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INCIDENCE AND DISTRIBUTION OF PISCINE ERYTHROCYTIC NECROSIS AND THE MICROSPORIDIAN, *GLUGEA HERTWIGI*, IN RAINBOW SMELT, *OSMERUS MORDAX*, FROM MASSACHUSETTS TO THE CANADIAN MARITIMES

Since the first discovery by Laird and Bullock (1969) of piscine erythrocytic necrosis (PEN) in the red blood cells of the Atlantic cod, *Gadus morhua*; seasnail, *Liparis atlanticus*; and longhorn sculpin, *Myoxocephalus octodecemspinosus*, 15 genera of fishes, including 17 marine species along the North Atlantic coast of North America have been found to be affected by PEN. Sherburne (1977) reported PEN in the alewife, *Alosa pseudoharengus*, and smelt, *Osmerus mordax*. Walker and Sherburne (1977) reported PEN in the Atlantic herring, *Clupea harengus harengus*; Atlantic tomcod, *Microgadus tomcod*; spot, *Leiostomus xanthurus*; tautog, *Tautoga onitis*; rock gunnel, *Pholis gunnellus*; sea raven, *Hemitripterus americanus*; fourspot flounder, *Paralichthys oblongus*; and winter flounder, *Pseudopleuronectes americanus*. Sherburne and Bean (unpubl. data) have found PEN in pollock, *Pollachius virens*; Atlantic menhaden, *Brevoortia tyrannus*; American shad, *Alosa sapidissima*; and blueback herring, *A. aestivalis*.

PEN has been confirmed by electron microscopy as an erythrocytic icosahedral cytoplasmic deoxyribovirus (EICDV) infection in two of the above species—the Atlantic cod (Walker 1971; Appy et al. 1976; Walker and Sherburne 1977) and the Atlantic herring (Philippon et al. 1977; Reno et al. 1978).

During our investigations of PEN in the Atlantic cod, other marine species were examined for evidence of PEN, especially those forming the diet of the cod. One of these was the rainbow smelt, *Osmerus mordax*. Smelt were examined for both PEN and the pathogenic microsporidian parasite, *Glugea hertwigi*.

This report shows the incidence and geographical distribution of PEN and *Glugea hertwigi* in smelt populations from Massachusetts to the

Canadian Maritimes and indicates that current management practices result in the transfer of PEN and *G. hertwigi* from one area to another via infected smelt.

Materials and Methods

For evidence of PEN, blood samples of 1,412 anadromous *Osmerus mordax* (12.7-27.3 cm total length, TL) were collected at 42 sites from 15 coastal smelt streams from Massachusetts to the Canadian Maritimes (Figure 1) between 3 November 1976 and 17 November 1977: Kittery, Maine (Spruce Creek); Boothbay, Maine (Hodgdon Cove and Boothbay Harbor); Bath, Maine (Whiskeag Creek); Dresden, Maine (Eastern River); Damariscotta, Maine (Damariscotta River); Wiscasset, Maine (Montsweag Creek); Warren, Maine (St. George River); Addison, Maine (Harrington River); Winterport, Maine (Penobscot River); Newington, N.H. (Great Bay); Kingston, Mass. (Jones River); Hingham, Mass. (Weir River); Quarryville, New Brunswick, Canada (Miramichi River); Portapique, Nova Scotia, Canada (Portapique River); and Oxford, Nova Scotia, Canada (Philip River).

In addition, blood samples from 256 landlocked *O. mordax* (7.6-27.0 cm TL) were collected in nine samples from five Maine lakes between 15 January and 5 May 1977: Damariscotta (the lake is approximately 10 mi long, begins at Jefferson, Maine, and empties into the Damariscotta River at Damariscotta); Wyman (Bingham, Maine); South Twin and Millinocket (Millinocket, Maine); and East Musquash (Topsfield, Maine).

For evidence of *G. hertwigi,* 1,692 anadromous *O. mordax* (12.7-27.3 cm TL) were collected in 42 samples from 16 localities (the above 15 plus Casco Bay, Maine) between 3 November 1976 and 17 November 1977. In addition, 254 landlocked *O. mordax* (7.6-27.0 cm TL) were collected in eight samples from the above five lakes between 15 January and 5 May 1977.

