THE LIFE CYCLE AND INCIDENCE OF BLACK SPOT PARASITE IN THE LAKE SHINER, *RICHARDSONIUS BALTEATUS*, IN BRITISH COLUMBIA

by

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Abstract

A description of a new strigeid metacercaria of the Neascus group responsible for the productionof black spot in the lake shiner, *Richardsonius balteatus* is given. The metacercaria is distributed over the body in melanistic integumentary cysts.

All attempts to obtain the adult flukes by feeding infected fish to pigeons, ducklings, rats,and cats failed.

Black spot is erratically though widely distributed throughout British Columbia. Thirty-six of the forty- four lakes examined showed the disease to be present in the shiners.

Ptycholeilus oregonensi, *Mylocheilus caurinus*, *Catastomus macrocheilus*, *Cottus asper*, *Salvelinus fontinalis*, *Salmo gairdneri kamloops* and *Prosopium williamsoni* are also infect by black spot.

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INTRODUCTION

Although "black spot disease" had been noticed on the North American continent more than thirty-five years ago, it is only during the last fifteen to twenty years that much interest has been shown in this disfiguring trematode infestation of fish. All the investigations have been carried out in eastern Canada and eastern United States by such men as Cameron, Lyster, Miller, Hunter, Lachance and Krull, who studied the disease in game fish. No investigation of the disease has been carried out in British Columbia, to the author's knowledge.

Black spot occurs on the skin below the scales or in the muscles of several species of fish. Elevated black spots about the size of a pinhead, are caused by the encystment of certain trematode larvae, the adults of which occur in fish-eating birds and mammals. After the cercaria has penetrated the fish and secreted its cyst the injured fish is stimulated to form a connective tissue cyst about which melanophores become concentrated, producing a black spot over the site of injury.

Examination in November 1949 of a large collection of lake shiners or red-sided bream (Richardsonius balteatus), from the Columbia Fraser watershed revealed the presence of black spot. Because of the large collection available for immediate study and the abundance of this fish in British Columbia, the shiner was chosen as the basis of the present study.

With the exception of a small number of fish from Erie lake on November 24, 1949 no fresh material was available for study until May 1950 when the fish began to move into shallow water. No shiners have been obtained during the winter months because of various difficulties in making collections.

The present study has been made along two lines; first, an investigation of the occurrence of black spot in the lake shiner and other species of fish associated with it and second, a study of the life cycle of the trematode.

SURVEY OF LITERATURE

A survey of the literature has shown that at least two groups of trematodes, the heterophyids and strigeids, are responsible for the production of "black spot" in the fishes of eastern Canada and the United States. Those strigeids whose life cycles have been worked out, unlike the heterophyids, show a distinct host specificity with regard to their definitive host.

Black spot infection first came into prominence during the late 1920's when LaRue, his colleagues and students began a thorough review of the Family Strigeidae (Holostomidae) in North America. There are earlier reports of the disease on this continent but it was not until the possible economic importance of the disease was recognized that thorough investigations were carried out.

In 1898 Linton reported the occurrence of *Neascus cuticola* (Van Nordmann) from *Lepomis auritus*, *Eupomotis pallidus* and *Choenobryttus gulosus*. He states it "exists in capsules under skin especially under serous membrane...." This strigeid metacercaria is responsible for black spot in a number of European fishes. In his description Linton fails to mention a pigmented cyst and his figure 2, Plate XLII shows an unpigmented cyst attached to the serous membrane by a narrow pedicle. Hughes (1928) is of the opinion that this metacercaria is Neascus vancleavei rather than Neascus cuticola.

Cooper (1915) reported Distomum sp. larva from pigmented cysts in the muscles of Perca flavescens and pigmented cysts of Cercaria sp. from the skin of an unidentified minnow. The worm from the perch is not figured but that taken from the minnow is shown in figure 26 A and B of Plate III of his paper. From these figures it would appear that Cercaria sp. is a strigeid; there being a distinct fore and hind body and an extremely large "ventral sucker", probably the holdfast. The true ventral sucker appears to have been overlooked. He recorded that "practically all of the small species of fishes together with the young and not a few adults of the larger species are more or less infected with this kind of cyst, which statement applies particularly to those frequenting the shallow weedy bays and shores; ...."

The first identification of an organism responsible for the disease in North America was given by Hughes (1927), who described Neascus amblоplites from Ambloplites rupestris, Micropterus dolomieu and Eupomotis gibbosus in New York State. In the following six years four more strigeid metacercariae were incriminated. These were the metacercaria of Cercaria hamata Miller 1923, found by McCoy (1928) in sunfish; Neascus wardi Hunter (1928) in Lepomis cyanellus; Neascus bulboglossa Hughes (1928) in Perca flavescens; and Neascus rhinichthysi; Hunter (1933) from Rhinichthys atronasus and Rhinichthys cataractae.

Cameron (1936) and Lyster (1940) reported the disease from Canada, the causal agent being two heterophyid metacercariae, Apophallus venustus and Apophallus imperator, Lyster 1940=Apophallus brevis Ransom 1920. A. venustus attacks several fish, Ictalurus punctatus and Micropterus dolomieu most frequently, while A.brevis attacks Salvelinus fontinalis and Salmo fario.

In 1940 Neascus cuticola Van Nordmann was again reported by Linton from Huro flordano, Micropterus dolomieu, Salvelinus fontinalis and Perca flavescens. His figure 323, Plate XXIV shows the worm enclosed in a pigmented cyst which he reports as being found "mostly on the fins but also under the skin and a few in the flesh...."

The disease has been found to occur sporadically in fishes of the southeastern portion of Ontario and in Quebec from "east of Levis county southeast and south along the United States border, across Montreal island, along and including the Ottawa river up to Pontiac county." (Lachance, 1947). The infestation continues southward into the United States where it has been reported from New York, Michigan, Wisconsin, Connecticut and Massachussetts.

There have been no reports of the disease from western Canada and the United States. Although no account has been given of the disease in Manitoba fishes, McLeod (1940) reports the presence of Cercaria bessiae (Cort & Brooks, 1928), the cercarial stage of one of the black spot organisms, Uvulifer amblopiites Dubois 1938.

Table I. Blackspot in fish--life cycles

Specific Name	Definitive Host	First Intermediate Host	Second Intermediate Host	References
Apophallus venustus (Cameron, 1936)	Ardea h. herodias Procyon lotor Cat) Dog) experimental	Goniabasis livescens	Ictalurus punctatus Micropterus dolomieu	Cameron, 1936, 1937
Apophallus brevis, Ransom, 1920 (Lyster, 1940)	Gavia immer Californian Gull (Larus californicus(?)	Amnicola limosa	Salvelinus fontinalis Salmo fario	Ransom, 1920 Lyster, 1940, 1946; Miller 1940, 1941, 1946.
Crassiphiala bulboglossa Van Haitsma, 1925	Ceryle alcyon	-	Esox lucius Perca flavescens Semotilus atromaculatus (Neascus bulboglossa, Hughes)	VanHaitsma 1925 Hughes, 1928.
Uvulifer ambloplitis (Hughes, 1927) Dubois, 1938.	Ceryle alcyon	Helisoma trivolvis (Say) H. campanulatum (Say)	Ambloplites rupestris Micropterus dolomieu	Hughes, 1927 Cort & Brooks, 1928 Hunter, 1933.

Table 1. Blackspot in fish--life cycles. Cont'd

Specific Name	Definitive Host	First Intermediate Host	Second Intermediate Host	References
Uvulifer amblo-plitis (Hughes, 1927) Dubois, 1938	Ceryle alcyon	(C. bessiae, Cort & Brooks)	Eupomotis gibbosus (Neascus amblopli-tes Hughes)	Hunter & Hunter, 1930 1935. Krull, 1932, 1934
			Lepomis cyanellus (Neascus wardi, Hunter)	Hunter, 1928 VanCleave & Mueller, 1934
			Rhinichthys atrona-sus. R. cataractae (Neascus rhinich-thysi)	Hunter, 1933
Posthodiplosto-mum cuticola (VanNordmann, 1832)	Ardea cinerea Egretta garzetta Nycticorax sp.		Huro floridana Micropterus dolmieu Salvelinus fontina-lis Perca flavescens (Neascus cuticola (VanNordmann))	Linton, 1898 1940, Hughes, 1928.
		Planorbis trivolvis Say (Cercaria hamata, Miller, 1923)	Helioperca incisor Apomotis cyanellus	Miller, 1923 McCoy, 1928 Todd, 1929.

