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## SEASONAL PREVALENCE OF SKIN TUMORS FROM WALLEYE (*STIZOSTEDION VITREUM*) FROM ONEIDA LAKE, NEW YORK

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**ABSTRACT:** A seasonal survey of skin tumor prevalence in walleyes (*Stizostedion vitreum*) was conducted during the ice-free period on Oneida Lake, New York in 1986. During the survey, 1,028 walleyes were collected and examined for the presence of lymphocystis disease, dermal sarcoma, discrete epidermal hyperplasia and diffuse epidermal hyperplasia. Skin growths were high in prevalence in early spring, low in prevalence during the summer, and again high in prevalence in the fall. Lymphocystis disease and dermal sarcoma were more frequently observed than either discrete or diffuse epidermal hyperplasia. Histologically, a moderate to severe inflammatory response was associated with dermal sarcoma in the early spring and late spring but not in the fall. Regardless of the time of year, varying degrees of inflammatory response were seen associated with lymphocystis disease. Samples were inadequate to assess seasonal trends in incidence of discrete and diffuse epidermal hyperplasia.

**Key words:** Walleye, *Stizostedion vitreum*, neoplasia, virus associated tumors, pathology, epidermal hyperplasia, dermal tumors, lymphocystis disease, dermal sarcoma, seasonal prevalence, survey.

### INTRODUCTION

Four virus-associated skin growths have been described for the walleye (*Stizostedion vitreum*). Lymphocystis disease, caused by an iridovirus, is characterized by enlarged non-neoplastic cells surrounded by a hyaline layer (Weissenberg, 1965). The virus has been isolated in cell culture and the disease can be transmitted experimentally (Dunbar and Wolf, 1966). Dermal sarcoma was first reported in walleyes by Walker (1969) in fish collected from Oneida Lake, New York. These tumors ranged in morphology from highly cellular to densely fibrous. Intercellular, budding, retrovirus-like particles, 135 nm in diameter, were common in these tumor cells. Epidermal hyperplasia, now termed discrete epidermal hyperplasia (Yamamoto et al., 1985a, b), was reported also by Walker (1969) in walleyes from Oneida Lake. These lesions appeared as broad, flat, sharply delimited plaques of up to 7 cm in diameter. Retrovirus-like particles, with an average diameter of 120 nm were as-

sociated with these hyperplastic cells. Since the early reports by Walker (1969), similar virus-associated skin growths have been reported by Yamamoto et al. (1976) in walleyes from several lakes in western Canada. Neither of the retrovirus-like particles have been cultured in vitro.

A herpesvirus has been found in a fourth type of skin lesion from the walleye and that virus has been cultured in a cell line of walleye origin by Kelley et al. (1980, 1983) in Canada. The lesion was termed as a "diffuse epidermal hyperplasia." The lesion did not exhibit the discrete border seen in discrete epidermal hyperplasia and was often observed to have an associated swelling of the underlying hypodermis. The two types of epidermal hyperplasia are difficult to differentiate without histologic examination (Yamamoto et al., 1985a).

Reports in the literature and observations made by fisheries researchers and sportsmen have suggested that walleye skin growths are more common in the spring than during the summer. The purpose of

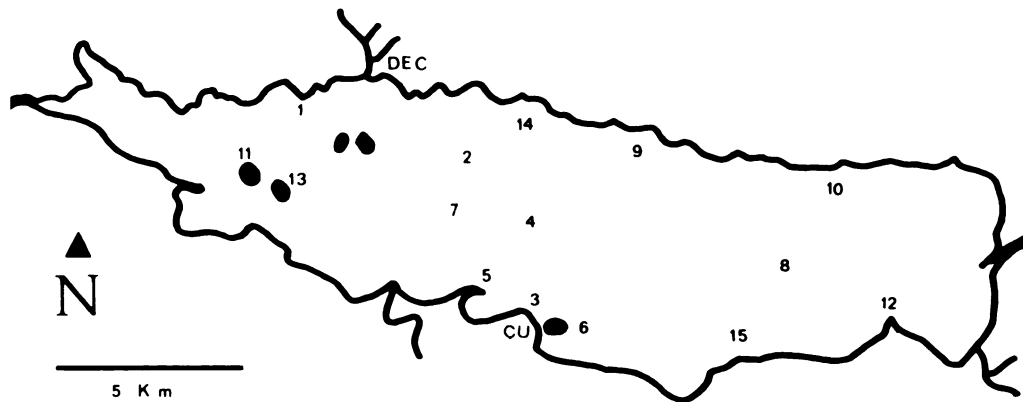


FIGURE 1. Locations of weekly gillnet sampling where fish were caught (numbers 1–15) on Oneida Lake, New York, during the period 2 June to 8 September 1986. DEC = N.Y. State Department of Environmental Conservation Fish Hatchery, Constantia, New York. CU = Cornell University Biological Field Station, Shackelton Point, Bridgeport, New York.

this study was to document the seasonal occurrence of walleye skin growths. The investigation was undertaken as an initial step to understand the apparent growth and regression of these lesions in a poikilothermic animal.