Smelt were obtained from our own catches, from those of fishermen and from Massachusetts Division of Marine Fisheries personnel. Depending

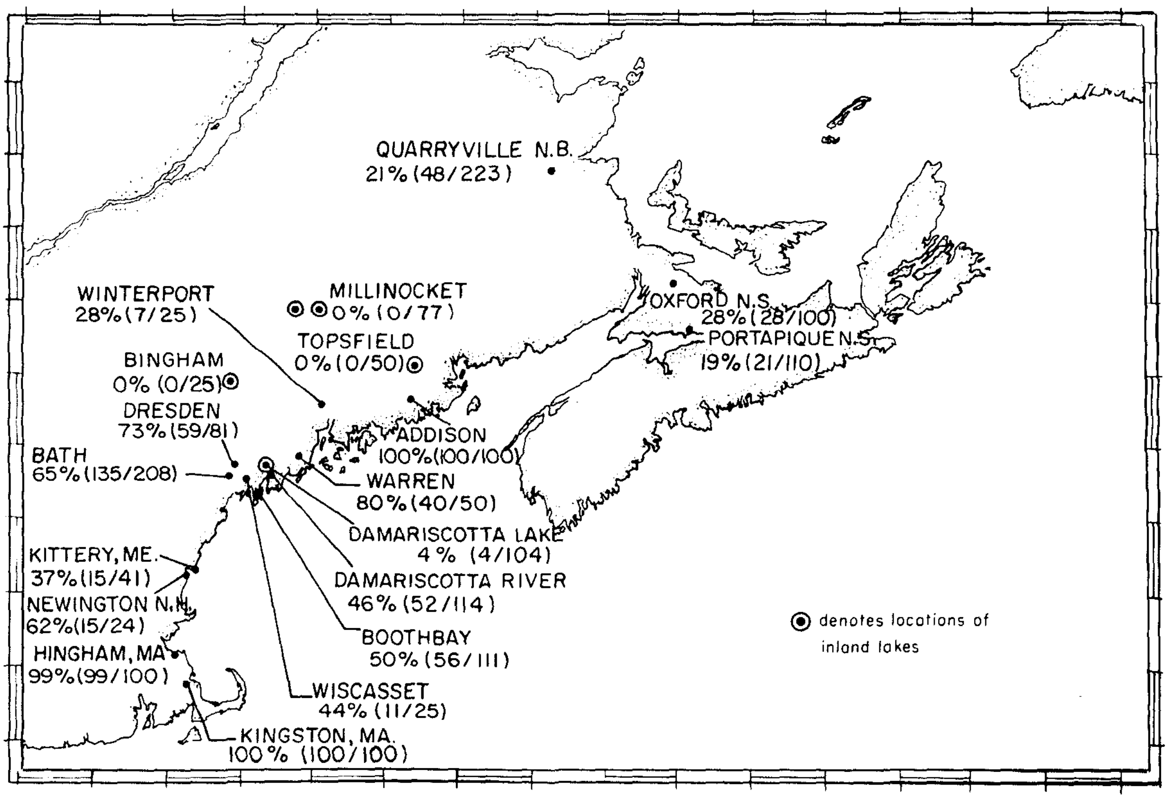

FIGURE 1.—Incidence and distribution of piscine erythrocytic necrosis (PEN) in anadromous rainbow smelt from the Canadian Maritimes to Massachusetts and landlocked rainbow smelt from five Maine lakes (two lakes are located at Millinocket).

upon the location and season, smelt were caught by handline and by dip, cast, bag, and gill nets.

Except for a few instances the same individuals were examined for both PEN and *G. hertwigi*. For evidence of PEN, live smelt were measured for total length, and the caudal peduncle was wiped free of water and mucus with a clean towel and then severed and a smear made from a small drop of blood placed on a clean slide. Smelt were sexed and given a gross external and internal examination for evidence of *G. hertwigi* and other abnormalities. Microscopic examination of unstained and Giemsa-stained spores from cysts was initially made to confirm the presence of *G. hertwigi*. Air-dried blood smears were fixed in absolute methanol for 3 min and stained with diluted Giemsa for 30 min. Smears were examined thoroughly under oil immersion at 1,000× to determine the presence of PEN.

