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Natural Occurrence of *Diplostomum* sp. (Digenea: Diplostomatidae) in Adult Mudpuppies and Bullfrog Tadpoles from the St. Lawrence River, Québec

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ABSTRACT: Adult mudpuppies (*Necturus maculosus*) and bullfrog tadpoles (*Rana catesbeiana*) infected with the eyefluke *Diplostomum* sp. in the lenses were collected from the St. Lawrence River, Québec, Canada. Respective prevalence and mean abundance of *Diplostomum* sp. were 100% and 3.1 ± 1.7 in Lake St. François, 58.3% and 1.5 ± 1.8 in Lake St. Louis, and 53.8% and 0.7 ± 0.8 in Lake St. Pierre. No eyeflukes were observed in mudpuppies from the Richelieu River. Prevalence and mean abundance of *Diplostomum* sp. were significantly higher in mudpuppies from Lake St. François than in those from other sites. The high prevalence and abundance in Lake St. François may be because the regulated water levels may enhance snail intermediate host habitats. There was a significant negative correlation between mudpuppy length and number of eyeflukes per host when samples were pooled from the 3 sites where *Diplostomum* sp. was found. Mean length of infected mudpuppies from those 3 sites was significantly smaller than uninfected ones. Twenty-four (28%) of 86 mudpuppies had cataracts associated with infections of eyeflukes. Prevalence and mean abundance of *Diplostomum* sp. in bullfrog tadpoles collected from Lake St. Pierre were 14.3% and 0.1 ± 0.4 parasite per animal, much lower than observed for mudpuppies from the same lake. Higher occurrence of eyeflukes in mudpuppies compared with tadpoles is attributed to the greater age and more sedentary benthic nature of mudpuppies. This is the first report of amphibians naturally infected with *Diplostomum* sp. and only the second with eyeflukes in general.

KEY WORDS: *Diplostomum* sp., eyefluke, *Necturus maculosus*, mudpuppy, *Rana catesbeiana*, tadpole, amphibians, prevalence, abundance, St. Lawrence River, Canada.

The eyefluke *Diplostomum spathaceum* (Rudolphi, 1819) (Digenea: Diplostomatidae) is among the most common parasites of freshwater fishes worldwide (Chappell et al., 1994) and infects more than 100 species of fish belonging to diverse taxa (Chappell, 1995). Diplostome metacercariae are the most important pathogens of the eyes of fish, cause blindness, and lead to poor growth, emaciation, and death (Williams and Jones, 1994; Chappell, 1995).

While the host spectrum of *D. spathaceum* is without question diverse, its actual extent beyond fishes is not clear. For example, Ferguson (1943) successfully infected tadpoles and adults of the northern leopard frog, *Rana pipiens* Schreber, 1782, in addition to painted turtles (*Chrysemys picta* (Schneider, 1783)), with metacercariae of *D. spathaceum*. Morphologically, worms appeared normal in these "abnormal" hosts. Development to adulthood occurred when worms from frogs were administered to chicks (Ferguson, 1943), indicating that amphibians and reptiles may be able to function as intermediate hosts. Sweeting (1974) successfully established infections of *D. spathaceum* in the African clawed frog, *Xenopus laevis* (Daudin, 1802), and observed what appeared to be normal development of metacercariae.

The occurrence of *D. spathaceum* in natural amphibian populations is not known. However, in Mountain Lake, Virginia, U.S.A., the red-spotted newt, *Notophthalmus viridescens* (Rafinesque, 1820), is naturally infected with the fish eyefluke *Tylodelphys scheuringi* (Hughes, 1929) in its humors (Etges, 1961). No frogs or other salamanders were found infected, and experimental infections of tadpoles and adult frogs were unsuccessful (Etges, 1961).

