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CENTRARCHID FISH AS PARATENIC HOSTS OF THE GIANT KIDNEY WORM, *DIOCTOPHYMA RENALE* (GOEZE, 1782), IN ONTARIO, CANADA

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ABSTRACT: Infective larvae of *Dioctophyma renale* were found in the hypaxial musculature of pumpkinseed (*Lepomis gibbosus* L.) from three lakes in Algonquin Provincial Park, Ontario, Canada. This represents the first report of *D. renale* in centrarchid fish. In the three lakes surveyed prevalence and mean intensity ranged from 5 to 23% and one to two larvae respectively. Larvae elicited a mild granulomatous reaction in pumpkinseed. Two ferrets were each given five larvae from pumpkinseed. Adult *D. renale* were recovered from the right kidney capsule of ferrets 108 and 134 days post-infection. An opening in the ventral surface of the right kidney capsule was present in one ferret. Chronic peritonitis was associated with eggs of *D. renale* and cellular debris which probably entered the abdominal cavity from the right kidney capsule.

INTRODUCTION

Dioctophyma renale or giant kidney worm is a parasitic roundworm found in the right kidney, mainly, of mink. Dogs, wolves and other mammals including man can be infected. Transmission of this parasite involves eggs being passed in the urine of an infected mink. Eggs develop in the aquatic environment and first-stage larvae hatch in the aquatic oligochaete, *Lumbriculus variegatus*, which is the intermediate host (Karmanova, 1959, 1960, 1962). Oligochaetes containing infective third-stage larvae of *D. renale* can infect mink directly or may infect suitable paratenic hosts such as fish or frogs which can then infect the final host if ingested (Mace and Anderson, 1975).

Species of fish implicated as paratenic hosts of *D. renale* include orfe (*Leuciscus idus* (L.)) in Rumania (Ciurea, 1921), bullheads (*Ictalurus* sp.) in Michigan, USA (Woodhead, 1950), black bullheads (*Ictalurus melas* (Rafinesque)) in Michigan, USA (Hallberg, 1953), northern pike (*Esox lucius* L.) in the USSR (Karmanova, 1961), and brown bullheads (*Ictalurus nebulosus* (Lesueur)) in Ontario, Canada (Mace and Anderson, 1975). In all but two cases, larvae of *D. renale* were reported encapsulated on the stomach, intestine or mesentery of fish. Mace and Anderson (1975) also reported larvae of *D. renale* on the stomach and in the abdominal and pectoral muscles of frogs (*Rana* spp.).

The present study reports the occurrence of third-stage larvae of *D. renale* in the musculature of pumpkinseed in Ontario, Canada. Histopathology of larvae encapsulated in pumpkinseed muscle and experimental infection of ferrets (*Mustela putorius* L.) with larvae from pumpkinseed are described.

MATERIALS AND METHODS

Fish were collected from three lakes in Algonquin Provincial Park, Ontario, namely Sasajewun (45°35'N, 78°30'W), Ryan (45°41'N, 78°05'W) and Billy (45°38'N, 78°07'W). Collections were made using trap nets, Windermere traps and gill nets from May to October 1983. Fish were killed by cervical dislocation. The body cavity was opened, viscera removed and placed in piscine saline. Fish were then cut sagittally and the peritoneum removed. Viscera and muscles were examined using a dissecting microscope. Myomeres were teased apart to facilitate location and removal of encapsulated larvae situated deep in muscle. Larvae were removed from capsules in piscine saline using dissecting needles and were fixed in hot glycerin-alcohol (1 part glycerin to 9 parts 70% al-

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cohol). Larvae were cleared by allowing the alcohol to evaporate.

Encapsulated larvae in muscle were fixed in Gendre's fluid (Humason, 1972) or in 10% buffered formalin. Tissue was then prepared for histologic examination using standard procedures. Sections were cut at 6 μm and stained with hematoxylin and eosin. Mallory's phosphomolybdic acid hematoxylin stain (Mallory, 1942) was used to detect collagen in capsules.