Results

PEN - Anadromous smelt—Of the anadromous smelt sampled, 55.7% (786/1,412) were infected with PEN (Table 1). By light microscopy, PEN lesions of smelt red cells resemble those of EICDV infected Atlantic cod (Figure 2). Infected smelt occurred in every stream sampled (Figure 1). The highest incidences were in Kingston (100/100), Addison (100/100), and Hingham (99/100). Lower incidences were evident from Nova Scotia and New Brunswick than from Maine, New Hampshire, and Massachusetts.

Individual infections were light; of the 786 infected smelt, the highest infection was 8% in a smelt from Kingston. Overall, 87.7% (689/786) of the infected smelt had <1.0% of their red cells infected; 12.3% (97/786) had from 1 to 8%.

The two areas with the highest incidences, Kingston and Addison, also had the highest individual infections and accounted for 87.6% (85/97) of the smelt in this study with 1% or more infected erythrocytes. Kingston had 64/100 smelt with infections >1%; Addison had 21/100. In contrast, Hingham with an incidence of 99/100 had only four smelt with infections >1%. From a total of 1,387 anadromous smelt sexed, 54.3% of the males and 58.3% of the females had PEN.

PEN - Landlocked smelt—Damariscotta Lake was the only lake with PEN infected smelt, 3.8% (4/104). Individual infections were <1%. Because of the design of the fishway at Damariscotta, anadromous smelt are unable to negotiate the fishway leading into the lake. However, there is a possibility that some of the smelt we sampled could have been from a coastal population. Live coastal smelt are used by ice fishermen as bait for salmon and togue, and there is a possibility that unused bait could have been released into the lake with a resultant intermingling of coastal and landlocked smelt.

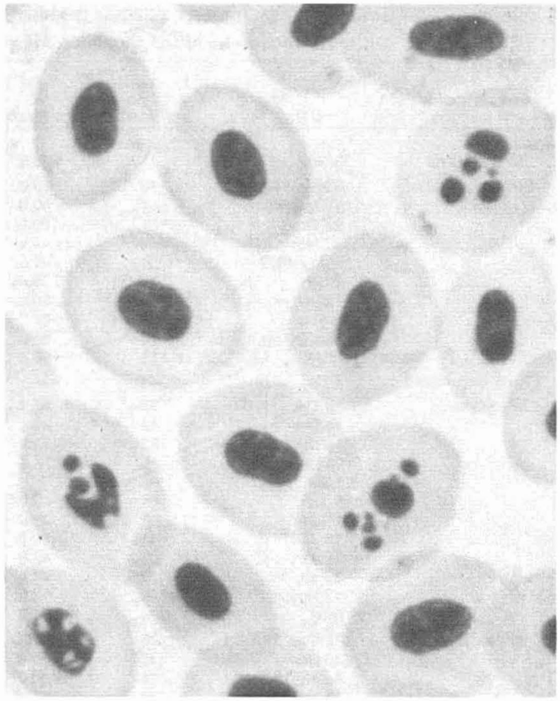

FIGURE 2.—Rainbow smelt erythrocytes with PEN lesions resembling those of PEN (EICDV) infected Atlantic cod. Infected cells show characteristic chromatin condensation and nuclear degeneration. Unlike cod, cytoplasmic virions were not visible by light microscopy in infected smelt erythrocytes.

Glugea hertwigi - Anadromous smelt—Overall, 8.0% (135/1,692) of the anadromous smelt were infected with *G. hertwigi* (Table 1). Infected smelt occurred in all 16 coastal areas sampled (Figure 3). Distinctive white, spherical cysts were found primarily along the intestinal tract but often on other internal organs such as liver and gonads. Cysts varied from pinhead size to 5 mm (Figure 4). Degree of infection varied from one cyst to severe infections where the abdominal cavity was nearly filled. Areas with highest incidences were at Kittery 49% (20/41) and Kingston 28% (28/100). The lowest incidence was at Boothbay with 0.9% (1/

TABLE 1.—Incidence of piscine erythrocytic necrosis (PEN) and *Glugea hertwigi* in anadromous and landlocked rainbow smelt from Massachusetts to the Canadian Maritimes, 3 November 1976-17 November 1977.

