LYMPHOCYSTIS DISEASE IN *CYNOSCION NOTHUS*, *CYNOSCION REGALIS* AND *STELLIFER LANCEOLATUS* FROM GEORGIA ESTUARIES[1]

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ABSTRACT

Lymphocystis disease is a viral disease of freshwater and marine fish. It was found in three species of Sciaenids along the Georgia coast. It was found in six of the eight estuaries sampled and appeared in three forms: cutaneous, visceral and ocular.

The cutaneous and visceral lesions were typical. An ocular site of infection, however, had not been previously described. The lymphocystis cells were found in the choroid coat of the eye near blood vessels and transport of the virus to the infection site via the blood was suspected.

The disease appeared in the fall and winter months in *Cynoscion regalis* and *Stellifer lanceolatus*. No conclusions could be drawn from the data on the seasonal appearance of the disease in *Dynoscion nothus*. Water temperature changes appear to be associated with the appearance and disappearance of the disease but additional factors need to be studied before a causal relationship can be established. There was no significant difference in the proportion of infected *S. lanceolatus* from lower salinity and higher salinity sample areas.

INTRODUCTION

Lymphocystis is a viral disease found in marine and freshwater fish. The disease was originally thought to be caused by a protozoan or to be an invertebrate animal's eggs deposited under the fish's scales (Wellings, 1970). This was discounted by Weissenberg (1965), who proposed that a virus was responsible. Recent studies have established that a DNA, polyhedral virus is found in the cytoplasm of infected cells (Walker and Weissenberg, 1965) and that homogenates of these cells will cause the disease 10 to 12 days after injection in susceptible fish (Nigrelli and Ruggieri, 1965). Tissue culture monolayers have been used to demonstrate the development of characteristic lymphocystis cells *in vitro* (Wolf, *et al.*, 1966).

Lymphocystis has wide distribution in North Atlantic waters (Nigrelli and Rugierri, 1965) but was not specifically reported in Georgia marine fish until 1970 (Smith, 1970). It has been identified in many species of fish including members of the family Sciaenidae (Christmas and Howse, 1970), however the findings reported here are thought to be the first records in three Sciaenids common along the Georgia coast.

Reviews of the literature of this disease have been published by Weissenberg (1965), Nigrelli and Ruggieri (1965, and Wellings (1970).

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MATERIALS AND METHODS

The survey began 1 October 1969, and continued until 31 December 1970. The fish were collected with hook and line, cast nets, seines and by the use of ten foot and twenty foot Otter trawls. Some specimens were contributed by colleagues at the Marine Institute. Most collections were made in the Doboy estuary. Seven other Georgia estuaries (Figure 1) were sampled in October and November of 1970.

