B L A C K   S P O T

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B A S S

by

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" BLACK SPOT OF BASS "

INTRODUCTION

In the past four years, considerable interest has been aroused by a condition in game fish known as " black spot ", a condition characterized by the presence on the fins, under and on the scales proper and in the myotomes of the fish of small black spots about the size of the head of a pin.

They are caused by the encystment of larval stages of certain trematodes the adults of which occur in the digestive tract of fish-eating birds or mammals. These encysted larvae or "metacercariae" do not all belong to a single species of trematode. They may occur in pigmented and non-pigmented cysts. This pigment, whenever it occurs, is due to the host's body reaction which produces pigment cells surrounding the encysted larvae.

The life histories of these parasites are similar to each other. In brief, three hosts are required; a snail, a fish and a fish-eating bird or mammal. The eggs, which are laid by the adult parasite in the intestine of their host, pass out into water. There they are either ingested by a fresh-water snail or they develop further, giving rise to a miracidium, which leaves the egg after hatching and penetrates into the snail. In both cases, the miracidium invades the digestive glands or liver of the snail, after which it metamorphoses into a sporocyst which eventually gives rise to hundreds of free living cercariae. These cercariae leave the body of the snail and penetrate certain species of fresh-water fish. After penetrating this second intermediate host, each cercaria changes into a metacercaria, encysting as soon as it reaches its final location. Sooner or later, pigment is usually laid down around the formed cyst, giving the well known appearance of black spot on fish.

When fish are infected with trematode larvae, their movements are impaired greatly, even to the extent that some of them will not eat and so become emaciated. Under these conditions, it is noted that they are more easily caught by the fish-eating bird in which development to the adult stage takes place, thus completing their life cycle. This cycle goes on as long as the required intermediate hosts are present, thus spreading disease to more and more fish and to, no doubt, new localities each year.

The black spots which occur in different fresh-water fish have been shown to be caused by various species of trematodes. At least two groups of trematodes have been demonstrated to parasitize fish, the strigeids and the heterophyids. The strigeids appear to have a very rigid host specificity (DuBois 1944), while the heterophyids have been shown to have very little host specificity (Cameron, 1937, 1937a, 1945). The life histories of certain heterophyids in Canada have been worked out, but so far of none the strigeids.

One of the most important of these parasites is the species which is found in fish of the family Centrarchidae. The first mention of this parasite is the description by Hughes in 1927 of its metacercaria as _Neascus amblopiitis_. The metacercaria was found encysted in 25 out of 29 rockbass [(_Ambloplitis rupestris_) Rafinesque,] (Jordan and Evermann,1902), taken from Douglas lake, Michigan, during the summer of 1926. Several specimens of black bass [(_Micropterus dolomieu_) Lacépède] (Jordan and Evermann,1902), revealed the presence of black spot and a brief study of the cysts suggested that the parasites belonged to the same species as those found in _A. rupestris_.

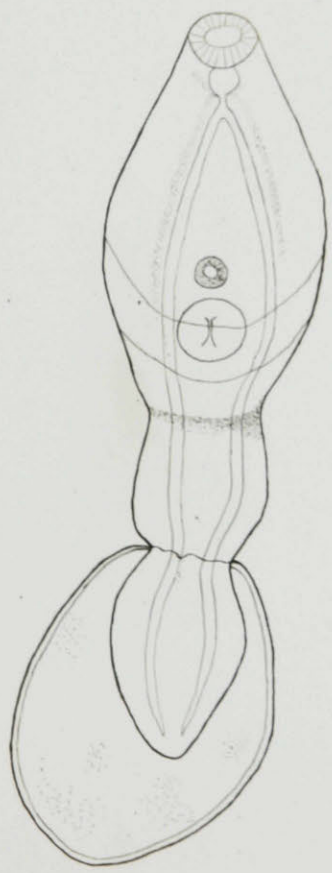
The cyst is composed of two parts, an outer black pigmented covering of host origin and an inner non-cellular hyalin layer of parasite origin. The inner cyst is thin, very tough and closely invests the parasite. Within the cyst the metacercaria is folded back upon itself. When the animal is freed from its cyst, it is seen to have a deep constriction dividing the body into two well defined regions, the fore- and hind-bodies. (figure 1.). Hughes chose this metacercaria as type of the new larval group " Neascus ".

This larval group has the following diagnostic characteristics; Strigeids with both fore- and hind-bodies well developed and distinctly set apart by a constriction; no lateral sucking cups; fore-body leaf like; holdfast organ well developed; reserve bladder highly developed; encysted.

In 1928, Cort and Brooks discovered a cercaria which they called _Cercaria bessiae_ , from a species of snail _Helisoma trivolvis_ (Say). collected from the Douglas lake region, in

FIGURE... 1.

Metacercaria of *Uvulifer amboplitis* escaping from its cyst.



the state of Michigan.

The specific diagnosis of Cercaria bessiae is that it is a holostome cercaria without a ventral sucker and with unpigmented eye spots; body about the same width as tail-stem and bent ventrad when hanging at rest in the water (figure 2.); digestive system very rudimentary; penetration glands six in number; tail-stem without caudal bodies; body spines on first third of the body; genital primordium large in size (figure 3.).