#### MATERIALS AND METHODS

Walleyes collected from Oneida Lake, New York (43°05' to 43°12'N, 75°44' to 76°09'W) were examined during the ice-free period of April through November 1986. Oneida Lake has a surface area of 20,700 ha, a maximum depth of 16.8 m and a mean depth of 6.8 m. Wind action and currents usually prevent thermal stratification during summer, although temporary stratification may occur during periods of extended calm weather. Ice normally covers the lake from mid- to late-December through March (Forney, 1977). Fish collected in trapnets by personnel of the New York State Department of Environmental Conservation and brought to the Constantia Fish Hatchery (Constantia, New York 13044, USA) for spawning were examined on 4 April 1986. Data collected from each fish included sex, total length and number and type of skin lesions. A representative sample of lesions were fixed in 10% neutral buffered formalin for light microscopic examination.

Walleyes were collected in experimental gillnets set once each week for 15 wk from 2 June to 8 September 1986. Each set consisted of 182.9 m of gillnet containing equal net length sections of 3.8-, 5.1-, 6.4-, 7.6-, 8.9- and 10.2-cm mesh netting. Nets were set at approximately 1930 hr and retrieved at 0730 hr the following morning.

The nets were placed at 15 randomly selected locations on the lake (Fig. 1). When retrieved, the nets were brought to the Cornell University Biological Field Station on Shackelton Point, Oneida Lake (RD 1, Bridgeport, New York 13030, USA), where fish were removed from the nets and the walleyes were examined for skin growths. Additional walleyes were collected on 15 October 1986 with a 5.5-m otter trawl and on 7 November 1986 from a trapnet set off Shackelton Point. Representative samples of the lesions were removed from the fish, preserved in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin for histopathological examination. Representative specimens have been deposited in the Registry of Tumors in Lower Animals (Smithsonian Institution, Washington, D.C. 20306, USA) under Accession Numbers RTLA 3967–3970. Water temperature was determined for collection dates by calculating mean temperature from a vertical temperature series taken at 1-m increments from surface to bottom measured at buoy 109 or 117 near the center of the lake. During 1986, 1,028 walleyes from Oneida Lake were examined during scheduled collections.

Tumor prevalence data was fit to the temperature data using a logistic regression (STATISTIX, NH Analytical Software, 1958 Eldridge Avenue, Roseville, Minnesota 55113, USA). Prevalence was defined as proportion of fish found in any one collection to have one or more of the skin lesions of interest. Grouped data at 5 C temperature increments were used for analyses. This resulting in five seasonal groupings of data: early spring (0.0–5.0 C), late spring (15.1–20.0 C), summer (20.1–25.0 C), early fall (10.1–15.0 C) and late fall (5.1–10.0 C).

TABLE 1. Seasonal prevalence of all skin growths, lymphocystis disease and dermal sarcoma on walleyes from Oneida Lake, New York during 1986.

	Temperature (°C)	n	All skin growths	Lympho- cystis	Dermal sarcoma	Discrete epidermal hyperplasia	Diffuse epidermal hyperplasia
Early spring	0.0–5.0	440	138 (32)*	50 (4)	60 (14)	41 (9)	16 (4)
Late spring	15.1–20.0	104	14 (14)	2 (2)	14 (14)	0 (0)	0 (0)
Summer	20.1–25.0	208	11 (5)	5 (2)	8 (4)	0 (0)	0 (0)
Early fall	10.1–15.0	93	24 (26)	5 (5)	23 (25)	0 (0)	0 (0)
Late fall	5.1–10.0	183	64 (35)	28 (16)	50 (27)	2 (<1)	0 (0)
Total		1,028	251 (24)	90 (9)	155 (15)	43 (4)	16 (2)

\* Number with lesions, percent in parentheses.

C). Non-transformed temperatures along with quadratic and cubic transformations were offered in stepwise fashion. A higher order term was included only if it added significantly to the model at the  $P < 0.10$  level.

Significant differences in tumor prevalence between male and female walleye was determined by Chi-square analysis. Tumor prevalence data was fit to total length data using an unweighted least square linear regression (STATISTIX, NH Analytical Software). Grouped data at 50 mm total length increments were used for analysis.