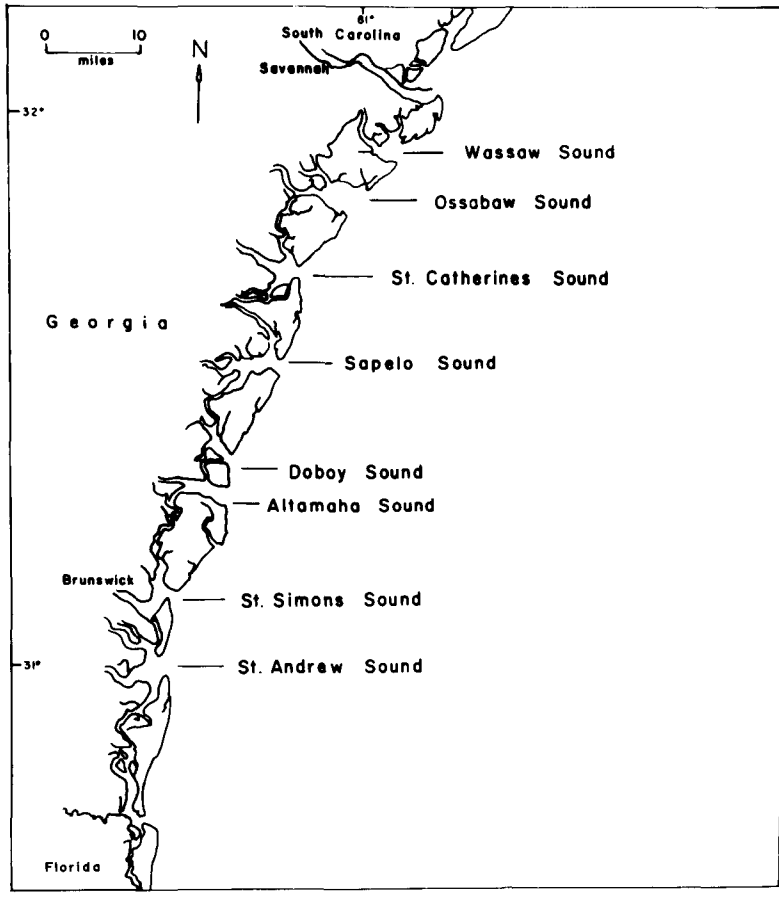

Figure 1. Map of the Georgia coast showing the sounds sampled during the survey.

Fish were fixed in formalin diluted approximately 1:9 with sea water for transport to the laboratory. A few specimens were received iced or frozen. In the laboratory all specimens were transferred to buffered 10% formalin for storage. Specimens were examined within three months of collection.

A five inch diameter illuminated magnifier was used to assist the post mortem examination. Each fish was examined for gross evidence of pathologic conditions. Tissues in which pathological changes were observed or suspected were removed to vials of fresh 10% buffered formalin until needed for further processing.

Tissues from suspected cases of lymphocystis disease were embedded in Paraplast[1] using graded ethanols and xylene for dehydration and clearing. Sections were cut at 8 μ thickness and stained with hematoxylin and eosin-phloxine for routine examination (Luna, 1968). Acridine orange fluorescent technique was used to confirm the presence of DNA in the cytoplasmic inclusions of the lymphocystis infected cells (Luna, 1968).

RESULTS

During the survey 11,852 fish were caught and examined. Seventy-seven percent (9190/11,852) of the fish were from the Doboy estuary. There were 62 species of fish represented in the collections but only three species, all in the family Scianidae, were found to have lymphocystis disease. All data that follows were based only on the catch of these three species.

The silver seatrout (A.F.S., 1970), *Cynoscion nothus* (Holbrook), was caught only during April, May and June of the survey (Table 1) when only the Doboy Sound estuary was being sampled. Lymphocystis was identified in this fish in the May sample (Table 1). The incidence of the disease was 3/135 silver seatrout or approximately 22/1000 fish.

Specimens of weakfish (A.F.S., 1970), *Cynoscion regalis* (Block and Sneider), were collected in each month of the year but were lacking in the December 1970 sample when no trawls were made. Lymphocystis appeared in September, October and November samples. It was found in the Doboy and Altamaha estuaries with an incidence of approximately 10/1000 fish (8/766) in this species.

Star drum (A.F.S., 1970), *Stellifer lanceolatus* (Holbrook), were not collected in the June sample nor in the one collection made in December 1970. All other months had samples of this fish. The star drum had lymphocystis lesions in September, October, November and January in this survey. There were 32 cases of the disease in the 4,763 specimens examined for an approximate incidence of 7/1000 fish in this species.

[1]Fisher Scientific Co., Atlanta, Georgia.

Table 1. Temperature, salinity and cases of lymphocystis disease for each month of the survey.

	1969			1970											
Month	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Temperature[1]	22	18	11	6	11	16	20	24	29	31	29	29	20	15	10[3]
Salinity[2]	22	24	23	23	20	20	15	22	23	27	26	25	29	27	25[3]
C. nothus[4]	-	-	-	-	-	-	+	3	+	-	-	-	-	-	-
C. regalis[4]	1	2	+	+	+	+	+	+	+	+	+	3	+	2	-
S. lanceolatus[4]	9	3	+	1	+	+	+	+	-	+	+	3	2	14	-

[1]°C

[2]°/oo

[3]estimated

[4]a - indicates none of this species were caught, a + indicates fish were caught but did not show lymphocystis lesions, and numerals indicate the number of cases of lymphocystis.