In 1930, Hunter and Hunter, published a note concerning their experiments on the development of Neascus ambloplitis Hughes into a hitherto unknown species of the genus Crassiphiala Van Haitsma, 1925, in young kingfishers. Two young kingfishers were fed a total of about 250 encysted metacercariae from the flesh of the small-mouthed bass. The droppings of the birds were examined at intervals and were positive some four weeks after feeding (Hunter, 1931, 1933,).

Krull in 1933, had observed the encystment of the cercaria of Uvulifer ambloplitis (Cercaria bessiae) and its transformation into Neascus ambloplitis after performing several experimental infections on the sunfish (Eupomotis gibbosus) Linneaus . The cercariae were obtained from a snail, Helisoma trivolvis (Say), collected near Cushing, Oklahoma. The first metacercariae matured in one month in heavily infected fish. Later in 1934, he studied the effects of parasitism and the reaction and resistance of the host according to their age. He found that when small fish were infected by large numbers

FIGURE...2.

Cercaria of Uvulifer amblopitis hanging at rest in water.

Note that the fore-body is bent ventrad upon itself.

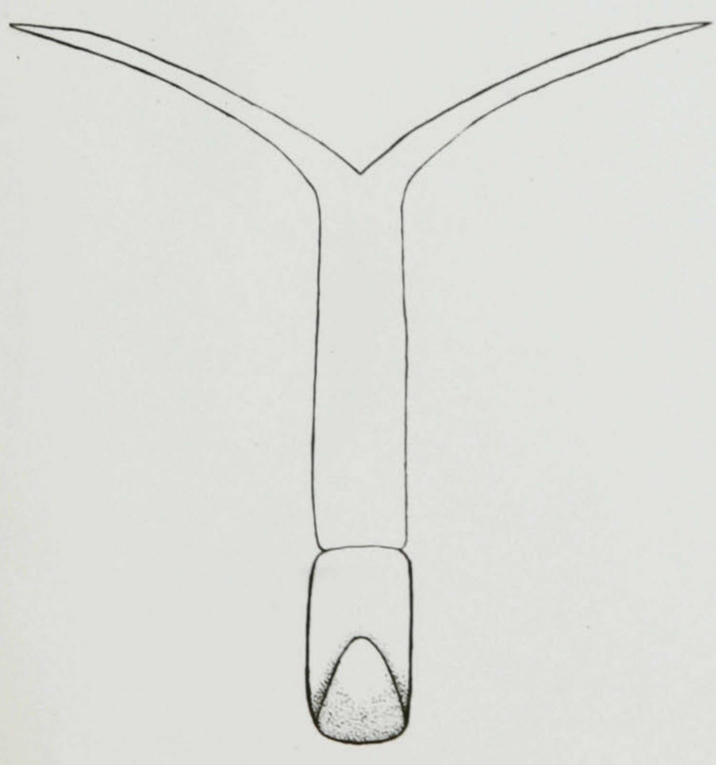
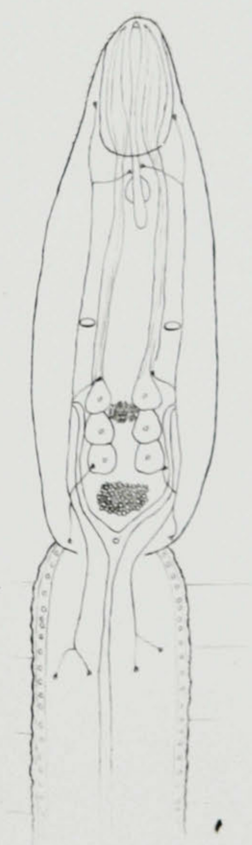


FIGURE...3.

Cercaria of Uvulifer amblopliitis showing internal anatomy of body and part of tail-stem.



of cercariae, they died; larger fish were more resistant. Fish of all sizes responded to the penetration of the cercariae immediately and in a very characteristic way. They were stimulated to activity. A small fish may acquire a sufficient number of developing cysts to produce permanent flexions in the body. Arrested development, particularly of the head, was observed in a small heavily infected fish. In 1933, Hunter and Hunter, (1934a, 1934b,) gave in detail the results of their experiments started in 1930, on the evolution of the metacercaria, together with a complete description of the morphology of the adult, to which they gave the name Uvulifer ambloplitis. They found that the metacercaria matured in the intestine of the belted kingfisher (Streptoceryle alcyon) L., in 27 ± three days. The adult trematode is covered by a smooth cuticula with the body constricted into distinct cephalic and caudal regions. The fore-body is bowl shaped and contains the ventro-terminal oral sucker. The acetabulum is situated in the center of the fore-body just anterior to the holdfast organ. Posteriorly, the fore-body passes into the hind-body which becomes distended dorsally and laterally by the uterus and two large testes lying dorsally in the last two thirds of the hind-body. Laurer's canal opens on the dorsal surface mid-way between the two testes. The vitelline reservoir lies adjacent to the Mehlis gland, between the testes and extends from the anterior level of the genital atrium to the base of the neck of the hind-body.

The ovary is small and lies to the left of the anterior testis.

Finally, Hunter and Hunter in 1934 published a résumé on the study of the life cycle of the parasite, the different phases of which they described in that same year.

