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Endemic Infections of *Parastrongylus* (=*Angiostrongylus*) *costaricensis* in Two Species of Nonhuman Primates, Raccoons, and an Opossum From Miami, Florida

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ABSTRACT: *Parastrongylus* (=*Angiostrongylus*) *costaricensis* was first reported in the United States from cotton rats, *Sigmodon hispidus*, in Texas in 1979. Here, we report the findings of *P. costaricensis* in a siamang (*Hylobates syndactylus*) from the Miami MetroZoo, in 2 Ma's night monkeys (*Aotus nancymaae*) from the DuMond Conservancy located at Monkey Jungle in Miami, in 4 raccoons (*Procyon lotor*) trapped near the MetroZoo, and in an opossum (*Didelphis virginiana*) trapped at the MetroZoo. These records are the first records of *P. costaricensis* from all 4 species of hosts. All of the primates were zoo-born, and the raccoons and opossum were native, indicating that this parasite is now endemic at these 2 sites.

Parastrongylus costaricensis (Morera and Céspedes, 1971) Ubelaker, 1986 (Nematoda: Angiostrongylidae) is a parasite of the mesenteric arteries of the cotton rat, *Sigmodon hispidus*, and other rodents in North, Central, and South America. It is also the causative agent of abdominal angiostrongyliasis in humans in Costa Rica (Morera and Céspedes, 1971), Honduras (Sierra and Morera, 1973), Mexico (Carrada-Bravo, 1980), Nicaragua (Duarte et al., 1991), Brazil (Rambo et al., 1997), Guatemala (Kramer et al., 1998), Colombia (Rodriguez, 2000), and in islands of the Caribbean such as Martinique (Jeandel et al., 1988) and Guadeloupe (Juminer et al., 1992).

The only records of *P. costaricensis* in nonhuman primates are from animals in captivity. Sly et al. (1982) reported 2 black-chested mustached tamarins, *Saguinus mystax*, infected at a research laboratory in Maryland; both animals had been imported from Iquitos, Peru. A black-tufted-eared marmoset, *Callithrix penicillata*, which was born in captivity in Brazil, died in a zoo in Germany and was found infected with *P. costaricensis* (Brack and Schröpel, 1995), and a Central American spider monkey, *Ateles geoffroyi*, was found infected at a zoo in Costa Rica (Berrocal et al., 1997).

Ubelaker and Hall (1979) reported *P. costaricensis* in 2 cotton rats from Texas, and this record remains the only endemic record of this species in the United States. Herein, we report on 3 cases of infections in nonhuman primates from 2 zoos in Florida, all of which were apparently locally acquired, and in raccoons (*Procyon lotor*) and an opossum (*Didelphis virginiana*).

In September 2002, a 3-yr-old male siamang (*Hylobates syndactylus*) at the Miami MetroZoo developed diarrhea and an increasingly large abdomen, and he became intermittently lethargic. It is important to note that this primate was born at the MetroZoo. Evaluation revealed numerous firm abdominal masses, a mild-to-moderate pulmonary infiltrate, anemia, and hyperproteinemia. An exploratory celiotomy was performed and numerous firm, multinodular masses were found attached to, and within, the walls of the small intestine and bowel as well as a large mass at the end of the appendix. Smaller nodules and tags were scattered over the intestinal serosa and extended into the adjacent mesenteries. The appendix mass and 2 small intestinal masses were resected, whereas some large bowel lesions were deemed nonresectable.

Histologic sections of the resected tissues revealed large numbers of nematode ova and larvae embedded in the deep lamina propria, submucosa, muscle layers, and serosa of the intestine (Fig. 1a, b). The ova were associated with granulomatous inflammation in all locations and with granulation tissue formation, sinus tracts, and fibrous tags (serosal adhesions) in the muscular tunics and associated serosa (Fig. 1c). A submucosal small artery in one section of the small intestine contained a luminal female metastrongyle (Fig. 1d), but identification to species was not attempted at that time. Segmental severe hemorrhage and partial infarction of the muscular and serosal tunics were noted in one section of the intestine associated with a submucosal arterial thrombus containing a degenerative nematode (Fig. 2). After treatment with anthelmintics, the siamang made a full recovery, was later transferred to the Memphis Zoo, and is still alive at this writing.