Table 1. Blackspot in fish--life cycles. Cont'd.

Specific Name	Definitive Host	First Intermediate Host	Second Intermediate Host	References
		Helisoma trivolvis (Cercaria flexicorpa Collins)	Apomotis cyanellus	Collins, 1935
			Helioperca macrochira	Hobgood, 1938

A list of organisms causing the disease in fresh-water fishes on this continent, together with pertinent data regarding their life cycles is given in Table I. Names in parentheses in the columns giving first and second intermediate hosts refer to the names given the cercarial or metacercarial stage before their relationship to the adult fluke was recognized. References cited are of papers dealing with larval or adult stages of the worm as well as descriptions of complete life cycles.

The following descriptions of the life histories of Uvulifer ambloplites and Apophallus venustus will serve as examples of strigeid and heterophid life cycles respectively.

The adult Uvulifer ambloplites is found in the small intestine of the belted kingfisher, Ceryle alcyon. In the water, the eggs, which pass out in the feces of the host, hatch liberating miracidia. These free swimming larvae penetrate the snail host (Helisoma trivolvis and H. campanulatum in the United States and H. anceps in Quebec). Within the digestive gland they give rise to elongate, motile, unbranched sporocysts which in turn give rise to cercariae, the last stage to develop within this host.

The fork-tailed cercariae break through the snail tissue to swim in the water until contact is made with the appropriate fish (sunfish or bass), whereupon they attach themselves to the integument, cast off their tails and burrow into the fish. Once they have reached their final destination, the connective tissue below the scales, the fins or the myomeres the larvae begin to form hylaline cysts around themselves. According to Hunter and Hunter (1935) there is laid down around this thin-walled cyst a thick syndesmous cyst of host origin. However, Krull (1932) believes that the larvae begin to grow while the fish forms the thick connective tissue cyst. Within this cyst the worm develops and when growth is completed, the inner closely investing hyaline cyst of parasitic origin is produced, after which the host cyst becomes pigmented. Pigmentation develops three weeks after penetration of the cercaria. Development of other strigeids may take longer and is dependent on the water temperature (McCoy, 1928 and Todd, 1929).

The life cycle is completed when the kingfisher eats an infected fish. The metacercariae are freed from their surrounding cysts in the bird's small intestine and in twenty-four to thirty days, according to Hunter (1933), reach maturity.

The adult of Apophallus venustus, whose metacercaria causes blackspot in catfish and bass, has been recovered from the small intestine of the cat, dog, racoon (Procyon lotor) and the great blue heron (Ardea h. herodias). Man, too, may be infected; one suspected case based on the recovery of ova from the feces of a patient in the Ste. Anne de Bellevue Military Hospital being reported by Cameron in 1937.

As was the case in U. amblopiltes the eggs pass from the host with the feces. However, the miracidia do not hatch until the eggs are eaten by the snail host Goniobasis livescens. No sporocyst stage has been described but all subsequent stages develop in the liver. Mother rediae give rise to daughter rediae which in turn give rise to cercariae.

The cercaria of A. venustus belongs to the pleurolophocercus group and is characterized by two pigmented eyespots and a long tail that has a continuous fin running the full length of its dorsal and ventral surface. When a cercaria comes in contact with a fish's fin it holds itself against the skin by rapid movements of its tail. Within ten minutes the cercaria has penetrated the host's skin leaving behind its tail. The cercaria usually enters a blood vessel and, by alternately contracting and expanding its body, eventually reaches the deeper tissue where it encysts; the encysted worm being known as a metacercaria.

Three to four weeks are required before the metacercariae become infective. Seven to ten days after being eaten by the definative host the flukes become mature, though eggs may not be recovered from the feces until twenty-four days after the infective feeding.

The effects of the parasites on the host have been neglected by most authors. The few that have been reported probably represent the results of extremely severe infections which only occur sporadically in nature. McCoy, (1928); Krull, (1934), and Hunter, (1937), record cases of the death of the host due to the parasite and Hunter and Hunter, (1936, 1938) record cases of the loss of weight by the host. The only reaction of the fish to the parasite which has been studied intensively is that of cyst formation, by Todd (1929), Krull (1934) and Hunter and Hamilton (1941). Hunter (1941) and Hunter and Hamilton (1941) present an hypothesis to explain the appearance of melanophores in the host cyst of some larval trematodes and their absence in others.

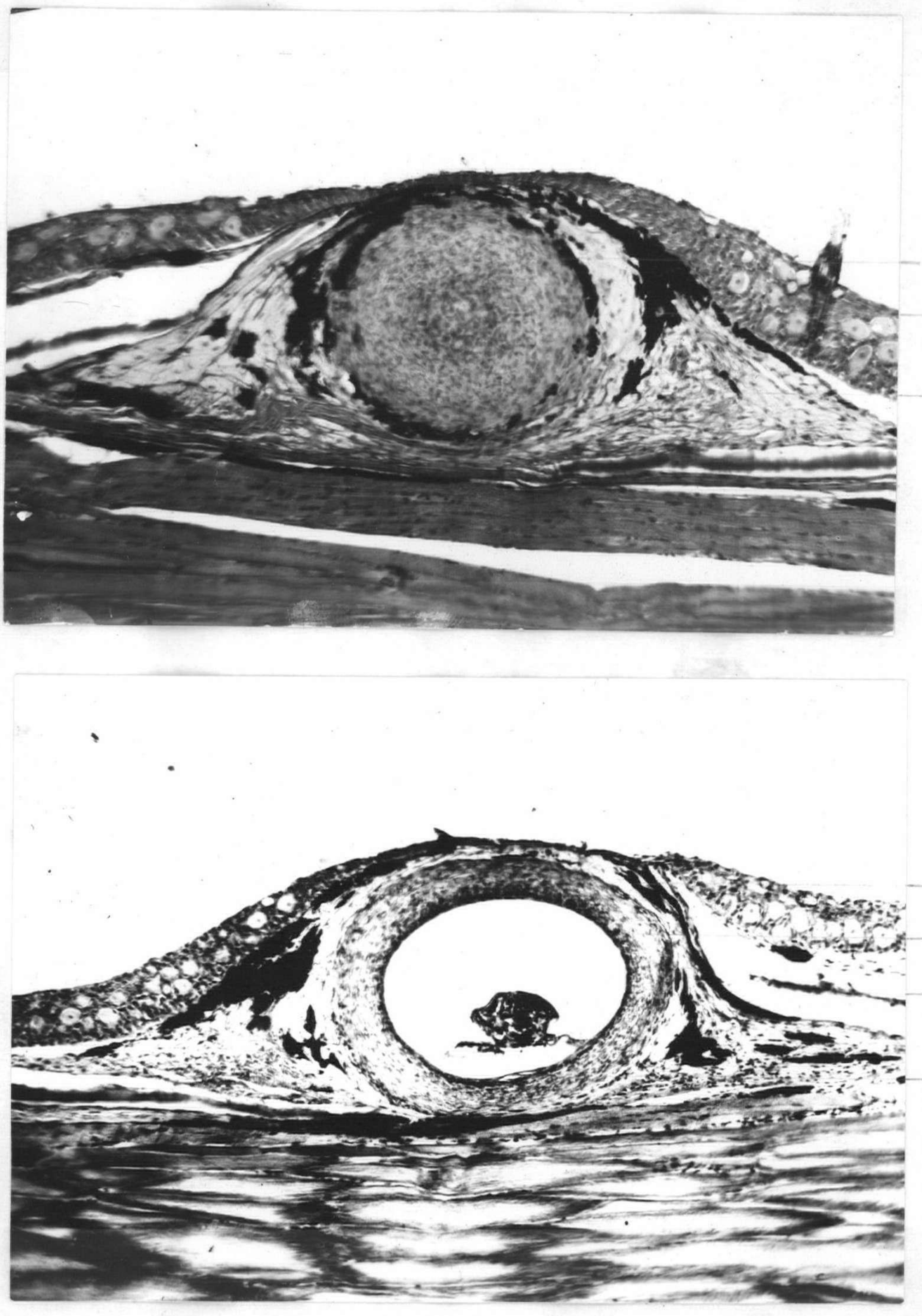

Fig. 1.
Upper:- Cross section through skin showing the two parts of outer host cyst.

Lower:- Cross section through skin showing metacercaria, its inner cyst and two parts of the outer host cyst.

Fig. 2. Two encysted metacercaria in skin of shiner. Prolificationof tissue causes displacement of scales.

