012		42 ^c	10	10	10	2	4	5	0	1		76.2
		2016–2017		0										0
Site 6	14.8	2002		2	1			1					100	
		2005		11	1	5	2	3					90.9	

^a16 additional first-year tadpoles of *L. clamitans* were examined, all without *L. cyprinacea*.

^bFour additional tadpoles of *L. clamitans* were examined, one with one *L. cyprinacea*.

^c10 additional first-year tadpoles of *L. clamitans* were examined, all without *L. cyprinacea*.

Tadpoles of *L. catesbeianus* in this study often showed subdermal hemorrhaging around the connective tissue surrounding the base of the attached female copepod. Development of the anchor associated with embedding and elongation of female *L. cyprinacea* was found to include not only the integument, but always affected some internal organ of *Lithobates pipiens* (Schreber) tadpoles (Tidd and Shields 1963). Tidd and Shields (1963) considered subdermal hemorrhaging and blood loss the probable causes of mortality of most tadpoles studied in the laboratory. Few to no *L. catesbeianus* tadpoles were captured at sampling sites in some years, and the population status of this species has not been monitored in these streams (Table 1). It is unclear if the infection of *L. catesbeianus* tadpoles by *L. cyprinacea* has an impact upon its status in these streams.

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