DIPLOSTOMULUM TRITURI (TREMATODA: DIPLOSTOMATIDAE), A LARVAL STRIGEOID TREMATODE IN THE BRAIN AND CRANIAL CASE OF THE NEWT, *NOTOPHTHALMUS VIRIDESCENS* (RAFINESQUE)

by

Suzanne Alice Whitlock

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APPROVED:

R. B. Holliman, Chairman

P. L. Dalby

D. F. Watson

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INTRODUCTION

The first diplostomulum was described in 1832 from the eyes of European fishes by vonNordmann. Braun fed the same parasite in 1894 as described by vonNordmann to a laughing gull (Larus atricilla) and found that it developed into the adult form known as Diplostomum volvens. Following the rules of zoological nomenclature, the generic name Diplostomulum was proposed for the larval stage.

Butler (1919) reported the occurrence of metacercariae of the genus Diplostomulum in the eyes of certain fish from Douglas Lake, Cheboygan County, Michigan. Later, La Rue, Butler and Berkhout (1926) reclassified the specimens as Tylodelphys Diesing. Hughes (1929) reexamined the original specimens of Butler and reported that they represent a new species which he named Diplostomulum scheuringi in honor of Professor L. Scheuringi of the Bayerische Biologische Versuchsanstalt für Fischerei of München, Germany.

Kelly (1934) first described Diplostomulum trituri mesocercaria from the eyes and brain of the newt, Trituris [= Notophthalmus] viridescens viridescens. After examining newts from locations in Pennsylvania, Ohio, Massachusetts, Michigan, South Carolina, and Virginia, Kelly reported the parasite only in the newts of western Pennsylvania. Rankin (1937) studied

several newt populations in North Carolina and failed to find D. trituri.

Lautenschlager (1956) first reported a fully developed strigeoid (member of Superfamily Strigeoidea) mesocercaria from the brain ventricles of this urodele.

Etges (1961) proposed and described the intramolluscan stages of D. scheuringi as a parasite of the snail Helisoma anceps. He experimentally infected newts and fish with cercariae of D. scheuringi and described the mesocercarial stage. He reported that D. trituri and D. scheuringi were two morphologically indistinguishable species. Since their taxonomic positions depend upon the identification of the adult forms it is not possible, at this time, to prove that they are synonomous. If D. trituri and D. scheuringi are proven to be synonomous; the term D. scheuringi will take precedence since it was first described by Hughes in 1929. Etges studied 71 adult newts collected from Mountain Lake, Giles County, Virginia, and recorded 100 per cent incidence of infection of this strigeoid mesocercaria.

It is possible for this species to have two developmental stages between the cercaria and the adult. The Genus Alaria of the Family Diplostomatidae has an extra larval stage that is interposed between the cercarial and metacercarial stages. This extra larval stage is termed the mesocercarial stage. It is characterized by having a less developed excretory system and by not being encysted. Schell (1970) reported that when

the mescocecaria is ingested by a suitable definitive host, it transforms to a diplostomulum type of metacercaria, which after some migration, finally settles in the intestine where it develops into the adult form. In some instances, the mesocercariae may be ingested by a collector or paratenic host in which they accumulate but cannot develop until this host is eaten by the suitable definitive host.

The term metacercaria is generally regarded as the larval stage interposed between the cercaria and the adult trematode. With the exceptions of the Genera Halipegus, Brachycoelium, and Panopistus, all metacercariae become encysted. Hoffman (1959) described the metacercariae of trematodes in the families Strigeidae and Diplostomatidae. The tetracotyle (Tetracotyle) encysts in the tissues of fishes, snails, and leeches. The cyst membrane is thick and transparent. Musculur pseudosuckers or cotylae are located on the ventral body surface. The adult trematode is an intestinal parasite of birds. The second type of metacercaria is the neascus (Genus Neascus) which encysts on the viscera of fishes. The cyst membrane is very thin and usually larger than the enclosed parasite. The body is divided into an anterior portion and a hindbody. The mature trematode is an intestinal parasite in piscivorous birds. The third metacercarial type is the diplostomulum (Genus Diplostomulum) which occurs in the eyes and central nervous system of fishes and amphibians. The host may produce a connective tissue layer or capsule to surround the parasite.

The life cycles that have been elucidated in the genus Diplostomum, as reported by Dubois (1936), Dawes (1956) and Yamaguti (1958), list a snail as the first intermediate host, an amphibian or a fish as the second intermediate host, and a bird or mammal as the definitive host. At the present time the first intermediate host and the definitive host are not known for D. trituri.

Etges (1961) exposed adult newts, Notophthalmus viridescens, to cercariae emerging from the snail Helisoma anceps. In 26 attempts to infect adult newts, cercariae were seen to crawl about on the skin but were unable to penetrate until they reached the eyes. Within 12 minutes, cercariae penetrated the cornea and entered the anterior chamber of the eye. Etges did not find any invasion of the retina, lens, or optic nerve. He also established that cercariae can penetrate the larval and red eft stage of Notophthalmus viridescens, and various fish (Lepomis gibbosus, Lepomis spp., Micropterus salmoides, and unidentified minnows). He also reported that cercariae can penetrate the skin of Notophthalmus larvae at any point when these immature newts are exposed to massive numbers of cercariae. This invasion causes small subcutaneous hemmorrhages. Migration by the cercariae via circulation carries them to all parts of the body, then they gradually accumulate in the brain. Etges did not find any worms outside the brain and eyes after six days. He noted that the growth of the unencysted parasite is rapid and that definitive

size was reached in 45-48 days. He noted no D. scheuringi in frogs and salamanders collected from Mountain Lake, Giles County, Virginia.

Lautenschlager (1963) did not find diplostomula in the eyes of the newts. However, he noticed that the intestinal lumen of some of the newts contained small snails or remains of snails. He suggested that some portion of the intestinal tract may serve as the site of infection and proposed a vascular route from the intestinal tract to the vessels of the meninges or the choroid plexus.

Other than the newt, D. scheuringi larvae have been reported in the vitreous humor of eyes of fishes in families Centrarchidae, Cyprinidae, Esocidae, Etheostomidae, Godidae, Percidae, Percopsidae, Salmonidae, and Siluridae by Hoffman (1959).

Three species of larval Diplostomulum have been recorded from urodeles. Kelly (1934) first reported D. trituri from newts. Kent (1940) found D. sirenis in sirens, and Rankin and Hughes (1937) described D. ambystomae from the coelom of Ambystoma opacum and A. maculatum.

An investigation of D. trituri was undertaken in order to determine by histopathology the sites of localization, gross effects, and neuropathological effects of its parasitism of the eastern spotted newt, Notophthalmus viridescens. The second phase deals with attempts to recover the adult stage from experimental feedings of the mesococercariae to various hosts.