Fig. 3.
Section through fin of shiner . Proliferation of tissue causes displacement of elements of the fin rays.

THE METACERCARIA

STRUCTURE OF THE CYST

The metacercaria is enclosed by an elastic, hyaline cyst of parasitic origin and surrounding this a thick syndesmous cyst of host origin consisting of two distinct layers. The outer layer contains melanophores lying in the loose connective tissue, while the inner layer is made up of more closely packed connective tissue and contains no pigment cells. (Fig.1.) The nuclei of the connective tissue cells are clearly visible, being larger and more prominent in the outer pigmented part. The two parts of the cyst cannot be completely separated by dissection, the innermost portion of the outer layer remaining attached to the denser inner area.

The cysts occur in the dermis and on the scales, and only rarely in the muscles beneath the skin. In very heavy infestations a few cysts may be found lying in the muscle septa, but were never found in sufficient numbers to blacken the flesh as has been reported by Cameron (1946) for bass.

Usually the parasite lodges in the dermis between two scales. The profuse proliferation of connective tissue causes the displacement of the scales that gives the skin its rough texture. (Fig. 2.) On the fins the cyst displaces the elements of the fin rays. (Fig. 3.) Pigmentation here, as on the body, is found concentrated in the immediate vicinity of the cysts.

There is little capillary involvement in the cyst. Where bloodvessels have been found in the cyst they have been limited to the outer pigmented layer.

The following table gives measurements of ten cysts from a shiner. All measurements were made with the aid of an ocular micrometer from serial sections stained with Harris' haematoxylin using alcoholic eosin as a counterstain.

Table II. Measurements in microns of ten integumentary cysts of a strigeid metacercaria from Richardsonius balteatus.

	Minimum	Maximum	Average
Total length of cyst	357.0	500.0	423.0
Diameter of pigment layer	26.0	59.0	40.0
Diameter of denser portion of cyst	19.0	46.0	33.0
Thickness of hyaline cyst	0.7	1.5	1.1
Size of cyst cavity	291.0	368.0	342.0

Inside the connective tissue cyst and separated from it by a fluid is the tough membranous cyst secreted by the parasite. The fluid gels on preservation in formalin and is easily discerned during manual dissection. Twenty inner cysts

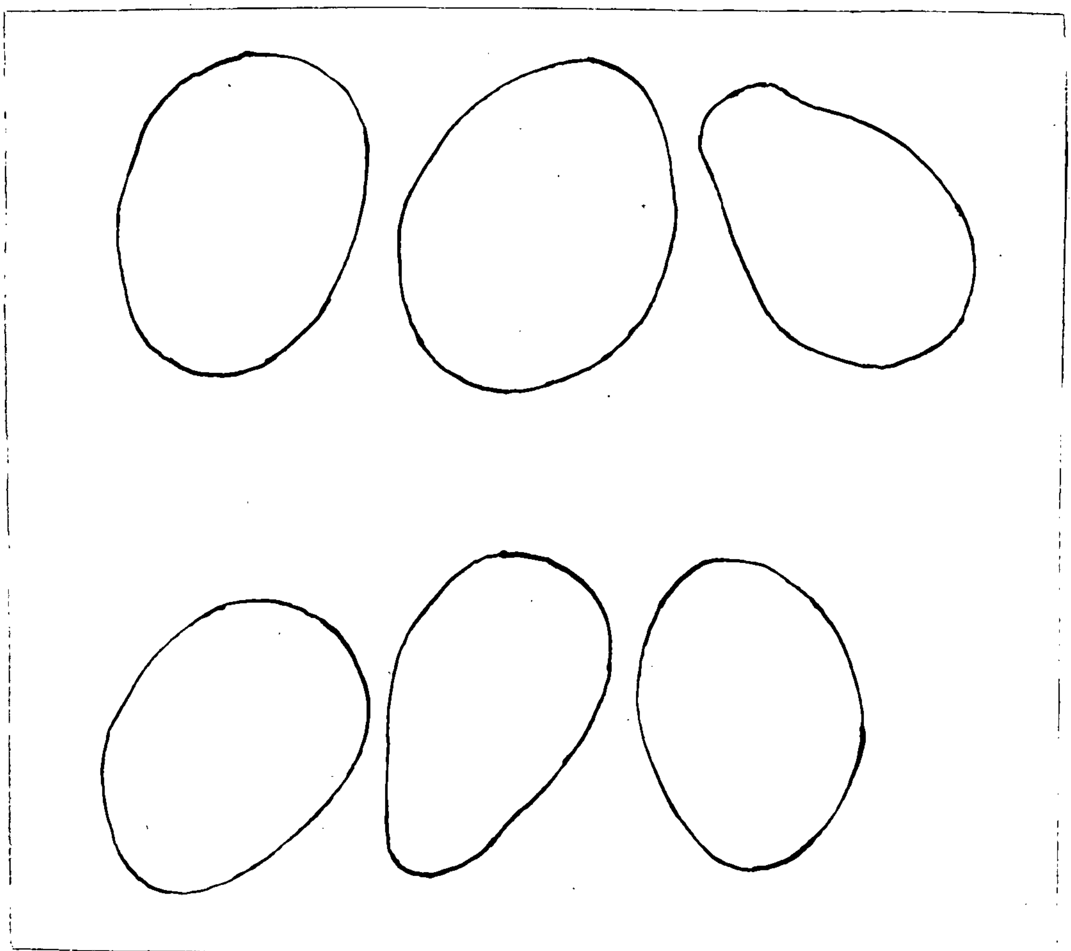

Fig. 4 Camera lucida drawings of inner cysts.

measured without pressure after the digestion of the host cyst by pepsin gave the following measurements. The cysts are oval with a length of 289 u to 391 u, average 331 u, with a breadth of 208 microns to 262 microns, average 222 microns. The largest cyst was 391 microns long by 262 microns wide, the smallest 289 microns long by 213 microns wide. Fig. 4 shows the outlines of some inner cysts drawn with the aid of a camera lucida.

Cysts containing moribund or dead metacercariae may be distinguished on sight from those containing living worms by their dull grey contents in contrast to the crystalline cytoplasm and large refractile granules seen in viable cysts. The cytoplasm of moribund metacercariae loses its clarity, becoming grey and bubbly in appearance. Cysts in which the metacercariae have been dead for some time are filled by a homogeneous grey gel.

DESCRIPTION OF METACERCARIA

The metacercaria is found lying somewhat bent within the cyst surrounded by a granular fluid (Plate I, fig. 1,2). In living specimens the contraction and expansion of the worms set these granules in motion. The anterior end of the worm is usually pointed with the oral sucker at the apex. The body then increases in width reaching its greatest dimension near the middle of the forebody. The oral sucker, ventral sucker and holdfast are visible. The remains of cystogenous cells

Explanation of Plate I

Fig. 1,2 - Metacercaria in inner hyaline cyst.

Fig.3 ---- Ventral view of living worm freed from cyst.

Fig. 4 --- Camera lucida drawing of fixed worm showing internal structure. Ventral view.

Fig. 5 --- View of worm showing longitudinal striations of cuticle.

Abbreviations

BC .... bursa copulatrix
CC .... cystogenous cell
CE .... intestinal caecum
EB .... excretory bladder
EC .... excretory canal
EP .... excretory pore
HB .... hindbody
HF .... holdfast
IC .... inner cyst
OS .... oral sucker
OV .... ovary
PH .... pharynx
T .... testis
UT .... uterus
VS .... ventral sucker