The adult , living in the intestine of the kingfisher, lays ovoid, operculated eggs which are passed out with the feaces of the host in an undeveloped stage. As soon as they reach the water, they develop rapidly and in about three days, a ciliated miracidium hatches out, which can penetrate two species of snails, Helisoma trivolvis (Say) and H. campanulatum (Say). Within this host, development takes about six weeks at a temperature ranging from 18° to 25° C. This miracidium in the snail soon gives rise to a mother sporocyst, and through parthenogenetic reproduction, to daughter sporocysts. These stages invariably occur in the digestive gland or liver of the snail where hundreds of cercariae are produced in about six weeks. These cercariae escape from the snail into the water and become free swimming. Within 48 hours (maximum 72) they must penetrate any species of centrarchid fishes, (Krull, 1932, and Hunter and Hunter 1934, 1934b) producing sometime during the third week after infection the well known pigmented black spot containing the metacercaria. Both the small and large mouthed bass, rockbass, common sunfish and the banded sunfish (Enneacanthus obessus) have all been successfully infected with the cercaria of U. amblоplitis. The sunfish (Apomotis cyanellus) was infected experimentally with the cercaria of U. amblоplitis (Krull 1934).

When the infected bass, rockbass or sunfish is eaten up by the kingfisher, the cyst is digested away, freeing the parasite which matures in 27 ± three days (Hunter 1933). Thus under ideal temperature conditions, 18° to 23° C., the complete life cycle of U. amblopliitis takes three to four months.

Following the classification of DuBois (1938), the original Neascus amblopliitis (Hughes, 1927) is now designated as Uvulifer amblopliitis; Crassiphiala amblopliitis (Hughes, 1927) Hunter, 1933 as it was first described, is now regarded as a synonym.

Black spot of bass is widely spread throughout the United States, particularly in the states of Michigan and New York, the Mohawk, Hudson and Raquette watersheds appearing to be the greatest foci of infection. Very little is known of its occurrence in Canada. It occurs in several parts of Ontario and its known distribution in Quebec , extends from East of Levis county, South-east and South along the U.S.A. border, across Montreal Island, along and including the Ottawa river up to Pontiac county. (figure 5.).

EFFECTS OF PARASITES ON FISH

Krull carried out penetration experiments with the cercaria of U. amblopliitis (Krull, 1934) on the common sunfish (Eupomotis gibbosus). He observed that heavy infections with this cercaria caused death of fish fry in two to four days.

FIGURE...5.

Partial map of the Province of Quebec showing the distribution of Black Spot of Bass in the Eastern townships and North-west Laurentians.

He showed however, that enormous infestations could be built up in small and medium size fish and that fish of all sizes responded to the penetration of the cercariae immediately and in a very characteristic way. They were stimulated to activity and went through the water by spurts in all directions. Soon after the initial attack of the cercariae, they attempted to detach these cercariae by rubbing their body against the bottom and sides of the aquarium or against objects in the aquarium. This activity subsided in about 15 minutes, being replaced by a flexing of the body while suspended in the water; the flexion to either side was extensive and lasted for several seconds before relaxing. Finally the exhausted fish came to rest in some particular spot near the surface of the water, remaining motionless for one hour or so before resuming normal activity.

In 1933, Cross made a study of perch and small-mouthed black bass and their parasites(cestodes, acanthocaphala and trematodes). These fish were collected from Wisconsin lakes. He showed that there was a decided difference in average length and weight when negative fish and those heavily infected are placed in separate groups, according to the actual age of each specimen. Incidentally, perch and bass, in a soft water lake were entirely free from trematode cysts in 1931 and 1932, but in 1933, after two summers of fertilization of that same lake with calcium and phosphorus fertilizers ,six percent of the fish examined were infected with strigeid cysts. This seemed to be paralleled by an increase in the snail population following fertilization of the lake.

Hunter and Hunter in 1936 and 1938 reported that a heavy infection with the metacercaria of U. ambloplitis in young small-mouthed bass caused a statistically significant loss in weight when compared to control fish. They suggested that this loss was due, in part at least, to disturbance of the normal metabolism of the host.

Hunter and Hamilton in 1941, gave a report of their studies on host parasite reactions to larval stages of U. ambloplitis; their findings are summarized as follows;

1.- The inner-hyalin cyst of parasite origin appears within two to four days of the time the parasite reaches its definitive location.

2.- The cysts appear to develop near the internal or external perimysium of the muscles.

3.- Pigment bearing cells resembling melanophores, appear during the third week and come to lie in the outer layers of the fibroblast of the portion of the cyst.

Hughes (1927) reported having found the cysts of Neascus ambloplitis in the myotomes, integument, bases of fins, around the eyes, in the mouth, under the surface of the head and in only a few cases, in the peritoneum. According to Krull (1934) the condition known as "pop-eye" may be caused by the parasite encysted in the eyes and in his experiments, he claims that the death in two of his infected specimens was due to the presence of this parasite.

Ward and Mueller (1926) found a similar pop-eye condition in the fry of the black spotted trout and attributed their death to the presence of an encysted metacercaria which they named Distomulum oregonensis. According to Price (1929), this is the metacercaria of Nanophyatus salmincola (Chapin, 1926) [= Troglotrema salmincola (Chapin)] .The minute cyst occured in great numbers at the bases of the fins,in the tissue of the orbit, around the eye capsule along the course of the eye muscle and within the optic nerve.