Two male ferrets (A and B) were purchased from Marshall Research Animals, Inc. (North Rose, New York 14516, USA). At 14 wk of age each ferret was given five larvae of *D. renale* encapsulated in muscle of pumpkinseed collected from Billy Lake in October. Food was withheld from ferrets 10–12 hr prior to infection. Ferrets were kept in an outdoor enclosure and given water and Co-op Purrfect Catfood (United Co-operatives of Ontario, 151 Center City Drive, Mississauga, Ontario L5A 3A4, Canada) ad lib. Diet was supplemented with lard, cooked liver and cooked fish. Patency was determined by weekly examination of urine for eggs of *D. renale*. Ferrets were killed with an intracardiac injection of Somnotol (MTC Pharmaceuticals Ltd., Hamilton, Ontario L4W 2S5, Canada) and examined for *D. renale* 108 and 134 days post-infection. Tissues were fixed in 10% buffered formalin and prepared for histologic examination using standard procedures. Sections were cut at 6 μm and stained with hematoxylin and eosin.

Specimens of larvae of *D. renale* from experimentally infected *Lumbriculus variegatus* (USNM Helm. Coll. No. 73805) and *Rana pipiens* (USNM Helm. Coll. No. 73806) deposited by Dr. T. Mace in the U.S. National Parasite Collection, Beltsville, Maryland 20705, USA, were examined.

Description of study areas

Billy Lake (93 ha, maximum depth of 9 m) and Ryan Lake (50 ha, maximum depth of 10 m) are shallow, oligotrophic lakes. Lake Sasajewun (44 ha, maximum depth of 12 m, but averaging less than 3 m) is a shallow, eutrophic lake. These three lakes are situated in Algonquin Provincial Park, Ontario. Billy Lake and Ryan Lake were treated in 1965 and 1962, respectively, with toxaphene to remove undesirable fish. One yr after treatment Billy Lake and Ryan Lake were stocked with hatchery-reared brook trout (*Salvelinus fontinalis* Mitchill), rainbow trout (*Salmo gairdneri* Richardson), and splake (*Salvelinus namaycush* Walbaum × *Salvelinus fontinalis*) (Fraser, pers. comm.). Pumpkinseed re-entered Ryan Lake when beaver (*Castor canadensis* Kuhl) activity facilitated movement of these fish from Border Lake to Ryan Lake. It is unknown how pumpkinseed re-entered Billy Lake. Lake Sasajewun was created by damming the North Madawaska River in the early 1900's. Lake Sasajewun has not been treated with toxicants or stocked with fish.

RESULTS

Examination of fish

Larval *Dioctophyma renale* were found in pumpkinseed from Ryan Lake, Billy Lake and Lake Sasajewun. One specimen of *D. renale* was also found on the stomach wall in one of 16 brown bullheads from Lake Sasajewun. There was no difference in prevalence between male and female pumpkinseed in each lake sampled (χ^2, $P < 0.05$, df = 1). Similarly there was no difference in prevalence between male pumpkinseed in each lake and between female pumpkinseed in each lake (χ^2, $P < 0.05$, df = 2) (Table 1). Intensities were similar in the three lakes studied. Mean total length of infected pumpkinseed was 11.4 (9.4–14.5) cm in Lake Sasajewun, 11.8 (11.0–12.9) cm in Ryan Lake and 14.0 (11.9–17.6) cm in Billy Lake. Infected pumpkinseed were approximately Age Class III and IV based on total length (see Cone and Anderson, 1977).

Larvae of *Dioctophyma renale* were within small (1 to 2 mm) oval, white capsules situated predominantly in the hypaxial musculature (Table 2). The longitudinal axis of capsules was oriented parallel to muscle fibers (Fig. 1). Capsules were composed of layers of tough, opaque, white tissue. A cloudy, white exudate was released when larvae were dissected from capsules. One larva was found free within the hypaxial musculature.

Larval *Dioctophyma renale* in capsules situated in the hypaxial musculature of pumpkinseed elicited a mild granulomatous reaction (Fig. 1). Capsules were composed of two layers. The outermost layer consisted of dense connective tissue with fibroblasts scattered between collagen fi-

TABLE 1. Prevalence and mean intensity of larval *Dioctophyma renale* in *Lepomis gibbosus* collected in Algonquin Provincial Park, Ontario.

