A STUDY OF THE SEASONAL CYCLE OF A TETRAPHYLLIDEAN CESTODE, <u>PROTEOCEPHALUS</u> <u>STIZOSTETHI</u> HUNTER AND BANGHAM, FOUND IN THE YELLOW PIKEPERCH, <u>STIZOSTEDION</u> <u>VITREUM</u> <u>VITREUM</u> (MITCHILL), WITH NOTES ON OTHER HELMINTHS FOUND IN THIS FISH

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"A Study of a Seasonal Cycle of a Tetraphyllidean Cestode, *Proteocephalus stizostethi* Hunter and Bangham, Found in the Yellow Pikeperch, *Stizostedion vitreum vitreum* (Mitchell), with Notes on Other Helminths Found in this Fish"

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A STUDY OF THE SEASONAL CYCLE OF A TETRAPHYLLIDEAN CESTODE, *PROTEOCEPHALUS STIZOSTETHI* HUNTER AND BANGHAM, FOUND IN THE YELLOW PIKEPERCH, *STIZOSTEDION VITREUM VITREUM* (MITCHILL), WITH NOTES ON OTHER HELMINTHS FOUND IN THIS FISH

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I. INTRODUCTION

A study has been made to determine the presence of a seasonal cycle of the tetraphyllidean cestode, Proteocephalus stizostethi, which occurs in the yellow pikeperch. This work was started in November of 1949 and specimens of this fish were examined for parasites at monthly intervals until April of 1951.

Juvenile and progressively more mature tapeworms of this species were obtained at nearly each examination during the fall and winter months, but specimens containing eggs were not found until the late spring and early summer. The fish host seemed to lose these parasites between late June and September.

In the literature of the parasites of fish there have been very few reports where a study was made throughout the entire year. Most studies have been made from material collected and preserved during the summer and brought to the laboratory for study during the remainder of the year. Because of this, it was thought that a study of a specific parasite, examined at intervals through two seasons, would be of value.

II. HISTORICAL REVIEW

A. A Brief History of the Genus Proteocephalus, Including some General Remarks on the Family Proteocephalidae

A detailed history of the family Proteocephalidae is included in the extensive Illinois Biological Monograph of 1914 by La Rue, a condensation of which follows:

> Rudolphi (1808-10) collected together the results of the systematic labors of helminthologists up to that time. His work is very complete and in it are to be found the diagnoses, synonymy, and descriptions of the species of this genus that were known at that time. These species are included in the genus Taenia Linnaeus 1758. ... It is needless to say that the works of these early investigators are almost entirely concerned with the study of external characters. There were no more important works which have to do with this group until the time of Dujardin (1845) and Diesing (1850). These investigators listed several new species and new host species.
>
> Investigators up to the time of Monticelli (1891) with the exception of Weinland (1858) considered this group of fish cestodes to belong to the genus Taenia. The latter author proposed the name Proteocephalus ... and the former proposed the name Tetracotylus. It was von Linstow (1891) who first pointed out that the fish species made up a closely related group within the genus Taenia. He made the first careful study of one of this group. ... Since this work of Monticelli, Lonnberg (1894) proposed the genus Ichthyotaenia and in this genus many species have been included. The range of hosts includes amphibians, snakes, lizards, and all the larger groups of freshwater fish. ...
>
> It has been found necessary to restrict the genus Proteocephalus by removing from it the species of amphibian and ophidian Proteocephalids. These make a fairly homogeneous group for which the writer proposed the name <u>Ophiotaenia</u> La Rue 1911.

Benedict (1900) under the direction of H. B. Ward was the first American investigator to make a careful and accurate study of the histological structure of two fish tapeworms, _Proteocephalus ambloplitis_ and _P. filicollis_. In his paper he says of the work preceding him:

> Zschokke ('84) was the first to make any study whatever of anatomical structure, and therefore his work is of much greater value than that of the older authors. ...
> By far the best work yet done on this group is found in the paper by Riggenbach ('96), who, in addition to a careful description of two new species, has for the first time made an accurate and discriminating summation of the present knowledge of this group. At the present time a great amount of confusion exists and the accuracy of the specific determinations is doubtful in over half of the species of _Ichthyotaenia_ so far described.