MATERIALS AND METHODS

The present investigation had a twofold objective: the distribution of black spot of bass in the Province of Quebec, and the identification of the snail host of U. ambloplitis in this country.

It was not until 1939, after inquiries were received from various fish and game clubs, that studies on black spot of fish were begun at the Institute of Parasitology. Both the black spot and yellow grub of bass, trout (Miller, M.J. 1940, 1942) and related fish were widely distributed throughout the Quebec lakes. These investigations have elucidated in several cases the cause of the disease, methods of infections,and certain factors influencing infection.

The studies on black spot of centrarchid fishes, were carried out during the summers of 1943 and 1944. As many lakes and streams as it was possible to investigate in that time, were visited, both in the Eastern townships and the Laurentians, and wherever possible, small-mouthed black bass, rockbass and sunfish together with hundreds of snails were sollected from these localities. However certain difficulties were encountered from time to time in various places.

On a few lakes and streams, there were no boating facilities while on others, the facilities were not sufficiently adequate to permit collections being made. This has rendered the fishing possibilities and collections of snails extremely difficult.It was also practically impossible to collect snails on windy days or when the waters were rough, due to the fact that snails of the genus Helisoma, which are fairly large in size, (figure 4.) are not congregated in a small area, but are very scattered. Moreover, they must be collected by hand instead of using a net. This net is usually made up of ordinary window screen netting.It consists of a rectangular metal frame, eight inches wide by 18 inches long, attached to the end of a five foot rod as a handle. The screen netting is cut out in the form of a box, about six inches deep, with the width and length as the rectangular metal frame on which it is fixed. Snails which live on weeds are collected by means of this net, by sweeping through these aquatic plants. The snails collected during this survey, were either examined on the spot or packed in containers with damp moss or grass and shipped to the labo-

FIGURE... 4.

Helisoma anceps, the natural intermediate host of *Uvulifer ambloplitis*.

These snails are one-third natural size.

ratory where they could more easily be examined for the presence of cercariae.

In the laboratory, the method found most efficient for the examination of escaping cercariae from these snails, was to place two or three snails in a half-pint milk bottle and with the aid of an eight X or ten X hand lens, to examine the water twice daily, in the morning and at night. Whenever cercariae were present, they could be seen suspended in the water, head downward with the furcae of the tail widely spread.

Tentative identification of escaping cercariae in the water were made with the hand lens and later they were checked by microscopical examination. All the morphological observations were made on living material. The details of morphology were first worked out as far as possible on temporary mounts of living specimens, studied with a low power objective,. With the use of a 50 X oil immersion lens, these were checked and further details added. Observations of these strigeid cercariae were made with both unstained and stained specimens. Certain points were observed with greater accuracy by the use of intra-vitam stains. The microscopical studies of living cercariae were best made on fresh, newly emerged cercariae, due to the fact that old specimens showed degenerative changes.

Several intra-vitam stains were tried. The best of these were those used by Talbot (1936) for the study of the schistosome cercariae. The cercariae are first stained with very dilute neutral-red for about five minutes, then two or three drops of nile blue are added. Due to the rapid penetration of the nile blue the cercariae were ready for study within a few minutes.

In some instances, cercariae are stained in neutral-red followed by orange G. This stain was found more useful in staining the excretory system.

Aqueous mounts of intra-vitally stained cercariae can be preserved for a considerable time if the coverglass is surrounded by a film of melted vaseline or glycerine jelly to prevent evaporation of the water.

Measurements of cercariae were made with dead specimens, due to the fact that live cercariae have too great a power of extention and contraction. The best method found in killing the cercariae was the following: water containing cercariae was placed in a small glass bowl and an equal amount of boiling ten percent formalin was quickly poured in. In this way, very few specimens were distorted.

The infected fish, when received in the laboratory, were kept in an ordinary house refrigirator until they could be examined. Two methods have been used for obtaining metacercariae free from their encystment. The first method consists in placing the flesh of the infected specimen in artificial digestion medium, which is prepared in the following manner:

15 grams of pepsin are dissolved in three liters of water. When the pepsin is completely dissolved, 21 cc. of HCl (sp. gr. 1.10-1.19) are added. Then the solution is rendered normal by the addition of 25.5 grams of NaCl. The flesh of the fish to be digested is placed loose in a large beaker with the digestive medium. A few hours are usually sufficient to free the metacercariae which are found at the bottom of the beaker.

The second method used, is a mechanical one. The cyst is first taken out of the tissues of the fish with fine forceps and, placed on a microscope slide with a drop ortwo of water. Then with the use of two very fine needles, the metacercaria is dissected out of the cyst under the low power compound microscope. Care must be taken not to injure the cyst by applying too much pressure. After performing this operation, a cover slip is placed over the living larval trematode, again without applying too much pressure, and the temporary mount is ready for study. Studies were made with both stained and unstained, living and dead specimens.

Intra-vitam stains as used above in the study of the cercariae, were not found more useful or efficient than unstained live material.