Sample source and category	Date	PEN Total no. examined	PEN % incidence	*G. hertwigi* Total no. examined	*G. hertwigi* % incidence	Mean length, SD	Range (cm)
Anadromous:							
[1]Kittery, Maine	3 Nov. 1976	41	36.6	41	48.8	16.6±1.8	13.8–22.0
Boothbay, Maine	6 Nov. 1976	10	50.0	10	—	18.3±1.3	16.0–21.3
	19 Nov.	4	50.0	4	25.0	20.7±1.3	19.7–22.7
	22 Nov.	1	100.0	1	—	18.8±0	18.8
	23 Nov.	65	44.6	65	—	18.9±2.1	15.2–25.0
	5 July 1977	4	50.0	4	—	16.2±1.4	14.2–17.5
	6 July	12	50.0	12	—	18.0±2.3	15.9–22.9
	7 July	5	60.0	5	—	16.9±0.7	15.9–17.9
	5 Aug	3	67.0	3	—	18.1±1.4	16.5–19.2
	25, 26 Aug.	7	86.0	7	—	19.9±1.8	17.8–23.0
[1]Boothbay Total		111	50.4	111	0.9		
Casco Bay, Maine	9 Dec. 1976	—	—	50	10.0	16.8±2.6	12.7–21.6
Bath, Maine	14 Dec. 1976	52	65.4	52	—	20.8±2.4	15.6–26.7
	15 Dec.	46	89.1	46	6.5	21.3±2.8	17.8–27.3
	16 Dec.	23	73.9	51	2.0	20.9±2.2	17.8–26.0
	20 Dec.	25	76.0	114	5.3	20.6±1.9	15.9–24.8
	6 Jan. 1977	—	—	29	3.5	20.5±2.6	15.9–26.7
	6 Jan.	4	50.0	4	75.0	17.0±1.9	14.6–18.5
	11 Jan.	8	37.0	12	8.3	19.8±1.8	15.9–21.6
	13 Jan.	15	60.0	15	6.7	18.3±2.3	13.4–22.1
	17 Jan.	7	42.8	7	14.3	20.4±1.6	18.1–22.9
	3 Feb.	9	44.4	9	22.2	20.3±2.1	17.8–22.9
	17 Nov.	19	15.8	19	15.8	20.3±2.1	16.3–24.3
[1]Bath Total		208	64.9	358	6.1		
Dresden, Maine	27 Dec. 1976	25	64.0	25	4.0	18.8±1.6	16.2–22.3
	30 Dec.	35	82.8	35	8.6	19.5±2.0	15.6–24.4
	4 Jan. 1977	15	60.0	15	6.7	17.8±1.8	15.2–20.7
	11 Jan.	6	83.3	6	16.7	18.5±1.5	16.5–20.3
[1]Dresden Total		81	72.8	81	7.4		
Damariscotta, Maine	17 Jan. 1977	3	—	3	—	18.1±2.7	15.6–20.9
	21 Jan.	70	48.6	70	12.9	19.7±2.8	15.0–26.6
	23 Jan.	27	51.8	27	18.5	20.4±2.0	17.1–24.1
	5 Feb.	1	—	—	—	16.2±0	16.2
	19 Feb.	13	30.8	—	—	19.1±1.3	17.8–22.9
Damariscotta Total		114	45.6	100	14.0		
Wiscasset, Maine	28 Jan. 1977	25	44.0	25	8.0	18.7±1.8	16.0–23.4
Warren, Maine	1 Feb. 1977	25	80.0	25	—	18.0±2.0	14.9–24.8
	8 Feb.	25	80.0	33	3.0	18.6±2.1	14.6–22.2
Warren Total		50	80.0	58	1.7		
[1]Addison, Maine	10 Feb. 1977	100	100.0	100	5.0	20.4±2.3	14.0–26.7
Newington, N.H.	27 Feb. 1977	24	62.5	24	8.3	18.9±2.0	15.2–21.6
Winterport, Maine	8 Mar. 1977	25	28.0	84	2.4	18.5±2.9	14.0–26.7
[1]Kingston, Mass.	15 Apr. 1977	100	100.0	100	28.0	18.5±1.3	12.7–21.9
[1]Hingham, Mass.	15 Apr. 1977	100	99.0	100	10.0	18.9±2.2	12.7–23.8
Quarryville, N.B.	30 Apr. 1977	100	23.0	100	—	19.7±1.8	15.6–25.4
	1 May	70	20.0	97	1.0	19.3±1.5	15.9–23.5
	2 May	53	20.7	53	3.8	19.6±1.6	16.5–24.1
[1]Quarryville Total		223	21.5	250	1.2		
Portapique, N.S.	3 May 1977	110	19.1	110	6.4	20.2±1.9	15.9–26.3
Oxford, N.S.	3 May 1977	100	28.0	100	7.0	16.4±1.5	12.7–20.3
TOTAL		1,412	55.7	1,692	8.0		
Landlocked:							
Jefferson, Maine (Damariscotta Lake)	15 Jan. 1977	7	—	—	—	19.8±2.0	17.8–23.5
	9 Feb.	10	—	10	10.0	21.0±2.9	18.4–26.7
	18 Feb.	12	8.3	12	8.3	18.0±1.4	16.5–20.3
	23 Feb.	18	—	18	—	19.5±2.8	16.5–26.3
	24 Feb.	32	—	32	—	18.9±1.6	15.9–23.5
	10 Mar.	25	12.0	25	8.0	22.1±2.6	17.1–27.0
Jefferson Total		104	3.8	97	4.1		
Bingham, Maine	19 Jan. 1977	25	—	25	56.0	13.9±1.1	11.4–15.9
[2]Millinocket, Maine	12 Feb. 1977	77	—	82	56.1	8.6±1.7	7.6–12.1
[2]Topsfield, Maine	5 May 1977	50	—	50	—	9.1±0.4	8.2–10.2
TOTAL		256	1.6	254	25.2		