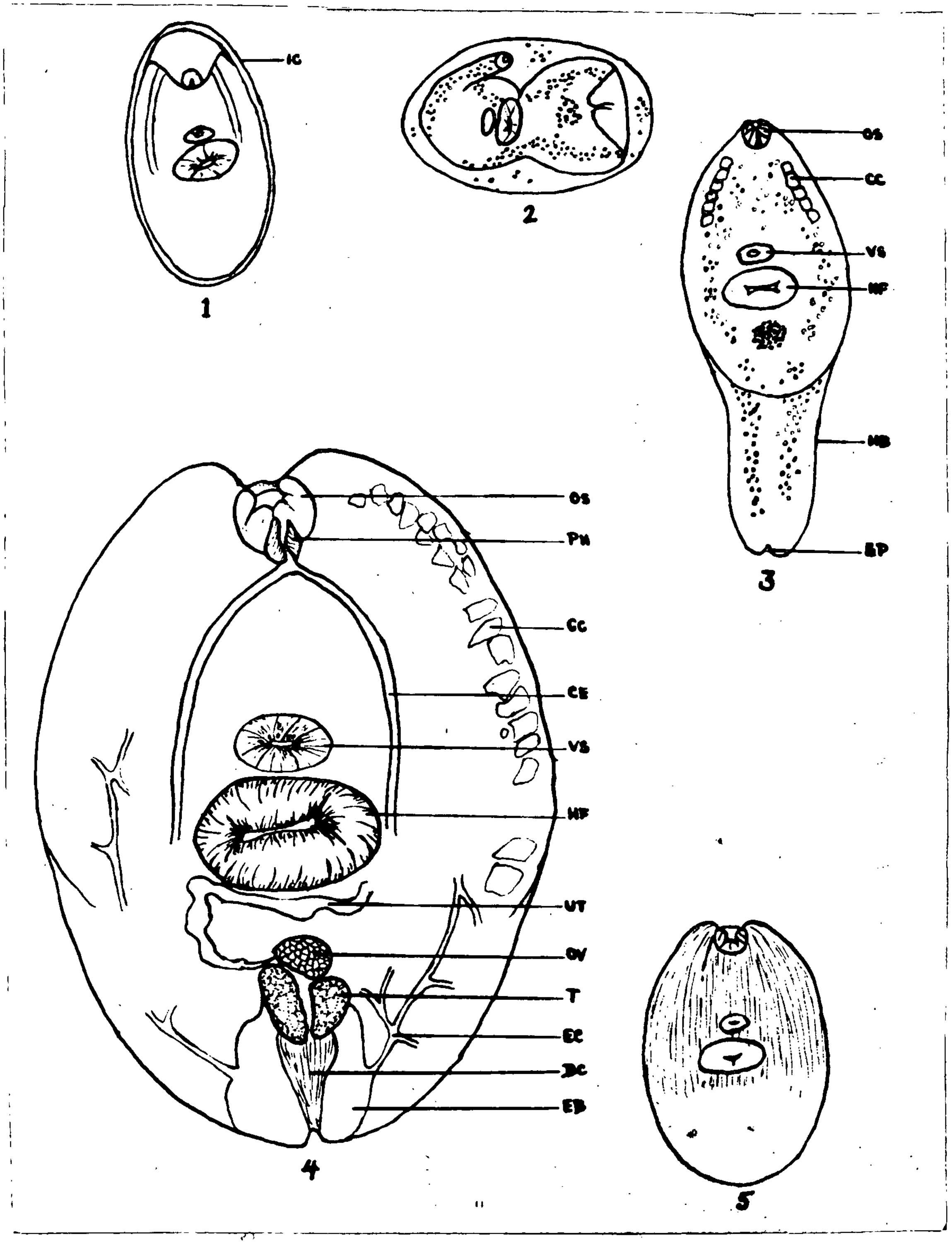

Plate I

can be seen, being particularly clear in fixed and stained specimens where they appear as large pink to orange hyaline cells arranged in rows along the sides of the forebody (Plate I, fig. 3, 4).

This description is based on two adolescaria freed from their cyst during pepsin digestion.

The broad forebody bearing the oral sucker, acetabulum and "holdfast" organ is separated from the narrower hindbody by a constriction (Plate I, fig. 3) in the living worm. After fixation evidence of the constriction was lost to a large degree due to extreme contraction on the part of the specimens (Plate I, fig. 4).

Accurate body measurements on living worms at any particular moment were impossible to obtain because of the animals' constant activity. General figures as to the length and breadth of the specimens will be given. The total length of the worms ranged between 0.6 mm. and 1.1 mm. while the width of the forebody at the level of the holdfast varied between 0.25 mm. and 0.35 mm. The hindbody measured 0.2-0.4 mm. long by 0.1 mm. wide.

The oral sucker, ventral sucker, "holdfast" and genital primordium can be identified, but the digestive and excretory systems are obscured by refractile droplets which fill most of the body. No flame cells were seen.

The cuticle of the fixed worms shows longitudinal striations (Plate I, fig. 5). There is no evidence of spines.

The oral and ventral suckers are well developed. The oval "holdfast" organ is found in the posterior third of the forebody with its long axis lying transversely across the body. In neither specimen is it seen to cover the ventral sucker. Remains of cystogenous cells are to be found lying between the intestinal crura and lateral margins of the body in varying numbers. The primordia of the reproductive organs show either as a single oval shaped fundament or as a lobed structure suggesting the formation of the future organs (Plate I, fig. 4).

The oral sucker measures 0.03 mm. by 0.03 mm. Decimal fourteen mm. posterior to the oral sucker is the ventral sucker measuring 0.04 mm. by 0.04 mm. The large "holdfast" lying directly behind the ventral sucker is 0.06 mm. long by 0.1 mm. wide in one specimen and 0.09 mm. by 0.09 mm. in the other.

The digestive system consists of a subterminal mouth opening into the muscular pharynx measuring 0.02 mm. long by 0.01 mm. wide. The prepharynx is either extremely short or lacking. The intestinal crura are given off from a short oesophagus and proceed posteriad to the posterior margin of the "holdfast". In one specimen the lumen of the intestine had not formed.

The reproductive system which, as stated previously, may consist of a single undifferentiated oval fundament or show the beginning of differentiation into the adult organs, lies in the hindbody. Both the worms show the beginning of this differentiation and rudiments of the ovary, testes and uterus may be seen (Plate I, fig. 4).

The ovary lies in the anterior portion of the hind-body directly behind the "holdfast" organ. It is oval in shape and measures 0.02 mm. by 0.01-0.03 mm. The oviduct and uterus are seen as a mass of denser tissue coming from the right of the ovary proceeding anteriorly and to the left in a loop between the ovary and "holdfast". The oötype may be represented by a small cluster of cells at the base of the oviduct. There is no indication of Laurer's canal.

The male reproductive system consists of two testes lying lateral to and slightly posterior to the more ventrally placed median ovary. The testes measure 0.03 mm. by 0.02 mm. No seminal vesicle or vasa efferentia are indicated. Bursa copulatrix is present.

Details of the excretory system, obscured during life by the granules floating in the circumambient fluid and by the remains of the cystogenous cells, became clearer in one of the specimens after staining (Plate I, fig. 4). The large median excretory bladder occupies approximately a third of the hind-body, lying posterior and dorsal to the genital primordia and opens to the exterior through a dorsally placed excretory pore. Two excretory canals, one on either side, are given off from the bladder and proceed anteriorly giving off lateral branches to form a loose network of vessels. These canals and their branches could only be traced to the level of the "holdfast" where their course becomes obscured by the denser structure of the forebody.

TAXONOMIC POSITION OF METACERCARIA

Strigeid metacercariae have been divided into five groups, each having a more or less definite location in their host. The three genera that concern us here are *Diplostomulum*, *Tetracotyle* and *Neascus*.

The genus *Diplostomulum* is usually found free within the eyes of fishes. The body is not clearly divided into two parts. An oral sucker, pharynx and two lateral cotylae are present. A small ventral sucker is found behind the midpoint followed by a large "holdfast" organ which has a small transverse "holdfast" gland at its posterior margin. The caudal lobe contains the genital primordia and the excretory bladder. The oesophagus is of varying length and the intestinal crura usually extend into the caudal lobe.

The genus *Tetracotyle* is characterized by having a thick round cyst and is found encysted in the muscles, mesenteries, and pericardium of the host. Hughes (1928) says that the name Tetracotyle should be applied to "only those strigeid metacercariae having lateral cotylae, a slightly developed hindbody and a reserve bladder consisting of an irregular, continuous coarse-meshed network of spaces, not in the form of definite vessels".

Neascus, the remaining larval genus, is described by Hughes (1927) as follows: "Strigeid metacercariae with both fore-and hindbodies, well developed and set apart by a constriction; no lateral cups; forebody leaflike; holdfast organ well developed; reserve bladder highly developed, the smaller branches of which usually anastomose; calcareous granules mostly free in the circumambient fluid; encysted." Neascids have a thin, ovate cyst and is found in the viscera mesenteries, skin, and frequently in the pericardium.

The metacercaria, responsible for blackspot in the shiner, believed to be a new species, is a strigeid of the Neascus group found in melanotic, integumentary cysts. The neascid larvae reported as causing blackspot in North American fresh-water fishes are as follows: Neascus bulboglossa Hughes, Neascus ambloplites, Neascus rhinichthysi Hunter, Neascus cuticola van Nordmann, Neascus wardi Hunter, and the metacercariae of Cercaria hamata McCoy and Cercaria flexicorpa Hobgood.