MATERIAL AND METHODS

A. Histopathology

For the initial phase of the study, thirty newts were collected from Mountain Lake, Giles County, Virginia. The newts were maintained in aerated aquaria with standard chlorinated tap water. The newts were fed either raw beef liver or mealworms every three days. Fifteen newts were observed for a period of six months for any abnormal behavior. The remaining newts were used in the histopathology study. Uninfected newts were collected from Pandapas Pond, Montgomery County, Virginia for use as controls.

Newts were anesthetized by immersion in one per cent tricaine methanesulfonate (MS-222). The newts were subjected to intracranial injection of very warm FAA (formol-acetic-alcohol) to fix the diplostomula in place. When FAA was omitted the mesocercariae in the brain cavity migrated to the incision and moved freely in the saline solution. The heads were removed by making a transverse cut just posterior to the angle of the jaws with sharp scissors. The brain was exposed by making cuts through the ventral and dorsal skull. As soon as the brain cavity was exposed, the presence of the worms was most obvious. The brain cavity was gently flushed with amphibian saline and examined under a dissecting

microscope. The brain was removed with the aid of the dissecting microscope. The eyes, brain, and viscera were examined for infection.

The brains were fixed in FAA for 24 hours and were dehydrated beginning with 70% alcohol to absolute alcohol and cleared in Xylol. The brains were infiltrated with paraffin under vacuum, thus reducing the time the brains were exposed to heat. The brains were sectioned into 8 to 15 microns thickness with a rotary microtome. When transverse sections were made, the paraffin block was trimmed in order that one slide could hold 20 sections of paraffin ribbon. After drying, the slides were deparaffinized with xylene followed by coating in parlodion dip. The standard technique was followed for staining in Harris hematoxylin and eosin. The slides were cleared in xylene and mounted with balsam damar.

Eyes were removed from five newts and prepared for sectioning. Eyes were fixed in Kolmer's fixative for 24 hours. After 24 hours a small cut was made in the side of the eye and allowed to fix for another 24 hours. The dehydration process was followed very slowly. The material was then infiltrated with paraffin. Sagittal sections were attempted through the optic nerve at thicknesses of 6 to 12 microns. The material was stained in Patay's triple stain. Photomicrographs were taken using Tri-X Pan and Panatomic film.

B. Experimental Feedings to Various Hosts

A number of different vertebrates were tested in an effort to locate the definitive parasite host and to find the adult trematode. Sixteen of twenty-one day old Japanese quail, Coturnix coturnix, were infected orally by using a glass pipette with rubber tubing attached at the end. Each quail was given 15 mesocercariae. The quail were examined at the end of two, twenty, thirty-six, forty-eight, seventy-two, ninety-six, and one hundred-twenty hours. Four control quail were examined at the end of thirty-six, seventy-two, and ninety-six hours. Three four-month old mallard ducks, Anas platyrgnchous, were used in the second attempt to find either the encysted larval stage or the adult. Two ducks were infected with 150 mesocercariae orally by using the glass pipette and rubber tubing. Immediately after giving the parasites, the ducks were given water as before to force the ducks to swallow the parasites. The third duck was maintained as a control. The ducks were examined at the end of forty-eight and ninety-six hours. A three-year old raccoon, Procyon lotor, was offered living newts. The raccoon consumed a newt; however, later newts were refused. Mesocercariae were counted and placed into a dish containing amphibian saline. Approximately 15 mesocercariae and saline were placed into each empty number three gelatin capsule. They were ingested readily by the raccoon when hidden in sardines. Two one-year old raccoons

were infected in the same manner. A two-year old raccoon was maintained as a control. The raccoons were examined six days post infection.

A three-month old kitten was obtained from the SPCA in Roanoke, Virginia. The kitten was infected by using both the capsule method and glass pipette with rubber tubing in each case receiving 25 mesocercariae. The kitten was examined four days post infection. A muskrat, Ondatra zibethicus, was anesthetized with ether and inoculated orally with 200 mesocercariae by using a syringe with rubber tubing attached to the needle. It was examined six days post infection. The Virginia opossum, Didelphis virginiana, was inoculated with 100 mesocercariae in the same manner as the muskrat. Examination was made six days post infection. Fish from the families Salmonidae, Catostomidae, Cyprinidae, and Centrarchidae were also tested. The various fish tested included Catostomus, Salmo gairdneri, Lepomis, and Carassius auratus. Immersion in one per cent tricaine methanesulfonate (MS-222) was used to anesthetize the fish. Rubber tubing attached to a syringe was used as a stomach tube for the passage of the mesocercariae directly into the stomach. Equally effective was injecting earthworms and salmon eggs with the mesocercariae in saline solution. The fish were given 100 mesocercariae in each of the methods employed. All were examined three, four, and 21 days post infection. A post-mortem examination included the intestinal tract, organs, muscles,

eyes, and brains of the experimental animals.

RESULTS

Measurements were made of living trematodes with a standard light microscope. The typical strigeoid body is divided into a large anterior portion and a tiny posterior portion containing a small genital primordium and two bilateral excretory bladders.

The average length of twenty mesocercaria was 1.102 mm, and the average width was 0.37 mm. The structures in the anterior portion included a terminal oral sucker, a short prepharynx, a small pharynx, excretory tubules, a large holdfast organ, and an acetabulum (Figure 1). The average measurement for the acetabulum was 0.05 mm long and 0.06 mm wide. The holdfast organ was 0.142 mm long and 0.092 mm wide. The ceca were evident in few specimens. The material in the living trematode ceca was black and was assumed to be ingested neural tissue (Figure 2). It was evident in several slides that the mesocercariae were attached to the brain tissue by their oral sucker (Figure 3).

Twenty newts had an average total length of 94.9 mm; the average trunk length was 45.8 mm, the average head width was 7.6 mm. There was no significant differences in the measurements of normal and diseased newts. This finding agrees with those of Lautenschlager (1963), who compared the mean