Permanent stains however were found more useful. The metacercariae are first killed in hot 7o percent alcohol, and stained in the usual way with Gower's alum-carmine stain, cleared in beechwood creosote, and mounted in Canada balsam, without pressure, other than that of the weight of the cover slip. All drawings of both cercariae and metacercariae were made with the use of a camera lucida, but the detail drawings of the cercaria presented in this work is a diagrammatic representation of the interpretations of the different structures from the studies of the large series of specimens used.

During the spring of 1945, it was impossible to obtain young hatchery bass. For this reason, the fish used in the experiments were taken from the Ottawa river at Ste-Anne de Bellevue by means of a trap. This fish trap consists of a three foot square frame work construction, covered with " minnow netting " so that it can be easily dismounted or carried. Two opposite sides of the trap have a funnel shaped opening, made up of 1/4 inch mesh wire netting. The narrow end of the funnels lead inside that trap. Bread was used as bait, and the trap was taken out of the water every day so as to remove the fish caught within. The fish used in the experiments were fed on earth worms and the snails on fresh lettuce changed once a week.

DISTRIBUTION OF BLACK SPOT

The following lakes and streams in the Province of Quebec are on record as containing small-mouthed black bass. 60 of these were visited and in 32 ," Black Spot " was discovered.

They are here recorded under the headings of counties and townships and those visited are underlined and the presence or absence of the disease indicated. While the disease has not been recorded from the others, there is no reason to believe it is absent or will remain absent from many of them.

COUNTY	TOWNSHIP	LAKE OR STREAM	
Pontiac	Dorion	Cayamant	Not examined
	"	Mcgoey	" "
	"	Lac du Moulin	" "
	Leslie	Clark	" "
	"	Farm lake	" "
	"	Hughes (McCauig)	" "
	"	Hughes N°2	" "
	"	Hughes N°3	" "
	"	Otter	" "
	"	Stevens	" "
	Thorne	Killorans	" "
	"	Thorne lake	" "
	Onslow	Lac la Pêche(Wilson)	Black spot present
	Bristol	Long	Not examined
	Clapham	Murray lake	" "
	"	Picanoc River	" "
	Mansfield	Serpentime river	" "
	"	Truite (Trout)	" "

COUNTY	TOWNSHIP	LAKE OR STREAM			
Gatineau	Aumond	Castor	Not examined		
	Hincks	Green lake	"	"	
	Northfield	Heney	"	"	
	Wright	Lac des Iles	"	"	
	"	Perreault	"	"	
	Cameron	Michel	"	"	
	"	Roddick lake	"	"	
	Dorion	Paterson	"	"	
	Masham	Gauvreau	Black	spot	absent
	"	Browns'lake	"	"	present
	"	Cameron lake	"	"	"
	Hull	Johnston	"	"	absent
	"	Harrington	"	"	present
	"	Meach	"	"	"
	Eardly	Phillippe	"	"	"
	Low	Bernard	"	"	"
Papineau	Mulgrave	Blanche lake	"	"	"
	Petite Nation	Papineau lake	"	"	"
	Templeton	Burnt Shanty	Not examined		
	"	Macgregor	"	"	
	Mulgrave	Coeur	"	"	
	Portland West	McArthur	"	"	
	" "	Mountain	"	"	
Argenteuil	Montcalm	Bark lake	"	"	
	"	Bates ville	"	"	

COUNTY	TOWNSHIP	LAKE OR STREAM	
	Arundel	Bevin (Beavan's)	Not examined
	Gore Wentworth	Clair	" "
	Harrington	Harrington	" "
	"	McDonald lake	Black spot present
	"	Long lake	" " "
	Grenville	McCullough	Not examined
	Howard	St-Joseph	" "
	"	Ste-Marie	" "
	Morin	Simon	" "
	"	Theodore	" "
Terrebonne	Abercromby	Achigan	" "
	"	Cornu	" "
	"	Round(Ste-Adèle)	" "
	Doncaster	Arpents	" "
	"	Long (Grand ludger)	" "
	Ste-Agathe	Brulé	" "
	Wexford	Carré	" "
	Beresford	Carré	" "
	"	Grisé	" "
	"	Lac des Sables	" "
	Morin	Daly or Millette	" "
	"	Paquin	" "
	"	Raymond	" "
Maskinongé	Calonne	Lambert	" "

COUNTY	TOWNSHIP	LAKE OR STREAM	
Arthabaska	Tingwick	Rivière des pins	Not examined
	"	Richmond	Black spot present
	Warwick	Rivière des Rosières"	" "
Wolfe	Weedon	Clear (Clair)	Not examined
	"	Lake Louise or Weedon	Black spot absent
	Dudswell	Silver or Mirror	" " "
	Shipton	Nicolet River	Black spot present
	"	Tor lake	Not examined
	Stratford	Lake Brochet	Black spot present
	"	Lake Aylmer at Disraeli	" " absent
Richmond	Brompton	Salmon brook	Not examined
	"	Brompton lake	Black spot absent
	Windsor	Windsor	Not examined
	Shipton	Francoeur	" "
Frontenac	Winslow	Lac aux Isles	Black spot absent
	Spalding	Chaudière River	Not examined
	Lambton	Lambton(Lac à Richard)	Black spot absent
	Stratford	Lac des Ours	Black spot absent
	Coleraine	Lac St-Francis (small and large)	" " "
	Gayhurst	Lac Drolet	" " present
	"	Three Mile Lake	" " absent
	Ditchfield	Lac Mégantic	" " "
	"	Spider lake	" " "
	"	Egg Pond	" " "
	Clinton	Rush lake	" " "