The site of the cysts in the host eliminates Uvulifer ambloplites (Dubois, 1938) which is found along the muscle septa. The other metacercariae are all integumentary but differ from the metacercariae found in the shiners in the following ways.

Neascus cuticola (1) is closely invested in a spherical cyst, (2) the forebody is not longitudinally striated (3) acetabulum is larger than oral sucker.

The metacercariae of Cercaria flexicorpa are characterized by (1) absence of reproductive fundaments, (2) presence of gland cells in hindbody, (3) oral sucker twice as large as the acetabulum.

Neascus wardi differs in having its reproductive fundaments in the posterior portion of the forebody rather than in the hindbody. The ovary is dorsal to the acetabulum and the testes are widely separated, paralleling one another posterior to the holdfast.

Neascus rhinichthysi is (1) readily freed from its inner cyst, (2) acetabulum weak and little more than half the size of the oral sucker.

Neascus bulboglossa is characterized by (1) lack of acetabulum, (2) surface of forebody and holdfast covered quincunically with minute spines, (3) oesophagus very long bifurcating just in front of the holdfast, (4) large pharynx slightly smaller than oral sucker.

Besides the above morphological differences the metacercaria in question also differs in size but this is not believed to be of primary importance in the identification of the parasite. Wardle (1932) mentions that the lack of pronounced host specificity may result in the production of larval morphae of certain parasites which are sufficiently unlike to appear as distinct species. Only with the completion of the life cycles of these neascids can the legitimacy of the species be finally established.

Table III. Distribution of cysts on Taylor lake shiners. (Ten at random.) August, 1950.

Body Area											Total for 10 fish	Per cent of total
Head	57	-	-	19	2	2	-	1	-	2	83	12.04
Trunk above lateral line	223	1	12	54	8	1	4	1	3	10	317	42.14
Trunk below lateral line	63	-	44	18	-	-	-	-	-	3	128	18.81
Base of tail fin	8	-	3	3	1	2	3	-	-	-	20	2.91
Tail fin	22	-	2	10	2	-	-	2	2	2	42	6.17
Dorsal fin	12	1	3	3	1	-	-	-	-	-	20	2.91
Pelvic fins	4	-	-	4	-	-	-	-	-	-	8	1.17
Pectoral fins	22	-	1	15	-	1	2	1	-	1	43	6.17
Anal fin	11	-	-	4	-	-	1	-	2	2	20	2.91

Average 68.1 per fish

Table IV. Distribution of Cysts on Snowshoe lake shiners. (Ten at random.) September 1949.

Body Area											Total for 10 fish	Per cent of total cysts
Head	-	-	1	1	27	-	2	2	1	6	40	7.54
Trunk above lateral line	1	7	-	3	68	3	2	11	-	31	126	23.82
Trunk below lateral line	1	10	-	2	97	6	9	13	-	50	188	35.54
Base of tail fin	-	-	1	1	2	2	-	4	-	5	15	2.85
Tail fin	1	1	-	-	15	4	-	4	-	-	25	4.54
Dorsal fin	-	-	1	2	10	7	1	12	-	8	41	7.73
Pelvic fins	-	-	3	-	5	1	-	5	-	4	18	3.40
Pectoral fins	-	-	2	1	20	1	-	9	-	14	47	8.88
Anal fin	-	-	2	1	13	2	1	6	-	4	29	5.48

Average 52.9 per fish

Table V. Distribution of cysts on Puntchesakut lake shiners. (Ten at random.) July, 1949.

Body Area											Total for 10 fish	Per cent of total cysts
Head	-	-	1	-	-	2	-	-	1	1	5	20.0
Trunk above lateral line	-	-	-	-	1	-	-	-	-	-	1	4.0
Trunk below lateral line	-	-	-	1	-	2	-	1	4	-	8	32.0
Base of tail	-	-	-	-	-	-	-	-	-	1	1	4.0
Tail fin	-	1	1	-	1	-	-	-	-	1	4	16.0
Dorsal fin	-	-	-	-	1	-	-	-	-	-	1	4.0
Pelvic fins	1	-	1	-	-	-	-	1	-	-	3	12.0
Pectoral fins	1	-	-	-	-	-	-	-	-	-	1	4.0
Anal fin	-	-	1	-	-	-	-	-	-	-	1	4.0

Average 2.5 per fish

DISTRIBUTION OF BLACKSPOT ON THE HOST

To facilitate the counting of cysts, the fish was marked off into nine parts, the number of cysts in each area being recorded. The divisions were as follows: the head, the trunk above the lateral line; the trunk below the lateral line; the caudal peduncle, the caudal fin; the dorsal fin; the pelvic fins; and the anal fins.

Table III, IV, and V show the distribution of cysts on ten shiners from Taylor and Snowshoe lakes, both with a high incidence of infection, and Puntchesakut lake, with low incidence.

There appears to be no preference for any particular site on the fish, the cysts being scattered over all parts of the body. ((Fig. 5.)). However, in light infections the body proper is infected more often than the fins. Besides the skin and fins of the fish the cysts have also been found in the eye, mouth and gills though these sites are not infected very often, usually only in heavily infested shiners.

There are indications that infested fish in lakes with a high percentage of infection have more cysts per fish than do fish from lakes of low incidence. Taylor lake, with 80.5 per cent of the fish infected shows an average of 58.1 cysts per fish; Snowshoe lake, 64.5 per cent infected, has an average of 52.9 cysts per fish while Puntchesakut lake, 17.65 per cent infected averages 2.5 cysts per fish.

Fig. 5.

Upper:- Shiner infected with black spot.
Lower:- Uninfected shiner

RELATIONSHIP OF BLACKSPOT TO AGE OF FISH

During the preliminary survey of the lakes it was noticed that fish of about forty millimeters or less were not infected as often or as heavily as were the larger fish. The results of a thorough examination of Taylor lake shiners gave the results seen in Table VI.

Table VI. The average number of cysts in eight length classes of shiners from Taylor lake.

Length Class	Average Number of Cysts per Fish	Size of Sample
0-20 mm.	0.10	28
21-30 mm.	0.92	40
31-40 mm.	3.5	73
41-50 mm.	6.4	38
51-60 mm.	10.7	41
61-70 mm.	39.9	40
71-80 mm.	20.1	21
81-90 mm.	46.5	2

The table shows an increasing number of cysts per fish with each increase in age except in the 61-70 mm. class. The average number of cysts per fish in this group was raised by the presence of two heavily infected fish, one having 204 cysts, the other 578. If the two heavily infected fish are omitted the average for this group is 21.4 cysts per infected fish.

EXPERIMENTS TO DETERMINE LIFE CYCLE

INTRODUCTION

The experiments recorded in this section describe attempts to determine the complete development of the strigeid metacercaria causing blackspot in shiners. Two plans of approach were utilized.

A. Cercariae:

Snails from Taylor lake were collected during May and July 1950 and placed in pint jars in the laboratory. Snails were observed every three hours ~~for~~ during the first week for emerging cercariae, then twice daily. All cercariae were studied alive with the aid of intra vitam staining with neutral red.

B. Adult worm:

In an attempt to obtain the adult stage of the fluke, infected shiners were fed to experimental animals, namely cats and pigeons. Prior to feeding, fecal examinations to determine the presence of parasites were made. Adult cats were fed eviserated shiners, while kittens were fed chopped shiners, or if unwilling to eat, were force fed. All the pigeons and the duck used were force fed. The animals were given one hundred cysts on two consecutive days.

The animals were sacrificed at various intervals following the last feeding with infected fish, at least seven days elapsing from the date of feeding to the date of sacrifice. During this period fecal examinations were made to determine the presence of eggs.

The abdominal and thoracic cavities of the sacrificed animal were opened by a ventral incision, the visera removed and placed in physiological saline. The digestive system was examined first--beginning with the oesophagus. The intestine was cut in three-inch lengths, washed, and washings examined under the binocular microscope. The intestine was then scraped and the sediment examined as before. Following the examination of the digestive tract, the liver, and the respiratory and urogenital systems were also examined.

RESULTS OF EXPERIMENTS

A. Cercariae:

No strigeid cercariae were found emerging from the snails while an examination of the snails tissues failed to show any developmental stages of a strigeid cycle. Two echinostome cercariae were found emerging from *Helisoma sp. subcrenatum* (Carpenter) from Taylor lake. These bear resemblance to *Cercaria trivolvis* Cort (1914) and to *Cercaria cita* Miller (1925).

