

## LYMPHOCYSTIS DISEASE IN THE WINTER FLOUNDER, *Pseudopleuronectes americanus*

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**Abstract:** Nodular lesions on the fins of winter flounder, *Pseudopleuronectes americanus*, from Casco Bay, Maine, were identified as lymphocystis disease. Hypertrophied, encapsulated connective tissue cells contained cytoplasmic inclusions composed of icosahedral virus particles. The winter flounder is a new host for lymphocystis disease and is the second flatfish in the western North Atlantic to have the disease.

### INTRODUCTION

Lymphocystis disease is probably one of the most conspicuous viral diseases of marine and freshwater fishes by virtue of the characteristic macroscopic lesions produced. The disease was first described in European plaice<sup>2</sup> and is characterized by the presence of multiple nodules in the dermis of the skin, particularly over the fins. Weissenberg<sup>6</sup> established the identity of lymphocystis nodules in European perch as hypertrophied connective tissue cells which enlarge in response to the presence of intracytoplasmic virus. Lymphocystis disease has been noted in many marine, estuarine and freshwater fishes.<sup>3</sup> In flatfish of the eastern Atlantic, the disease is limited to the plaice, *Pleuronectes flesus* and *P. platessa*, and the dab, *P. limanda*. In the western Atlantic, the disease has been found in American plaice, *Hippoglossoides platessoides*,<sup>5</sup> and southern flounder, *Paralichthys dentatus*.<sup>4</sup> This report documents another flatfish, the winter flounder, *Pseudopleuronectes americanus*, as a host for lymphocystis disease.

### MATERIALS AND METHODS

Winter flounder were obtained from Casco Bay, Maine. The first fish examined histologically was obtained by ot-

ter trawl on June 4, 1974, and together with approximately 40 other flounder was placed in a 2000 l seawater holding tank (flow-through) for 2 weeks. Several flounder, including the one subsequently examined histologically, were then placed in separate 110 l aquaria containing artificial seawater (recirculating). Ambient temperature at the time of capture was 10.4 C; aquaria water temperature was maintained at approximately 15 C. Lesions were noted after 4 months' captivity and the fish was sacrificed for histopathologic examination.

The second fish examined histologically was obtained by otter trawl on November 18, 1974, when the ambient water temperature was 7.4 C. Together with approximately 40 other flounder, the fish was placed in a 2000 l seawater holding tank. Lesions were noted and the fish was sacrificed after 4 weeks in the holding tank. No lesions were noted on either of the fish before confinement; however, early lesions easily may have been overlooked.

A sample of 64 winter flounder from Casco Bay was obtained by otter trawl on March 19, 1975. The fish were examined carefully in the field and nodules consistent with lymphocystis disease were noted. Six affected fish, ranging in size from 312-325 mm (SL), were selected for histopathologic examination.

Tissues were fixed in 10% seawater-formalin. Subsequent to fixation, fin rays were decalcified in RDO (DuPage Kinetic Laboratories\*) for 2-4 h (or when soft enough to cut satisfactorily). Tissues were processed routinely, cut at 6  $\mu$ m, and stained with hematoxylin and eosin, a Feulgen stain,<sup>1</sup> Mallory trichrome, and periodic acid Schiff (PAS).

#### RESULTS AND DISCUSSION

Histopathologic examination of both winter flounders held in laboratory aquaria confirmed the gross diagnosis of lymphocystis disease. Grossly, fin lesions of laboratory-held flounders consisted of either single nodules (Fig. 1) or groups of nodules in a raspberry-like cluster. Of the 64 winter flounder captured in March, 1975 in Casco Bay, 44 (68.7%) had nodules characteristic of lymphocystis disease. In contrast to the laboratory-held fish, these fish usually had single nodules on either the anal or dorsal fin. It is not known whether the laboratory-held fish contracted lymphocystis while confined or whether the disease merely progressed to a more recognizable state in the laboratory. Since trawled fish had single nodules and the laboratory-held fish had multiple nodules, the disease in the first two fish examined may have been present at the time of capture, only to progress within the laboratory aquaria.

Microscopically, all the lymphocystis lesions were similar. Nodules grossly appearing to be single cells often contained more than one lymphocystis cell (Fig. 2). Lymphocystis cells were ovoid to circular and varied in size from 0.22 x 0.37 mm to 1.48 x 1.81 mm. The granular cytoplasm of the cells contained abundant peripheral Feulgen-positive inclusions. Inclusions were ovoid, circular or "C" shaped, and when examined by electron microscopy contained abundant icosahedral virus. Nuclei were not discernible in

the cells observed; a central, faintly eosinophilic area containing basophilic granules suggested nuclear karyorrhexis. The basophilic granules, however, were not Feulgen-positive. Each cell was surrounded by three distinct layers readily resolved by staining with PAS. The outermost layer consisted of collagenous tissue and the middle and inner layers constituted the hyaline capsule which was visible with hematoxylin and eosin. The hyaline capsule consisted of an outer band of strongly PAS-positive material and an inner band which stained less intensely.