The classification within the family is still confused, and the usual arguments exist about generic synonymy. La Rue (1914) was, and still is, a proponent of the generic name Proteocephalus. He believes this because the genus Proteocephalus Weinland, 1858, and the genus Ichthyotaenia Lonnberg, 1894, are based on the same species and the earlier name should be retained. Meggitt (1927) prefers the generic name Ichthyotaenia because the term Proteocephala was first used by de Blainville, 1828. Meggitt believed unnecessary confusion was courted by the retention of Proteocephalus for a genus of Tetraphyllidea while Proteocephala was used for a group of Pseudophyllidea. Woodland (1925), in a paper containing criticisms of the existing classification, would place all specimens of the family Proteocephalidae in a single genus (Proteocephalus), which would then contain an assemblage of some 100 species. Harwood (1933) revised the genera of the family Proteocephalidae in an attempt to bring some degree of order to the departures from the classification by La Rue. In general, the generic name Ichthyotaenia is retained by the European investigators including Fuhrmann (1931), Joyeux and Baer (1936), and Baer (1951). American writers, seemingly without exception, since the paper by Benedict (1900) and the monumental work by La Rue (1914) have used the generic name Proteocephalus. The new work by Libbie Hyman (1951) elevates members of this group of cestodes to the new order, Proteocephaloidea, and retains only those forms found in the intestine of elasmobranchs in the order Tetraphyllidea.

The proteocephalid under consideration in this investigation was first described by Hunter and Bangham (1933) following their survey of the Lake Erie fish parasites and was named by them, *Proteocephalus stizostethi* nov. sp. The first mention of this worm, as cited by the above authors, is as follows: "Cooper (1919) in the description of *Bothriocephalus cuspidatus* mentions finding a worm of the genus Proteocephalus often associated with his new species in the intestinal tract of the wall-eyed pike." The above authors felt that since their new species and *B. cuspidatus* were often found together in the specimens they examined from Lake Erie, it appeared probable that the forms referred to by Cooper were *P. stizostethi*.

Fischthal also found this parasite in his 1944 survey of Northwest Wisconsin Fishes.

B. A Review of the Known Life Histories in the Cestode Family Proteocephalidae

Most of the work of the German authors prior to 1925 was taken from the paper by Kuczkowski (1925).

August Gruber in 1878 was the first to discover a larval cestode in the body cavity of a Cyclops. He found and described a procercoid from *Cyclops brevicaudus* which he believed to be the larva of *Proteocephalus torulosus* (Batch). Although Gruber's findings were published in Leuckart's text in 1879, the leading parasitologists of the day, among them Fuhrmann, von Linstow, and Zschokke, were inclined to doubt the existence of an intermediate host and taught that the mature intestinal parasites of fish pass through their larval stages in the liver of the same fish. No experimental work seems to have been done on the first intermediate hosts of fish cestodes until the work of Schneider, 1903, who infected *Gammarus locusta*, an amphipod, with the eggs of a species of Proteocephalus. La Rue (1909) proved experimentally that plerocercoids in the tissues of salamanders were larval stages of proteocephalids which, upon ingestion by other salamanders, became adults in their intestines. In 1914 Meggitt in England did a very complete study of the anatomy of *Proteocephalus filicollis* and, in addition, traced the complete development of this species by experimental methods. *Cyclops varius* was found to be the first and only intermediate host. *P. filicollis* is parasitic in the stickleback (*Gasterosteus aculeatus*). The latter is infected by ingesting the Cyclops which contains the mature procercoids of *P. filicollis*. Wagner, 1917, made an experimental study of the developmental cycle of *Proteocephalus torulosus*, in which he discovered *Cyclops strenuus* and *Diaptomus castor* to be the first intermediate hosts. The fish host, *Cyprinus orfus*, is infected with *P. torulosus* by feeding on copepods.

The literature appears to contain no further studies on the life cycles of proteocephalids of fish until the paper by Kuczkowski (1925). This worker succeeded in infecting *Cyclops strenuus*, *C. serrulatus*, and *C. oithonoides* experimentally with the eggs of *Proteocephalus percae* which is parasitic in *Gasterosteus aculeatus*, and likewise *C. strenuus* and *C. serrulatus* with the eggs of *Proteocephalus longicollis* which is parasitic in *Coregonus albula*, an European whitefish. In this study Kuczkowski gave particular attention to the development of the bladder appendage, or "cercomer," and its bearing on the "cercomer theory." The "cercomer theory" was originated in 1921 by Janicki and proposes the origin of both the digenetic trematodes and the cestodes from the Monogenea. The validity of this theory has been recently attacked by Libbie Hyman (1951).