Infection levels of diplostomatid eyeflukes in fish from the St. Lawrence River are believed to be high, given the frequency of cataracts and blindness in fish from the river (Fournier et al.,

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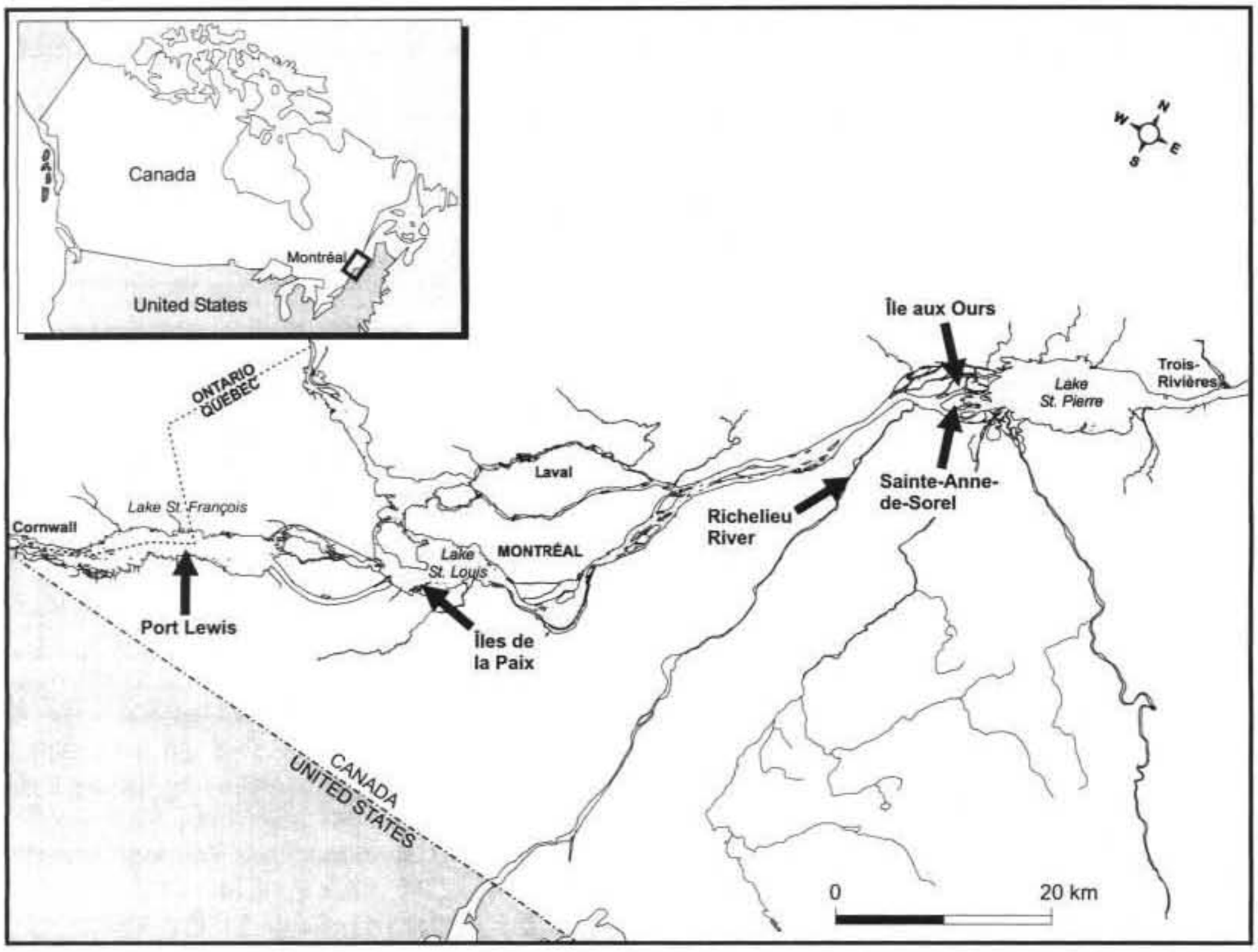

Figure 1. Map of the St. Lawrence River, Québec, Canada, depicting sampling localities and areas mentioned in the text. Adult mudpuppies (*Necturus maculosus*) were collected from Port Lewis, Îles de la Paix, Sainte-Anne-de-Sorel, and the Richelieu River during winter 1998. Bullfrog tadpoles (*Rana catesbeiana*) were collected from Île aux Ours in August 1998. Insert: Location of the sampling region is indicated on the map of Canada by a rectangle encompassing Montréal and the St. Lawrence River.