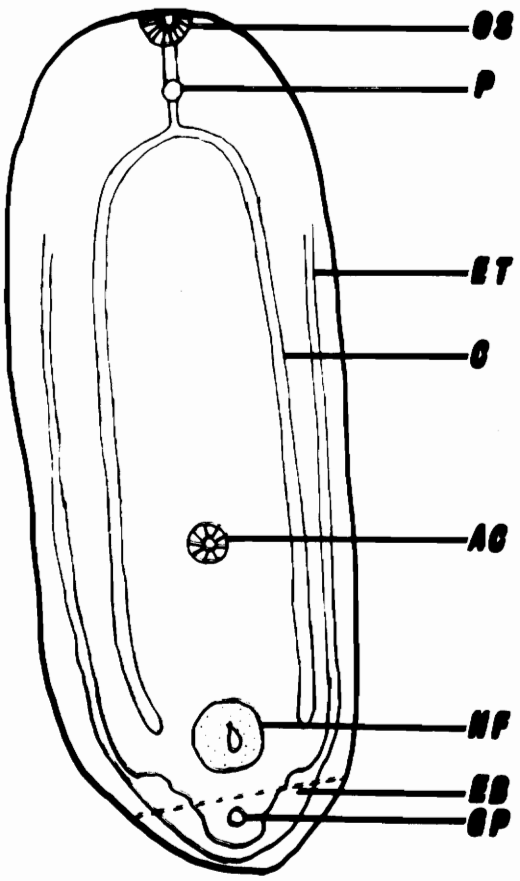


Fig. 1. Drawing of living specimen of Diplostomulum trituri. X 50

OS - oral sucker

P - pharynx

ET - excretory tubule

C - ceca

AC - acetabulum

HF - holdfast organ

GP - genital primordium

EB - excretory bladder

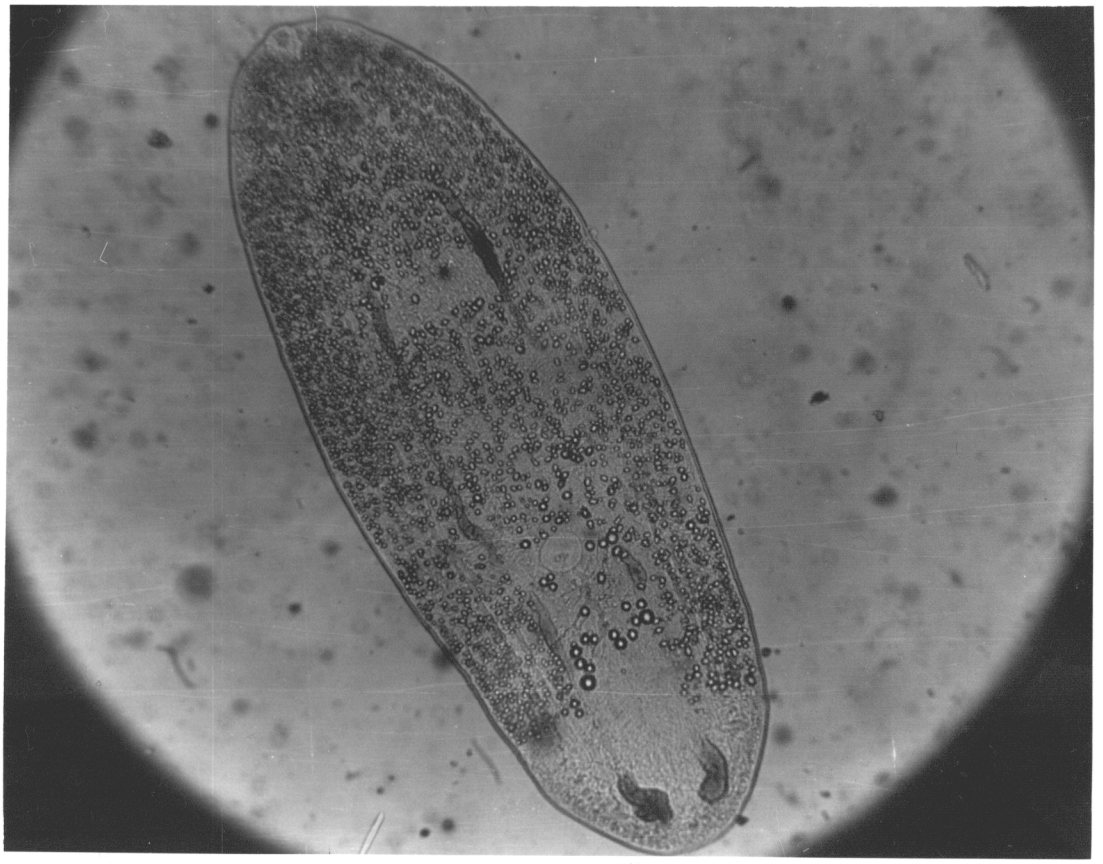


Fig. 2. A living Diplostomulum trituri showing black material in ceca. X50

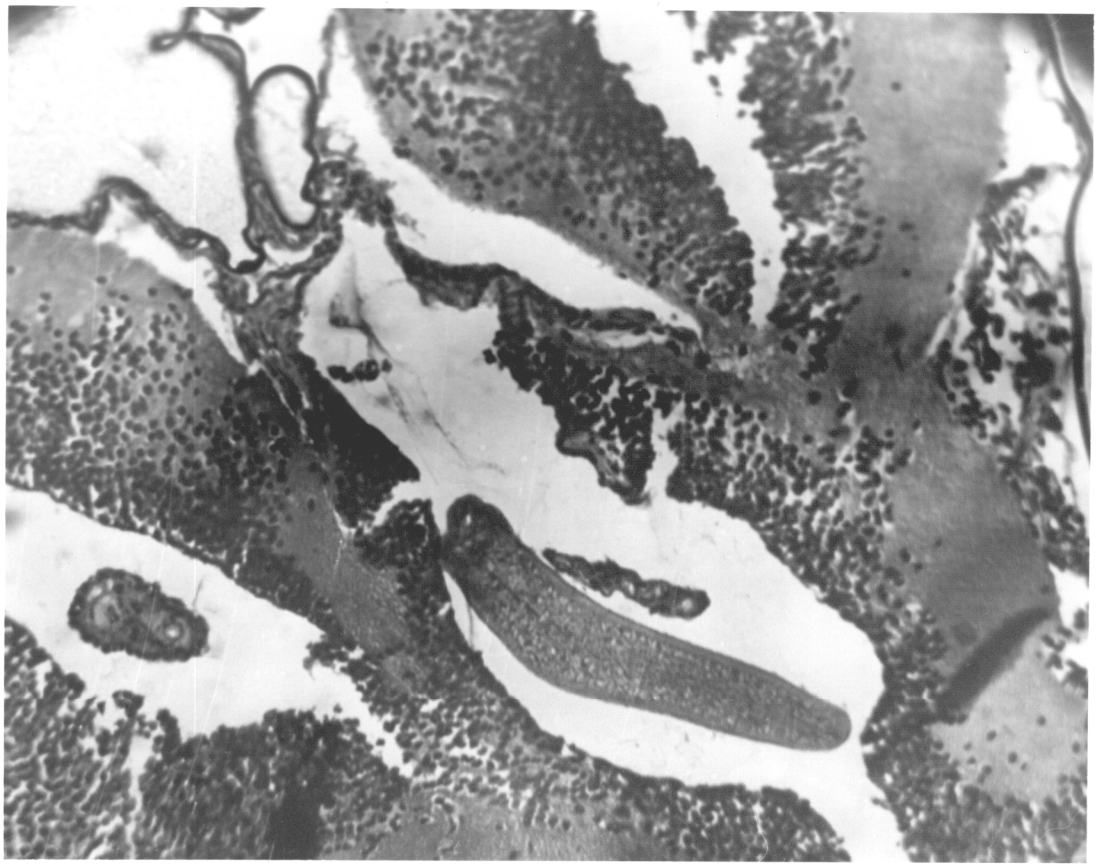


Fig. 3. Obliquely transverse section through the rostral border of the chiasma ridge and the middle of the eminentia thalami of the newt showing Diplostomulum tri-turi attached to the neural tissue. X100

measurements of 230 infected and 150 uninfected newts.