Bangham (1925), in his studies of the cestode parasites of the black bass, reported that the procercoids of *Proteocephalus pearsei* were found in a species of Cyclops and also in *Epischura lacustris*; however, no experimental work was done to establish the identity of the larvae.

The life cycles of two new species, Corallobothrium giganteum and C. fimbriatum, which belong to the family Proteocephalidae, were described by Essex (1927). The adults were found in the intestine of catfish, Ictaluris punctatus, Amieurus melas, and Leptops olivaris. The infection was produced experimentally by feeding eggs of C. giganteum to Cyclops serrulatus and C. prasinus. Positive results were obtained by feeding to Cyclops bicuspidatus, C. serrulatus, and C. prasinus the eggs of Corallobothrium fimbriatum. Cyclops infected with the mature procercoids of C. fimbriatum were fed to minnows (Notropis blennius) and larvae were recovered from the body cavity of the minnows after three days. Sections of an infected minnow showed the presence of the larvae in the intestine, within the coelom, and in the musculature. The infected minnows were fed to Ameiurus melas and the larvae of C. fimbriatum were recovered from the intestine. The evidence indicated that catfish may acquire the parasite either by ingesting infected Cyclops or infected minnows.

Hunter (1928a) contributed to the life history of Proteocephalus amblopliitis by feeding its eggs and establishing infection in Cyclops albidus and C. prasinus. Later, the infective Cyclops were eaten by the fry of large-mouth black bass, and in three days the fry were examined and found to contain procercoid larvae in the liver, mesentery, and gonads. Two weeks later, yearling bass were introduced to the aquaria and the infected fry were all eaten. After three days the yearling bass were found to harbor young plerocercoid larvae of _P. amboplitis_ in the intestine. Thus, the black bass was proved to be both the first vertebrate host and the final host for this parasite. Because the larval stages of _P. amboplitis_ damage the testes and ovaries of the bass and cause sterility, this parasite is a limiting factor in the production of this fish, especially in hatcheries, and seemingly of greater importance than are many other fish tapeworms. Further experimental studies on _P. amboplitis_ were done in 1928 by G. W. Hunter and W. S. Hunter to determine the number and variety of intermediate hosts of this parasite. This same summer, Hunter (1928b), worked on the life history of _Proteocephalus pinguis_ and found various species of Cyclops to be the first intermediate hosts. These infective Cyclops established larvae in _Perca flavescens_ and _Notropis atherinoides_, and the infected fish were in turn fed to various species of Esox from which advanced plerocercoids were recovered.

Thomas (1934) studied the life history of a frog cestode _Ophiotaenia saphena_, another member of the Proteocephalidae. Frog tadpoles were infected by feeding them _Cyclops vulgaris_ containing procercoids of _O. saphena_. The finding of juvenile tapeworms in the adult frogs seemed to indicate they had eaten either parasitized copepods or tadpoles. Mature tapeworms in newly metamorphosed frogs suggested that the cestodes underwent either a gradual development during the tadpole stage or a more rapid development as the tadpoles began to metamorphose.

Although the above life histories differ in details, in each case the first intermediate host is a Cyclops, wherein the parasite migrates from the digestive canal into the body cavity and growth ensues. A small fish eats the crustacean and the procercoid larva bores its way into the body cavity of the second intermediate host where it encysts. The life cycle is completed when this small fish is devoured, thus bringing the larva to the intestine of the larger carnivorous fish where proglottid formation takes place and the parasite becomes an adult. However, the necessity for a second intermediate vertebrate host is not mandatory, as shown by Meggitt (1914), since a more mature procercoid from Cyclops was found to reach adulthood in the intestine of a small fish which then became the final or definitive host for the parasite.

The life history of P. stizostethi appears not to have been worked out at the present time, but it seems fairly safe to assume that it would follow one of the above. Also, the presence of a second vertebrate intermediate host seems certain, since, as Eschmeyer (1950) pointed out, fish constitute up to 99 per cent of the volume of food taken by yearling pikeperch and the most common forage fish were young perch and the fry of white suckers. These two fish may possibly be a proposed second intermediate host for P. stizostethi.