Lake	Number of fish examined and sex	Prevalence (%)	Mean intensity ± SD (range)	Mean total length (range) cm of fish examined
Sasajewun	48 ♀	6.3	1.7 ± 1.2 (1–3)	10.2 (8.9–11.8)
	40 ♂	5.0	1.0	10.5 (8.8–12.4)
Ryan	24 ♀	16.7	1.8 ± 0.9 (1–3)	10.7 (6.1–12.9)
	73 ♂	16.4	1.2 ± 0.4 (1–2)	12.3 (6.8–12.8)
Billy	56 ♀	23.2	1.5 ± 0.9 (1–4)	13.0 (10.1–18.4)
	38 ♂	13.2	1.6 ± 1.3 (1–4)	12.8 (10.7–17.7)

bers. The inner layer consisted primarily of macrophages but some lymphocytes were present (Fig. 2). An occasional giant cell was observed in the inner layer. Necrotic cells which contained nuclear debris and pale vacuolated cytoplasm were present in the inner layer near apices (Fig. 3). Connective tissue fibers proliferated into some necrotic areas. Muscle tissue surrounding capsules appeared normal except for some mild compression.

Specimens of *Dioctophyma renale* in pumpkinseed from the three lakes were third-stage larvae. There was no significant difference (Mann-Whitney test, $P < 0.05$) between total length of male and female larvae (Table 3). The genital primordium of female larvae was monodelphic and opisthodelphic. The vagina was evident, but the vulva was not yet patent (Fig. 4). The vulva was located near the esophageal-intestinal junction. From the vulva the female genital tube extended posteriorly and the distal tip was enlarged. The male genital primordium was diorchic and opisthorchic and the bifurcation of the male genital primordium was evident. One branch of the male genital primordium ended blindly anterior to the rectum; the other branch connected ventrally with the rectum. Two cuticles were evident in the anal region of larvae (Figs. 5, 6).

Specimens of *Dioctophyma renale* from pumpkinseed were similar to those from experimentally infected *Lumbriculus variegatus* and frogs deposited by Dr. Thomas Mace in the U.S. National Parasite Collection.

Specimens of larval *D. renale* from pumpkinseed have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNM Helm. Coll. No. 78096). Specimens of larval *D. renale* from pumpkinseed have also been deposited in the National Museum of Natural Sciences, Ottawa, Ontario, Canada (NMCIC(P) No. 1984-0324).

