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Original Manuscript

No. 782

A report on the investigation of cysts under the fins and in the fillets of the winter flounder (*Pseudopleuronectes americanus*) with notes on the parasites' biology and incidence in the Maritimes

By

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April, 1951

Gratitude is expressed to Dr. W. R. Martin for his advice and encouragement as well as to Mr. Frank McCracken for criticism and aid in the project. Thanks is also expressed to technical staff and workers at the Station for their co-operation. Special thanks is due to Mr. William Carson for his patient and energetic assistance.

Special permission has been granted by Dr. T. W. M. Cameron and McGill University to carry on this work as partial requirement for the degree of Ph. D. in Parasitology.

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A report on the investigation of cysts under the fins and in the fillets of the Winter Flounder (*Pseudopleuronectes americanus*) with notes on the parasite's biology and incidence

The cysts which infest the fins and fillets of flatfishes of the Maritimes and especially the Bay of Fundy region are caused by penetration of an intermediate larval stage of the trematode *Stephanostomum (Distomum) histrix* Dujardin, 1845. This infestation is effectuated either by penetration of the epidermis or by penetration of the gut after ingestion of the intermediate form. All species of flatfishes commonly caught in the Bay of Fundy area were found infested excepting the brill (*Lophopsetta aquosa*). The heaviest incidence is noted in the winter flounder which is the most important commercial fish locally and consequently the species on which the greatest amount of data were obtainable.

The worm, a species of the trematode family Acanthacolpidae, matures in scavenger fish; the commonest in Passamaquoddy Bay, N. B., is *Hemitripterus americanus*, the sea raven. Other hosts have been recorded for this species by Stafford (1904): *Lycodes* species (arctic eelpouts), *Cryptocanthodes maculatus* (wrymouth) as well as the species recorded above. Manter (1925) reported the species as rare in the halibut off Mount Desert Island, Me. The parasite is common in England, encysting in flatfishes and facultatively in the angler (*Lophius piscatorius*), and is recorded as maturing there in the halibut (*Hippoglossus hippoglossus*) and the father lasher (*Cottus scorpius*), a related host to the sea raven. It is interesting to note that Dawes (1944-45) states that the status of the genus *Stephanostomum* Looss, 1900, in European waters is rather uncertain. The species in referred to in Britain and North America as *Stephanostomum baccatum* Nicoll, 1907. From the description of the cyst in the subcutaneous tissues of the fish in Europe it seems probable that it is the same species as that found in Canadian flounders.

Stephanostomum histrix (Dujardin, 1845) is described as a cystic stage of trematode found in flatfish (*Pleuronectes* sp.) in European waters. His description is close to that of *St. baccatum* and is probably a synonym. Levinsen (1881) described *Stephanostomum (Distomum) sobrinum* from the intestine of *Cottus scorpius* which is probably the adult of *St. histrix*. Since Dujardin's description (1845) was the earliest, it takes precedence over all later descriptions.

The genus has been described over a wide geographical range, there being species described from all save south American waters. Manter has contributed much information regarding distribution in his papers on surveys off Florida and the Galapagos Islands. The species, as is expected, have their greatest abundance in tropical waters. Linton has described three species of Stephanostomum from the Woods Hole region. Manter holds two of these as valid: St. dentatum Linton, 1889, and St. tenue Linton, 1910. Another species of doubtful position is St. caducus (Looss, 1901). Immature species are referred to frequently but their validity has been questioned by a number of authors. Linton has always included them under the heading of Stephanostomum valde-inflatum. This species encysts in viscera and pleura of scavenger fish and all or part of them are almost certain to be Stephanostomum tenue metacercariae as seen by a review of Linton's host records.

W. E. Martin (1938-39) has worked out the life history of St. tenue which pursues the following general pattern:

1) A snail (Nassa obsoleta) bears the redia (an intermediate larval stage parasitic in the tissues of the mollusc) which is an organism having a pharynx and simple sac-like gut. It gives rise to numerous cercariae by growth of primordial cells at its posterior end. The cercaria, an active free-swimming stage, is born from the redia, ruptures the parasitized tissue of the snail and migrates to the mantle cavity.

2) The cercaria is of the ophthalmoxiphidio type, that is, having eye spots and an oral sucker spine plus a short, stout tail which enables it to swim upright in the water while moving from place to place.

3) According to Martin it is swallowed at this stage by a puffer (Spheroides maculatus) and migrates into the viscera and visceral pleura where it encysts. The encysted stage is known as a metacercaria and is often compared to a cocoon stage in which modifications are carried out in the physiology and morphology of the worm which enable it to take up an adult existence in the final host.

4) The life cycle is completed when the puffer or any other fish bearing the metacercaria in its viscera is eaten by a sea bass (Roccus lineatus). Within this host the cyst wall is digested off and the metacercaria attaches to the intestinal wall, becomes fully mature and lays eggs which are passed out with the faecal matter.

5) The final stage is not described by Martin. It includes the development of the miracidium, a pre-redial stage, within the egg. It is this embryo which, upon entering the tissues of the snail, gives rise to the redia. Cable and Hunnien (1943) working with an allied genus Deropristis found that the egg developed a miracidium but did not report that it hatched. They assumed that the egg was eaten by the snail.

There seems to be no reason to assume that the stages are different in number for St. histrix and St. tenue but the way in which the cycle is carried out is much different since the metacercariae are subcutaneous instead of visceral, making an external penetration almost a necessity.

The Incidence

Incidence was studied by examining catches of flounders from various regions at different depths in order to get as wide a range of infection as possible. The data are made up of exploratory surveys in 1949 by F. D. McCracken and D. N. Fitzgerald together with more extensive examinations during the summer of 1950 in both Passamaquoddy Bay, N. B., and St. Mary Bay, N. S.

Method of Examination

Fish were measured, filleted and inspected both externally and internally. In no case was found in the viscera or pleura a cyst which could be identified as Stephanostomum species. Several times cysts in these locations were found which belonged to a genus of Acanthocephala. These were confused at first because of spinous structures but familiarization with the location and shape of the cysts made further identification a simple matter.

Fish were regarded as uninfected when no cysts could be seen after filleting, lightly infected when there were few cysts, numerously infected when the cysts were obvious without searching and heavily infected when the numbers were so great that they filled the inter-finray regions both above and below the skin level.

The following table demonstrates the incidence per area studied throughout the Maritimes together with the numbers of animals involved. The majority of specimens were taken in the Bay of Fundy area and are not directly comparable to samplings taken outside the bay area but per number of fish examined. The percentages show a definite trend toward a peak incidence in the middle and lower bay region.

The incidence of Stephanostomum in the Maritimes

Place	Date	Total No.	Infested	Uninfested	Source of Data
Bras D'Or ** Lakes, N. S.	9/5/50	53	30	70	Scott
Pubnico-Argyle, N. S.	5/12/50	97	41	59	Wolfgang
St. Mary Bay, N. S.	20/5/50	317	70	30	Wolfgang
Passamaquoddy Bay, N. B.	June, July/50	1071	74	26	Wolfgang
Annapolis * Basin, N. S.	1949	165	80	20	Fitzgerald
Minas Basin, N. S.	June, July/50	187	4	96	Sollows Wolfgang

* This collection is not directly comparable to the 1950 one but is added to give an indication of the incidence.