III. MATERIALS AND METHODS

In an attempt to have the fish host available throughout the year for study, fish from the market were used. While this source of fish was not ideal, due to post-mortem migration of the parasites within the host and other changes in the host tissues, it seemed the only solution for a continuous and available supply of parasites. The pikeperch is not regularly caught in any of the lakes or streams adjacent to East Lansing. All of the fish examined were of legal size and were said to come from Lake Erie, with two exceptions which came from inland Canadian lakes. The fish examined were thought to have been dead for about three days; yet, in most cases the parasites were found to be vigorously alive. None of the fish had been held in live-tanks previous to their sale. Whole fish were examined at the start of this study, but in most cases only the viscera were available.

The heart and larger blood vessels were searched separately for blood flukes. The intestine, stomach, swimbladder, liver, and other internal organs were placed in transparent glass dishes in 0.7 per cent sodium bicarbonate solution, teased apart, and inspected over a good light source. The larger parasites were removed to fresh bicarbonate solution, and the washed and decanted residue was explored with a binocular dissecting microscope for any small parasites not seen grossly. Nematodes and acanthocephalans were fixed in hot glycerin-alcohol. Cestodes were washed well in bicarbonate solution to remove any debris adhering to them, and placed in a refrigerator for a few hours to relax. They were then fixed in a hot solution of A.F.A. fixative. After some experimentation, the method used with good results to fix the cestodes was to pick up the worm near the posterior end with a broken wooden applicator stick and dip it for a moment in the hot, but not boiling, A.F.A. solution. The worm was then removed to either a dry glass plate or a flattened strip of non-absorbent paper by means of a medium-sized soft camel hair brush. The worm was straightened with the brush, which had been dipped in the hot fixative. The parasite was uniformly extended and killed immediately by the dip in the hot fixative. If absorbent towelling was used to straighten the cestode, there was likely to be distortion of the proglottids because of too much stretching. After a few minutes the fixed and straightened worms were removed to a flat bottomed dish of the fixative and allowed to remain for a period of 12 to 24 hours. They were then washed in 70 per cent alcohol and stored in fresh 70 per cent alcohol.

The cestodes were stained with Semichons' acetic carmine (1924), or with a mixture of Delafield's and Ehrlich's hematoxylin after the method of Thomas (1950). More consistent results were obtained with the carmine stain, but better definition of the internal structure, and especially the smaller ducts, was obtained with the hematoxylin. The same observation on the usefulness of these two stains was made by La Rue (1914). Synthetic oil of wintergreen was used as a clearing agent. Terpineol, La Rue (1950), was also used as a clearing agent in some staining, and although it did not harden the parasites as the oil of wintergreen did, the final result was not as brilliant a stain.

Because of the rather dense dorsal and ventral longitudinal musculature of these cestodes, the best, but most time-consuming, method of preparation was to make dorsoventrad serial sections of the proglottids. These were made 20 micra in thickness and stained with Harris' hematoxylin and eosin. The clearest and most useful preparations were made by this method.

Trematodes were fixed with hot A.F.A. solution. The larger ones were flattened between glass slides and fixed in this position. The smaller trematodes were introduced directly into the fixative. Bouin's fixative was used for some of the more delicate trematodes, and while the results were excellent, the difficulty in removing the yellow color from the specimens did not warrant its use routinely. The A.F.A. fixative was very satisfactory for trematodes, and the Semichons' carmine gave excellent staining results.

The nematodes and Acanthocephala were not mounted on slides, but were cleared and examined in lacto-phenol solution. Meggitt (1924) suggested this method for the rapid examination of cestode scolices, gravid proglottids, and eggs. The cestode was placed alive in the solution, without any previous treatment. The results with this method were not satisfactory, as the cestodes did not clear sufficiently for good observation of the internal structures.

IV. PRESENTATION AND ANALYSIS OF DATA

A. The Occurrence and Seasonal Variation of the Tapeworm, (*Proteocephalus stizostethi*), in the Pikeperch

Material from 75 fish, including whole fish and viscera only, was examined in this study. Except for the nematodes, whole mounts were made of most of the helminths found, and a total of 189 "in toto" slides were made. Serial sections were prepared from *P. stizostethi* in progressive stages of maturity for a total of 36 slides. Table I shows the observations of the seasonal variation and number of *P. stizostethi* found. It seemed evident from the data presented in Table I that the adult form of this parasite appeared only in the late spring and early summer and disappeared from the pikeperch some time between the first part of August until mid-September. No specimens of *P. stizostethi* were found in pikeperch during this time. Small immature forms were then found until the last of September, when parasites showing the first rudiments of genitalia were observed. Progressively larger and more mature specimens were seen throughout the fall and winter months, but no eggs or uterine development were observed during this time. The first outlines of uterine pouches, devoid of eggs, were seen in March, and some outlines of embryos were seen in these uterine pouches late in