Table 2. Catch and incidence of lymphocystis disease for each species of fish in each estuary sampled.

	Wassaw Sound	Ossabaw Sound	Saint Catherines Sound	Sapelo Sound	Doboy Sound	Altamaha Sound	Saint Simons Sound	Saint Andrews Sound	Sub-Total[1]	Sub-Total[2]
C. nothus	-	-	-	-	3/135	-	-	-	-	3/135
C. regalis	10	13	11	1	7/701	1/7	14	9	2/44	6/722
S. lanceolatus	1/22	5/324	230	2/160	16/3438	3/123	5/155	309	12/794	20/3969

[1]Total of high salinity stations.
[2]Total of low salinity stations.

Denominator - number of fish caught
Numerator - cases of lymphocystis disease

Table 3. Area of collection, standard length and lesions in each case of lymphocystis.

Case Number	Estuary	Standard Length (mm)	Lymphocystis Disease Lesions
		Cynoscion nothus	
601	Doboy	71	Trunk and operculum
602	"	72	Dorsal fin (3)
603	"	89	Caudal fin, trunk and head
		Cynoscion regalis	
57	Doboy	73	Dorsal fin and trunk
65	"	133	Causing exophthalmia (2,3)
112	"	90	Trunk
1257	"	56	Caudal peduncle (3)
1259	"	88	Trunk and fins
1263	"	72	Trunk (3)
2169	"	90	Trunk, fins and head
2230	Altamaha	120	Peritoneum posterior to stomach (2,3)
		Stellifer lanceolatus	
3	Doboy	65	Trunk, fins and head (1,2,3)
14	"	70	Entire body surface
16	"	40	Anal and dorsal fins
30	"	36	Head
45	"	50	Mesentary (2,3)
46	"	45	Mesentary
59	"	47	Head and fins
60	"	45	Trunk and fins
61	"	44	Causing exophthalmia (3)
90	Doboy	44	Head
92	"	43	Head (2)
119	"	52	Dorsal fin
216	"	50	Peritoneum (1,2,3)
1723	"	43	Pelvic fins (3)
1724	"	46	Fins (3)
1797	"	--	Caudal fin
2013	Wassaw	68	Anal fin
2051	Ossabaw	45	Peritoneum along stomach (2,3)
2052	"	50	Dorsal fin (3)
2053	"	35	Right pectoral fin (3)
2067	"	80	Trunk and fins
2077	"	55	Pectoral fins
2167	Sapelo	60	Trunk and caudal fin
2168	"	45	Dorsal fin
2205	Altamaha	60	Dorsal fin (2,3)
2268	"	50	Trunk and fins
2274	"	70	Dorsal fin (1,2,3)
2297	St. Simons	40	Anal fin (3)
2319	"	55	Dorsal and caudal fins (3)
2320	"	50	Head and fins (3)

Table 3. (continued)

Case Number	Estuary	Standard Length (mm)	Lymphocystis Disease Lesions
2321	"	55	Trunk, fins and head (2,3)
2322	"	95	Trunk and fins

Numbers in parenthesis indicate lymphocystis cell types:
1 — early
2 — developed
3 — degenerating

No attempt was made to determine the age of any of the fish, but the standard length (length in mm from tip of snout to the posterior extent of the caudal peduncle) of the preserved fish was recorded for each fish having lumphocystis (Table 3). Hildebrand and Cable (1934) estimated *C. nothus* to be about 75 mm in length at 7 to 8 months of age, *C. regalis* o be 135 to 250 mm in length at one year, and *S. lanceolatus* to be 100 to 125 mm at one year. Larger fish were seldom collected in this survey.