** Although the Bras D'Or Lakes' sample shows a higher incidence than the Minas Basin sample, individual specimens were always infected with only one or two cysts whereas the Minas Basin collection had fewer fish more heavily infected. (This may indicate a widespread low grade infection in Bras D'Or Lakes and an isolated heavy infection in Minas Basin. More collections are necessary, however, to establish this as a fact.)

Although the Sollows' examination of the Minas Basin infection was made at a different time and under different conditions from my own, it seems significant to note that our results were almost identical.

The main reason for the small sample in Bras D'Or Lakes was the apparent scarcity of the winter flounder in that region.

New Brunswick

The most extensive study of cyst incidence was carried out in Passamaquoddy Bay and the adjoining St. Croix River. Dragging and seining were the main methods of securing material and no area was considered sampled with less than fifty specimens.

It will be seen in the accompanying Tables II and III that the incidence with regard to distance from the open sea is graded in a decreasing order. Starting with the Waweig River and working down the St. Croix and into Passamaquoddy Bay there is a change in salinity, depth and current. The percentages in the table are for the various ranges used in the survey and no combination is made here. However, in the adjoining graph samples were lumped into two groups: "few and none" and "numerous and heavy". This grouping seemed to provide the best gradient.

In Graph I are represented fin infestation and fillet infestation. In general, the fin infestation is higher than the fillet infestation for any given area. The correlation between the two types of infestation is not graded although there is an increase in one with an increase in the other for the area as a whole. From the arrangement two possibilities present themselves:

1) The infestation is progressive, building first in the fillets and later in the fins. (Corresponding infections with other fish and with various size gradients bear this out.) There is a possibility of a tissue reaction too which sensitized the muscle and confined later infection to the fins.

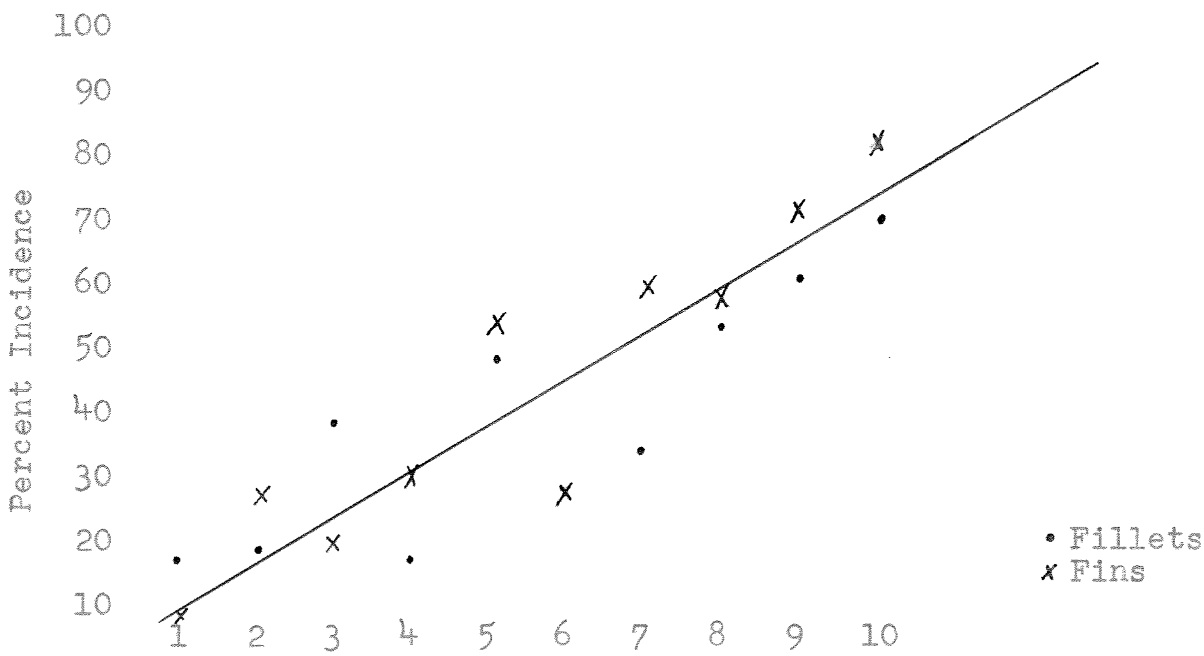
2) The infestation may be acquired in a number of locations, an early infection being followed by a later one. This would correspond to the migratory habits of the fish. (Ages of cysts in the fins and flesh of the winter flounder indicate that the infections are acquired at different times.)

Below is a key showing what sampled locations are indicated by the following numbers on both the map of Passamaquoddy Bay (at the end of this section) and in Graph I.

1. Waweig River
2. Oak Bay
3. Brandy Cove
4. St. Andrews Harbour
5. Minister Island
6. Bocabec Bay
7. Hardwood Island
8. Station 848
9. Pendleton-Deer Islands
10. Mascareen

Note - Number 6 (Bocabec Bay) is represented only by the fin infection. The material for this location was caught by hand-line and since it was not cold stored the fillets were in no condition to be examined.

<u>Graph I</u>



Tables II and III on the following pages summarize the data for samples taken in Passamaquoddy Bay.