TABLE I

SEASONAL VARIATION IN THE STATE OF THE SEX ORGANS AND IN THE NUMBER OF *PROTEOCEPHALUS STIZOSTETHI*

Date examined	Number of fish examined	Number of *P. stizostethi* per fish	Sexual development
1949:			
Nov. 18	2	4	Genital organs developing
Dec. 23	2	14	Mature genitalia; no eggs found
1950:			
Jan. 31	5	9.8	Mature genitalia; no eggs found
Feb. 8	2	7	Mature genitalia; no eggs found
Mar. 23	2	6.5	No eggs in uteri; outlines of uterine pouches seen
Apr. 11	4	9.5	No eggs in uteri; uterine pouches further developed
May 10	5	4.6	Some eggs in uteri; embryos not yet outlined
June 30	3	4.3	Gravid proglottids; outlines of embryos seen
Aug. 3	1	0	No proteocephalids found
Aug. 16	1	0	No proteocephalids found
Sept. 13	3	0	No proteocephalids found
Sept. 18	2	2	Immature forms found; no trace of genitalia
Sept. 21	2	5.5	No trace of genitalia
Sept. 29	3	12	First rudiments of genitalia found

TABLE I CONTINUED

Date examined	Number of fish examined	Number of *P. stizostethi* per fish	Sexual development
Oct. 6	2	0	No parasites found; intestine of host liquified
Oct. 10	2	1	Genital organs developing
Oct. 20	3	2	Genital organs developing
Oct. 27	2	1.5	Genital organs developing
Nov. 3	2	0	No proteocephalids found
Nov. 10	2	8	Genitalia further developed
Nov. 17	2	9	Genitalia mature; no eggs found
Dec. 8	2	1.5	Genitalia mature; no eggs found
Dec. 20	3	6	Genitalia mature; no eggs found
1951:			
Jan. 16	2	4	Genitalia mature; no eggs found
Jan. 23	3	6	Genitalia mature; no eggs found
Feb. 9	2	6.5	Genitalia mature; no eggs found
Feb. 23	2	4.5	Genitalia mature; no eggs found
Mar. 9	3	11.3	Outlines of uterine pouches evident; no eggs seen
Mar. 23	3	6.6	Outlines of uterine pouches evident; no eggs seen
Apr. 20	3	3	Outlines of embryos visible in uterine pouches

April, 1951, and in early May, 1950. As late as June 30, 1950, embryos were found in these cestodes; however, the embryos were still immature as the six-hooked oncospheres had not developed by this time.

The above observations also appear to confirm the opinion of La Rue (1914) that the outpocketing or lateral branching of the uterus occurs prior to the discharge of eggs into the uterus and is not due to crowding by these eggs.

The size and maturity of the tapeworms secured from fish on any particular date was quite consistent, except for a period from early October until late in November. During this time tapeworms from the same fish varied in size and in maturity from small forms showing only the rudiments of genital organs to larger worms with well developed ovaries and testes. This variation in the size and maturity of the cestode during a period in the fall was due possibly to intermittent feeding on the infective forage fish by the pikeperch. Later in the winter the pikeperch no longer seemed to receive new infections of the parasite, which suggested that the forage fish was not available in the diet of the pikeperch at that time. It was not thought that the tapeworms matured and were lost from the fish host at any time excepting the late spring and summer months, as evidence of egg production was not found at any other season. Also, it was not probable that proglottids or sections of the strobila containing eggs were lost from the host during other times of the year since the distinctive end proglottid was found on each specimen of _P. stizostethi_ examined. This end proglottid was described by La Rue (1914) as retaining certain of the characteristics of the posterior end of plerocercoid which was not lost in the passage of the young tapeworm through the stomach of the host. It is pointed posteriorly and has at the tip a median excretory pore through which the excretory products are discharged.

There seemed to be no correlation between the number of _P. stizostethi_ found and the season. Quite possibly, this could be explained by the relatively small number of fish examined at any one time.