Monkey Jungle (MJ) is a private reserve of 30 acres in Miami, which was founded in 1933, and is now home to 400 primates of 30 species, most running free. It is located approximately 5 km southeast of the MetroZoo. In October 2003, a 6-yr-old female Ma's night monkey, *Aotus nancymaae*, which had been born in captivity in the DuMond Conservancy located at the MJ, died suddenly and was found on necropsy to have irregularly thickened areas of the ileum and cecum, with areas of hemorrhage. Histologic sections of the intestine had extensive multifocal inflammation of the muscular tunics consisting of lymphocytes, plasma cells, numerous eosinophils, multinucleate giant cells, and macrophages. Numerous larvated eggs with an eosinophilic shell as well as free larvae were found in the tunics and also in the portal triads of the liver. One complete female metastrongyle was teased out of a mesenteric artery.

In November 2004, a 7-yr-old male Ma's night monkey, also born in the DuMond Conservancy, started showing signs of mild anorexia and apparent abdominal pain, and died despite being given fluids and antibiotic therapy. At necropsy, it was found to have an intussusception in the distal ileum. In the area of the intussusception, chronic inflammation and marked smooth muscle proliferation were noted surrounding groups of nematode larvae. An area of fibroplasia and necrosis was found on the serosal surface, along with an infiltrate comprised primarily of neutrophils and scattered macrophages. There seemed to have been some sort of a perforation of the intestine with subsequent peritonitis. Nematode larvae also were found in the liver, which was diffusely congested. Five adult nematodes (1 male and 4 females) were extracted from arteries along the cecum and ascending colon.

In April and May 2005, a number of raccoons were captured in an area adjacent and contiguous to the MetroZoo as part of a control program and killed. A large, apparently healthy male raccoon was found to have a mass in its colon, and sections of the colon showed a marked eosinophilia and numerous nematode ova and larvae in the submucosal and muscularis layers. Fragments of at least 7 female nematodes and 1 intact male were teased from the mesenteric arteries. Subsequently, 3 other raccoons from the same area were found infected, 1 of which harbored 21 females and 14 males.

In June 2005, an opossum was found dead in a live-trap inside a bird enclosure at the MetroZoo and necropsied. A localized area of peritonitis was found in the small intestine with adhesions from the omentum, and 24 female and 12 male nematodes were teased from the mesenteric arteries.

According to Ubelaker (1986), *Rodentocaulus* is distinguished by an archlike cuticularized telamon supporting the cloaca. Because this structure was absent in our specimens (Fig. 3), this genus was eliminated from consideration. *Parastrongylus* is distinguished from *Angiostrongylus* by having the 3 lateral rays arising from a common trunk in the former versus the externolateral ray being separate from the others in the latter. The only species of *Parastrongylus* described from the mesenteric arteries are *P. costaricensis* and *Parastrongylus siamensis* (Ohbayashi, Kamiya, and Bhaibulaya, 1979) from *Rattus sabanus* in Thailand; the remaining species are all found in the pulmonary arteries. The bursal rays and spicules of the males from the night monkeys,

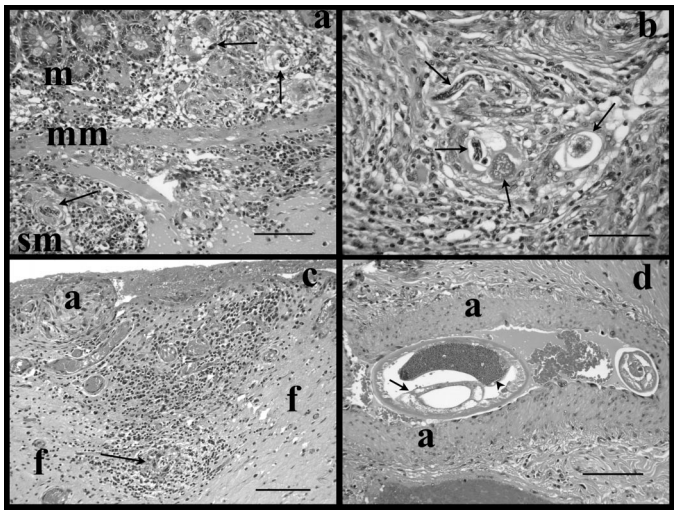

FIGURE 1. Intestine of siamang. (**a**) Mucosa (m), muscularis mucosa (mm), and submucosa (sm). Note microgranulomas containing oblique sections of nematode larvae (arrows). Bar = 260 μm. (**b**) Granuloma in the muscular tunic containing numerous larval nematode stages (arrows). Bar = 150 μm. (**c**) Serosal tunic of intestine. Note microgranuloma containing nematode larva (arrow), expansion of the serosal by fibrous tissue (f) and adhesion (a) on external surface of serosa. Bar = 250 μm. (**d**) Artery (a) in submucosa containing a mature nematode. Note coelomyarian musculature, intestine lined by vacuolated cells (arrow), and uterus containing sperm (arrowhead). Bar = 275 μm.