COUNTY	TOWNSHIP	LAKE OR STREAM			
Compton	Lingwick	Moffat	Black	spot	absent
	"	McGill	"	"	"
Stanstead	Magog	Magog river	"	"	present
	"	Magog lake	"	"	"
	Barnston	Lyster	"	"	absent
	North Hatley	Lake Massawippi	"	"	"
	Stanstead	Tomifobia river	"	"	"
	"	Crystal lake	"	"	"
	"	Memphremagog	"	"	present
Sherbrooke	Orford	Fraser lake	"	"	absent
Mount Bruno		Seigneurial lake	"	"	"
		Mill or Daisy	"	"	"
Drummond	at Drummondville-	St-Francis river	"	"	"
Brome	Brome	Brome lake	"	"	present
	Bolton	Lake Orford	"	"	absent
	Bolton Center	Lake Nick	"	"	"
	Sutton	Missisquoi river	"	"	present
	Bolton Center	Trouser's lake	"	"	"
	" "	Trouserleg lake	"	"	absent
	" "	Long Pond	"	"	present
Shefford	North Stukely	Stukely or Bonelli	"	"	absent
	Shefford	Waterloo lake	"	"	"
	"	Libby's lake	"	"	present
	Rivière N° 2., 1 1/2 mile east of Foster station at the bridge. Address.- Foster Route 2.		"	"	"

COUNTY	TOWNSHIP	LAKE OR STREAM			
Missisquoi	Frelighsburg	Pike river	Black	spot	present
	"	Selby lake	"	"	absent
	at Cowansville	Yamaska river	"	"	present
Huntingdon	at Huntingdon	Châteauguay river	"	"	"
Vaudreuil	- - - - - - - -	Coulé St-Lazare	"	"	"
	- - - - - - - -	Rivière St-Jean at Bellevue, near lake St-Louis	"	"	"
	- - - - - - -	Rivière Raquette	"	"	"
Soulanges	- - - - - - - -	Rivière Beaudette	"	"	"
Jacques Cartier	- - - - - -	Lac St-Louis	"	"	"
		Ottawa river at Ste-Anne de Bellevue	"	"	"

SNAILS FOUND AND EXAMINED IN THE SURVEY

The following nine species of snails (Ward and Whipple, 1918) were examined for cercaria infection:

Helisoma trivolvis macrostomum (Whiteaves)

H. campanulatum (Say)

H. cf infracarinatum F.C. Baker

H. anceps latchfordi (Pilsbry)

H. anceps (Menke)

Amnicola limosa

Lymnea stagnalis

Bythinia tentaculata

Physa sp.

The snails were collected from a few inches to about three feet of water. The majority of them were located on muddy bottom, usually covered with dead organic material. Very few of them lived on rocks, aquatic plants and rotten logs; this is particularly true of the genus *Helisoma*.

The snails were identified by Mr.A. LaRocque of the Geological Survey, Ottawa. In this survey *H. anceps latchfordi* was found in lac LaPêche (Wilson's lake)in the Gatineau district, and Mr. LaRocque notes that this is a new location for this species.

Five species of snails were found to be infected with cercariae as follows:

a.- *H. trivolvis macrostomum* infected with a furcocercous cercaria identified as *Cercaria wardi* (Miller, 1923).

b.- *H.anceps* infected with a xiphidiocercaria sp.

c.- Several snails belonging to the genus *Physa* sp., infected with a furcocercous cercaria identified as *Cercaria douglasi* (Cort and Brooks, 1928).

d.- *Lymnea stagnalis* infected with an unidentified furcocercous cercaria.

e.- *Bythinia tentaculata* infected with an unidentified cercaria.

Helisoma anceps was the only snail found infected with *C. bessiae*. The percentage of *C. bessiae* in the snails collected was only 0.4 percent. This species has not previously been recorded as a host for C. bessiae.

BEHAVIOR OF C. BESSIAE IN WATER

The cercariae can be seen suspended in the water, head downward. In this position, the furcae are held at a slightly obtuse angle with the tail-stem, the tail-stem being well extended and the fore-body being bent on itself very much like a closed finger.(figure 2.). After the free suspended cercariae have remained motionless for a while, they become suddenly active and move quickly upward for a short distance, until they regain their original position. They always move backward with the spiral wriggling movement characteristic of the holostome cercariae.

INFECTION EXPERIMENTS

Five species of fish were exposed to infection with the cercaria of U.ambloplitis.

1.- Ambloplitis rupestris --rockbass

2.- Lepomis gibbosus -- common sunfish

3.- Simotilus atromaculatus -- horned dace

4.- Catostomus catostomus -- white sucker

5.- Lebistes reticulatus -- guppy

The cercariae used in these experiments were obtained from snails collected in stream No 2., one and a half miles east of Foster, Quebec.