In the 150 newts examined from Mountain Lake, the incidence of infection was 100 per cent. The number of mesocercariae ranged from 2 to 300 within the ventricles of the brain, under the meninges, free in the cranial cavity, and in the eye chamber (Figures 4 and 5). Forty of the above newts harbored more than 200 mesocercariae each. Each of three newts apparently had an eye destroyed due to the parasite. The average number of parasites found within the remaining newts was 50. The average number found within the eye in newts with eye infections was 6. Lautenschlager (1963) noted that in 21 infected brains, the number of mesocercariae ranged from 5 to 35 in the submeningeal tissues, ventricles, and brain case.

There was a marked increase in the size of the infected ventricles of the cerebral hemispheres when compared to uninfected brains (Figure 6). Lautenschlager (1963) noted only 2 larvae in the ventricles, whereas in this study the number ranged from 5 to 30 in the ventricles (Figures 7 and 8). Lautenschlager also reported the absence of the choroid plexus of the ventricles of infected newts. In 5 newts a very distinct brown pigmentation was noted in many cells of the ependymal layer which is the membrane lining the ventricles of the cerebral hemispheres. Those showing the dark pigmentation in the ependymal layer contained over 200 parasites. Lautenschlager (1963) tested the pigmented material for hemoglobin

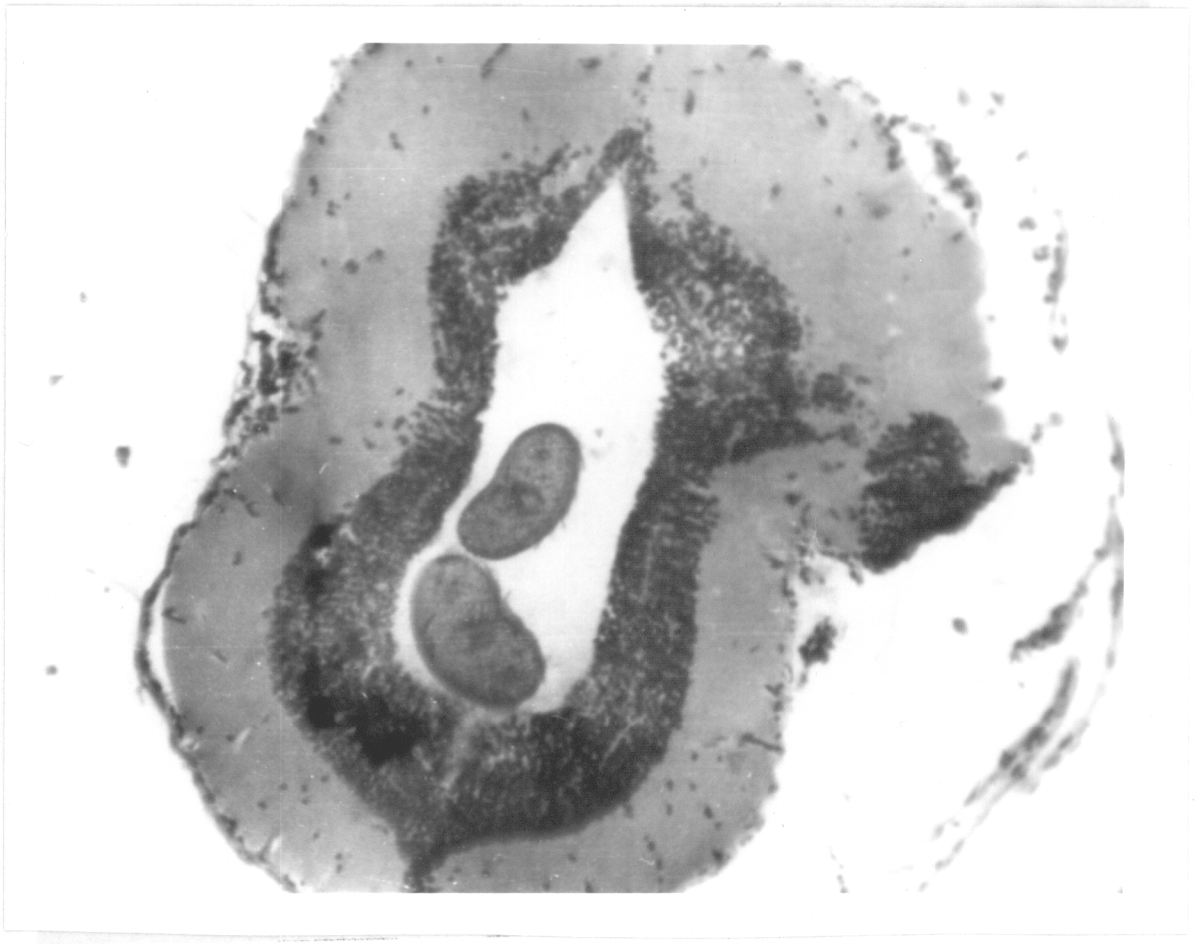


Fig. 4. Transverse section of the fourth ventricle of the newt showing two Diplostomulum trituri. X40