Table VII. Infection trials with experimental animals.

Host	Period of Infection	Result of fecal examination	Result of post-mortem examination	Remarks
Rat	33 days	negative	negative	Metacercariae few, approx. 20
Cat	14 days	--	negative	Fed two lots of 100-200 cysts in eviserated shiners 2 days apart.
Cat	21 days	--	negative	One hundred-200 cysts in eviserated shiners.
Cat	14 days	--	negative	Fed eviserated Eastern Brook trout containing 100 cysts.
Pigeon	15 days	Positive for Trichurus eggs	1 fluke-Psilostomum ondatrae	Fed skin containing cysts.
Kitten	10 days	--	Negative	Fed 60 cysts by hand
Kitten	12 days	--	Negative	(eviserated (shiner. force
Kitten	13 days	--	Negative	(fed 60 cysts (on 2 consecu-(tive days.
Cat	20 days	--	--	
Pigeon	10 days	negative	negative	Fed approx. 150 cysts
Pigeon	13 days	negative	negative	Fed approx. 150 cysts.
Duck	7 days	negative	negative	100 cysts.

Lymnaea palustris Müller collected from Okanagan lake were infected by an unidentified xiphidiocercaria.

B. Adult worm:

The results of the feeding experiments are summarized in Table VII.

No successful infections resulted--all the animals being free of trematodes except one pigeon which was infected with a single specimen.

DISCUSSION

The shiners used during June to infect two cats as well as an Eastern Brook trout fed to another cat, were shipped dead from Summerland. A sample of cysts removed from these fish for examination showed that the metacercariae were living. However, as the fish had been dead for at least twenty-four hours it is possible that there may have been some toxic effect upon the parasite due to the decomposition of the host. This toxic effect, though not great enough to kill the worm, may have been great enough to alter the physiological condition to such an extent that the worm was unable to establish itself in the experimental host.

The one worm recovered from the proventriculus of a pigeon used for the feeding experiments was not a strigeid. There was no evidence of a pathological condition in the proventriculus. A description of the fluke follows.

Explanation of Plate II

C ....cirrus

CI ....intestinal caecum

CS ....cirrus sac

EP ....excretory pore

OS ....oral sucker

OV ....ovary

PH ....pharynx

SG ....shell gland

T ....testis

UT ....uterus

VF ....vitalline follicles

VS ....vemtral sucker

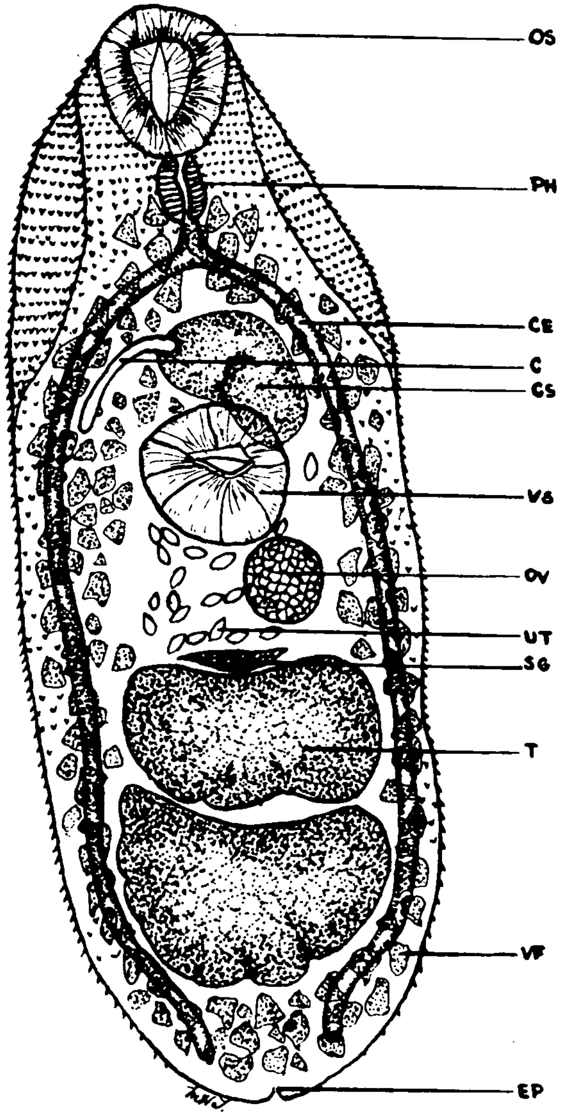

Plate II

<u>Psilostomum</u> <u>ondatrae</u> Plate II

Host: Pigeon (experimental)

Site: Proventriculus

The ovoid body of the fluke measures 2.16 mm. in length by 0.81 mm. in width at the level of the acetabulum and is flattened dorso-ventrally at the anterior end. Cuticular spines are distributed over the entire body in alternate transverse rows, thinning out from the level of the posterior testes to the posterior extremity of the body. Spines average 18.9 u in length. Oral sucker is subterminal, 0.210 mm. by 0.217 mm.; oral aperature slit-like. Ventral sucker strongly muscular and almost circular measuring 0.273 mm. by 0.252 mm. Distance between the center of the oral sucker and that of the acetabulum 0.77 mm. Prepharynx short. The muscular pharynx is 0.14 mm. long by 0.07 mm. wide. The oesophagus branches to form the intestinal caeca just in front of the anterior margin of the cirrus sac. Intestinal caeca are simple, extending almost to the posterior margin of the body. The excretory pore is terminal. Other parts of the excretory system could not be followed because of the density of the reproductive system. The large, transversely elongated testes are tandem in position. The anterior testes measure 0.49 mm. by 0.287 mm., the posterior testes o.504 by 0.308 mm. The cirrus pouch is large and bladder-like with its posterior margin extending dorsal to and slightly beyond the anterior edge of the ventral sucker. The long slender cirrus is unarmed. The ovary (0.161 mm. by

0.147 mm.) lies anterior to the testes and to the left of the midline. The vitellaria is composed of large follicles extending from the posterior margin of the pharynx to the posterior tip of the body. The follicles meet medianly both in the anterior portion of the body where they obscure the oesophagus and in the posterior portion of the body behind the testes. The uterus is relatively short and lies between the testes and ventral sucker. The uterus contains approximately thirty yellowish-brown eggs which measure 0.08 mm. by 0.35 mm. Shell gland is dorsal and anterior to testes. Genital pore is situated to the right of the median line anterior to the ventral sucker.

The measurements of this fluke fit the descriptions of Psilostomum ondatrae described by Price (1931), Law and Kennedy (1932) and Beaver (1939). Beaver describes the worm and its life cycle. The two anterior intestinal diverticulae pictured by Beaver were not seen, nor are they pictured by Price or Law and Kennedy. The density of the follicles could easily obscure these diverticulae and as no serial sections were made of the fluke it is impossible to state definitely whether or not they are present.

The metacercariae of this fluke are found encysted in a thin-walled cyst along the lateral line canal, and nares of a number of fishes and amphibians, including bullheads (Ameiurus), perch (Perca flavescens), rock bass (Ambloplites rupestris), smallmouth black bass (Micropterus dolomieu), pumpkinseed (Eupomotis gibbosus), the bluegill (Lepomis pallidus), tadpoles of several species, and salamanders. It is probable that the pigeon became infected from cysts that were situated in the lateral line of the shiners fed to it during the feeding experiments. The snail host for this fluke is Helisoma antrosum percarinatum. The Helisoma sp. subcrenatum (Carpenter) found so common in Taylor lake could very likely harbour the cercariae.

Table VIII. Incidence of blackspot in *Richardsonius balteatus* in British Columbia.

Locality	Size of Sample	Per cent Infection
Allison lake	237	1.38
Baptiste lake	51	9.80
Box lake	109	7.34
Cardew lake	58	0.00
S. Champion lake	288	3.49
Chimney lake	18	0.00
Cultus lake	362	3.30
Douglas lake	228	7.00
Duck lake	30	0.00
Erie lake	438	38.58
Erie pond	45	31.10
Erie pothole	116	34.30
Erie slough	53	66.04
Fleming lake	105	0.00
Garnet Valley dam	180	17.75
Horseshoe lake	63	0.00
Hyas lake	164	45.12
Heffley lake	125	22.40
Inonoaklin river	51	3.92
Jewel lake	93	100.00
Kalamalka lake	140	28.11

Table VIII. Incidence of blackspot in *Richardsonius balteatus* in British Columbia. Cont'd.