Table II

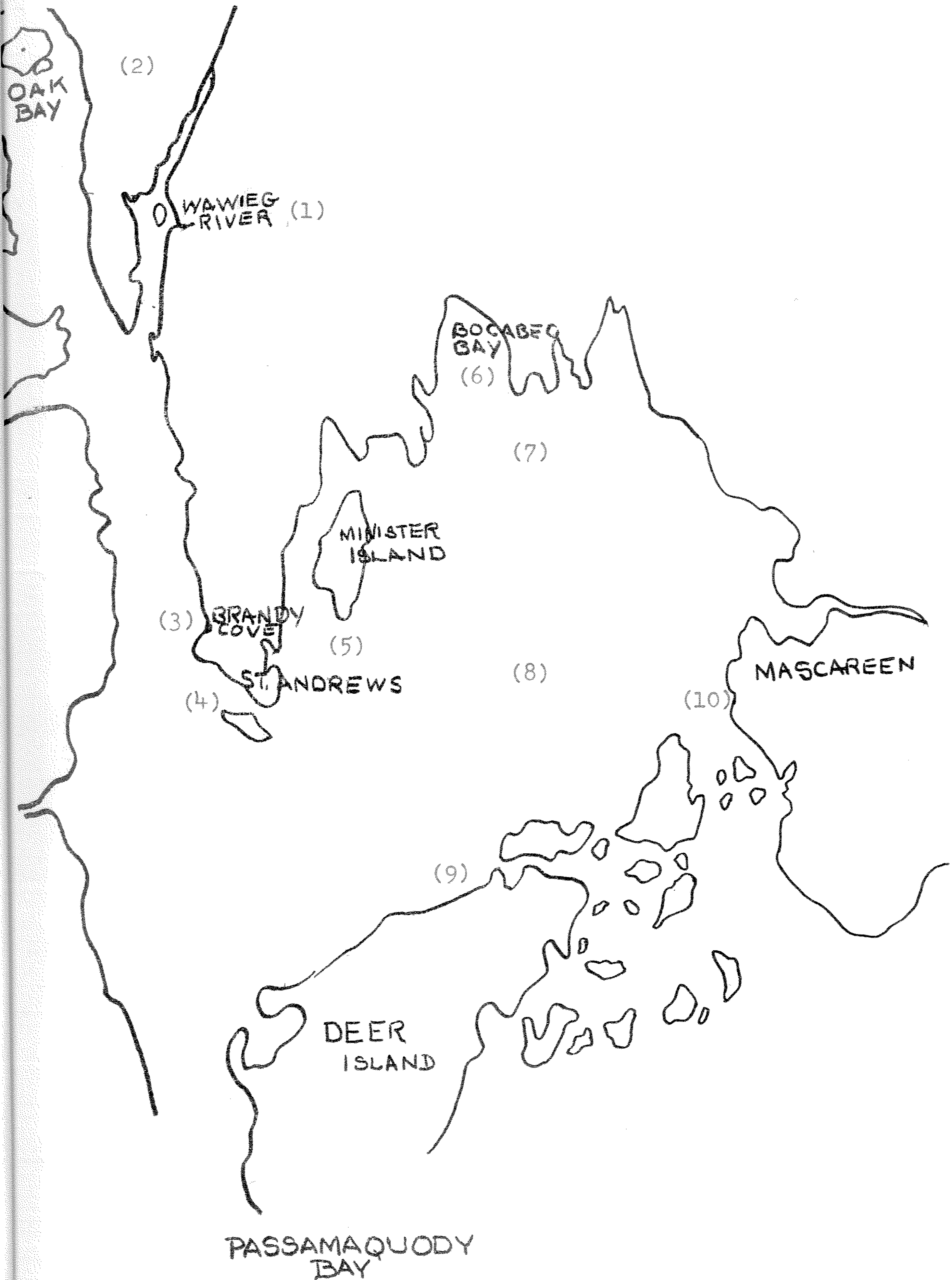
The incidence of cysts in the fins of the winter flounder

Locality	Date	Depth Fath.	None	Percentages Few	Numerous	Heavy	Total Fish	Total Uninfected	Percentages Infected
Waweig River	July	inter-tidal	63	30	5	2	92	63	37
Oak Bay	August	3-12	32	41	19	8	184	32	68
Brandy Cove	July	inter-tidal	59	22	5	14	83	59	41
St. Andrews Harbour	July	1-2	31	37	20	12	50	31	69
Minister Island	July	3-16	9	31	10	50	116	9	91
Bocabec Bay	July	1-3	31	33	14	14	78	39	61
Hardwood Island	July	11-16	8	30	23	39	204	8	92
Station 848	June-July	22	28	15	5	52	54	26	74
Pendleton-Deer Islands	July-August	12-25	9	7	8	76	136	9	91
Mascareen	August	28-30	3	8	17	72	65	3	97

Table III

The incidence of cysts in the fillets of the winter flounder

Locality	Date	Depth Fath.	None	Percentages Few	Percentages Numerous	Heavy	Total Fish	Total Uninfected	Percentages Infected
Waweig River	July	inter-tidal	73	14	7	6	98	73	27
Oak Bay	August	3-12	47	37	8	8	184	47	53
Brandy Cove	July	inter-tidal	24	38	19	19	47	24	76
St. Andrews Harbour	July	1-2	49	33	9	9	45	49	51
Minister Island	July	3-16	30	25	14	31	110	30	70
Bocabec Bay		No data available							
Hardwood Island	July	11-16	26	37	11	26	194	26	74
Station 848	June-July	22	23	26	30	21	43	23	77
Pendleton-Deer Islands	August	12-25	9	33	14	44	123	9	91
Mascareen	August	28-30	6	26	9	59	65	6	94



(2)
OAK BAY
WAWIEG RIVER
(1)
BOCABEC BAY
(6)
(7)
MINISTER ISLAND
(3)
BRANDY COVE
(5)
ST. ANDREWS
(8)
(4)
MASCAREEN
(10)
(9)
DEER ISLAND
PASSAMAQUODY BAY

Note - In the preceding tables the data do not correspond exactly in that the number of fish for the fillet examination may be less than for the fins. Conditions of fish did not always allow for complete examination.

Nova Scotia

Examination of winter flounders in St. Mary Bay was carried on in conjunction with tagging experiments on the motor vessel "J. J. Cowie" during the month of May, 1950. St. Mary Bay is a different type of bay from Passamaquoddy Bay. It is long and narrow and opens into the Atlantic Ocean parallel with the Bay of Fundy whereas Passamaquoddy Bay opens into the Bay of Fundy at right angles to its shore line.

It has been shown, however, that the flounder populations in the Bay of Fundy are of a different type from those in Passamaquoddy Bay with respect to growth and movement. The two populations are not known to mix. In the discussion of incidence and size of fish to follow it will be seen that flounders in St. Mary Bay were larger than those sampled from Passamaquoddy Bay. This difference is due to the confinement of the project there to a narrower range of depths and locations as well as the fact that a large mesh cod-end was used to make these particular drags.

Table IV which discusses the incidence with relation to location to depth shows the same sort of upper to lower bay gradation that was demonstrated in Passamaquoddy Bay. This is indicated in Graph II for the fins; the fillets showing the same erratic distribution as discussed previously.