1996; Lair and Martineau, 1997; Mikaelian and Martineau, 1997). During a population study of mudpuppies, *Necturus maculosus* (Rafinesque, 1818), from the St. Lawrence River, we observed an individual with cataracts, and subsequently, an infection of *Diplostomum* sp. We then examined adult mudpuppies from 4 areas in the St. Lawrence River and 1 of its tributaries for eyeflukes. The mudpuppy is a long-lived, bottom-feeding, and strictly aquatic salamander, studied as a bioindicator of the St. Lawrence River (Bonin et al., 1995; Gendron et al., 1997). In addition, a single sample of bullfrog tadpoles (*Rana catesbeiana* Shaw, 1802) was collected from an area of high diplostome intensity (Marcogliese and Compagna, 1999) and examined for eyeflukes.

Materials and Methods

Mudpuppies were collected during a live trapping program with a small hoop net baited with dead fish placed at a depth of 1.5–2.5 m (Bonin et al., 1994) between the end of January and March 1998 from Port Lewis in Lake St. François (45°10′N; 74°17′W), Îles de la Paix in Lake St. Louis (45°20′N; 73°50′W), Sainte-Anne-de-Sorel in Lake St. Pierre (46°04′N; 73°03′W), and the Richelieu River (45°53′N; 73°09′W). The 3 lakes are formed from expansions of the St. Lawrence River, and the Richelieu River composes 1 of its tributaries (Fig. 1). Animals collected from Lake St. François (N = 36), Lake St. Louis (N = 27), and the Richelieu River (N = 23) were examined live for cataracts. Subsamples from these collections were examined directly for eyefllukes as follows. Animals from Lake St. François (N = 13) and the Richelieu River (N = 10) were transported live to the laboratory, where they were euthanized by cervical dislocation. The eyes were removed from the freshly killed animals, dissected, and examined with a stereomicroscope for parasites. Animals from Lake St. Louis (N = 12) were euthanized by cervical dislocation, fixed, and stored in 10% neutral buffered formalin, and their eyes removed, dissected, and examined with a stereomicroscope for parasites. No animals from Lake St. Pierre were examined for cataracts, but a sample

Table 1. Number (*N*), prevalence (P), and mean abundance (A ± SE) of *Diplostomum* sp. in the lenses of adult mudpuppies (*Necturus maculosus*) and bullfrog tadpoles (*Rana catesbeiana*) collected from localities in the St. Lawrence River and 1 of its tributaries in 1998.

	Lake St. François			Lake St. Louis			Lake St. Pierre			Richelieu River		
	N	P (%)	A ± SE	*N*	P (%)	A ± SE	*N*	P (%)	A ± SE	*N*	P (%)	A ± SE
Necturus maculosus	13	100	3.1 ± 1.7	12	58.3	1.5 ± 1.8	13	53.8	0.7 ± 0.8	10	0	0
Rana catesbeiana	—	—	—	—	—	—	35	14.3	0.1 ± 0.4	—	—	—

(N = 13) was collected and processed as that from Lake St. Louis.

Tadpoles (N = 35) of bullfrogs (*R. catesbeiana*) were collected from Île aux Ours in Lake St. Pierre (Fig. 1) using a beach seine measuring 22.6 m long by 1.15 m high, with a 3-mm mesh, in August 1998. Animals were euthanized by an overdose of anesthetic (MS 222), and their eyes were removed, dissected, and examined with a stereomicroscope for parasites.

Classification used herein adheres to that described by Gibson (1996). Metacercarial stages of diplostomes are difficult to identify to species, and resolution of the group's taxonomy is required before specimens can be assigned to species (Chappell, 1995; Gibson, 1996). In North America, metacercariae found in the lens of fish usually are considered to be *D. spathaceum*, and those in the vitreous humor to be other species, but these identifications must be regarded with caution (Gibson, 1996). The parasites found in this study correspond in terms of morphology and site within the host to *Diplostomum* sp. Experimental work where similar metacercariae were recovered from various fishes collected in the St. Lawrence River and fed to ring-billed gulls (*Larus delawarensis* Ord, 1815) suggests that 2 types of diplostomes occur in the river (*Diplostomum spathaceum indistinctum* (Guberlet, 1923) and *Diplostomum huronensis* (La Rue, 1927)) (J. D. McLaughlin, Concordia University, pers. comm.). The former typically occurs in the lens and the latter in the humor of the eyes of fish (Gibson, 1996). Unfortunately, the specific identification of eyeflukes from fishes and other vertebrates is problematic because most researchers working on surveys of these parasites do not have the capacity to rear these parasites in definitive hosts in the laboratory, especially when large numbers of metacercariae are involved. In addition, rearing metacercariae to adults in laboratory hosts such as chicks is problematic for 2 reasons. First, body dimensions of diplostomatid metacercariae can be affected by the species of host where they reside (Niewiadomska, 1987; Graczyk, 1991; Field and Irwin, 1995). Second, to minimize host-induced morphological variations, all hosts used by a parasite, including snails, fish, and birds, over the course of its life cycle must be identical (Field and Irwin, 1995). However, with metacercariae from wild-caught organisms such as fish or amphibians, it is often impossible to determine which snail hosts participated in their life cycles. For the various reasons listed above, numerous surveys simply record the parasites as *Diplostomum* sp. or *Diplostomulum* sp. (see Margolis and Arthur, 1979).