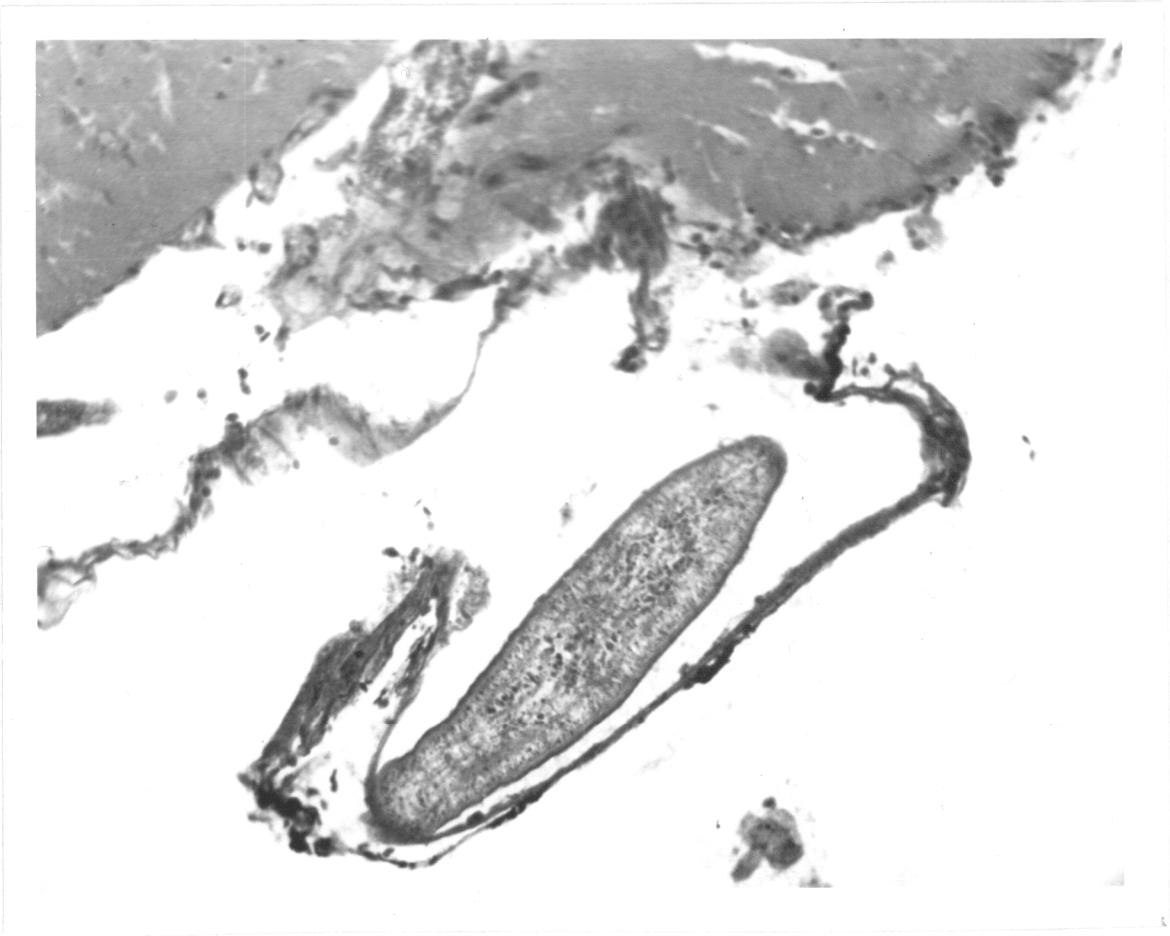


Fig. 5. Diplostomulum trituri under the meninges of the brain in the newt. X100

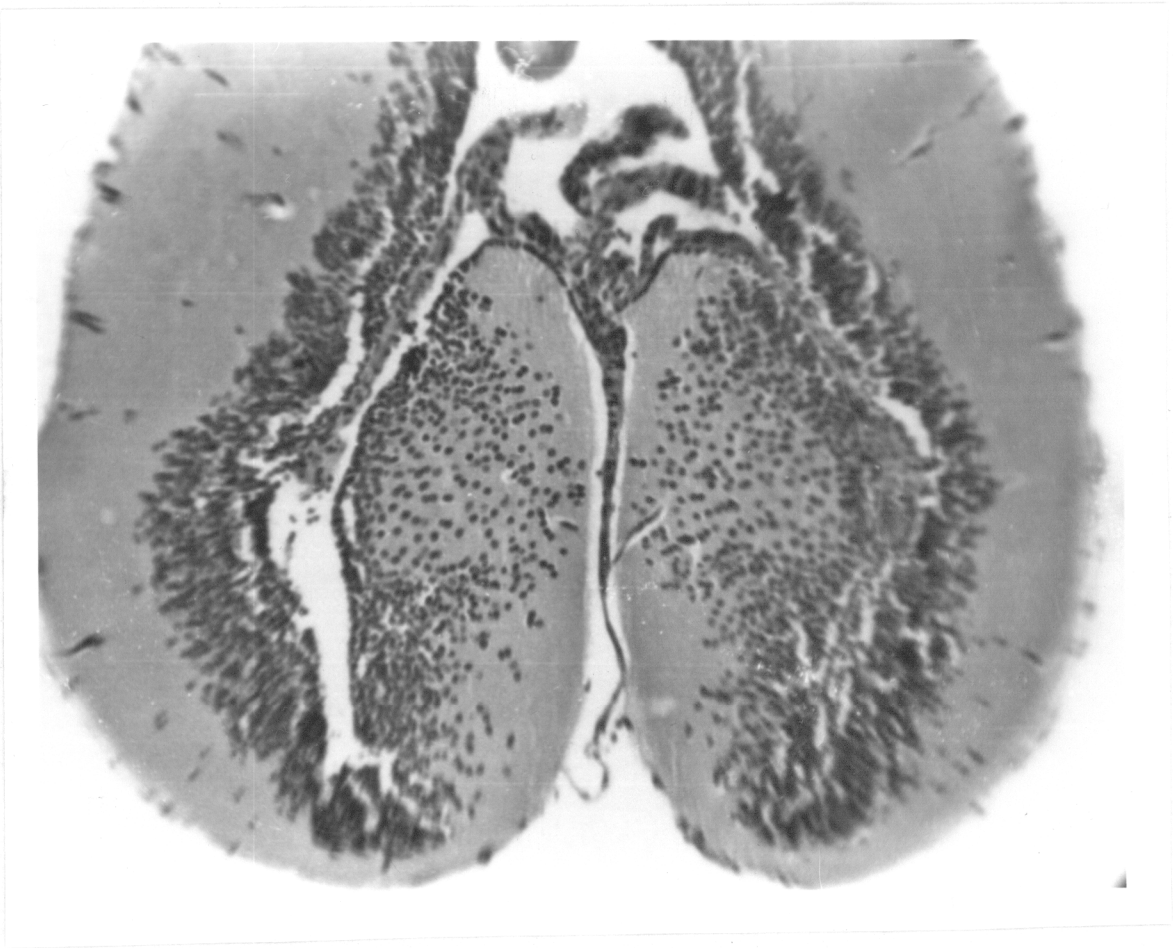


Fig. 6. Longitudinal section through cerebral hemispheres of an uninfested newt. Note the size of the ventricles as compared with Figure 7. X40