Table IV

The incidence of the cyst in the fins of the winter flounder

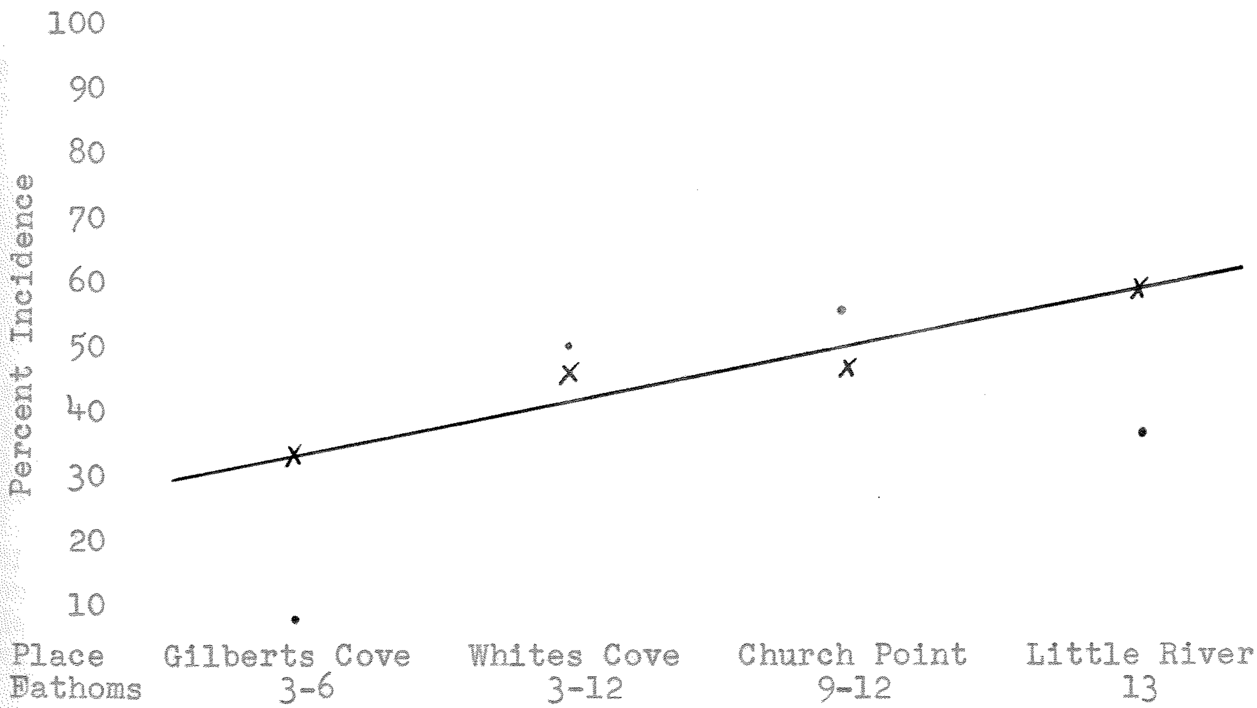
			Percentages					Total Percentages	
Place	Date	Depth Fath.	None	Few	Numerous	Heavy	No. of Fish	None-Few	Num.-Heavy
Gilberts Cove	May	3-6	24	44	14	18	50	68	32
Whites Cove	May	3-12	20	31	17	32	281	51	49
Church Point	May	9-12	18	30	15	37	124	49	51
Little River	May	13	20	24	20	36	50	44	56

Table V

The incidence of the cyst in the fillets of the winter flounder

Place	Date	Depth Fath.	None	Percentages Few	Numerous	Heavy	No. of Fish	Total Percentages None-Few	Num.-Heavy
Gilberts Cove	May	3-6	58	38	2	2	50	96	4
Whites Cove	May	3-12	24	21	11	44	140	45	55
Church Point	May	9-12	23	14	22	41	79	37	63
Little River	May	13	31	37	17	15	48	62	38

Graph II



Discussion

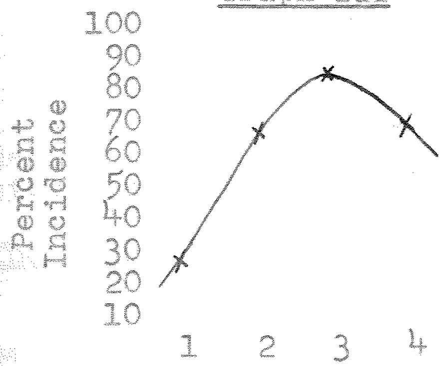
The samples in St. Mary Bay were collected between three and thirteen fathoms while those in Passamaquoddy Bay were taken from intertidal to 30 fathoms. If the incidence were to run as it does in Passamaquoddy Bay it is almost certain that there are areas in St. Mary Bay with a higher incidence than reported in the above tables in deeper waters.

It will be noted that the incidence continues to rise toward the 13 fathom mark without dropping. Deeper water toward the mouth of the bay might show a declining incidence.

<u>The incidence with regard to depth</u>

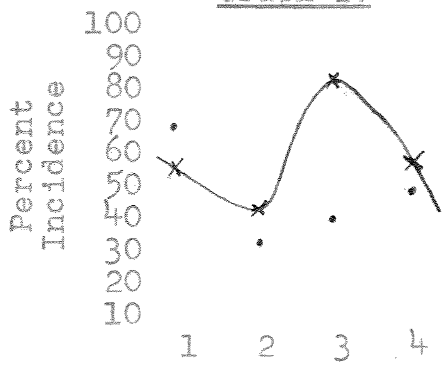
There is some indication that not only location but depth is a determining factor in the rate of incidence among winter flounders. The following graphs and discussion are given to show the trends in infection with regard to the depth of the water in Passamaquoddy Bay. St. Mary Bay depths are discussed above.

<u>Graph III</u>



The incidence of the cysts in winter flounders without regard to location in Passamaquoddy Bay.

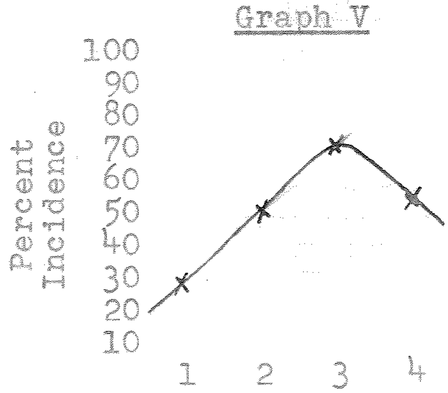
<u>Graph IV</u>



The incidence of the cysts in winter flounders in the region of Minister Island (Pass. Bay) working toward mid-bay.

Graph III is a comparison between incidence and depth in the bay region. The following numbers represent the depths on the graph: 1 1-3 fathoms, 2 6-11 fathoms, 3 11-16 fathoms and 4 over 20 fathoms. On the horizontal axis the peak of infection occurs at 6 to 11 fathoms. Graph IV is similarly constructed but based on samplings from the area of Minister Island toward the deeper mid-bay region. The numbers represent essentially the same depths as Graph III. Here the peak of infection is again in the 11-16 fathom range with erratic incidences for other points.