Locality	Size of Sample	Per cent Infection
Kootenay river	155	0.00
Liard lake	193	51.31
Madden lake	229	10.50
Mara lake	33	0.00
McBains lake	94	54.25
Mitchell lake	39	0.00
Monte lake	34	38.28
Nicola lake	86	0.00
Okanagan lake (Okanagan Landing)	168	6.43
Paddy Ryan lake	52	59.60
Paul lake	197	2.10
Pemberton lake	167	35.71
Pinantan lake	298	4.38
Puntchesakut lake	65	6.16
Rock lake	125	95.20
Rosebud lake	962	24.40
Shuswap lake	70	5.71
Skaha lake	124	7.26
Snowshoe lake	423	64.48
Surveyor's lake	50	96.00
Taylor lake	339	80.47
West lake Beaverly creek	63	52.37
Cluculz creek	159	64.15

GEOGRAPHIC DISTRIBUTION, BRITISH COLUMBIA

Examination of some six thousand fish from forty-four localities on the Columbia-Fraser watershed provides the information on the geographical distribution of black spot. The fish were caught and preserved in formalin during the summers of 1947, 1948, 1949, and 1950, with the exception of one sample from Garnet Valley Dam belonging to the Ontario Museum of Zoology which was taken on July 6, 1928.

Blackspot is erratically though widely distributed throughout British Columbia. Thirty-six of the forty-four lakes and streams represented in the collection showed the disease to be present in the shiners. These waters are listed alphabetically along with the sample size and percentage of infected fish in Table VIII.

The incidence varies from almost complete freedom from the disease in Skaha, Allison, Champion, Okanagan and Paul lakes to infections involving fifty per cent or more of the fish as in Jewel, Rock, Taylor and Snowshoe lakes. In Jewel lake every one of the ninety-three fishes examined was infected. Fig. 6 is a map of British Columbia showing the distribution of infected lakes and the percentage of fish with the disease.

The constancy of infection has been noticed in lakes where collections are available for a number of years. However, more data are necessary before a definite statement

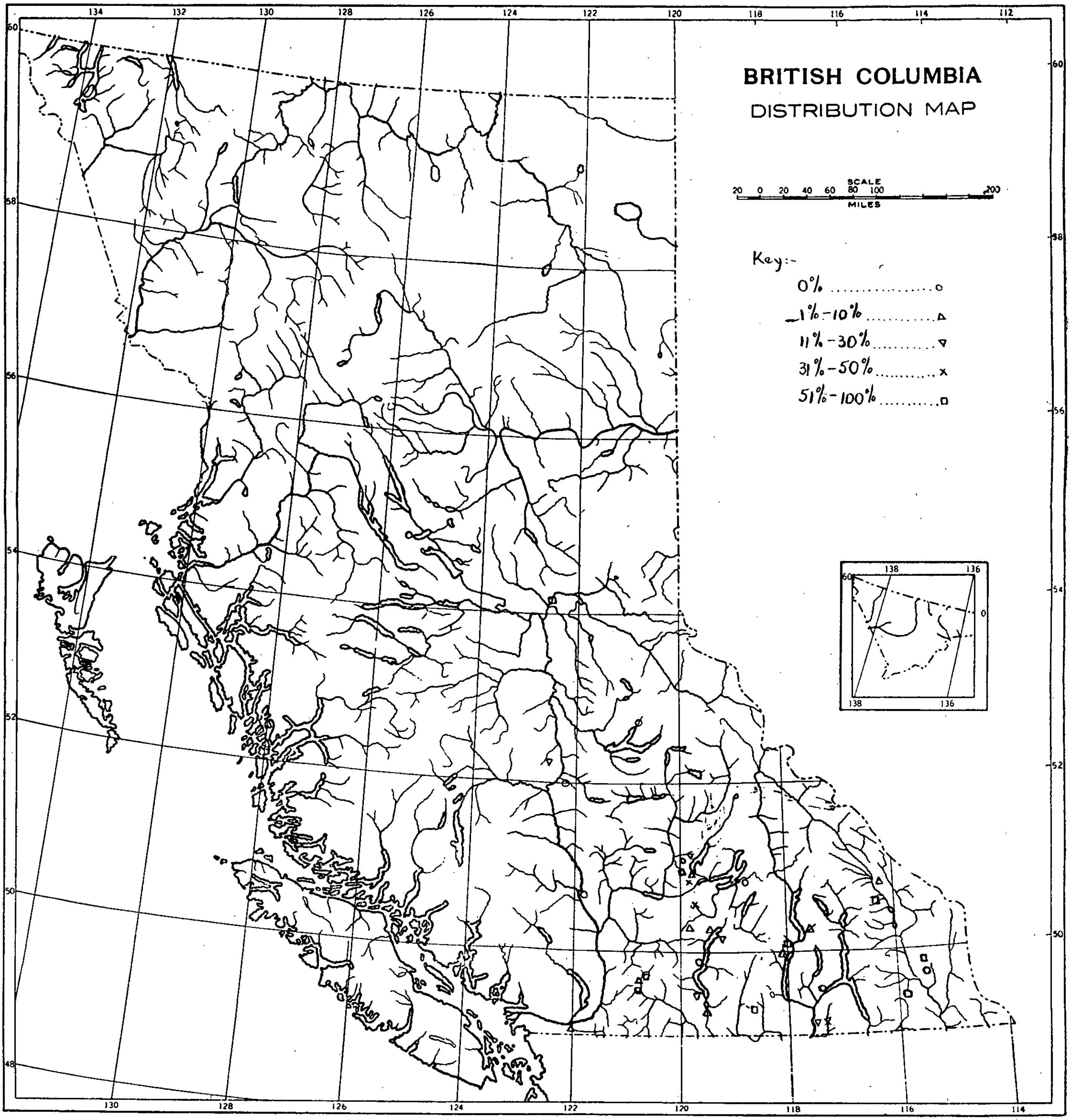

Fig. 6. Distribution and incidence of black spot in the shiner, <u>Richardsonius</u> <u>balteatus</u>.

may be made. It would be interesting to follow, for a number of years, the infestation in a lake such as Paul lake where the shiners have recently been introduced to see if there is a gradual increase in the percentage of the fish infected. The table below gives the per cent infection of four lakes for which data are available for a number of years.

Table IX. Per cent infestation of shiners in four lakes for several years.

	1928	1948	1949	1950
Garnet Valley Dam	18.3			17.2
Cultus lake			3.3	2.1
West lake			61.1	58.3
Taylor lake		81.4	80.1	80.8

Samples of shiners are available from May to August 1949 from Rosebud and Taylor lakes. Rosebud shows an increase in percentage of infected fish from May till August, a phenomenon not found among samples from Taylor lake. The percentage of fish infected and the size of the sample for the months of May, June and August for each lake follows.

Rosebud lake

	Percent Fish Infected	Size of Sample
May	12.6	445
June	16.4	328
August	46.3	200

Taylor lake

	Percent Fish Infected	Size of Sample
May	80.3	163
June	80.5	203
August	81.7	159

Several fishes other than the lake shiner were found to be infected with blackspot; squawfish, *Ptychocheilus oregonensis* (Richardson); chub, *Mylocheilus caurinus* (Richardson); coarse-scaled sucker, *Catastomus macrocheilus* Gerard; prickly sculpin *Cottus asper* Richardson; Eastern brook trout *Salvelinus fontinalis*; Kamloops trout, *Salmo gairdnerii* kamloops and the Rocky Mountain whitefish, *Prosopium williamsoni* (Gerard). The squawfish were found to be as heavily infected as the shiners in most localities, all other species being relatively free of the disease. The disease in these fish may or may not be caused by the metacercaria responsible for the disease in the shiner.

Eastern Brook trout from Garnet Valley Dam and Mabel lake are heavily infected, while the Kamloops trout from the same lakes are apparently free from the disease. Fishermen from this area have made several complaints regarding the disease in the Eastern Brook trout to Mr. Art Higgs of the Summerland Hatchery.

DISCUSSION

The sporadic distribution of blackspot observed in the shiner on the Columbia-Fraser watershed is also noticeable in eastern Canada and United States. This cannot be attributed to the distribution of the definitive host, a fish-eating bird (the kingfisher for both the strigeids whose life cycle have been determined) for the bird will be found wherever there is a body of water containing enough fish to furnish it with food. It appears then that the snail is the factor upon which the presence or absence of the disease depends.