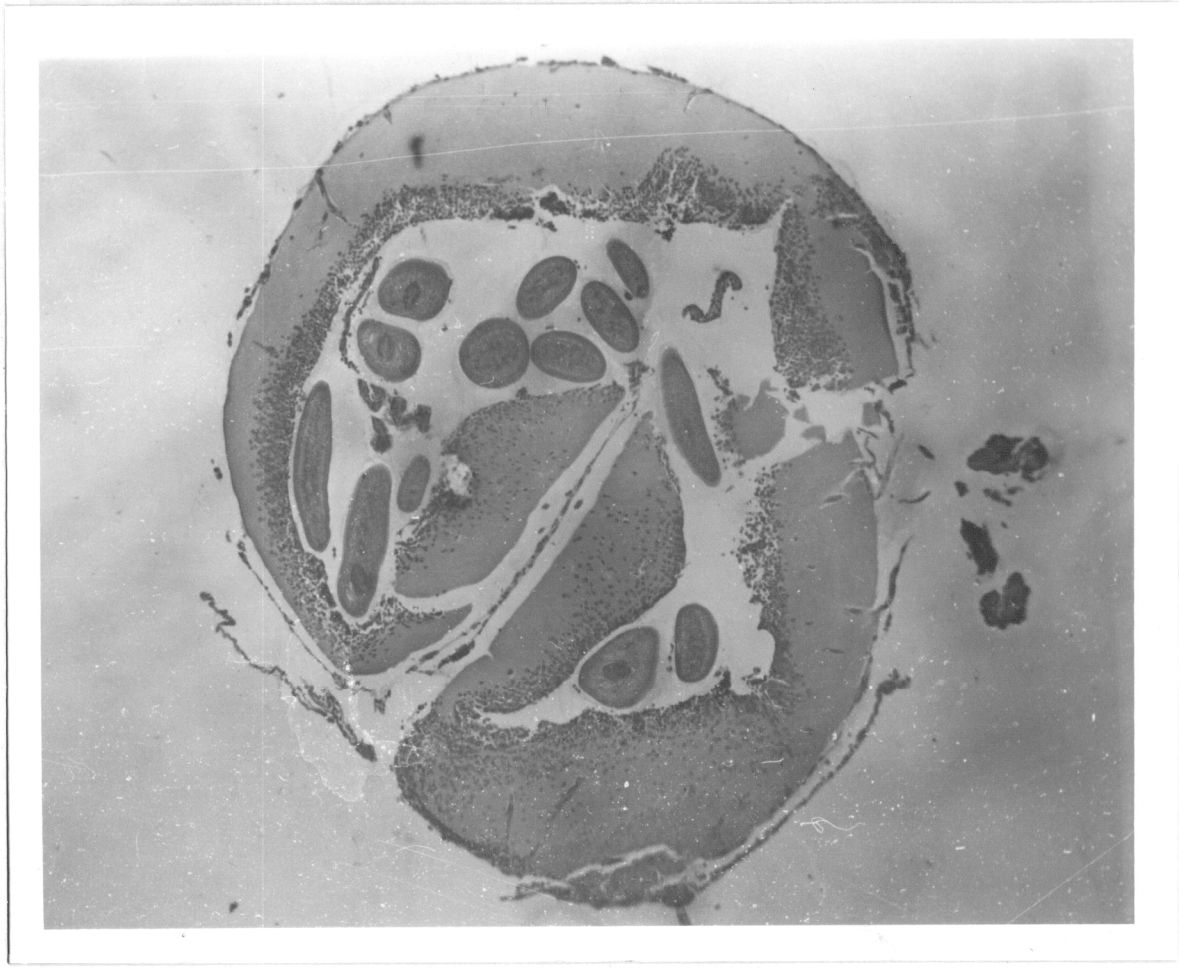


Fig. 7. Longitudinal section through cerebral hemispheres of the newt showing thirteen Diplostomulum trituri inside the ventricles. Note the marked increase in size of ventricles as compared to Figure 6. X40

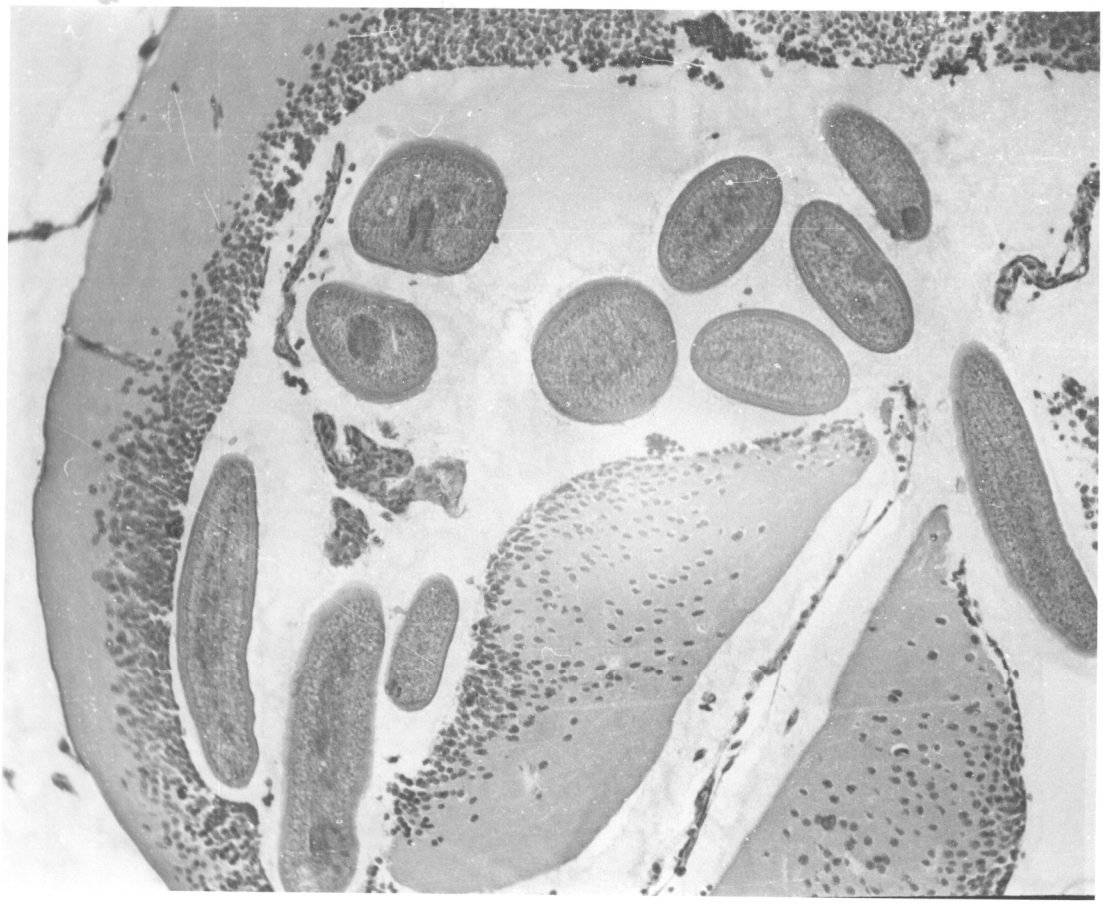


Fig. 8. Longitudinal section through cerebral hemispheres of the newt showing enlarged view of lower right quadrant of Figure 7. Note the enlarged size of the ventricles and loss of neural tissue. X100.

and found it was negative (with Turnbull's blue for organic iron [Glick, 1949].)