Proteocephalus stizostethi is the only proteocephalid known to reach maturity in the pikeperch. Van Cleave and Mueller (1934) reported one adult worm from an Oneida Lake pikeperch which they thought to be _P. macrocephalus_, a parasite of the eel (_Anguilla rostrata_). As this work was done between 1929 and 1931, and the original description of _P. stizostethi_ was not published until 1933, it seems likely that this latter worm was the one found. The fact that the above authors in their summer examination of 56 pikeperch did not find the rather distinctive _P. stizostethi_ is further evidence of the loss of this parasite from the pikeperch during the summer months. Larval forms of _P. ambloplitis_ and _P. pearsi_ have been reported in the pikeperch; however, these seem to be transients and they probably do not become adult in this fish.

In support of the above data, which gives evidence of a seasonal cycle of *P. stizostethi* in the pikeperch, the works of the following authors can be cited. Meggitt (1914) in his study of *P. filicollis* states: "In winter the number of infected fish was considerably smaller, and adult parasites were rare; while in spring the proportion of adults again increased." Wagner (1917), as quoted from Essex (1927), makes the following statement regarding *P. torulosus*: "As reported by different authors (Zschokke, von Linstow, Kraemer, Riggenbach), the mature embryos of fish tapeworms occur between spring and autumn. In the winter only immature forms are found, which my results with *Ichthyotaenia torulosa* confirm." Essex (1927) found in his studies on *Corallobothrium giganteum* and *C. fimbriatum* that the adult form of Corallobothrium appeared in the late spring or early summer, reached its maximum during June and July, and disappeared entirely in the latter part of October or the first part of November. In her studies of *Trianophorus crassus* in *Esox lucius* from Canadian lakes, Ekbaum (1937) found gravid parasites from May 10 until May 28 and immature parasites from June 28 until the following spring. Miller (1943) in his extensive work on *T. crassus* and *T. nodulosus* from northern pike taken from Lesser Slave Lake, Alberta, observed a seasonal cycle in the parasites. He found the majority of *T. crassus* to lay their eggs during the first half of May, and during a period of about a month from mid-May to mid-June, the pike were almost free of this parasite. Of T. nodulosus, this same author found egg laying to occur about one month later than T. crassus. Miller (1945) described a new species of cestode from pikeperch, which he named Triaenophorus stizostedionis. This species was found in fish taken from Lake Superior and from Greater and Lesser Slave Lakes. He goes so far as to refer to the time the eggs are shed as the spawning period of the tapeworm, and estimated the state of ripeness of the tapeworms by placing the worms in water; ripe worms released a cloud of eggs from their uterine pores, while unripe worms released no eggs or only a few after several hours. He states: "If the spawning time of the worms is close at hand, these liberated eggs show clearly defined embryos, which move within the egg shell." The main spawning period of this parasite was found to be the first two weeks in June, one month later than T. crassus, and one to two weeks later than T. nodulosus.

The findings of this study on P. stizostethi are thought to approximate the results of these other authors very closely. Egg production was found only in the late spring and early summer, and the parasites were not found in the hosts during the late summer or early fall months. Immature forms were then found in the intestine, and increases in size and maturity were noted during the winter and early spring.

In any discussion of the reasons for a seasonal cycle such as has been described, only hypothetical answers can be given, for there seems to be no experimental work on this subject. The demonstrable presence of a seasonal cycle in the life history of a parasite involving two or more hosts may involve some of the following points.

Changes in temperature of the water at various seasons may be an important factor. Eschmeyer (1950) found the spawning behavior of pikeperch to be sharply restricted by the weather and a variation in different years due to this cause was found. However, temperature alone does not seem to be the only reason for the production of eggs by this tapeworm, as the cestode, Bothriocephalus cuspidatus, was found to contain eggs in December and January, although these eggs were not shed when the tapeworms were placed in water.

The longevity of the parasite in the final host may be a determinative factor, for some parasites probably mature faster than others.

Seasonal changes in the food habits of the final host, or active migration of the host to and from active sources of infection, along with the extent of time in which the infection may occur in the intermediate hosts are also important considerations.

An interesting possibility may be the effect of the endocrines of the host on the maturation of the tapeworm. The times at which the eggs were shed, as reported by various investigators, seem to coincide near the spawning times of the hosts under consideration. In the work by Kuczkowski (1925), and mentioned by Fuhrmann (1931), the proteocephalids from whitefish, which is a fall spawner, were observed to contain eggs in December and one month later Kuczkowski could not find the parasite at all and was required to stop his investigation until the end of April when eggs were recovered from a different species of the genus and from the stickleback, a different host, which is a spring spawning fish. Turner (1948) relates the spawning period of the fish of the northern hemisphere coincident with the appearance of secondary sex characters, which are dependent on the physiology of the testis. The testis in turn is influenced by the secretions of the anterior pituitary. Both light increments and variations in temperature may stimulate the pituitary. It would be interesting to attempt to get egg production in the cestode at a time other than that of the normal seasonal occurrence by stimulating the host with pituitary extracts.