Each of the strigeids responsible for the disease in North America whose cercarial stage is known, has been found to utilize some species of Helisoma as its first intermediate host. The lake in which black spot occurs must provide the type of habitat necessary for the survival of these snails. In Taylor lake and Summit lake, the two lakes examined by the author, Helisoma of subcrenatum (Carpenter) is the most abundant snail found. These snails were found on the waterplants (sedge, bulrush, and water lily) and submerged logs near the edge of the lake, being most abundant where there is a muddy bottom (fig. 7). They were never found along the rocky stretches of shoreline (fig. 8). Shiners were both among the reeds and along the rocky areas.

An attempt was made to correlate the disease with

Fig.7. Snail habitat at Taylor lake.

Fig. 8. Barren shoreline free of snails, Taylor lake.

the temperature and degree of hardness of the water in lakes and streams where the disease was known to occur, however, no relationship could be established.

All other factors being equal, snails will be more abundant in waters with high calcium and phosphorus concentrations. An increase in the amount of calcium and phosphorus in a soft water lake through the addition of chemical fertilizers will bring about conditions favouring the support of a larger snail population and hence a tendency for an increase in black spot incidence. Cases of this nature have been noticed by some workers, among whom are Cross, Cameron and Lachance. Cross (1933) found that smallmouthed black bass and perch in a small soft water lake in northern Wisconsin were entirely free from trematode cysts in 1931 and 1932. However, in 1933, after two summers of calcium and phosphorus fertilization, six per cent of the fish were found to be infested with strigeid cysts. A similar situation was described by Lachance (1948) in Quebec.

RELATION OF INFECTION TO AGE OF HOST

The effect of parasitism on the host depends primarily on the number of parasites present and the site of infection. Initial infection may lead to partial immunity to future attacks by the same parasite, but this does not appear to be the case in shiners infected by blackspot. Earlier in the paper it was pointed out that there appears to be a significantly larger number of cysts in old fish than in young fish.

Also fish in their second year, or older, show cysts in various stages--unpigmented, newly formed; fully pigmented and others in various stages of disintegration indicating super-imposed multiple infections. Hunter (1928) working with Lepomis cyanellus noticed that the larger fish showed more cysts than did the smaller fish and came to the conclusion that this was due to multiple exposures. McCoy's experiments in 1928 with Cercaria hamata showed that the fish did not acquire resistance to future attacks.

EFFECT OF PARASITE ON HOST

From the information available in the literature the penetration of the cercaria into the fish is the period of greatest irritation. The author did not find any strigeid cercariae during examination of snails from Taylor lake, thus no experiments on the penetration of cercariae into shiners could be carried out. McCoy (1928), Krull (1934) and Hobgood (1938) found that experimental fish became highly irritable during attacks by cercariae. Krull gives the fullest description and states as follows: "They are stimulated to activity and go through the water by spurts in all directions. Soon after the initial attack they attempt to detach the cercariae by rubbing against the bottom and sides of the aquarium." This activity is replaced by a flexing of the fish's body to one side or the other for several seconds. Activity ceases after about fifteen minutes and "the exhausted fish collect .....near the surface of the water, remaining motionless for an hour or more except for profound respiratory movements, before resuming their normal activity."

Such extreme exhaustion would make the fish easy prey for predators. However, the extreme concentrations of cercariae used in the experiments may not occur in nature and at the same time the violent activity of the fish caused by the penetrating cercariae would probably soon take them outside the range of the larvae.

The wounds made by the cercariae may provide a port of entry for bacteria and/or fungi but no such infections have been observed. Some authors have reported the loss of a few drops of blood from the skin and the development of a few small haemorrhagic areas in the tissue at the time of cercarial penetration.

Small fish with large numbers of cysts in their muscle tissue may have their body permanently flexed or stiffened to such an extent that their swimming speed is decreased according to Krull (1934). This latter effect was noticed by the writer while working at Taylor lake and again in the fish hatcheries at Summerland and the University of British Columbia, being particularly evident in some of the larger shiners which were completely covered by blackspot. These fish, however, had the cysts confined to the skin except for the occasional one found in the muscle. Therefore, unless the presence of the cysts in the skin reduces the elasticity of the skin to the extent where movement is hampered, it is more likely that the retardation in speed is the result of lowered or upset metabolic activities of the fish. The cysts in the fins displaced the ray elements, but this was of little importance as the body is used in swimming, the fins merely acting as stabilizers.

Retardation in development of young fish has been reported by Hubbs (1927) and by Krull (1934). This aspect of the problem was not investigated but a heavy infection of any parasite during a period of rapid growth would exert a severe physiological strain on the fish. It was noticed in lakes infested by Schistocephalus sp. that infected fish were retarded in growth and body deformed, but here the size of the parasite is an important factor, a factor which is of little consequence in blackspot except in very heavy infestations.

The eye was sometimes a site of infection especially in the heavily infected fish, the cysts being confined to the socket and the conjunctiva near the periphery of the socket. No signs of "pop-eye" were noticed as reported by Krull in C. bessiae infections. Blackspot has been reported by Hsia (1941) to have caused blindness in the common cod Gadus callaris L. but this is without doubt another instance of the exception rather than the rule. The presence of the metacercariae in the shiners' eyes would reduce the amount of light entering the eye and cause some distortion of the visual image though they were never found in sufficient numbers to cause blindness.

ECONOMIC CONSIDERATIONS

The shiner, Richardsonius balteatus, is not of direct economic value in that it is not a commercial or sport fish. Blackspot disease in it is therefore not of direct economic significance. However, the shiner may serve as reservoir from which the infestation of the definitive host may be maintained at a high level. This would mean an increase in the number of eggs reaching the snail resulting in an increase in the number of cercariae available to infect game and commercial fish.

To date the only complaints of black spots in game fish have come from Garnet Valley Dam and Mabel lake near Summerland where the Eastern Brook trout are heavily infected. Whether the metacercaria in this fish is the one responsible for the disease in shiners remains to be determined, but those examined by the author show the same morphological characteristics as those in the shiners. Life history studies will have to be completed before a definite decision can be reached. The author, as stated earlier, has found the disease to be present in the Kamloops trout and Rocky Mountain whitefish, both of which are important game or commercial fish.

Control of the disease through the eradication of snails may not be practical because of expense. Copper sulphate is the chemical generally used to destroy snails but has the disadvantage of being highly toxic to fish and of being rapidly lost from solution by run-off and convection currents. A mixture of copper sulphate and copper carbonate in the ratio of 2:1 by weight is more effective according to Cort (1950). Cost may be prohibitive in large bodies of water where repeated application would be required.

As the reduction of parasite populations is attained by effectively interrupting the chain of events necessary to complete the life cycle, the destruction of shiners should they prove to be a reservoir would also help control the disease. If the shiners can be removed or reduced in sufficient numbers without upsetting the economy of the lake to any great extent, control would result from the reduction of infective metacercariae.

Control by killing the definitive host is hardly feasible as it will likely prove to be either a fish-eating bird or mammal. Possibly numbers of the mammal could be limited but those of birds would be more difficult because of their migratory habits. Effective control of the disease will have to stem from the reduction of the snail population, or the shiner population, or both.

SUMMARY

1. A description of the strigeid metacercaria responsible for black spot in <u>Richardsonius</u> <u>balteatus</u> is given. underline

2. The host cyst consists of two layers, an outer area with melanophores in the loose connective tissue and an inner more dense, unpigmented connective tissue layer.

3. Cysts are found in the skin, fins, eye, mouth, and gills.

4 Lakes with a high percentage infection show more cysts per infected fish than do lakes with a low percentage infection.

5. Young fish have fewer cysts per fish than do old fish.

6. Attempts to obtain adult strigeids by feeding cysts to pigeons, ducklings, cats, and rats failed. One worm, <u>Psilostomum</u> <u>ondatrae</u> was recovered from a pigeon.

7. Black spot is widely though erratically distributed in British Columbia. Thirty three of forty two lakes examined were infected.

8. <u>Ptycholeilus</u> <u>oregonensi</u> ; <u>Mylocheilus</u> <u>caurinus</u>; <u>Catastomus</u> <u>macrocheilus</u>; <u>Cottus</u> <u>asper</u>:, <u>Salvelinus</u> <u>fontinalis</u>, <u>Salmo</u> <u>gairdnerii</u> <u>kamloops</u>, and <u>Prosopium</u> <u>williamsoni</u> were also infected by black spot.

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