The disease was found in all but Saint Catherines Sound and Saint Andrew Sound collections (Tables 2 and 3). If the *S. lanceolatus* samples were grouped into those fish which were caught at stations near the mouth of the sounds (higher salinity, Table 2) and those which were caught further in the sounds and rivers (lower salinity) there was no significant difference ($z = 1.01$) even at the 5% level in the proportions of diseased fish. The number of *C. nothus* and *C. regalis* was insufficient for statistical analysis (Ferguson, 1966, p. 177).

The lesions seen in those fish which were found to have lymphocystis were variable sized masses containing white nodules. Smaller masses were composed of one or two nodules and the adjacent tissue. In other cases much of the fish was covered by the masses and many nodules could be seen (Figure 2). The histological appearance of the lesions in all cases of lymphocystis was similar. Characteristic cells between 10 and 800 μ in diameter were surrounded by a hyaline membrane from 2 to 10 μ thick.

Lymphocystis was found in one of three forms in these fish: cutaneous, visceral or ocular. Only one form was found in any one specimen. From Table 3 it can be determined that there were thirty-six cases of cutaneous, five cases of visceral and two cases of ocular lymphocystis disease found in this survey.

The gross appearance of the cutaneous lymphocystis lesions was characteristic. "The lesions have a granular appearance due to numerous white, spherical or oval, tremendously enlarged connective tissue cells, lying singly or in groups in lymph spaces below the stratified epithelium" (Nigrelli and Ruggieri, 1965).

The visceral lesions were small, white, discrete nodular masses in the peritoneum adjacent to the organs as listed in Table 3. Lymphocystis cells were not found in the parenchyma of any of the viscera in this survey (Figure 3).

The ocular lymphocystis was detected by the exophthalmia which the masses caused (Figure 4). Only one eye was affected in each of the two fish having this lesion. The internal structure of the eye appeared normal when viewed through the cornea but obvious thickening and distortion of the intraorbital tissues could be seen when the eye was enucleated.

The choroid coat, normally a thin layer of vascular connective tissue, was filled with a mass of lymphocystis cells that was 3 to 4 mm thick in some areas. The cartilage of the sclera was destroyed for approximately one-fourth of the circumference of the globe in the deep intraorbital area. The more flexible

retrobulbar muscle layers remained intact over the mass. There was a marked infiltration of lymphocytes in the area where the scleral cartilage was penetrated. Pockets of lymphocytes were found between the lymphocystis cells and around the blood vessels in the lesion.

The retina appeared normal in its peripheral areas where there was no lymphocystis. The area around the optic papilla, where the lymphocystis was thickest, had obvious retinopathy. The inner layers of the retina appeared normal but the bacillary layer (Bloom and Fawcett, 1968) was disrupted and filled with melanotic granules. The inner segments of the bacillary layer became more acidophiolc in the area of the lesion and then disappeared where the bacillary layer was disrupted. The nuclei became karyolytic where the inner segments of the rod and cone cells were absent. The inner plexiform layer contained many more nuclei in the area adjacent to the optic papilla.

It was convenient to divide all lymphocystis cells into three overlapping categories. Most lesions contained a mixture of the three cell types (Table 3). The larger, type 2 and 3, cells were seen most often in this survey.

Type one cells were the smaller and presumably the immature stages of the infection. The nucleus was pyknotic and adjacent to the cell membrane in most cases. The cytoplasm was more deeply staining than in later cell types. Often there was a perinuclear light area and occasionally small dark-staining granules in the cytoplasm.

Type two cells were the intermediate sized cells (100 to 500 μ in diameter) containing distinct chromatic networks developed within the cytoplasm. The nucleus often contained a large nucleolus and considerable nuclear sap. The nucleus was beginning to lyse in some cases. This was similar to Alexandrowicz's (1951) stage of virus "transformation".

Type three cells were apparently degenerating. They were the largest of the cells in these sections and had a light blue, granular cytoplasm. There were usually peripheral spaces containing a reticular network of fine granules. The nucleus was usually karyolytic and often only a space remaining in the cytoplasm marked its previous location. Alexandrowicz (1951) described similar cells as those which were infected but had failed to develop the virus particles to completion.