Because the rockbass and sunfish were collected in the Ottawa river, they were kept in the laboratory for three weeks before being exposed to infection, to ensure that they did not carry a natural infection and, to acclimatize them to laboratory conditions. The white suckers, horned dace and guppies had been kept in the laboratory for two years. Five rockbass, two sunfish and a large number of white suckers, horned dace and guppies were used as controls.

The methods and time exposure in carrying out the experimental infections with the species of fish mentioned above, were quite varied, so that each experiment requires individual description. Newly emerged cercariae were used in all the experiments, the water was kept at a temperature varying between 18° to 22° C., except for two days when the temperature dropped to 16° C. The temperature in all cases was taken at 11 o'clock a.m. and p.m. The pH of the water in which the snails were kept was at all times, exactly the same as the water in which the experimental fish were kept.

Three rockbass were placed in water with varying numbers of cercariae. The first one was exposed to about 300 cercariae for eight hours, the second to several hundreds for eleven hours and the third for 24 hours. This procedure was repeated three times at one day intervals.

A sunfish was exposed to infection in the same manner as the third rockbass.

In these experiments , soon after the cercariae and the fish had been placed together, the fish became very excited and showed great nervousness. They moved by quick and sudden jumps and spurts, rubbing their body on the sides and bottom of their aquarium. However, after about an hour, they became quiet,remaining in one corner of the aquarium, near the surface of the water, but each time I approached their tank, during the infection period, they would begin their characteristic movements.

Another rockbass was exposed by placing it and the cercariae in 60 cc. of water. Using the binocular dissecting microscope with a magnification 16 diameters, the penetration of the cercariae was observed. This fish was exposed to infection for 60 minutes, then removed to its original aquarium. This procedure was repeated four times in two days.

The cercariae were seen penetrating under the scales of the fish, but it was very difficult to observe which part of the fish they penetrated most. This difficulty could hardly be overcome, due to the fact that the fish was in almost constant motion. However, the cercariae were seen to throw off their tails within two minutes after coming in contact with the fish. The actual time they took to penetrate and disappear from view, after throwing off their tails could not be ascertained, partly due to the fact that the cercariae do not attack the fish all at the same time.

Four sunfish were exposed to infection on different occasions in the same manner as the rockbass mentioned above. The penetration of the cercariae could not be observed because there was too much débris introduced in the small aquarium with the cercariae. After 40 minutes of exposure, the fish were removed. The water containing the cercariae in which the fish had been exposed was examined under the low power of a compound dissecting microscope. A few tailless cercariae were present, crawling on the bottom of the small aquarium, many tails, however were seen floating about in the water. Among the tailless cercariae that were seen on the bottom several were degenerating quickly. It is interesting to note that not one complete cercaria was noticed.

On the 18th and 19th day after the initial penetration of the cercariae, in all the experiments mentioned above, some very small whitish spots could be seen on the rockbass and the sunfish, particularly at the base of the caudal fin, which was found later to be the heaviest infected part of the fish. On the days following, these whitish areas were more easily seen, being larger and numerous. On the 22nd and 23rd day very dim pigmented black spots were observed in these areas. These black spots increased in size and number from day to day. On the 28th and 29th day, they were quite large and very obvious. These results confirm the findings of Krull

(1934) and Hunter and Hunter (1934, 1934b) who reported that the fully developed cysts appear in about three weeks if the organisms are kept between 18° and 21° C. Black pigment-bearing cells appear in the loose connective tissue of the outer coat sometime during the third week.

The location of the cyst in the experimentally infected fish varied considerably. In one of the small rockbass, the cysts were found mostly at the base of the caudal and dorsal fins, while in another larger rockbass, the cysts were concentrated mostly at the base of the caudal fin, around the eyes and in the mouth cavity. In most cases, cysts were recovered also from the myotomes, integument, and between the fin rays.

Hughes (1927) and Krull (1934) have both reported finding cysts under the surface of the head in addition to the above mentioned areas,while, Hughes (1927) has also recovered them from the peritoneum.

The final experiments employed the white suckers, horned dace and guppies. These three species of fish were kept in separate tanks of two gallons each, throughout the experiments. They were exposed to infection, by introducing hundreds of cercariae in the aquarium each time. These experiments proved that these three species of fish could not be infected with the cercariae of _U. amblоplitis._

YELLOW GRUB OF BASS

During the first summer of the present survey, several snails of the genus _Helisoma_ collected at lake LaPêche (Wilson's lake), were found to be infected with cercariae. These were identified as the cercariae of _Clinostomum marginatum_, the metacercaria of which is known to encyst in several species of fresh-water fish causing the condition commonly referred to by fishermen as the " yellow grub disease " (Hunter and Hunter, 1933, 1934c, 1935). Two small healthy sunfish which had been kept in the laboratory, for two years, were subjected to infection by placing the snails and the fish in the same aquarium.

Both sunfish became very heavily infected. One of these died six weeks after it was exposed to infection and there is no doubt that death was due to the heavy infection obtained. The yellow cysts covered the entire body of the fish.

The snail which carries the intermediate stage of _C. marginatum_ in Canada was identified as _Helisoma trivolvis macrostomum_. In the United States the natural snail carrier is _H. antrosum_, while _H. campanulatum_ was experimentally infected.