B. Notes on Other Helminths Found in This Fish

Nematoda

Three specimens of *Camallanus oxycephalus* were found in the intestine of a pikeperch examined in March, 1951. This was the only finding of this parasite during the study. Van Cleave and Mueller (1934) found immature specimens in *Perca flavescens* and said the genus seems rare in Oneida Lake. Bangham and Hunter (1939) found this nematode to be the most common and widely distributed species in their Lake Erie studies, especially in the western area of Lake Erie.

Three larval nematodes of *Contracaecum* sp. were found in one fish examined in January, 1951. One was found on the viscera before the intestine was opened, and two were found in the heart. These worms were not encysted. Other workers have found this form in the digestive tract, and the abnormal location of these parasites is due possibly to post-mortem migration.

Three specimens of *Philometra* sp. were found in the stomach of a fish examined during December, 1950. These were closely affixed to a partially digested forage fish and were, themselves, partially digested and did not withstand fixation. Because of their location and the fact that other parasites of this pikeperch were alive and active, it was thought the *Philometra* sp. were parasites of the forage fish.

Cestoda

The pseudophyllidian cestode, *Bothriocephalus cuspidatus*, was invariably found in each examination of fish having any parasites. It was by far the most plentiful parasite found, and as many as 115 of these cestodes were found in one fish in September, 1951. It was common to find both adult and very immature specimens in the same fish during all seasons. Egg production was noted as late as February 9, 1951. It would seem that the source of infection for the pikeperch is present throughout the entire year.

One immature cestode was found in the intestine of a pikeperch in October and has been tentatively classified as *Proteocephalus ambloplitis*. This specimen had a well developed end organ, or fifth apical sucker, and agreed closely with the descriptions of this form by Cooper (1915) and by Bangham (1925). According to Hunter (1928a), this structure degenerates and decreases in size as the parasite becomes more mature. Adult *Triaenophorus stizostedionis*, a pseudophyllidian cestode, first described by Miller (1945), was found in the present study during March, April, and May of 1950. It was not found again until September when immature specimens were present. Worms progressing in size and maturity were noted during the fall and winter, and egg production was again found in parasites examined on March 9, 1951. When these tapeworms were placed in water they discharged their eggs, as described by the above author. Miller found this worm to shed its eggs between May 16-31, 1942, and June 19-28, 1943, while in this study, eggs were discharged from worms

March 9, 1951. This earlier date may be due to the difference in geographical distribution of the host or, possibly, to climatic variation from year to year.

Trematoda

Adult specimens of Azygia augusticauda were found in the stomach of pikeperch on three different occasions. Two were found in September, 1950, and two in April, 1951. These robust and muscular trematodes have a reddish flesh color which makes them stand out in contrast to the pale mucosa of the stomach. The size of flattened and fixed specimens varied from 6.5 mm. to 17.5 in length. Van Cleave and Mueller (1934), noted a specimen of this species 20 mm. in length taken from a pikeperch, and remarked on the greater length the parasite attains in this host, as the usual length of this species is under 5 mm.

A cluster of nine encysted larval trematodes was found in material examined in February, 1951. These cysts were translucent and non-pigmented and contained calcareous granules free in the cyst fluid. The larvae were teased free of the cysts and were classified as Neascus sp.

Starting in November, 1950, a special effort was made to examine the chambers of the heart and the bulbus arteriosus for blood flukes. From two to seven specimens of the minute, delicate trematode, Sanguinicola occidentalis, were found in each examination. The size of these parasites agreed exactly with the original description by Van Cleave and Mueller

(1932) and were from 1 to 1.33 mm. long, with a greatest transverse diameter of about 0.17 mm. Owing to the minute size of the worms and because of the unusual location in the host, it is probable they were overlooked in earlier examinations.