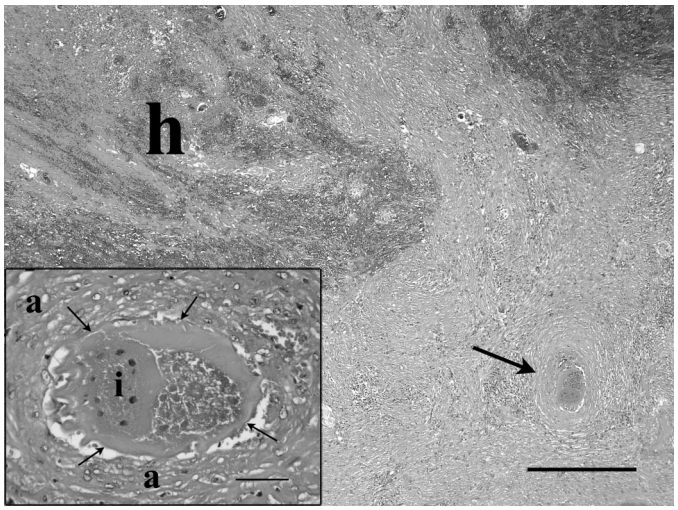

FIGURE 2. Muscular tunic of intestine, siamang. Note artery containing thrombus (arrow), and a large focus of hemorrhage (h). Bar = 500 μm. Inset, note arterial (a) thrombus is comprised of degenerative nematode (arrows). The intestine (i) of the nematode is discernible. Bar = 100 μm.

raccoons, and the opossum most closely resemble previous descriptions of *P. costaricensis*. The ventral rays are fused except for the terminal fifth; the lateral rays arise from a common trunk; the externodorsal rays are short, thin, and straight; and the dorsal ray is short and thick, terminating in 2 tips (Fig. 3). The spicules are 275–300 μ long, with simple points and a simple, lightly chitinized gubernaculum is present. Comparison of females from all 4 species of hosts showed them to be identical. The vulva of a 20.7-mm-long female from a night monkey is 260 μ, and the anus is 88 μ from the end of the tail.

Besides *P. costaricensis*, the only other species of *Parastrongylus* reported from North America are *Parastrongylus schmidti* (Kinsella, 1971) from rice rats (*Oryzomys palustris*) in Florida (Kinsella, 1988) and *Parastrongylus cantonensis* (Chen, 1935) from various mammals, including the opossum, in Lousiana (Kim et al., 2002), and from a nonhuman primate at the Miami MetroZoo (Duffy et al., 2004). Adults of both species exclusively inhabit the pulmonary arteries; in addition, the spicules of *P. schmidti* are 215–279 μ long and those of *P. cantonensis* are 1,000–1,400 μ long. We, therefore, suspect that all of the infections found here are *P. costaricensis* and that abdominal angiostrongyliasis is now endemic in 2 locations in Miami. These records are the first records of this nematode from all 4 species of hosts. Specimens from owl monkeys, raccoons, and the opossum were deposited as vouchers at the U.S. National Parasite Collection at Beltsville, Maryland, as Accession 95925, 96989, and 96990, respectively.

The origin of these infections is open to speculation. The most widespread reservoir host of *P. costaricensis* in Central and South America is the cotton rat, although *Rattus* spp. and other rodents are also commonly infected (Morera, 1973; Tesh et al., 1973). The first-stage (L1) larvae passed in the feces of the host are infective to various species of aquatic snails, land snails, and slugs, wherein they undergo 2 molts to the infective L3 (Morera, 1973). Subsequent hosts are infected when they ingest the snails or slugs or when they ingest the L3 larvae left on vegetation in the mucus trail of these molluscs. Although *P. costaricensis* does not develop to viable L1 stages in humans, it apparently does in nonhuman primates (Sly et al., 1982; Brack and Schröpel, 1995; Berrocal et al., 1997). The possibility that this parasite was historically endemic in south Florida in cotton rats, opossums, raccoons, or a combination seems to be remote because all of these hosts have been extensively surveyed for helminths in Florida (Kinsella, 1974; Forrester, 1992).