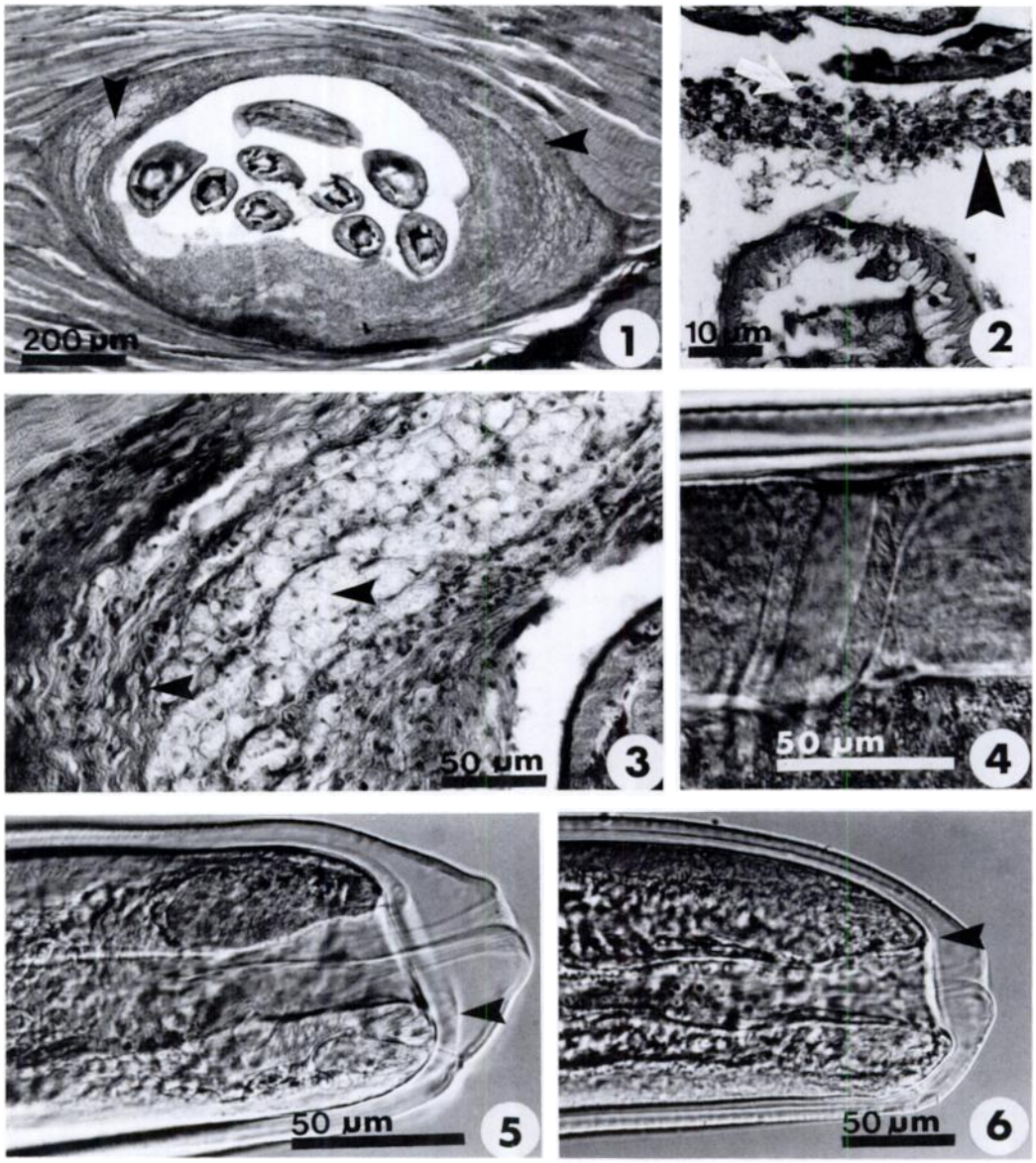
Transmission to ferrets

Fifteen minutes post-infection Ferret A given infected pumpkinseed muscle became agitated. Vomition began 30 min post-infection, was violent and intermittent and lasted for 45 min. Ferret B given infected pumpkinseed muscle started vomiting 30 min post-infection. Vomition

TABLE 2. Location of larval *Dioctophyma renale* in *Lepomis gibbosus* from Algonquin Provincial Park, Ontario.

	Number of infected fish			
	Number of larvae			
Location	1	2	3	4
Hypaxial musculature	22	4	2	2
Epaxial musculature	5	0	0	0
Stomach wall	2	0	0	0
Liver	1	0	0	0
Swim bladder wall	1*	0	0	0
Mesentery	2*	0	0	0

* One larva was also found in the hypaxial musculature.



FIGURES 1–6. 1–3. Capsule containing larvae of *Dioctophyma renale* within the hypaxial muscle of *Lepomis gibbosus*. (Hematoxylin and eosin.) 1. Encapsulated larval *D. renale*. Arrows denote outside layer of dense connective tissue and area of necrotic cells at apex of capsule. 2. Inner layer of capsule containing macrophages (arrows) between cross-sections of larval *D. renale*. 3. Area of capsule apex showing necrotic cells (top arrow) and infiltration by collagen fibers (bottom arow). 4–6. Third stage larvae of *Dioctophyma renale*. 4. Vulvar primordium of female larva. Cuticle of larva is to the top of the photograph. 5. Posterior extremity of male larva. Arrow denotes third-stage larval cuticle within the second-stage larval cuticle. 6. Posterior extremity of female larva. Arrow denotes third-stage larval cuticle within the second-stage larval cuticle.