Lautenschlager (1963) reported distinct tissue masses on the surface of the cerebral hemispheres which showed pathological characteristics similar to benign meningiomas. In several cases, he observed the typical hyperplasia of the meninges and choroid plexus to be associated with the tissue mass. Such tissue masses were not observed material in this study.

There was an increase in the pigment of the meninges in the infected newt. The cells of the ependymal layer of the brain were larger when intraventricular parasites were present.

The control newts showed no comparable pathological conditions.

DISCUSSION

The morphological descriptions of the mesocercaria of D. trituri are very similar to D. scheuringi. We must first recover the adult of D. trituri and of D. scheuringi before we can assume that they are the same or different species. It is of interest to note that Etges (1961) reported no natural infection of D. scheuringi in frogs or salamanders at Mountain Lake, Virginia. If these two species are truly synonomous, one would expect to find the larvae of D. scheuringi in frogs or salamanders.

Lautenschlager's (1963) report of the benign meningioma may be questioned due to the fact that the tissue mass was not observed in all of the parasitized newts studied by him and due to the fact that this writer did not observe the tumor mass. Other reports of tumor formation associated with trematode infection includes the work of Hoffman and Hoyme (1957) who experimentally demonstrated the "tumor" on the brain of a Stickleback caused by Diplostomum baeri eucaliae (Hoffman and Hundley, 1957). The "tumor" was an outgrowth of the columnar epithelium of the choroid plexus which surrounds the mesocercariae at the postero-lateral aspect of the optic lobes. The function of the choroid plexus is the secretion of cerebrospinal fluid.

The source of nourishment for D. trituri was suggested to be from cerebrospinal fluid by Lautenschlager (1963). He did not report any indication of particulate material in the lumen of the intestinal ceca. On the basis that particulate material was observed in the intestinal ceca and in several cases where the mesocercariae were attached to the host tissue, it is the conclusion of the author that the source of nourishment of the parasite also includes cells from the brain. There were several newts that showed loss of brain tissue. As stated earlier, the case of the one-eyed newts indicates that there may be resorption of the neural tissue due to the presence of the parasite. Hoffman and Hundley (1957) noted severe loss of brain tissue in the Stickleback infected with D. baeri which was due to resorption. In their report they assumed that the mesocercariae were eating macrophages. The "tumor" as described earlier was filled with cells that appeared to be macrophages. They did note cellular elements in the ceca.

Observations were made on infected and uninfected newts to determine if the parasites were causing neurophysiological symptoms. Newts maintained in the lab were casually observed for a period of six months. Aggressive behavior was noted for the one-eyed newts and other infected newts. Other observations were made on abnormal swimming patterns consisting of very sporadic movements. When attempts were made to hand catch the lab newts, the controls were very responsive and

difficult to catch while the infected were sluggish and easy to capture. Feeding behavior appeared normal in infected animals. In a study by Herrick (1965) he noted that the more sluggish fishes and, especially mudfishes living in stagnant water, have enormously enlarged and highly vascular choroid plexes, the ventricles are dilated, and the walls of the brain are thin. This ensures a supply of oxygen to the brain which is adequate for their quiescent existence.

The sluggish behavior and poor reflexes were thought to be associated with the increased size of the ventricles in the cerebral hemispheres. The cerebral hemispheres govern all motor activities and are the instigators of voluntary acts and exert a controlling force on reflex acts.

The attempts to recover either the encysted larval stage and/or the adult failed after experimentally infecting Japanese quail, mallard ducks, raccoons, muskrat, opossum, domestic cat, and fish from families Salmonidae, Catostomidae, Cyprinidae, and Centrarchidae with mesocercariae of D. trituri.

Etges (1961) fed fully grown diplostomula from both fish and newts to 22 one-day old chicks but failed to produce encysted larvae and/or adults. He also inoculated 31 mice and chicks orally and at the end of 2 hours post infection, dead larvae were found in the upper small intestine in both chick and mouse. Etges did get encysted worms along the axillary muscles due to the fact that he had administered

the worms with a syringe into the trachea instead of the esophagus. The diplostomula will encyst slightly in warm saline. He then suggested that the parasite's entire life history involves four obligate hosts and that the encysted stage must be in a poikilothermous host. Previous attempts made in this laboratory to discover the definitive host in snakes also have failed.

The high incidence of infection at Mountain Lake, Virginia, indicates that the definitive host is present in considerable numbers. It is possible that the newt is an unnatural host for the parasite.

With newts, as with other salamanders, there are few predators due to the fact glands in the skin produce a toxin which is very distasteful and potentially dangerous to the predator. The defense mechanism of salamanders was studied by Brodie (1968) who tested the susceptibility of 30 vertebrate species to toxin from a European newt, Taricha granulosa. He reported that all animals tested with the Taricha skin toxin displayed symptoms of muscle weakness, convulsions, gasping, gaping, regurgitation, flaccid paralysis and depression of blood pressure. The toxin was described by Brown and Mosher (1963) to be a nonproteinaceous neurotoxin, "tarichatoxin." The empirical formula was established as $C_{11}H_{17}N_3O_8$ and was identical to tetrodotoxin which comes from ovaries and liver of Sphoeroides rubripes, the puffer fish.

In fishes there appears to be a difference in toxic

response between species. It is of interest to note that Brodie found bluegill, large-mouth bass, and channel catfish to be susceptible. He did report cases where rainbow trout, Salmo gairdneri, were collected alive with T. granulosa in the stomach. Fishermen use salamanders as bait for large-mouth bass very successfully (Garrison, 1974). For turtles, Ernst and Barbour (1972) described their eating habits, and reported that salamanders were included in the diet of many. The eastern garter snake, Thamnophis sirtalis, was one of the few snakes which ate newts readily with no apparent ill effects.

The mesocercariae were removed from the newt before infecting the animals in this study. One raccoon did eat one newt and no adverse effects were noted. The animal did not show any interest when offered a newt as food on the following day. The parasites in the fish that have been described as D. scheuringi may be the only possible way for this life cycle to be completed. However, due to the fact that the parasite was removed from the newts before infection of an experimental host was attempted eliminated the potential toxic aspect of the skin and if the normal definitive host had been among the experimental hosts, the adult form of D. trituri would have been found. The only way we can determine if D. trituri and D. scheuringi are the same species is to produce the adult stage in some definitive host and compare their morphology.

Etges (1961) found three strigeoid infections among 107

specimens of Helisoma anceps (Menke) and identified them as cercariae for D. scheuringi. He did not find cercariae for D. trituri in the trematode study of snails.