It is interesting that the only other natural record of *P. costaricensis* from a carnivore was from a procyonid, the white-nosed coatimundi (*Nasua narica*), presumably trapped in the wild in Costa Rica, although not specifically stated in the report (Monge et al., 1978). Significantly, the coatimundi was a patent host, which was proven by infecting laboratory-reared snails from first-stage larvae in the feces, and inoculating the third-stage larvae, which developed in cotton rats.

Possible scenarios for the introduction of the parasite include (1) infected primates may have been introduced into the MetroZoo and MJ and subsequently infected endemic molluscs, and, in turn, raccoons and opossums; (2) infected *Rattus* spp. may have entered through the seaport of Miami and infected molluscs; or (3) L3 larvae from molluscs may have contaminated imported food supplies of the primates. In an outbreak of human abdominal angiostrongyliasis in Guatemala, mint leaves were implicated as the likely vehicle of infection (Kramer et al., 1998). Studies are underway to determine whether local populations of *S. hispidus* and *Rattus rattus* are infected. Snails and slugs also should be examined for *Parastrongylus* larvae, and any larvae found fed to laboratory rodents to identify the species, because *P. cantonensis* also has been recently found in a primate at the MetroZoo (Duffy et al., 2004). It has yet to be determined whether viable L1 larvae are present in the

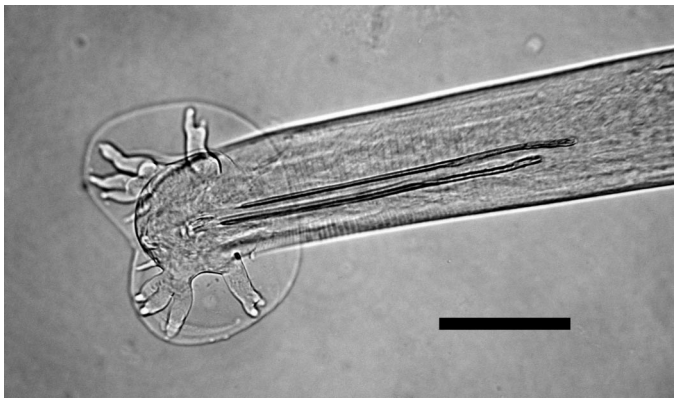

FIGURE 3. Bursa and spicules of *P. costaricensis* from a raccoon. Note typical shape of ventral, lateral, and externo-dorsal rays; dorsal ray only partially visible. Bar = 100 μm.

feces of infected raccoons or opossums. If so, the possibility exists for rapid expansion of the range of this parasite within the United States by these ubiquitous and wide-ranging hosts, much more so than if only rodents were infected. Risk to the human population at this time seems minimal, but there is at least the possibility of south Florida vegetable and fruit crops being contaminated with L3 larvae by infected mollusks.

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Helminths of Pond-Reared Walleye From Wisconsin

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ABSTRACT: One hundred extended growth walleye fingerlings, *Sander vitreus* (Percidae), collected on 6 October 2004, from 1 rearing pond at the Lake Mills State Fish Hatchery in Lake Mills, Wisconsin, were examined for parasites. *Diplostomum* sp. (Trematoda: Diplostomatidae), *Contracaecum* sp. (Nematoda: Anisakidae), *Bothriocephalus cuspidatus* (Cestoda: Bothriocephalidae), and *Proteocephalus* sp. (Cestoda: Proteocephalidae) were the only parasites found. The first 3 species occurred as larval stages. *Diplostomum* sp. and *Contracaecum* sp. had similar prevalences, mean intensities, and mean abundances (72%, 2.2, and 1.6 and 71%, 2.1, 1.5, respectively) in walleye fingerlings. A headlamp technique used by the Wisconsin Department of Natural Resources personnel generated a prevalence of *Diplostomum* sp. in the lens that overestimated the prevalence of *Diplostomum* sp. found in a random subsample of 100 fingerlings examined with a microscope. *Proteocephalus* sp. and *B. cuspidatus* infected 14 and 12 fingerlings, respectively.