DISCUSSION

The morphology of the lymphocystis cells in these three species of fish is similar to that described by Alexandrowicz (1951) and by Nigrelli and Ruggieri (1965). Based on the descriptions of Alexandrowicz (1951) and Walker and Weissenberg (1965) it may be surmised that what are here called type one cells are recently infected portions of the mass. Type two and three cells are later developmental stages and may indicated those cells where active virus production is taking place (two) and those cells where the production process was disrupted (three). The later two stages would be most easily detected by the gross examination procedure used in this survey because of the larger size of the lymphocystis cells.

The anatomical sites of infection of the lymphocystis virus in these fish differs from previously described cases in two ways: (1) there were no cases of the disease found in the gills of these fish and (2) the eye was infected. It is possible that there were cases of the disease in the gills of these fish but that none happened to be collected or detected during this survey.

The ocular infections in *C. regalis* and *S. lanceolatus* may be unique conditions, however. The location of the lymphocystis cells deep within the orbit and adjacent to the blood vessels of the choroid coat indicates the virus probably was transported to the site of infection via the blood. It appears the ocular

lesions were primary sites of infection as there were no other lymphocystis lesions found in these particular fish.

Lymphocystis disease is seasonal in *C. regalis* and *S. lanceolatus* along the Georgia coast. The disease appears as the water temperature begins to drop in the fall of the year and is present throughout the winter months. As the water temperature rises in the spring gross evidence of the disease disappears from the fish populations. Because *C. nothus* is collected for only a short time in the inshore waters of Georgia no conclusions could be drawn on the seasonality of lymphocystis in this fish.

The seasonal appearance of the disease seems to be linked to the water temperature. But several environmental parameters not measured during this survey may prove to be the actual controlling factor or factors. Some of these are dissolved oxygen levels, suspended organic matter, parasite loads and seasonal changes in the schooling tendencies of the fish.

Lymphocystis is found in both freshwater and marine fish. It might be suspected that there would be a greater proportion of infected fish in either the low salinity or high salinity areas of the estuary if either area was more favorable for this virus. However, no significant difference in infection rate was found in *S. lanceolatus* from low salinity and high salinity areas. The possibility that infection takes place in one area of the estuary and the fish subsequently distribute evenly throughout the sample area remains.

Lymphocystis disease seems an important disease for study in our estuarine areas because it is found in commercially important species of fish (e.g., weakfish) and may serve to demonstrate many of the basic principles of disease development in large semiclosed systems such as the estuary.

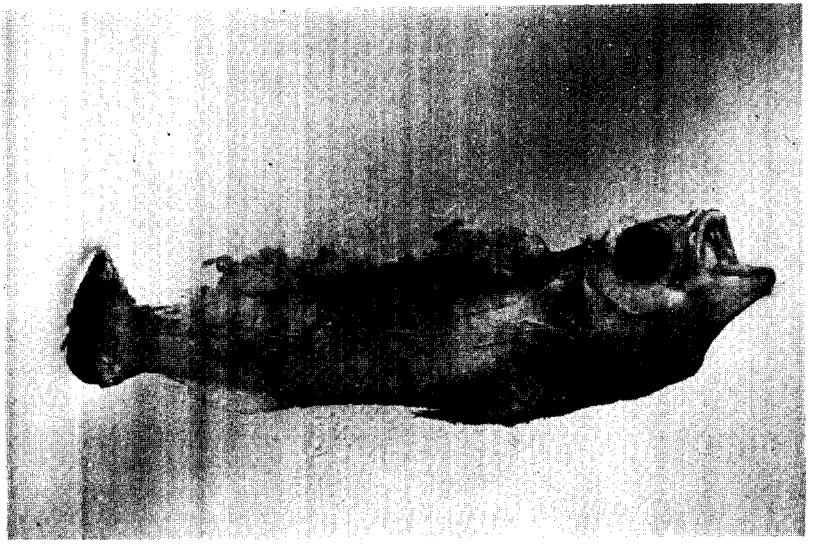

Figure 2. *Cynoscion regalis* with extensive cutaneous lymphocystis lesions (Case no. 2169, 90 mm standard length).