## RESULTS

Prevalence of lymphocystis disease, dermal sarcoma, discrete epidermal hyperplasia and diffuse epidermal hyperplasia on walleyes from Oneida Lake was high in spring, low in summer, and high in fall (Table 1). All four lesions were observed in samples taken during early spring (4 April 1986). Diffuse epidermal hyperplasia was observed only during the early spring (4 April 1986) collection, while discrete epidermal hyperplasia was only detected in early spring and the late fall (7 November 1986) collections. Lymphocystis disease and dermal sarcoma were more prevalent than either discrete epidermal hyperplasia or diffuse epidermal hyperplasia. There were several instances in which more than one type of skin growth was observed on an individual fish. It was not unusual to observe large numbers (>20) of lesions on an individual fish in the spring. During the summer, lesions were fewer in number (one or two per fish) and relatively

small in size (<5 mm). Compared to fish observed during the summer, fish collected in the fall were found with larger numbers (>5 per fish) of lesions.

Prevalence of walleyes with skin growths declined from 32% at water temperatures of 0–5 °C to 5% in samples taken when water temperatures were 20.1–25.0 °C (Table 1). The inverse relation with temperature was evident for both lymphocystis disease and dermal sarcoma tumors. For overall skin lesion prevalence, the fitted model included both temperature and temperature-squared, yielding the equation  $Y = -8.5659E-03T^2 + 1.0493E-01T - 0.9813$ , where  $Y$  = prevalence of tumors and  $T$  = temperature in degrees centigrade. The calculated significance of temperature on prevalence for this model was  $P = 0.0590$ . For lymphocystis infection, the fitted model included only temperature, yielding the equation:  $Y = -8.3259E-02T - 1.6838$ , where  $Y$  = prevalence of lymphocystis disease and  $T$  = temperature in degrees centigrade. The calculated significance of temperature on prevalence for this model was  $P = 0.0202$ . For dermal sarcoma, the fitted model included temperature and temperature-squared. The equation for this model was:  $Y = -1.5263E-02T^2 + 3.0750E-01T - 2.4985$ , where  $Y$  = prevalence of dermal sarcoma and  $T$  = temperature in degrees centigrade. The calculated significance of temperature on prevalence for this model was  $P = 0.0254$ .

TABLE 2. Prevalence of skin growths in male and female walleyes from Oneida Lake, New York at different water temperatures during 1986.

Temperature (C)	Male		Female		Total		Chi-square values of tumors present, male versus female
	<i>n</i>	Number with skin growths	<i>n</i>	Number with skin growths	<i>n</i>	Number with skin growths	
0.0–5.0	239	86 (36) <sup>a</sup>	201	52 (26)	440	138 (31)	5.18* 1 df
15.1–20.0	47	5 (11)	57	9 (16)	104	14 (14)	0.59 1 df
20.1–25.0	109	10 (9)	99	1 (1)	208	11 (5)	6.90** 1 df
10.1–15.0	53	17 (32)	40	7 (18)	93	24 (26)	2.53 1 df
5.1–10.0		ND <sup>b</sup>		ND	183	64 (35)	—
Total	448	118 (26)	397	69 (17)	1,028	251 (22)	9.80** 1 df

<sup>a</sup> Number with skin growths, percent in parentheses.<sup>b</sup> ND = not done; fish collected within this temperature range were not sexed.\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

Prevalence of skin growths found in male versus female walleyes is summarized in Table 2. When data from all temperature increments were combined, lesion prevalence in males (26%) was significantly ( $P < 0.01$ ) higher than prevalence in females (17%). Within specific temperature increments, prevalence of lesions was higher in males within the 0–5 C (36% versus 26%,  $P < 0.05$ ) and 20.1–25.0 C (9% versus 1%,  $P < 0.01$ ) increments. No significant differences ( $P > 0.05$ ) in prevalence of lesions between male and female walleyes were observed within other temperature increments.

Data describing prevalence of skin growths versus fish size are provided in Table 3. Total length of walleyes examined ranged from 172 to 641 mm. Only one fish <300 mm total length had a skin growth. As total length increased, prevalence of lesions increased and then appeared to plateau in the larger size groups of fish (>400 mm). An unweighted least squares linear regression of skin lesion prevalence versus

total length of fish indicated that total length had a significant effect on prevalence of lesions ( $r^2 = 0.8987$ ,  $P = 0.0012$ ).