Host response to lymphocystis in the winter flounder appears to be minimal. Most hypertrophied cells were surrounded by a zone of hemorrhage (Fig. 3). The thickness of the red blood cell layer varied considerably and was located between the hyaline capsule and the collagenous layer. A focal inflammatory response, consisting entirely of mononuclear cells, sometimes was present. The epidermis covering larger nodules was hyperplastic (Fig. 4) and the cells were highly vacuolated. Numerous eosinophilic granule cells (EGC) were present in the hyperplastic epidermis. Presently, it is not known whether the EGC is an inflammatory cell.

Recently, winter flounder with apparent lymphocystis nodules were obtained from the New York Bight (Murchelano, unpublished). In one fish, nodules were not limited to the fins but were present in the dermis of the pigmented and non-pigmented sides. The course of the disease in winter flounder is unknown. As is the case with other marine and non-marine species, the major significance of the disease lies in the unsightly appearance of affected fish. The reason for the unusually high prevalence of the disease in Casco Bay winter flounder is not known. The seasonal occurrence of lymphocystis disease in this economically important fish warrants further study.

\*Reference to trade names does not imply endorsement of commercial products by the National Marine Fisheries Service.

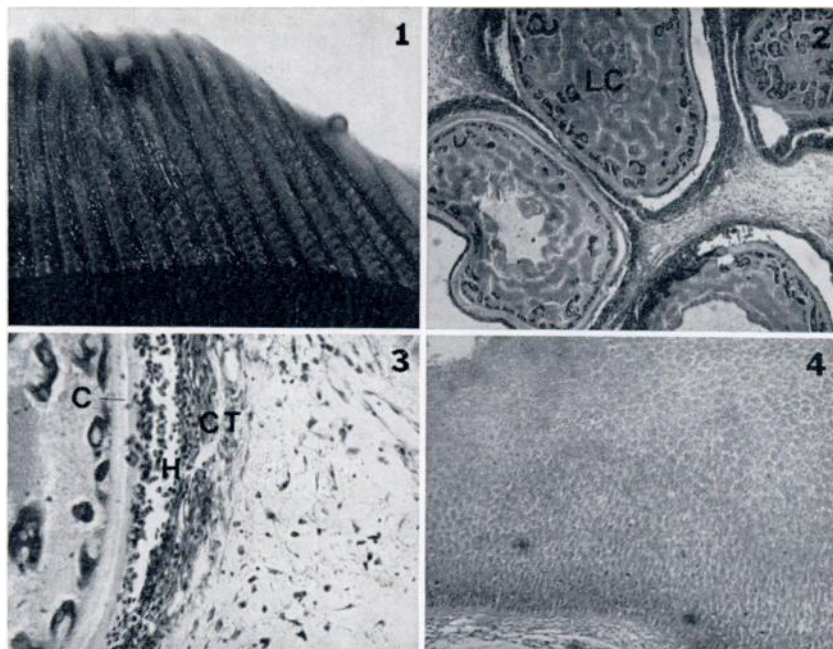


FIGURE 1. Dorsal fin of winter flounder with two lymphocystis nodules (collected in March, 1975).

FIGURE 2. Group of lymphocystis cells (LC) from nodule of laboratory-held winter flounder. Hematoxylin and eosin. x 100.

FIGURE 3. Margin of lymphocystis cell from laboratory-held winter flounder showing hyaline capsule (C), hemorrhagic zone (H), and adjacent connective tissue (CT). Hematoxylin and eosin. x 400.

FIGURE 4. Hyperplastic epidermis from laboratory-held winter flounder with lymphocystis nodules. Hematoxylin and eosin. x 100.

#### LITERATURE CITED

1. FARLEY, C. A. 1969. Probable neoplastic disease of the hematopoietic system in oysters, *Crassostrea virginica* and *Crassostrea gigas*. Natl. Cancer Inst. Monogr. 31: 541-555.
2. LOWE, J. 1874. Fauna and flora of Norfolk. Part IV. Trans. Norfolk Norwich Nat. Soc., Fishes pp. 21-56.
3. NIGRELLI, R. F. and G. D. RUGGIERI. 1965. Studies on virus diseases of fishes. Spontaneous and experimentally induced cellular hypertrophy (lymphocystis disease) in fishes of the New York Aquarium, with a report of new cases and an annotated bibliography (1874-1965). Zoologica 50: 83-96.
4. STICKNEY, R. R. and D. B. WHITE. 1974. Lymphocystis in tank-cultured flounder. Aquaculture 4: 307-308.
5. TEMPLEMAN, W. 1965. Lymphocystis disease in American plaice of the eastern Grand Bank. J. Fish. Res. Board Can. 22: 1345-1356.
6. WEISSENBERG, R. 1914. Über infektiöse Zellhypertrophie bei Fischen (Lymphocystiserkrankung). Sitzungsber. Kgl. Preuss. Akad. Wiss., Physik. Mathem. Kl. 30: 792-804.

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