Yellow grub disease in bass was found in the following lakes:

Trouser's lake

<u>Long Pond</u>

<u>Yamaska river</u>

<u>Lac LaPêche</u>

<u>Blanche lake</u>

<u>Ottawa river</u>

<u>Richmond lake</u>

The yellow grub is more widely distributed throughout the Province than is the black spot due to the fact that almost any species of fish can be infected. I have also found the disease in lac à la Carpe north-west of the Laurentide National Park. The metacercariae were encysted in trout, <u>Salvelinus</u> <u>fontinalis</u>.

In the late summer of 1945, I found the adult, <u>C</u>. <u>marginatum</u>, under the tongue and in the mouth cavity of two great blue herons, (Taverner, 1934). One of these was caught at Long Pointe (Montreal Island) and the other at Lake La-Pêche in the Pontiac county.

WHITE GRUB OF THE LIVER

During this survey, I have found that all the bass in Libby's lake had the liver infected with the encysted meta-cercaria of Posthodiplostomum minimum, (Hughes, 1928).

The adult trematode is known to live in the mouth of the great blue heron.(Hunter, 1937). The eggs are passed in the water with the feaces. After hatching they penetrate certain species of snails belonging to the genus Physa. Cercariae are produced and eventually invade the liver of the bass where they encyst. The infection found was so heavy that it was almost impossible to count the number of metacercariae encysted in the liver.

DISCUSSION

Black spot in bass, rockbass and sunfish was found in 32 lakes out of 60 which were examined. This distribution is far from being complete, when we consider that the snails of the genus Helisoma occur in practically every lake in this province. In spite of the fact that Helisoma anceps was the only snail found infected in nature with the cercaria of U. amblopitis in the province and that H. trivolvis and H. campanulatum, the naturally infected hosts in the United States were found free from infection, there is a possibility that the latter two may or even do carry the disease in nature here. Several thousands of Helisoma snails were examined during the present survey, but many thousands more need be collected from a wide area over a long period of time, and examined for the presence of escaping cercariae. This area should include both the Eastern townships and the Laurentians. A new distribution for H. anceps has been recorded for the first time in lac LaPêche where the disease is quite prevalent on bass.

The kingfisher, which is known to be the definitive host, both here and in the United States, has been seen frequenting all the lakes visited. This observation was extended beyond the areas examined during this survey; they include the Counties of Timiskaming, Roberval, Chicoutimi and Charlevoix-Saguenay.

Small-mouth black bass were not used in the experimental infections because it was impossible to obtain these small hatchery fish last year. However, it is hoped that during the summer of 1946, experimental infections will be carried out in the laboratory with hatchery bass free from black spot.

Both the black spot and the white liver grub were found in all the bass caught in Libby's lake, while in Brown's lake all the bass and sunfish were found infected with black spot but were free from the liver grub. This coincides with the occurrence of snails of the genus Physa which are the carriers of the white liver grub. Physa was found in Libby's lake but was not found in Brown's lake. At this point, it is worthy to mention that the bass in Libby's lake are very small in size; they seldom exceed nine inches in length. These two natural infections occur simultaneously in the same fish and may be responsible for the stunted growth of the bass, by upsetting their normal metabolism. There are other factors which may increase greatly this abnormal condition of these fish, such as the presence of both the adult and the plerocercoid stages of the fish tapeworm, Proteocephalus ambloplitis (Hunter and Hunter 1933). The adults were found in the intestine while the plerocercoid larvae were found in great numbers in the liver, spleen, mesenteries and gonads.

The yellow grub disease is one which, unlike the black spot, does not show a rigid host specificity. It was found by several workers, to occur in a variety of fish, including bass. For this reason, the distribution of this species is more extensive but on the other hand the percentage of infection is very much lower than the black spot. The snail carrier here was found to be _H._ _trivolvis macrostomum_. This is the first record of the natural occurrence of the disease in that snail. In the United States, _H._ _antrosum_ is the natural carrier, while _H._ _campanulatum_ was experimentally infected by Hunter and Hunter (1934b). _H._ _campanulatum_ was collected during this survey but was found free from this infection.

SUMMARY

Black spot in bass in Quebec is caused by the metacercarial stage of _Uvulifer ambloplitis_, a trematode parasite of the Belted Kingfisher. A survey of 60 lakes and streams in the Eastern townships and Southern Laurentians showed that the disease was present in 32 of these. Not only were small mouthed black bass and rockbass found to be infected, but sunfish were, for the first time, shown to suffer, in nature, from the same disease.

The snail intermediary was shown experimentally to be _Helisoma anceps_. _H._ _trivolvis_ and _H._ _campanulatum_ which had been found to be infected in New York and elsewhere, were not found infected here.

The cercaria encysts at the base and between the rays

of the fins,(especially the pectoral and caudal) around the eyes, in the mouth cavity, in the skin and in the myotomes. The encysted metacercaria stimulates the production of a black pigment in the third week after infection and causes the characteristic black spot. It can live in a dead fish for from 12 to two months.

During the survey, yellow grub was discovered in seven lakes. This is the metacercarial stage of a trematode living in the mouth of the Great Blue Heron. Its snail vector in Quebec was shown to be <u>Helisoma trivolvis macrostomum</u>.

White liver grub was found to be common in Libby's lake.

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