[1]Areas that include individual PEN infections of 1% or greater.
[2]Smelt from Millinocket and Topsfield, Maine, were within normal size ranges for populations in these lakes and were sexually mature.

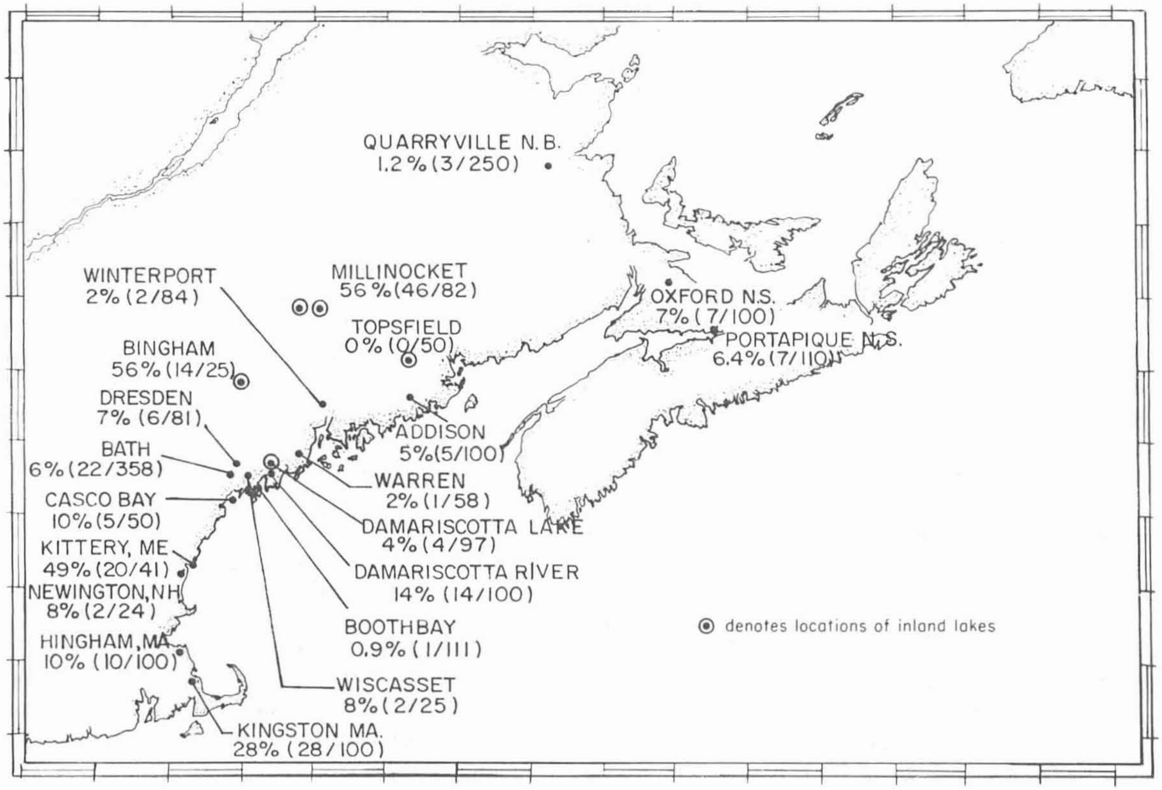

FIGURE 3.—Incidence and distribution of *Glugea hertwigi* in anadromous rainbow smelt from the Canadian Maritimes to Massachusetts and landlocked rainbow smelt from five Maine lakes (two lakes are located at Millinocket).

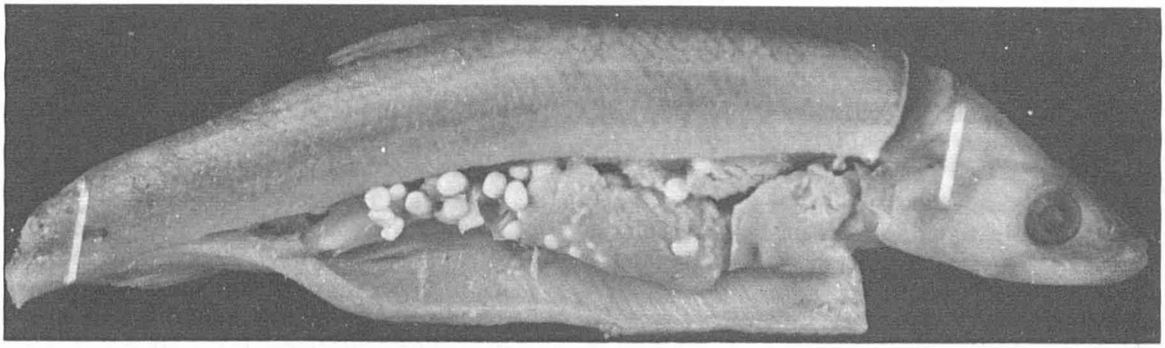

FIGURE 4.—A rainbow smelt infected with *Glugea hertwigi* showing large (up to 5 mm) white spherical cysts associated with this infection.

111). From a total of 1,663 anadromous smelt sexed, 7.8% of the males and 8.4% of the females had *G. hertwigi*.

Glugea hertwigi - *Landlocked smelt*—Infected smelt were found in four of the five lakes sampled. Overall, 25.2% (64/254) were infected. Heavy incidences occurred at Wyman, South Twin, and Millinocket Lakes—each lake had 56% of the sampled smelt infected, (14/25), (5/9), and (41/73), respectively. Infections varied from one cyst to severe infections.

Discussion

PEN and *G. hertwigi* infected smelt will undoubtedly be found in other areas, both within and beyond the geographical range sampled in this study. Management practices involving anadromous alewives have inadvertently contributed to the spread of PEN within the State of Maine (Sherburne 1977). Haley (1954b) reported similar circumstances