<u>Graph V</u>



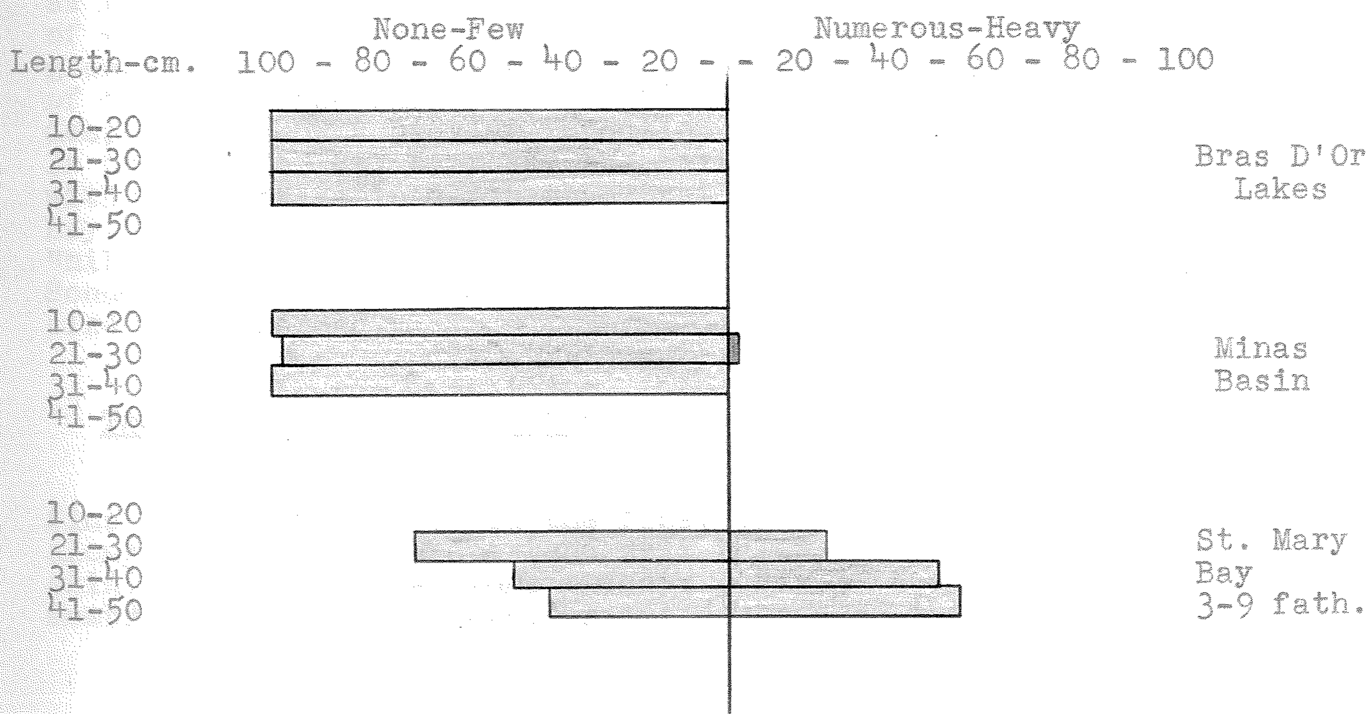
Graph V still using the same numerical depths demonstrates the relationships between depth and incidence Bocabec Bay (Pass. Bay) extending past Hardwood Island toward mid-bay. It will be noted that this graph follows the same general pattern as Graph III with a smooth climb to the 11-16 fathom mark and then a decline on the other side of that depth.

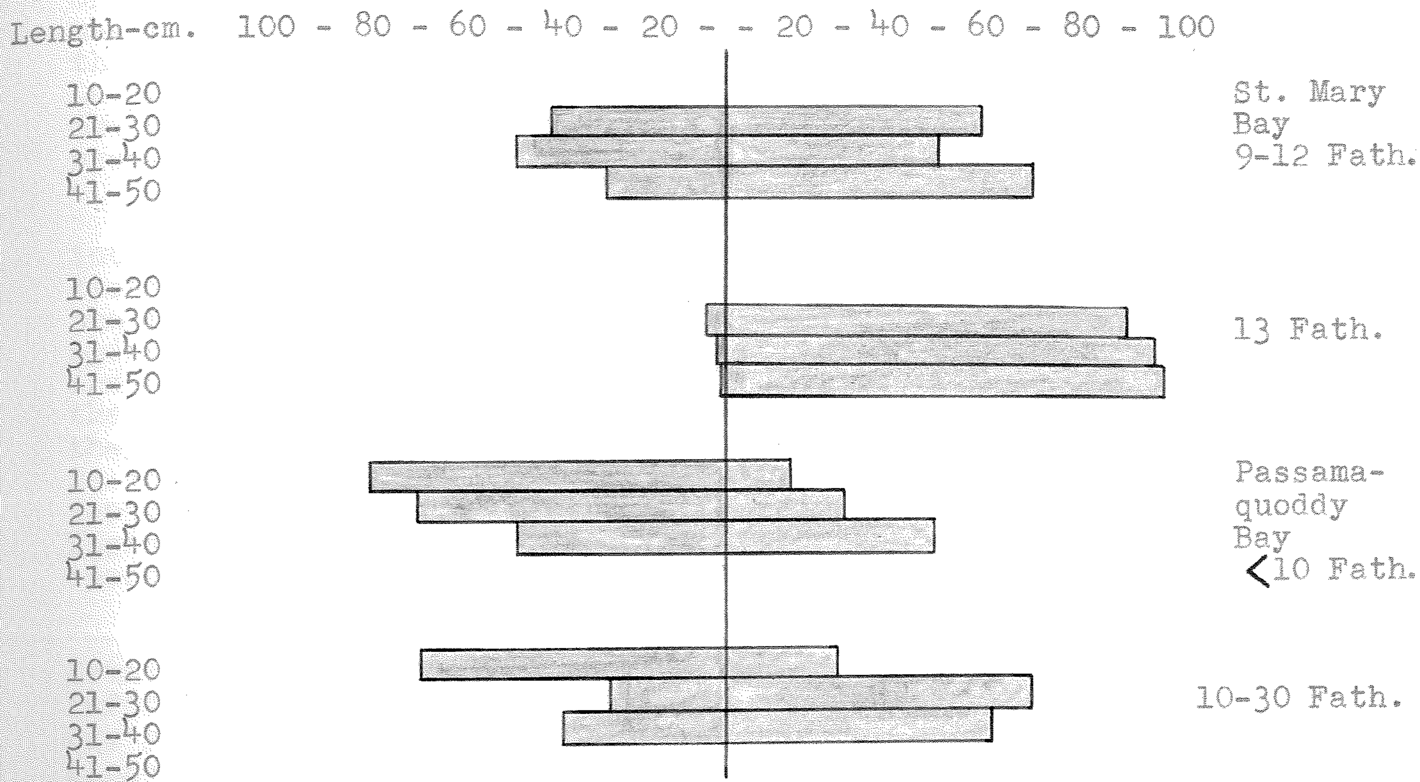
Note - only fin incidences are used in these graphs since the fillet infections are similar although generally lower.

The only inference that can be drawn from these graphs is that there seems to be an increasing incidence up to approximately 16 fathoms. It is probably this factor which causes the disturbance in the distribution of points between Bocabec Bay and Hardwood Island in Graph I, so that although the incidence appears to be graded with regard to location, this incidence is affected by depth when those areas have depths ranging over 10 and under 16 fathoms. A similar situation exists at point 2 (Oak Bay) in Graph I.

<u>The size of the fish and its relationship to incidence</u>

Although it has been possible to collect a fair amount of data from individual stations, the data are massed rather on the general locations from which the flounders were taken. That is to say a sample from Minas Basin is compared with a sample from Passamaquoddy Bay, etc. It was found with the help of Dr. Martin that these figures are best represented graphically by percent incidence per size rather than by tables.