Bangham (1955) and Muzzall et al. (1990) reported on the parasites of walleye fingerlings from a hatchery and ponds, respectively. Muzzall (1996) reviewed the parasites and diseases of pond-reared walleye and yellow perch, *Perca flavescens*. Besides these reports, there are no other published studies on the parasites of cultured walleye in North America. During a routine parasitological examination of walleye fingerlings at the Lake Mills State Fish Hatchery before their stocking, they were found to be infected with eye flukes, *Diplostomum* sp. These fish were reared for a walleye restoration project in the Milwaukee River, Wisconsin. The present study reports on the occurrence of *Diplostomum* sp. and other helminths in 100 extended growth walleye fingerlings from 1 pond in Wisconsin. Furthermore, 2 techniques (microscopic examination and visual examination by a small, light-focusing headlamp) used to detect *Diplostomum* infections are evaluated. The infection data of *Diplostomum* sp. and *Contracaecum* sp., the 2 most common species, also are discussed in relation to other studies.

One hundred extended walleye fingerlings (age 0+) from the Lake Mills State Fish Hatchery in Lake Mills, approximately 40 km east of

TABLE I. Prevalence (P), mean intensity (MI) ± SD, and mean abundance (MA) ± SD of helminths found in 100 *Sander vitreus* fingerlings (age 0+) from a Wisconsin pond in 2004.

Parasite	P	MI ± SD (maximum)	MA + SD	Site of infection
Digenea				
Diplostomum sp.*	72	2.2 ± 1.4 (7)	1.6 ± 1.6	E‡
Nematoda				
Contracaecum sp.*	71	2.1 ± 1.6 (8)	1.5 ± 1.6	M, G, L, S, SB
Cestoda				
Bothriocephalus cuspidatus†	12	1.4 ± 1.2 (5)	0.2 ± 0.6	C, I
Proteocephalus sp.*	14	1.0 (1)	0.1 ± 0.6	L, M

* Larval stages.
† Gravid adults.
‡ E, lens; M, mesentery; G, gonads; L, liver; S, stomach; SB, swim bladder; C, cecum; I, intestine.

Madison, Wisconsin, were examined for parasites. Two earthen rearing ponds were used to produce extended growth fingerlings, meaning fish were reared to a larger than normal size. These ponds were filled with filtered water from a nearby lake via a gravity flow pipeline. The filter is a rotating drum with a 1,000-m screen. Both ponds were fertilized with organic fertilizer to promote algae and zooplankton growth. The first pond was stocked with fry on 4 April 2004, and they were harvested on 9 June 2004. These fish were then stocked at lengths of 35 to 45 mm in the grow-out pond, approximate surface area of 0.44 ha, with volume between 4,000 and 4,300 m^3, on this date. Fathead minnows, *Pimephales promelas*, obtained from an out-of-state vendor, were stocked into these ponds and served as food for the walleye. Although the perimeter of the grow-out pond was fenced off and a grid of bailing twine and flagging covered its top, a few aggressive great blue herons, *Ardea herodias*, and gulls, *Larus* sp., still fed on the walleye. Minnows from these ponds were not available for examination. There were no other fish species in the pond.

After the fingerlings were harvested by seine on 6 October 2004, Wisconsin Department of Natural Resources (DNR) personnel visually examined each walleye eye lens for the presence or absence of *Diplostomum* sp. with a small, light-focusing headlamp. If an opaque area was observed or if a larva could be seen in the lens, it was considered infected. If the lens was clear or a larva could not be seen, it was not infected.

Before visual examination by the Wisconsin DNR personnel, a random subsample of 100 fingerlings was put on ice and frozen. Total length (millimeters) and sex of these fish were recorded before the entire fingerling was necropsied in the laboratory. The total mean length ± SD of examined walleye was 207 ± 16.7 mm (range 159–241 mm). Use of prevalence, mean intensity, and mean abundance is consistent with that recommended by Bush et al. (1997). Population numbers of helminth species in fingerlings were estimated as (prevalence) × (mean intensity) × (number of walleye) harvested from the pond, and helminth numbers also were estimated in walleye stocked in nature. A voucher specimen of *Bothriocephalus cuspidatus* Cooper, 1917 has been deposited in the USNPC Helminthological Collection (USDA, Beltsville, Maryland) as USNPC 97052. Larval stages of *Diplostomum* sp., *Contracaecum* sp., and *Proteocephalus* sp. have been retained by the senior author.