for *G. hertwigi* infected freshwater smelt in New Hampshire. *Glugea hertwigi* was found in rainbow smelt from Lake Winnisquam, N.H., as well as in other localities where smelt populations were established from the Winnisquam stock. Smelt that were being transported by the Massachusetts Division of Marine Fisheries from Hingham to Cape Cod, Mass., to initiate new runs during the time of this study had incidences of 99% (99/100) PEN and 10% (10/100) *G. hertwigi*.

Because of the known pathogenicity of *G. hertwigi* (Haley 1954a, b; Chen and Power 1972; Nepszy et al. 1978), localities with relatively high incidences may warrant further investigation. At the time of the decline of the smelt population in the Great Bay region of New Hampshire in the 1950's, 23.3% (308/1,323) of the smelt examined had *G. hertwigi* (Haley 1954a). Although our samples were considerably smaller, 33.8% (22/65) of the smelt sampled from Kittery and Great Bay were infected with *G. hertwigi*. The high incidences of *G. hertwigi* (56%) at Wyman, South Twin, and Millinocket Lakes were unexpected. However, Chen and Power (1972) reported that of 1,691 smelt sampled from Lake Erie 62.7% were infected with *G. hertwigi*. They reported that apart from actual mortality the real significance of *G. hertwigi* infection lies in its effect on smelt fecundity. In females, parasitic cysts replaced ovarian tissue, causing a serious reduction in the number of maturing eggs.