It will be seen that for the two areas with the greatest amount of data, St. Mary Bay, N. S., and Passamaquoddy Bay, N. B., that the percentages are graphed according to depth since the depth shows the greatest degree of constancy as far as size of the fish is concerned when studying normal populations of winter flounders. In general for Passamaquoddy Bay the greater the depth the greater the incidence of infection. Fish of the same size when compared for infection from shallow and deep water show a heavier infection in the deeper water. In the case of St. Mary Bay there is a more graded relationship with an increase in depth than that noted from Passamaquoddy Bay. In Minas Basin and Bras D'Or Lakes the incidence is "nil" for all sizes. Data on Annapolis Basin and Argyle Sound-Pubnico Harbour were incomplete insofar as incidence to size is concerned.

The character of the infection in winter flounders

The cysts of the trematode are arranged along the fins and the fillets. In the fins, cysts tend to line up along the fin rays and extend well below the surface of the skin. In heavy infections the cysts may be found in the fin membrane between the rays. A lot depends on the size of the fish as to how many cysts may be accommodated. Cysts are often found at the base of the fin rays lined up in the meaty portion just below the point where the skin stops and the fillet begins. Fin infections are the most noticeable. Later under the incidence as it concerns other flatfishes the variation in incidence and its manifestations in different species will be discussed.

The fillet infections are almost always near the integument. Cysts in heavily infected fish are found in the skin itself and present a bumpy or measely appearance. Deeper in the fillets the cysts can be found outward from the vertebral spines and fin rays. They tend to collect along the myomeres and decrease in numbers both deeper into the fillet and toward the lateral line. This would seem to support a theory that they enter via the fins. The major portion of the incidence is concentrated in the posterior half of the fish, in the caudal fin, dorsal and ventral fins and the ventral fillet. There have been few cysts recovered from the gills, the eyes, operculum, pectoral fins or the head and mouth.

While the dorsally located cysts are not commonly found in these infections they are encountered in very heavy infections. A report by Fitzgerald on flounders of St. Mary Bay gives the ratio as about 60 percent for belly infection alone, 34 percent both mixed belly and dorsal fillet infection and 16 percent dorsal fillet infection. I have never found a flounder infected in the dorsal fillet without it also being infected in the belly fillet as well. In fish which are infected orally, the infection spreads evenly throughout all the tissues which it comes into contact. <u>Stephanostomum tenue</u> infects the viscera and pleura of the puffer fairly evenly, dorsally and ventrally. The cod-worm (a poor comparison since it belongs to an entirely different phylum) is shown to encyst in a fairly even distribution throughout the fillets of cod and other species of fish. Examples of other parasites known to penetrate the skin at preferential points are <u>Cryptocotyle lingua</u> (Creplin) of the family Heterophyidae, common black spot on marine fish with littoral habits in the Maritimes; <u>Apophallus venustus</u> of the same family attacks about the fins of fresh water fish. Both the preceding parasites show an uneven distribution pattern in the host.

The cyst will live for a long time after the fish is dead and under various conditions. One series of well rotted fish, several days old, had viable cysts in the fins. In cold storage the cysts remain viable for as long as two weeks. No attempts were made to test their viability after freezing so that the limits of viability are not known. By dissecting the cysts from the fish and holding them in 0.7

percent saline they live well for two weeks. Only if the cyst is broken will the larval trematode inside it die rapidly.

A double envelope encloses the trematode. The outer wall which is the cyst seen with the naked eye appears to be fibrous in character. Inside the fibrous wall is a transparent membrane within which the immature worm lies in fluid. The outer membrane is tough and resistant to both pressure and decomposition of the surrounding tissue. The inner sac is elastic and pliable. As the trematode inside grows this sac becomes tight about the worm so that it looks like a second skin. The entire cyst is easily dissected from its *in situ* position. The simplest method for studying the metacercaria was found to be smashing the outer cyst between two glass slides. The fibrous coat cracks audibly and like an egg cracks clean exposing the transparent sac containing the worm. Within this sac the larva can be seen to move. Separation of the transparent sac must be done by teasing with a needle or sharp instrument. The larva has all the characters of the adult in an undeveloped state.

There is some indication that heavily infected fish react more slowly to stimuli than uninfected ones but no controlled experiments were carried out to determine pathological effects.

Incidence of the infection in flatfish other than the winter flounder in Maritime waters

The cysts of *Stephanostomum* species have been reported from flatfish almost universally except for the southern and temperate waters of the United States. Here the genus matures in the flounder *Paralychthes dentatus* as the adult worm *Stephanostomum dentatum* Linton, 1940. From the study of material from Maritime fish it seems almost certain that all cysts from flatfishes belong to the same species *Stephanostomum histrix* (Dujardin, 1845).

Only the brill (*Lophopsetta aquosa*) was found to be free of the cyst. Specimens of this fish have been examined in all of the regions surveyed and none were found to bear the cyst. In Passamaquoddy Bay the brill is heavily infected with *Cryptocotyle lingua*, the black spot disease.

The species examined are tabled on the following page according to their location and incidence of infection.

Yellowtail (Limanda ferruginea)

Specimens of this fish were taken in St. Mary Bay, N. S., during the month of May, 1950.

	Percent				
	None	Few	Numerous	Heavy	Total No. of Fish
Fins	18	36	23	23	22
Fillets	7	13	20	60	15

D. M. Scott examined one specimen in the region of Boisdale (Bras D'Or Lakes) and found it infected.

It will be seen from the above examination that the heaviest infection is in the fillets. The incidence seems to be about the same as that cited for the flounder (P. americanus) for a given area.

Plaice (Hippoglossoides platessoides)

Records have been made in Passamaquoddy Bay, N. B., Bras D'Or Lakes, N. S., and Tignish, P. E. I.

Tignish, P. E. I. (Data collected by F. D. McCracken)

	Percent				
	None	Few	Numerous	Heavy	Total No. of Fish
Fins	4	96	-	-	21
Fillets	48	44	8	-	21

Bras D'Or Lakes, N. S. (Data collected by D. M. Scott)

Fourteen fish were examined and none were found infected.

Passamaquoddy Bay, N. B.

	Percent				
	None	Few	Numerous	Heavy	Total No. of Fish
Fins	71	21	-	8	14
Fillets	1	61	15	23	12

The infection in plaice as judged from this data would not indicate a high incidence of infection. Offshore catches have not been examined by the author and it appears that a true picture can be obtained only by examination of the flesh as well as the fins. In none of the areas examined were the numbers of fish large. It should be noted, however, that only in the case of Passamaquoddy Bay were infections of considerable size even in the limited material.

Witch (*Glyptocephalus cynoglossus*)

Very few specimens of this fish were taken. The highest number collected was three off Bliss Island (Passamaquoddy Bay). Since only a few are available the percentages are not presented.