TABLE 3. Dimensions[a] of larval *Dioctophyma renale* from the muscle of *Lepomis gibbosus* collected in Algonquin Provincial Park, Ontario.

	Male	Female
Number	10	10
Length	5.9 (4.1–7.4) mm	6.5 (5.1–8.6) mm
Nerve ring[b]	100 (70–130)	105 (90–120)
Esophageal length	2.3 (1.6–3.1) mm	2.4 (2.0–3.3) mm
% of total length	38 (34–42)	37 (33–40)
Width at esophageal-intestinal junction	150 (110–190)	179 (160–200)
Vulvar primordium[b]	—	3.2 (2.5–5.0) mm
% of total length	—	49 (43–58)
Length of rectum	194 (150–250)	177 (110–230)
Width at anus	90 (70–130)	74 (50–90)

[a] Values are means (ranges).
[b] From anterior extremity.
Measurements in micrometers unless otherwise indicated.

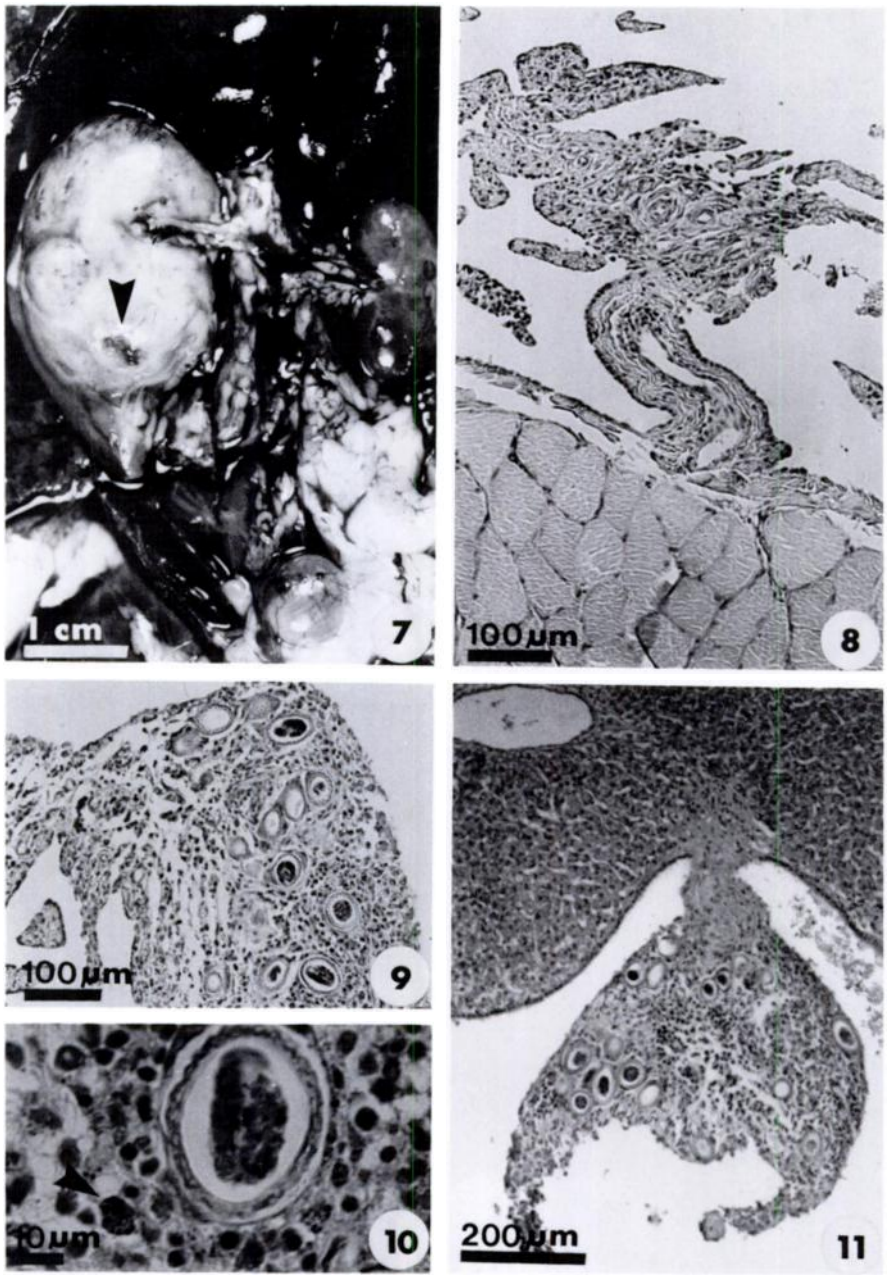
ceased 40 min post-infection. Between vomition episodes gagging and dypsnea were observed in both ferrets. No further clinical signs were observed during the course of the infection.