Figure 3. Lymphocystis lesion in peritoneum adjacent to the intestine showing glands (G), hyaline membrane around lymphocystis cells (H) and cytoplasmic DNA inclusions (I) (Case no. 45, Acridine Orange fluorescence, Original magnification X160).

Figure 4. Ocular lymphocystis showing retina (R), lymphocystis cells in choroid coat (L) and cartilage of the sclera (C) (Case no. 65, Hematoxylin and eosin-phloxine, Original magnification X160).

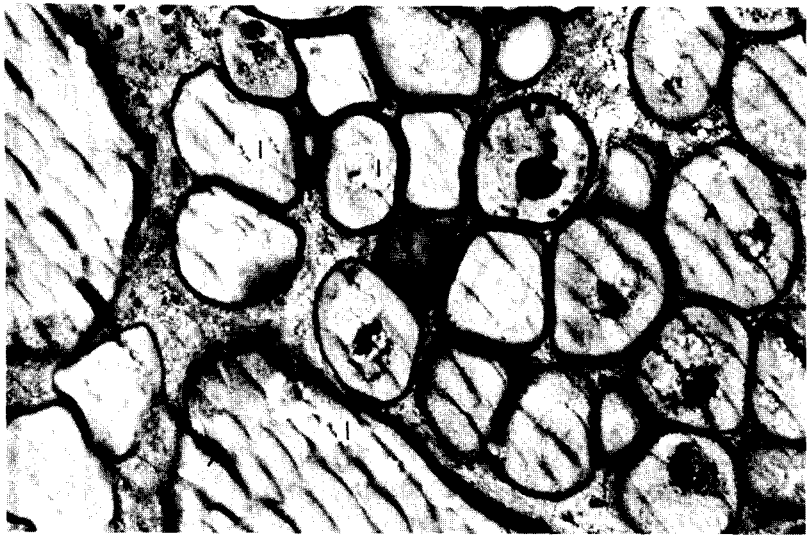

Figure 5. Lymphocystis cells from peritoneum showing nucleus (N), cytoplasmic DNA positive inclusions (I) and sectioning artifacts frequently seen in lymphocystis cells (A) (Case no. 2051, Acridine Orange fluorescence, Original mangification X160).

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A STUDY ON THE BIOCHEMISTRY OF ALARM SUBSTANCES IN FISH

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ABSTRACT

Two cyprinid fishes, *Clinostomus funduloides* and *Notropis cornutus* were tested with naturally occurring substances including some well-known biogenic amines. Behavioral responses to histamine were similar to those observed in previous tests with natural alarm substance extracts. A response threshold was obtained at 0.01 ppm. Spectrophotofluorometric emission spectra also indicated that the natural alarm substance known to exist in many species of fish may be a ringed or double ringed compound.

INTRODUCTION

Alarm substances and fright reactions in fishes have been studied by several workers (Von Frisch, 1941; Schutz, 1956; Pfeiffer, 1960, 1962, 1963, 1966, 1967; Reed, 1969). The alarm substance has been shown to be present in the skin of several families and at least three orders of fishes (Pfeiffer, 1967; Reed, 1969). Pfeiffer (1960) suggested that the alarm substance is contained in club cells present in the epidermis of fish skin and that this substance can only be released by damage to the skin. The substance is water soluble and is perceived by olfaction (Von Frisch, 1941b). Purrman (1947) stated that ichthyopterin, which is contained in the skin, was identical with the alarm substance but Schutz (1956) found that synthetic ichthyopterin did not produce a fright reaction although it had certain chemical properties similar to those of the natural alarm substances.

Huttel (1941) suggested that the alarm substance was a purine or pterin-like compound and showed that the substance is not volatile, although extremely soluble in water.

To provide additional information concerning the chemical nature of the alarm substance in fishes, a study was initiated using several qualitative and quantitative biochemical techniques.