	None	Few	Numerous	Heavy
Fins	3	2	0	0
Fillets	1	3	1	0

A very low incidence was observed. All concentration of cysts was in the flesh along the base of the fin rays with an occasional cyst deeper into the fillet. Winter flounders which have been taken in the same area are always more heavily infected.

One large witch was examined in St. Mary Bay. The cysts were all in the belly fillet and were orange to yellow in colour. The colour was due to pigmentation in the fibrous coat. The transparent cyst wall inside was the same as that described from the winter flounder.

Smooth Flounder (*Liopsetta putnami*)

All specimens of this fish were taken in the region of the Biological Station at St. Andrews, N. B. They were caught by seine hauls in shallow water in the rivers entering the bay.

	Percent				
	None	Few	Numerous	Heavy	Total No. of Fish
Fins	100	-	-	-	21
Fillets	64	36	-	-	18

All specimens were classed in the none-few range. None were found with cysts in the fins and those with cysts in the fillets were found farther offshore than the completely negative ones. The few infection never rose above two cysts per fish. This fish vies with the brill for lowest incidence.

It seems necessary to mention at this point that small smooth flounders (6-8 cm.) collected in the Waweig

River were occasionally infected in the flesh at the base of the fin rays. Never more than one cyst was found. It is possible that the flounder acquires the infection very young and has no more contact with it.

Discussion

Fish like the yellowtail and the witch are thin-bodied fish. In these species the infection is heavier in the flesh than in the fins as opposed to results for the winter flounder. A possible explanation is that the heavier body and skin of the latter species may prevent easy penetration.

It should be noted that the smooth flounder is also a heavy skinned and heavy bodied fish but it is not found in the regions of heaviest incidence. Winter flounders taken with smooth flounders also show a light incidence of infection.

The above flounders overlap in their distributions. Sea ravens, the known definitive host, have been found in every location discussed and, as will be seen later, infected ones have been found in every area as well. It must be considered, therefore, that every flatfish save the brill is a potential reservoir capable of perpetuating the infection either in its immediate area of habitat or elsewhere when carried by scavenger fish as the adult.

Examination of possible final hosts

The final host is an animal in which the metacercaria, encysted in the flatfish, becomes adult laying its eggs and living an active parasitic life. In the case of the family Acanthocolpidae adult flukes are found in the alimentary tract of teleost fishes.

In Passamaquoddy Bay a considerable amount of the collection was made up of possible scavenger fishes which might serve as definitive hosts for the cystic stage in the flounder. In St. Mary Bay examination of fishes was incidental to the examination of flatfishes for cyst incidence. Although fragmentary data are available from Nova Scotian waters at a variety of points, it can serve no other purpose than to indicate the existence of the adult in these waters.

The following Tables VI and VII give the number of specimens and species examined in Passamaquoddy and St. Mary Bays. None of the species noted in these tables was infected.

Table VI

Fishes examined for adult trematodes in St. Mary Bay, N. S.

Species Name	Common Name	Number Examined
Raja stabuliforis	Barndoor skate	1
Raja scabrata	Small skate	7
Anarhichas lupus	Ocean catfish	6
Macrozoarces americanus	Eelpout	18
Myoxocephalus octodecimspinosus	Long-horned sculpin	25
Lophius piscatorius	Angler fish	1
Gadus morhua	Codfish	4
Melanogrammus aeglefinus	Haddock	3
Pseudopleuronectes americanus	Winter flounder	50
Glyptocephalus cynoglossus	Witch	1
Limanda ferruginea	Yellowtail	12
Lophopsetta aquosa	Brill (Windowpane)	3

Table VII

Fishes examined for adult trematodes in Passamaquoddy Bay, N. B.

Species Name	Common Name	Number Examined
Raja scabrata	Small skate	3
Squalus acanthias	Dogfish	4
Macrozoarces americanus	Eelpout	52
Urophycis tenuis	White hake	10
Lumpenus lampetraeformis	Snake blenny	1
Pholis gunnellus	Rock eel	3
Lophius piscatorius	Angler fish	1
Myoxocephalus groenlandicus	Short-horned sculpin	2
Myoxocephalus octodecimspinosus	Long-horned sculpin	40
Gadus morhua	Codfish	8
Melanogrammus aeglefinus	Haddock	10
Pollachius virens	Pollock	7
Pseudopleuronectes americanus	Winter flounder	50
Hippoglossoides platessoides	Plaice	8
Liopsetta putnami	Smooth flounder	11
Glyptocephalus cynoglossus	Witch	3

The sea raven, *Hemitripterus americanus*, was the only fish found infected with adult *Stephanostomum* trematodes in Maritime waters. The following table gives the number of infected sea ravens per area examined.

Table VIII

Sea Raven infection in Maritime waters

Place	Total No. Infected	Total No. of Fish
Passamaquoddy Bay, N. B.	30	43
St. Mary Bay, N. S.	2	6
Annapolis Basin, N. S.	1	3
Bras D'Or Lakes, N. S.	1	3
Canso, N. S.	2	9

Passamaquoddy Bay is the only area from which sufficient numbers of sea ravens have been collected to determine the percentage incidence. Out of 43 specimens, 30 were infected or about 70 percent infection. The infection ranged from one to over 300 worms per infected fish. The incidence does not appear to depend upon the size of the fish since all sizes are infected at varying degrees.

Specimens of Stephanostomum examined which were collected by Stafford (1904) came from Canso, N. S., Malpeque, P. E. I., and St. Andrews, N. B. These worms appear to be identical with the adults taken from the sea raven during the summer of 1950. They are, however, in rather poor condition, the spines having been lost and the stain poor. Stafford reports this species occuring in the sea raven as well as the wrymouth (Cryptocanthodes maculatus) and the Arctic eelpout (Lycodes reticulatus). No specimens of the latter two species were examined by the writer.

Considering that all three of the above species were definitive hosts, the worm would have a great range of location though nothing is known of the first intermediate host. It seems probable under the circumstances that more species than have been here indicated might act as definitive hosts. In the areas surveyed Hemitripterus americanus, the sea raven, is the major host for the adult worm.

Stephanostomum histrix is found in the rectum of the sea raven. In very heavy infections a few worms are found in the intestine close to the rectal sphincter. They appear to bury their heads in the mucosa and to hold on by a double ring of oral spines. No lesions could be seen in the mucosa.

One codfish was found during the summer with several hundred cysts but no adults of _Stephanostomum_ in its rectum. The outer fibrous coat was still intact, however; in the sea raven the outer coat is digested off before the metacercaria reaches the rectum.

Artificial Infestation

Three species of fish were used for the artificial infection with cysts from the winter flounder: the sea raven (_Hemitripterus americanus_), the eelpout (_Macrozoarces americanus_) and the long-horned sculpin (_Myoxocephalus octodecimspinosus_). These are the most common scavenger fish found among the flounders in the drags. Previous to the experiments, the sea raven was found to have a parasite in the rectum which was identified as belonging to the genus _Stephanostomum_ and which resumbled as far as spines, size of suckers and arrangement of the genitalia the metacercaria found in the flesh of the flounders.