At necropsy 106 days post-infection eggs of *D. renale* were found in the urine of Ferret A and a 34 cm long adult gravid female was found in the right kidney capsule. Eggs of *D. renale* were found in the urine of Ferret B at 133 days post-infection. An 18 cm long adult male and a 36 cm long gravid female were found in the right kidney capsule of Ferret B. Eggs released by the gravid female worm from Ferret A failed to develop when incubated at 22 C for 1 mo. Eggs released by the gravid female worm from Ferret B developed into first-stage larvae when incubated.

Kidney capsules contained red fluid consisting of erythrocytes, cellular debris and eggs of *D. renale*. The capsule in Ferret A had a 3 mm opening on the posterior ventral surface (Fig. 7). A thin, flexible staghorn was observed on the dorsal wall of each kidney capsule. The kidney capsule was divided into two chambers in Ferret B. The proximal chamber, near the ureter, contained worms. The distal chamber contained no worms. The staghorn formation was present in the chamber containing worms.

Yellow-brown foliate masses (4–5 mm long) were adherent firmly to the peritoneum of the diaphragm and of the right lateral abdominal wall of both ferrets. On histologic examination a proliferative chronic inflammatory reaction was evident on the parietal peritoneum (Fig. 8). The underlying abdominal muscles were not affected. The lesions on the mesothelium consisted of fibrous tags containing fibroblasts and macrophages surrounding eggs of *D. renale* (Fig. 9). Plasma cells, erythrocytes and lymphocytes were also present. Dark brown granules were present within some macrophages and also on the mesothelium in some areas (Fig. 10).

Small (0.5–1.5 mm) firm, white to yellow depressed lesions were on the capsule of the visceral surface of the liver, mainly on the right lobes. White to yellow foci were also observed within the liver and again were more prevalent in the right lobes. These lesions indicated chronic persistent hepatitis with lymphocytes and plasma cells in portal tracts and occasionally around terminal hepatic veins. Random, small foci of mononuclear cells were also present throughout the parenchyma. Small pedunculate masses containing eggs



FIGURES 7–11. 7. Abdominal cavity of Ferret A experimentally infected with *Dioctophyma renale*. Note enlarged infected right kidney capsule with ventral opening (arrow). 8–11. Tissue from Ferret B experimentally infected with *D. renale*. (Hematoxylin and eosin.) 8. Extension of the peritoneum into the abdominal cavity. 9. Fibrous proliferative lesion on the peritoneum. Note eggs of *D. renale*. 10. Eggs of *D. renale* surrounded by macrophages. Note granules within macrophages (arrow). 11. A pedunculate mass of mononuclear cells, fibrous connective tissue and eggs of *D. renale* attached to the liver.

of *D. renale*, macrophages, lymphocytes and plasma cells were present (Fig. 11). Occasionally unorganized fibrin containing a few neutrophils was present at the distal tip of these masses. Flattened mesothelial cells were present on the surface of these masses.

Specimens of adult *D. renale* from experimentally infected ferrets have been deposited in the U.S. National Parasite Collection (USNM Helm. Coll. No. 78248).