Data were not normally distributed and were compared using a nonparametric Kruskal-Wallis rank sum test, followed by pairwise comparisons using Tukey-Kramer HSD tests, using the JMP® version 3.2.1 statistical package (SAS Institute, 1997). Statistical significance was set at a value of $P < 0.05$. Prevalence is defined as the proportion of animals infected in a sample, expressed as a percentage, and mean abundance is expressed as the mean number of parasites per host, infected and noninfected, in a sample (Bush et al., 1997).

Results

Adult mudpuppies from 3 of the 4 localities were infected with *Diplostomum* sp. in the lens of the eye. All animals from Lake St. François were infected, and abundance was significantly higher than at the other 3 localities ($\chi^2 = 24.65$, df = 3, $P < 0.0001$) (Table 1). Prevalence was similar in mudpuppies from lakes St. Louis and St. Pierre, though mean abundance was higher in those from Lake St. Louis (Table 1). None of the mudpuppies examined from the Richelieu River was infected with eyeflukes. Multiple infections were much more common in mudpuppies from Lake St. François, with 10 of 13 animals having 2 or more parasites, and a maximum of 7 per host. In contrast, 5 of 12 from Lake St. Louis and 1 of 13 from Lake St. Pierre had 2 or more worms, with maxima of 6 and 3 worms per host, respectively. Prevalence of cataracts was 56% in mudpuppies from Lake St. François (N = 36), 11.1% in those from Lake St. Louis (N = 27), and 4.3% in those from the Richelieu River (N = 23). No mudpuppies from Lake St. Pierre were examined purposely for cataracts.

The correlation between total length of mudpuppies and total number of metacercariae per host was not significant ($P > 0.2$) at any of the 3 sites where *Diplostomum* sp. was found. There was a significant negative correlation between total number of parasites per host and total length of mudpuppies when animals were pooled from those 3 sites ($r^2 = 0.303$, P =

0.0003). Mean total length of infected hosts (246.0 ± 11.3 mm) was smaller than that of uninfected ones (306.2 ± 17.8 mm) ($\chi^2 = 6.88$; df = 1; $P = 0.0087$) when mudpuppies were pooled from the same 3 sites.

Five of 35 bullfrog tadpoles from Lake St. Pierre were infected, each with a single worm, giving a mean abundance of 0.1 ± 0.4 and a prevalence of 14.3% (Table 1).

Discussion

This is only the second report of amphibians from North American waters naturally infected with eyeflukes. Previously, red-spotted newts from Mountain Lake, Virginia, were found infected with *Tylodelphys scheuringi* at a prevalence of 100% (Etges, 1961). Adult mudpuppies and bullfrog tadpoles were infected with *Diplostomum* sp. at various localities in the St. Lawrence River. Mudpuppies were infected to a much greater degree than were tadpoles, probably due to their more sedentary benthic nature and their greater age. All mudpuppies collected were reproductive, making them at least 5 yr of age for males and 6 yr for females (Bonin et al., 1994). Among fishes, benthic species tend to be more heavily infected than pelagic ones. Cataracts are more prevalent in benthic fishes in the St. Lawrence River compared with pelagic foragers (Lair and Martineau, 1997). In addition, *D. spathaceum* metacercariae accumulate from year to year in hosts (Chappell et al., 1994), so older hosts tend to be more heavily infected. Benthic fish in the St. Lawrence River are more heavily infected than mudpuppies. Mean abundance of *Diplostomum* sp. in the white sucker (*Catostomus commersoni* (Lacépède, 1803)) aged 2–6 yr was 69.5 in Lake St. Louis and 22.0 in Lake St. Pierre, whereas in fish aged 7 yr or older, it was 167.0 and 62.9 in the 2 lakes, respectively (Marcogliese, unpubl.).