Materials and Methods

Specimens for the tests were taken from various depths throughout Passamaquoddy Bay and the St. Croix River. After being caught the eelpouts, sea ravens and sculpins were placed in a tank aboard the motor vessel "Clupea H.". The tank was equipped with running water so the fish could be kept alive until they could be placed in tanks at the Station. Attempts to mark the fish and to keep them in a single tank met with failure as the fish, being predators, fed upon one another. A divider was used for a comcrete tank which separated it into sixteen small compartments. It was constructed of a wooden frame over which was stretched hardware cloth (about ¼" mesh). The pens were numbered and the fish placed in separate pens. Some difficulty was net in trying to keep the sea ravens alive after capture since they engorge air and float when taken from depths. This problem was overcome by seining and taking only shallow water forms or by keeping the fish under water during transfer. Once in the tanks, they became quiet and would not eat. Only a few were kept alive for the full time required for the test. The eelpouts and sculpins on the other hand ate voraciously once a proper food was found. The best feeding material proved to be fresh clams.

Three methods of infection were tried. The first consisted of starving the host and offering to it the flesh, fins and skin of infested flounders. The fish, however, would not touch the bait even after three weeks of starvation. Occasionally a sculpin would strike but often regurgitated the meal. The second method met with more success and was the only successful one attempted with sea ravens. Fish were placed in an approximately .5% solution of Eurethane, a standard anaesthetic. When signs of quiet were noted, the fish was removed and cysts which had previously been dissected from the fins and fillets of infected flounders were forced into the stomach of the host by means of a rubber-bulbed pipette with a smooth glass tip to prevent injury. Once the cysts were placed in the gut the fish was returned to the tank and allowed to recover. The results of the tests may be found in Table IX. Amount of infection was determined by examining the worms for degree of maturity. In the case of the eelpouts no adult worms were found either in nature or during the test. In the case of the sea ravens the adult trematodes were easily distinguished from the immature worms by the size, thickness and degree of maturity (presence of vitellaria, eggs, etc.). Only mollusc shells have been found in the gastro-intestinal tract of these hosts, whereas the sculpins and sea ravens have many bones, otoliths and scales of herring, pollock and flounders.

The pipette method has its limitations. As the fish is not fully conscious it tends to gulp water and to regurgitate violently when recovering so that the takes although based on the number of cysts given are totally dependent on luck. Since the sea raven was a hard animal to handle and since it serves as the natural host, a fact which complicated the counting and evaluation of the artificial infection, only eelpout was retained as an experimental host.

The third method utilizes gelatine capsules, the classic method used in inducing infection. Starving the fish to permit maturation of worms was complicated because starving fish often shed their intestinal linings thus disrupting their physiological state. Instead, clams were fed to eelpouts for about two weeks prior to infection. To overcome force feeding, the capsules (size 0) were selected so that they would fit the syphon of the clam. Cysts were dissected out as in the manner before mentioned. About four to five hundred cysts were placed in a capsule and capsules in the clams were fed to the eelpouts. No trouble was encountered in getting the fish to eat the capsules.

There is no method available at the moment to determine the percent of the cysts that were used which might be injured beyond survival or which might be unable to develop. Therefore, only arbitrary numbers were used and the total survivals must determine the success of infection.

At the termination of the test all surviving animals were removed from the tank and allowed to die by suffocation. Worms were removed by slitting open the abdomen and removing the intestine and stomach. The intestine was cut opposite the mesentery and the contents washed out with a gentle stream of water into glass syracuse watch glasses. Worms were easily seen and collected against a dark background. Those desired for staining were placed in fresh water and allowed to relax

Table IX

Results of the experimental infestation of sculpins, eelpouts and sea ravens

Pen No.	Type of Host	Method of Infestation	Developmental Time	Number of Cysts	Number of Recoveries
1	Sea Raven	Pipette	7 days	300	9
2	Sea Raven	Pipette	4 days	520	123
3	Eelpout	Pipette	9 days	750	20
4	Sea Raven	Pipette	10 days	840	98
5	Sea Raven	Pipette	10 days	735	15
6	Eelpout	Capsule	19 days	200	11
7	Eelpout	Capsule	17-16-15 days	300	86
8	Sculpin	Fins	15 days	?	3
9	Eelpout	Pipette	22 days	300	1

Note - A large number of the fish used showed no infection at the end of the treatment or died before the experiment was begun. Only those fish which showed a positive infection at the end of the developmental time are tabled above.

after which they were fixed either with bouins fluid, formal-saline or a solution of 70 percent alcohol.

Artificial Maturation Tests

An attempt was made to raise the metacercaria to adult stage in artificial media. Li and others have kept blood flukes alive for 22 days in horse serum and saline. Smyth has used horse serum and amphibian ringer to raise to maturity pleurocercoids of a cestode occuring in fish.

The experiment was conducted with the advice of Dr. V. B. Engelbert of Toronto University. Eight percent horse serum in amphibian ringer was innoculated into 10 c.c. serum bottles by using sterile syringes. Cysts were dissected from flounders antiseptically, broken and the freed worms placed in the horse serum. The media was changed every two days and the cultures held at approximately 16 degrees centigrade. The worm lived for 32 days at the end of which time the bottle seal was broken and the trematode removed. Very little development was evident but the metacercaria was still active. Further tests are planned using sea raven serum.

Examinations of molluscs for cercaria

Snails were collected in both St. Mary and Passamaquoddy Bays. This work was incidental and not concentrated until the end of the summer. Collection included *Littorina littorea* L., the common periwinkle, and *Thais (Purpura) lapilla.* Cercaria were recovered from both snails but were of the wrong type. *L. littorea* shed cercaria of *Cryptocotyle lingua*, the black spot of litteral fish and *T. lapilla* shed *Cercaria purapurae*, both of which parasites have been reported by Stunkard, Lebour and others as being common in European waters. *Polinices heros*, a common snail of the area, was examined to a limited extent but found to be uninfected. *Nassarius trivitata*, common mud snail, was found occasionally in drags. *Nassarius obsoleta* in Massachusetts bears the cercaria of the related species of trematode *Stephanostomum tenue*. Although the snail is reported as common on mud flats at estuaries, none was found except at Belliveau's Cove, N. S., by Marco Giglio, who did not find them to be infected with cercaria. This snail has a sub-species which lives in deep water but dragging methods were never adequate to recover large numbers. Many empty shells have been found off Pendleton Island, the area of greatest incidence of the cyst. Live *Nassa* have also been recovered from eelpouts in this area. The snail is so active that it burrows at the slightest disturbance.

A small mussel, *Yoldia* sp., was found in large numbers associated with flounders throughout the bay as well as in the flounder stomach contents. Although large numbers were examined, none was infected. A more thorough controlled examination of all mollusc forms in the infected areas should indicate the host. Experimental infections using matured embryonated eggs should facilitate the finding of a primary host.