The prevalence, mean intensity, and mean abundance of the 4 helminth species are in Table I, with *Diplostomum* sp. having the highest values. There were no significant differences in the prevalences, intensities, and abundances of *Diplostomum* sp. between left and right lenses of walleye (chi-square analysis and Student's *t*-test). The prevalence, mean intensity, and mean abundance of *Contracaecum* sp. were similar to values for *Diplostomum* sp. Identification of larvae of *Contracaecum* infecting walleye was based on the presence of the intestinal caecum and appendix, and the excretory pore entering near the ventral interlabium (Deardorff and Overstreet, 1980). There were no significant differences in the prevalences, intensities, and abundances of the 2 most common species, *Diplostomum* sp. and *Contracaecum* sp., between female and male fish (chi-square analysis and Student's *t*-test). *Bothriocephalus cuspidaus* and *Proteocephalus* sp. infrequently infected the fingerlings and had low and similar mean intensities and mean abundances.

Wisconsin DNR personnel examined 6,100 walleye fingerlings from the grow-out pond with the headlamp and determined that 5,919 fingerlings (97%) were infected with *Diplostomum* sp. These fish were killed because they did not want to stock infected fish. The remaining 181 fish were stocked into the Milwaukee River. The headlamp technique generated a prevalence that overestimated the prevalence of *Diplostomum* sp. found in the random subsample of 100 fingerlings examined by the microscope. This prevalence led to an additional 1,527 uninfected walleyes that were killed by the Wisconson DNR personnel, instead of being stocked.

Ninety-three walleye fingerlings of 100 were infected with 1 or more helminths. The mean helminth species richness (number of helminth species) for the 100 walleye examined was 1.7 ± 0.8, range 0–3. Fifty-five walleye were simultaneously infected with *Diplostomum* sp. and *Contracaecum* sp. The estimated population numbers of *Diplostomum* sp., *Contracaecum* sp., *Proteocephalus* sp., and *B. cuspidatus* based on overall infection values in walleye fingerlings at harvest (6,100 individuals) on 4 October, 2004 from this pond were 966,240, 909,510, 85,400, and 102,480, respectively. The estimated numbers of *Contracaecum* sp., *Proteocephalus* sp., and *B. cuspidatus* based on overall infection values in the 181 walleye fingerlings stocked into the Milwaukee River were 26,987, 2,534, and 3,041, respectively. An estimated number of *Diplostomum* sp. was not included because the 181 stocked fingerlings were uninfected.

Of the helminth species found in the present study, *Contracaecum* sp. and *Diplostomum* sp. had high prevalences and deserve further discussion. Dick et al. (1987) reported that a stocking program with rainbow trout, *Salmo gairdneri*, failed in a lake because of infections with large numbers of *Contracaecum* sp. Prevalence was 100% in 4 separate years, and intensities varied from 1 to 100. Histological observations in these infected rainbow trout included dark hemorrhagic areas associated with some larvae coiled within the body musculature; in heavy infections, the anus was blocked on occasion, and some larvae had penetrated through the intestinal wall. None of these conditions were observed in infected walleye in the present study.

Diplostomum spp. have been found in several fish species raised in hatcheries and culture conditions. Muzzall et al. (1990) reported that the prevalences of *Diplostomum* sp. in walleye from Michigan ponds varied from 11 to 47% and that mean intensities varied from 1.1 to 2.4 flukes per fish. Prevalence of *Diplostomum* sp. in walleye in the present study was much higher than that reported by Muzzall et al. (1990), but mean intensity in the present study was within the range reported. Bangham (1955) only found *Diplostomum* sp. in 2 hatchery walleye fingerlings. Betterton (1974) reported that the prevalence of *Diplostomum spathaceum* in rainbow trout was 100% and mean intensity varied from 27 to 331 flukes per fish in a hatchery. Juvenile rainbow trout were blinded by heavy infections with *D. spathaceum*. Other reports of blindness, cataracts, emaciation, deformities, and death of fish because of eye fluke infections are common, including Ferguson and Hayford (1941), Davies et al. (1973), Shariff et al. (1980), Chappell et al. (1994), and Chappell (1995). High mean intensities of *Diplostomum* sp. reported in these studies were not found in walleye in the present study.