Acanthocephala

In the examination of five fish in May, 1950, a total of 54 specimens of *Leptorhynchoides thecatus* were recovered. This was the only time this species was found during this study. These worms were found with their probosises firmly attached to the mucosa of the intestine, with a characteristic reddened area surrounding the place of attachment. The intestine of fish examined the following month showed evidence of these same reddened areas, although none of these parasites were found.

Two specimens of *Neoechinorhynchus cylindratus* were found in October and November. This species was not as firmly attached to the intestine as was *L. thecatus*, and could be removed without dissecting a piece of the intestine along with the worm to avoid breaking the proboscis. Van Cleave and Mueller (1934) found this species to be the commonest representative of the genus on this continent.

Five *Pomphorhynchus bulbocolli* were found in April. This parasite was distinguishable from all the other fish parasites by the presence of a long cylindrical neck. At the end of this neck there was usually a large spherical enlargement. Van Cleave (1934) says this bulb is an accessory attachment organ, for though it lacks hooks or spines, the tissues of the host intestine grow around it and serve as an effective anchor to prevent dislodgment of the worm. Particular care was taken to avoid breaking this worm, and the best results were obtained by fixing the worm and the attached piece of intestine and then dissecting away the host tissue. A few specimens of this species were also obtained from carp (Cyprinus carpio) in the spring of 1950.

Table II summarizes the reported helminth parasites of the pikeperch as found by various authors. While not all of these forms were found in the present study, 37 per cent of the reported species appeared.

TABLE II

A SUMMARY OF THE REPORTED HELMINTH PARASITES OF THE YELLOW PIKEPERCH

The following number code refers to the references given in this table

NEMATODA

Agomonema sp.	2
Camallanus oxycephalus	2 ; 3 ; 4
Capillaria catenata	1 ; 3
*Contracaecum sp.	3 ; 4
Dichelyne cotylophora	1 ; 2 ; 3
*Philometra sp.	4
Philometra cylindracea	3
*Spinetectus sp.	1
S. carolini	3

CESTODA

Bothriocephalus cuspidatus	1 ; 2 ; 3 ; 4
*Dibothriocephalus latus	7
*Proteocephalus ambloplitis	3 ; 4
P. macrocephalus	1
P. stizostethi	2 ; 3 ; 4
Triaenophorus nodulosus	1 ; 3
T. stizostedionis	4 ; 6

* Larval forms only
1 Van Cleave and Mueller, Oneida Lake, New York 1934
2 Bangham and Hunter, Lake Erie 1939
3 Fischthal, J. H., Northwest Wisconsin Lakes 1944
4 This present study, Lake Erie 1951
5 Cameron, T. W. M., Canadian Lakes 1937
6 Miller, R. B., Lesser Slave Lake, Alberta 1945
7 Chandler, A. C., no locality given 1950

TABLE II CONTINUED

The following number code refers to the references given in this table	
TREMATODA	
Ancyrocephalus aculeatus	1
*Apophallus americus	1
*A. venustus	5
Azygia augusticauda	1 ; 2 ; 3 ; 4
Bucephalopsis pusilla	1 ; 2 ; 3
Centrovarium lobotes	1 ; 2
*Clinostomum marginatum	3
*Diplostomum scheuringi	2
Gyrodactyloidea	3
*Neascus sp.	2 ; 4
N. vancleavei	2
Phyllodistomum superbum	1
*Posthodiplostomum minimum	3
Sanguinicola occidentalis	1 ; 3 ; 4
ACANTHOCEPHALA	
Leptorhynchoides thecatus	1 ; 2 ; 3 ; 4
Neoechinorhynchus cylindratus	1 ; 2 ; 4
N. tenellus	3
Pomphorhynchus bulbocolli	3 ; 4

V. SUMMARY AND CONCLUSIONS

It is believed that enough data have been presented to allow the formulation of the following general statements:

1. A study has been made of a seasonal variation in the fish cestode, Proteocephalus stizostethi, taken from the intestine of the pikeperch.

2. The adult form of this parasite was found only in the late spring and early summer months, and worms of this species seemed to disappear entirely from early August until mid-September. The tapeworms increased in size and maturity during the fall and winter, and eggs were again produced the following spring.

3. The observation was made that the outpocketing or lateral branching of the uterus occurs before eggs are produced.

4. The only observed inconsistency between the size and maturity of specimens taken from the same fish occurred during October and November.

5. There seemed to be no correlation between the season and the number of worms found.

6. No unreported helminths were found in this study. Of the total number of helminths found by various authors in this host, 37 per cent were found in this investigation.

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