SUMMARY

1. Diplostomulum trituri, a mesocercarial larval strigeoid, parasitic only in the eye and brain of the newt, Notophthalmus viridescens, is associated with pathological damage which includes increased size of the ventricles and loss of brain tissue due to resorption of the neural tissue.
2. Neurophysiological symptoms of parasitism include sluggish behavior and poor reflexes. This was assumed to be the result of the increased size of the ventricles in the cerebral hemispheres. Aggressive behavior was noted for the one-eyed newts and other infected newts.
3. Black material was observed in the lumen of the intestinal ceca. Several mesocercariae were observed to be attached to the neural tissue by their oral suckers.
4. In 150 newts examined from Mountain Lake, Giles County, Virginia, the incidence of infection with D. trituri was 100 per cent. The total number of parasites in the brain and surrounding area ranged from 2 to 300. The number of mesocercariae in the ventricles of the brain was approximated to range from 5 to 30.
6. The attempts to recover the encysted larval stage and/or adult failed using Japanese quail, mallard ducks, raccoon,

muskrat, opossum, domestic cat, and fish from families Salmonidae, Catostomidae, Cyprinidae, and Centrarchidae as experimental definitive hosts for *D. trituri*.

LITERATURE CITED

Brodie, E. D. 1968. Investigations on the skin toxin of the adult rough-skinned newt, Taricha granulosa. Copeia. 2:307-313.

Brown, M. S. and H. S. Mosher. 1963. Tarichatoxin: isolation and purification. Science 140:295-296.

Butler, E. P. 1919. Notes on the presence of larval trematodes in the eyes of certain fishes of Douglas Lake, Michigan. Ann. Report Mich. Acad. Sci., 21-116.

Dawes, B. 1956. The Trematoda. Cambridge Univ. Press., Cambridge.

DuBois, G. 1936. Monographic des strigeida (trematoda). Mem. Soc. Neuch. Sci. Nat. 6:1-535.

Ernst, C. H. and R. W. Barbour. 1972. Turtles of the United States. Univ. Press of Kentucky.

Etges, F. 1961. Contributions to the life history of the brain fluke of newts and fish, Diplostomulum scheuringi. Hughes, 1929 (Trematoda: Diplostomatidae). Jour. Parasit. 47:453-458.

Garrison, C. 1974. The 'dog that sniffs out bass.' True's Fishing Yearbook. 25:64-65.

Glick, D. 1949. Techniques of histo- and cytochemistry. Interscience Publishers, Inc., New York.

Herrick, C. J. 1965. The brain of the tiger salamander Ambystoma tigrinum. Univ of Chicago Press., Chicago.

Hoffman, G. L. 1959. Synopsis of Strigeoidea (Trematoda) of fishes and their life cycles. Fish. Bull. (175) U.S. Fish and Wildl. Service 60:439-469.

Hoffman, G. L., and J. B. Hoyme. 1957. The histopathology of the "tumor" on the brain of the stickleback caused by Diplostomum baeri eucaliae. Hoffman and Hundley, 1957. J. Parasit. 43 (5) Sec. 2:33.

Hoffman, G. L. and J. B. Hundley. 1957. The life-cycle of Diplostomum baeri eucaliae (Trematoda: Strigeida). Jour. Parasit. 43(6), 613-627.

Hughes, R. C. 1929. Studies on the trematode family strigeidae (holostomidae). XIX. Diplostomulum scheuringi sp. nov., and D. vegrandis (LaRue). Jour. Parasit. 15(4), 267-271.

Kelly, R. S. 1934. The trematode parasites of Trituris viridescens viridescens (Rafineque). Univ. Pitt. Bull. 31(1); 201-210.

LaRue, G. R., Butler, E. P. and P. G. Berkhout. 1926. Studies on the trematode family strigeidae (Holostomidae) No. IV. The eye of fishes, an important habitat for larval strigeidae. Trans. Amer. Micr. Soc., 45:282-288.

Lautenschlager, E. W. 1956. A study of Diplostomulum sp. (Trematoda: Diplostomatidae) in the brain and cranial case of Trituris viridescens viridescens (Rafineque). Master's thesis, Univ. of Virginia.

_____. 1963. Diplostomulum trituri, larval strigeid trematode. Univ. Microfilms, Ann Arbor.

Rankin, J. S. 1937. An ecological study of parasites of some North Carolina salamanders. Ecol. Manag. 7:169-269.

Rankin, J. S., Jr., and R. C. Hughes. 1937. Notes on Diplostomulum ambystomae n. sp. Tr. Am. Micr. Soc. 56: 61-66.

Schell, S. C. 1970. How to know the trematodes. Wm. C. Brown Comp., Dubuaue.

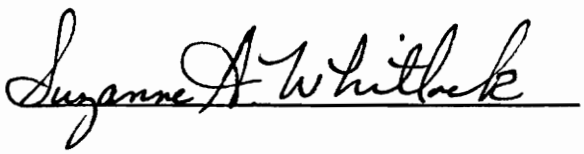
von Nordmann, A. 1832. Mikrographische Beiträge zur Naturgeschichte der Wirbellosen Thiere. I. 118 pp. Berlin.

Yamaguti, S. 1958. Systema Helminthum, Vol. 1. Interscience Publishers, Inc., N.Y.

VITA

Suzanne Alice Whitlock was born January 7, 1950 in Roanoke, Virginia to Walter Bernard and Margaret Obenshain Whitlock. She attended Troutville Elementary School and was graduated from Lord Botetourt High School in Daleville, Virginia in 1968. She attended Madison College in Harrisonburg, Virginia and was awarded a B.S. degree in Biology in June 1972. She was accepted at Virginia Polytechnic Institute and State University for graduate studies in September, 1972. She received her M.S. degree in Zoology from that university in June, 1974.

Her permanent address is Box 205, Troutville, Virginia, 24175.



DIPLOSTOMULUM TRITURI (TREMATODA: DIPLOSTOMATIDAE) A LARVAL STRIGEOID TREMATODE IN THE BRAIN AND CRANIAL CASE OF THE NEWT, *NOTOPHTHALMUS VIRIDESCENS* (RAFINESQUE)

by

Suzanne Alice Whitlock

(ABSTRACT)

Diplostomulum trituri is associated with pathological damage which includes increased size of the ventricles and loss of neural tissue. Neurophysiological symptoms of parasitism include sluggish behavior and poor reflexes. Black material was observed in the lumen of the intestinal ceca. Several mesocercariae were observed to be attached to the neural tissue by their oral sucker.

In 150 newts examined from Mountain Lake, Giles County, Virginia, the incidence of infection with *D. trituri* was 100 per cent. The total number of parasites in the brain and surrounding area ranged from 2 to 300. The number of mesocercariae in the ventricles of the brain was approximated to range from 5 to 30.

The attempts to recover the encysted larval stage and/or adult failed using Japanese quail, mallard ducks, raccoon, muskrat, opossum, domestic cat, and fish from families

Salmonidae, Catostomidae, Cyprinidae, and Centrarchidae as experimental definitive hosts for *Diplostomulum trituri*.