There is little information on geographic variation in infection levels within the St. Lawrence River system. In a survey of young-of-the-year fishes, no significant differences were found among sites (Marcogliese and Compagna, 1999), but among older fishes, infection levels were much higher in those from Lake St. Louis compared with Lake St. Pierre and near Québec City (Marcogliese, unpubl.). Data presented herein demonstrate that infection levels in mudpuppies from Lake St. François were significantly higher than in lakes St. Louis and St. Pierre. Moreover, there is a gradient in abundance declining downstream from west to east in the river. This cannot be directly correlated to the distribution of the definitive hosts, gulls and terns, as a large colony of ring-billed gulls consisting of 6156 pairs in 1997 is located near the sampling site in Lake St. Louis, but 3 larger colonies of ring-billed gulls, each consisting of more than 10,000 pairs, are situated downstream east of Lake St. Louis (P. Brousseau, Canadian Wildlife Service, pers. comm.). In addition, small colonies of common terns (*Sterna hirundo* Linnaeus, 1758), totaling 85 pairs in 1989, 108 pairs in 1997, and 138 pairs in 1997, as well as colonies of black terns (*Chlidonias niger* (Linnaeus, 1758)) occur in Lake St. François, Lake St. Louis, and Lake St. Pierre, respectively (Chapdelaine et al., 1999). Habitat in Lake St. François may be more suitable for the first intermediate hosts, lymnaeid snails. One important difference between Lake St. François and the other lakes is that water levels in this lake are heavily regulated, and do not fluctuate as much as in the other lakes. This stability may enhance snail populations and productivity. No worms were found in mudpuppies from the Richelieu River, although 1 mudpuppy was observed with cataracts. There is no information available on whether fish are infected with *Diplostomum* sp. in that river. Characteristics of that river may make it particularly unsuitable for the completion of the parasite's life cycle, in that definitive hosts or snail intermediate hosts are rare. There are no colonies of gulls or terns located on the river.

There was no relationship between body length and number of parasites among mudpuppies at any of the sites. When data were pooled from the 3 sites where *Diplostomum* sp. was found, there was a significant negative correlation between mudpuppy length and the number of parasites per host. Moreover, mean length of uninfected mudpuppies from those 3 sites was significantly greater than that of infected ones. These observations suggest that infections with *Diplostomum* sp. may be detrimental to mudpuppy growth, as was observed with infections in fish (Williams and Jones, 1994; Chappell, 1995). However, this conclusion may be premature. Our sample sizes are small. In addition, size of mudpuppies may be affected by pollution levels. For example, concentration of contaminants in mudpuppies varies with location in the

St. Lawrence River watershed (Bonin et al., 1995). Differences in mudpuppy size also could reflect some other aspect of habitat quality or age differences among the populations.

Prevalence of cataracts was high in Lake St. François, but extremely low in Lake St. Louis and the Richelieu River. This can be attributed to the higher prevalence and abundance of *Diplostomum* sp. in Lake St. François compared with the other sites. Yet, in both Lake St. François and Lake St. Louis, the prevalence of cataracts was much lower than the prevalence of eyeflukes. Thus, the presence of cataracts is not a reliable indicator of infection with eyeflukes, at least in mudpuppies. It is not known if the single mudpuppy possessing cataracts in the Richelieu River was infected with eyeflukes, or whether the cataracts resulted from another cause. Cataracts are caused by metacercariae, by dietary deficiency or excess, or by excessive exposure to sunlight, cold, or injury (Ferguson, 1989). In any case, the possibility of the presence of *Diplostomum* sp. in the Richelieu River cannot be dismissed.

The results demonstrate that animals other than fish become infected with metacercariae of *Diplostomum* sp. Given that amphibians develop cataracts (Ferguson, 1943; this study), concern for the health of aquatic fauna susceptible to blindness resulting from infection with eyeflukes must be extended beyond fish to include amphibians, especially in areas where *Diplostomum* sp. levels are high.

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