There was no apparent relations between *G. hertwigi* and PEN in the populations sampled in this study. Although smelt at Kingston had high incidences of both PEN and *G. hertwigi* (100/100 and 28/100, respectively) other populations with high incidences of PEN did not have high incidences of *G. hertwigi*, i.e., Hingham had 99/100 with PEN, 10/100 with *G. hertwigi*; Addison had 100/100 with PEN, 5/100 with *G. hertwigi*; Warren had 40/50 with PEN, only 1/58 with *G. hertwigi*.

There was no apparent relation between *G. hertwigi* and PEN infections in individuals. Of 135 andaromous smelt with *G. hertwigi*, 71 (52.6%) likewise had PEN.

Chen and Power (1972) reported seasonal fluctuations in *G. hertwigi* infection from Lakes Ontario and Erie, with the highest incidence during the winter, when smelt were undergoing the most active phase of gonadal maturation. Most of our sampling was confined to winter; therefore, we have no evidence of seasonal fluctuations of PEN and *G. hertwigi* infections in this study. However, since different areas were sampled at similar times and all fish sampled were adults, the data obtained should afford a representative comparison between areas.

This study has determined that PEN and *G. hertwigi* are widely distributed in rainbow smelt populations along the North Atlantic coast from Massachusetts to the Canadian Maritimes, that the incidence of PEN in each population is high but the intensity of individual infections is low, and that higher incidences of *G. hertwigi* occur in inland lakes of Maine than in coastal populations. These findings differ from previous studies on the Atlantic cod and Atlantic herring where lower incidences of PEN have been evident but individuals have had nearly every red cell infected (Walker and Sherburne 1977; Sherburne 1973).

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A SIMPLE METHOD TO OBTAIN SERUM FROM SMALL FISH

It is desirable to obtain blood serum information from small fish due to their extensive use in pollutant and disease studies (Snieszko et al. 1969; Snieszko 1974; Mulcahy 1975). It is well known that gross observations cannot detect subtle changes in blood chemistry caused by environmental factors such as stress, diet, or inflammation (Mulcahy 1975) and some pesticides (Walker 1963).

Techniques to obtain fish blood for study have been described in reviews by Hesser (1960) and Blaxhall (1972). Cardiac and venous puncture are the most commonly used techniques for fish >150 mm, while severance of the caudal peduncle and insertion of a capillary tube to draw blood is usually employed for smaller fish. Fish <60 mm present problems because the quantity of blood obtainable is small (generally <0.2 ml), coagulation time is quick, and tissue fragments or clots can clog collecting tubes, causing loss of serum in the transfer from one container (or collecting tube) to another for centrifugation. In most cases anticoagulants are used to eliminate some of these problems.

Sodium oxalate, heparin, or dipotassium ethylenediaminetetraacetate (EDTA) are the most commonly used anticoagulants. Unfortunately, oxalate and EDTA anticoagulants can interfere with serum ion determinations, such as calcium, and produce misleading data (Tietz 1976). When many blood serum components are to be measured, especially on instrumentation such as an amino acid auto analyzer, a quantity of serum (at least 0.5 ml and preferably free of anticoagulant) must be obtained for the numerous tests these analyzers can do. Heparinized tubes, excellent for single serum component tests, are limited because the volume of serum they can obtain is generally not enough for use with sophisticated instrumentation. This note describes a simple method to obtain pooled serum samples, without anticoagulants, from fish <60 mm when heparinized tubes are not practical.

Materials and Methods

Small fish <60 mm fork length should be anesthetized, if desired, and blotted to remove excess water on the fish's body. A dry Kimwipe[1] is wrapped around the fish, covering the vent to prevent contamination of the sample, leaving approximately 2.5 cm of the tail exposed (Figure 1B). A small portion of the Kimwipe is allowed to overlap the fish's head. The caudal peduncle is severed with sharp scissors, leaving a slight point at the caudal region (Figure 1C). The fish is rapidly in-

[1]Reference to trade names does not imply endorsement by the University of Southern Mississippi or by the National Marine Fisheries Service, NOAA.