DISCUSSION

This study represents the first report of larval *D. renale* in centrarchid fish. Ciurea (1921) described larvae in the muscle of orfe in Rumania and dogs given these fish developed infections of *D. renale*. However, the larvae described by Ciurea were not *D. renale*. Bangham and Hunter (1939) reported larval *D. renale* in the body cavity of smallmouth bass or largemouth bass (*Micropterus* sp.) from Lake Erie. Bangham and Hunter (1939) did not describe the larvae found in bass, thus it cannot be determined whether they were *D. renale* or *Eustrongylides* sp. The latter occurs commonly in fish (Karmanova, 1961; Mace and Anderson, 1975; Fastzkie and Crites, 1977; Lichtenfels and Madden, 1980).

Woodhead (1950) and Hallberg (1953) reported *D. renale* larvae on the mesentery of bullheads. Description of capsules agrees with that of the present study. Karmanova (1961) described *D. renale* encapsulated in the tunica musculosa of the small intestine of pike in the USSR. Mace and Anderson (1975) reported *D. renale* in five of 451 (1.1%) brown bullheads examined from Lake Sasajewun. Larvae were encapsulated in the wall of the stomach or on the mesentery and one was in abdominal muscles. Capsules in bullheads were not described. However, capsules in experimentally infected frogs (*Rana pipiens, R. clamitans*) were similar to those described in the present study; most capsules were located within the stomach wall or abdominal and pectoralis muscles 25 or more days post-infection. These authors also found that 25 of 504 (5.6%) wild frogs (*Rana* spp.) collected near Washago, Ontario were infected with *D. renale*. Most encapsulated larvae were within the stomach wall (47%) or abdominal (28%) and pectoralis (5%) muscles (Mace and Anderson, 1975). In the present study 85% of larvae were in the epaxial or hypaxial muscles of pumpkinseed.

Pumpkinseed eat aquatic invertebrates, some vegetation and in older age classes small fish which may comprise a significant part of the diet (Scott and Crossman, 1973). It has been suggested that fish such as bullheads and pike may acquire dioctophymatoid larvae by ingesting small fish infected with larvae (Woodhead, 1950; Karmanova, 1961, 1962; Cone and Anderson, 1977).

In the present study measurements of third-stage larvae from pumpkinseed were similar to those from pike reported by Karmanova (1961). However, measurements of third-stage larvae from experimentally infected *Lumbriculus variegatus*, experimentally infected frogs and wild frogs reported by Mace and Anderson (1975) were greater than those of the present study. Size of larvae may be affected by the host species.

The prepatent period determined in ferrets in the present study is similar to that observed by other workers. Woodhead (1950) infected ferrets and obtained gravid specimens of *D. renale* 156 days post-infection. Karmanova (1962) observed eggs of *D. renale* in urine of infected dogs 135 days post-infection. Mace and Anderson (1975) observed eggs of *D. renale* in urine of an infected mink 154 days post-infection.

Vomition episodes observed in ferrets in the present study were also described by Mace and Anderson (1975) in experimentally infected mink.

Mace (1976) described chronic peritonitis associated with adult worms in the

body cavity of mink. In the present study adult worms were found only within the right kidney capsule. There was an opening in the right kidney capsule of Ferret A which probably permitted eggs of *D. renale* and cellular debris to enter the peritoneal cavity. No opening on the kidney capsule was observed in Ferret B although chronic peritonitis seen in this animal was associated with the presence of eggs. Dark brown granules seen histologically were probably hemosiderin from lysis of erythrocytes.

Mace and Anderson (1975) suggested that frogs may be important paratenic hosts in some enzootic areas (Lake Sasajewun, Washago) while fish such as bullheads may be important paratenic hosts in other enzootic areas (Lake Sasajewun). The present study indicated that centrarchids may be more important paratenic hosts than bullheads or frogs in some enzootic areas (Lake Sasajewun, Ryan and Billy lakes). Fish, including centrarchids, are the predominant source of food of mink especially in spring, fall and winter (Hamilton, 1940; Sealander, 1943; Wilson, 1954; Gerell, 1967).

Dioctophyma renale can cause morbidity in man if infective stages are ingested (Mace, 1975; Beaver and Theis, 1979). The present report of infective larvae of *D. renale* in the musculature of pumpkinseed suggests a human health risk in enzootic areas if pumpkinseed are poorly cooked or eaten raw. Other centrarchids, such as bass preferred by sportsmen, may also be suitable hosts.

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