Opacity of the eyes of fish has been studied in relation to the occurrence and number of *Diplostomum* sp. in the eyes, with varying degrees of success reported. Marcogliese et al. (2001) reported that a visual index developed to measure the degree of lens opacity was not a reliable indicator of levels of eye fluke intensity. Karvonen et al. (2004a, 2004b), by using slit-lamp microscopy, found that cataract intensity was strongly dependent on the burden of eye flukes and provided a quantitative analysis of cataract development. However, Mikaelian and Martineau (1997), working with several fish species, reported that the presence of cataracts was not necessarily indicative of the presence of eye flukes, whereas the absence of cataracts did not reflect the absence of eye flukes. Furthermore, the number of walleye found infected with *Diplostomum* sp. via the 2 techniques (headlamp and microscope ex-

amination) used in the present study were significantly different ($\chi^2 = 189.3$, $P < 0.001$).

Diplostomum spp. use a variety of snail species as first intermediate hosts, a variety of fish species as second intermediate hosts, and several piscivorous bird species as definitive hosts (Hoffman, 1960). Definitive hosts of *Contracaecum* spp. are piscivorous birds and mammals, and invertebrates including copepods serve as first intermediate hosts and fish serve as second intermediate hosts (Anderson, 1992). Several species of crustaceans, including copepods, serve as first intermediate hosts for *Proteocephalus* spp., and fish serve as transport and definitive hosts (Hoffman, 1999). Copepods serve as intermediate hosts, and fish can serve as transport and definitive hosts for *Bothriocephalus* spp. (Hoffman, 1999)

Although *Diplostomum* sp., *Contracaecum* sp., *Proteocephalus* sp., and *B. cuspidatus* were found in walleye in the grow-out pond, it does not necessarily mean that fish became infected there. Cercariae of *Diplostomum* sp. from snails could have infected walleye when they were in the first or second pond. Muzzall et al. (1990) discussed general recommendations to control *Diplostomum* sp. at the snail intermediate host level in ponds and pointed out that complete control is extremely difficult and does not seem possible. There are several potential sources or ways walleye could have become infected with *Contracaecum* sp., *B. cuspidatus*, and *Proteocephalus* sp. Walleye could have become infected in their previous pond before they were transferred to the grow-out pond. Water used to fill the rearing ponds could have brought in copepod intermediate hosts for these helminth species. This is doubtful, however, because the filter should have removed the copepods. Last, walleye might have become infected in the ponds when they ate infected fathead minnows.

It is suggested that before walleye are raised in culture conditions, the pond should be investigated for parasites that might infect the fish. A sample of fish that will serve as food for the walleye also should be examined for parasites. These activities have constraints and may be time-consuming, but they are worthwhile when the fish are ready to be stocked. Furthermore, measures should be taken to keep piscivorous birds, definitive hosts of *Diplostomum* and other parasites, from feeding at the ponds, but this is difficult to do, as exemplified by the present study.

Regarding *Diplostomum* sp. infecting walleyes that are going to be stocked, microscopic examination of the lens is precise in detection and revealing intensities, but it is not practical to examine a large number of fish in the field and the fish are lost when examined. This method assumes that the subsample of fish examined provides a prevalence and mean intensity that are similar to those values in the fish being stocked. The headlamp technique, in contrast, can examine a large number of fish and is not lethal. Although its prevalence estimate was higher compared with the microscope technique, resulting in a larger number of fish being killed, this method provides confidence that stocked fish are not infected with *Diplostomum* sp. Perhaps microscopic examination of some fish in the pond should be performed first and throughout the time the fish are in the pond. If prevalence and intensity are low in fish examined through the culture process, the fish can be stocked. However, if these values are high, the headlamp should be used to remove the infected fish at harvest. Using one method, or a combination, should allow for all, some, or a few fish to be stocked when concerned with infections of *Diplostomum* sp., but the entire sample does not have to be destroyed.

The infected walleye from this rearing pond are not playing a role in introducing new parasite species into the receiving waters because *Diplostomum* sp., *Contracaecum* sp., *Proteocephalus* sp., and *B. cuspidatus* have already been found in walleye and other fish species in Wisconsin (Pearse, 1924; Fischthal, 1947, 1952; Amin, 1975). Comparison of infection values of the helminths found in the present study to those of the same helminths in walleye from other studies cannot be made because of temporal and habitat differences, and walleye age differences. Many articles dealing with the parasites in cultured or hatchery raised fish do not mention whether infected fish were destroyed or stocked. The stocking of infected fish depends on a variety of factors, such as the parasites found, their prevalence and intensity, and whether the parasite(s) is already present in the receiving waters.

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