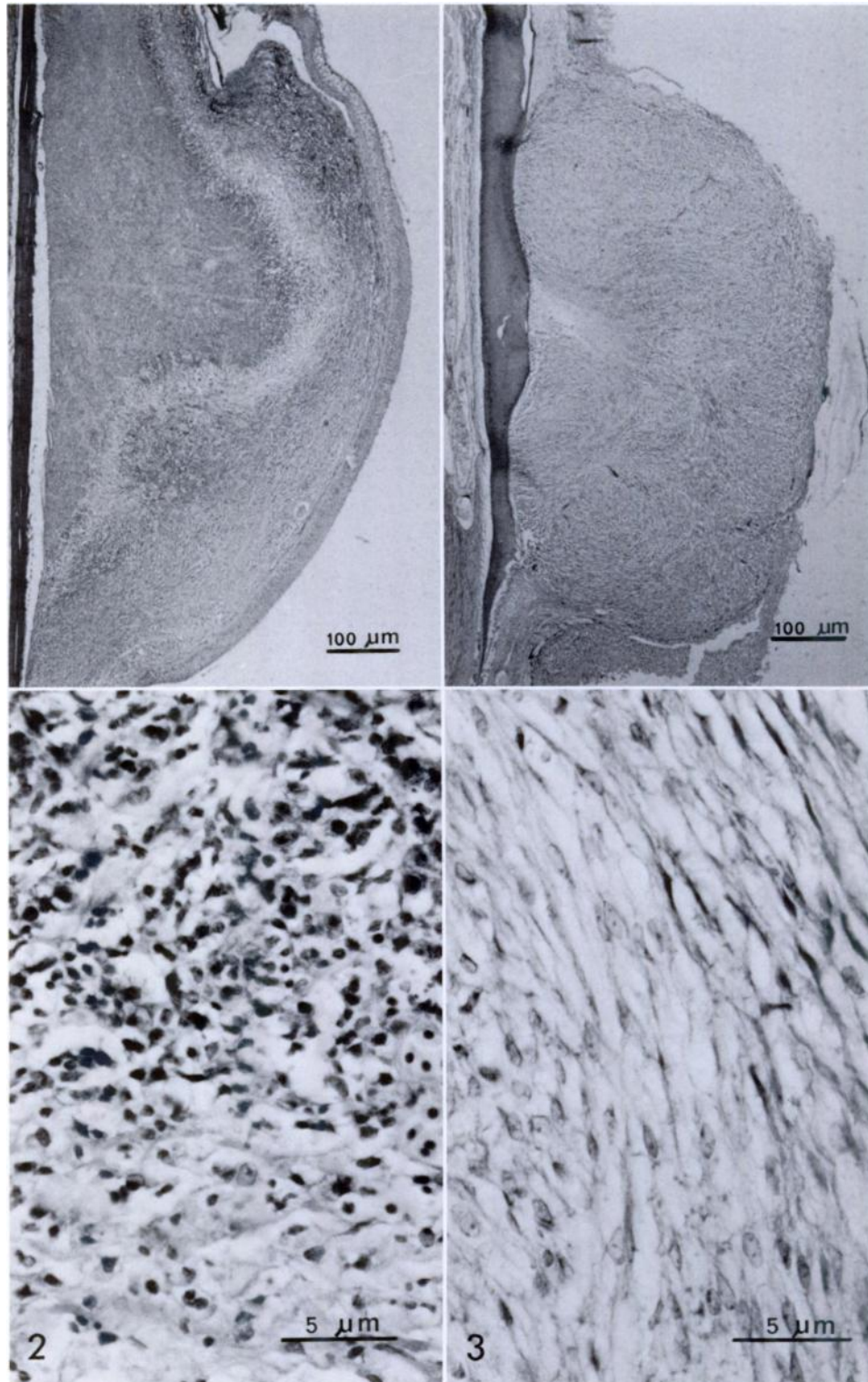
Histologic examination of sectioned materials revealed seasonal differences in dermal sarcoma. A moderate to marked inflammatory response was observed in dermal sarcoma from fish collected in the spring and summer (Fig. 2). Dermal sarcoma from fish collected in the fall showed little or no associated inflammatory response (Fig. 3). Examination of lymphocystis disease did not reveal the same temporal trend in inflammatory response. Regardless of time of collection, a moderate to marked inflammatory response was associated with lymphocystis observed on walleyes from Oneida Lake. Inadequate numbers of diffuse and discrete epidermal hyperplasia lesions prevented similar seasonal comparisons.

## DISCUSSION

A seasonal prevalence of skin growths in walleyes was observed during this study.

TABLE 3. Prevalence of skin growths in walleyes from Oneida Lake, New York versus total length (mm) of the fish.

Size	<250	251–300	301–350	351–400	401–450	451–500	>500	Total
Number examined	30	17	85	420	365	84	27	1,028
Number with tumors	0	1	7	104	103	27	9	251
Percent	0	6	8	25	28	32	33	24



FIGURES 2, 3. 2. Dermal sarcoma from a walleye from Oneida Lake, New York collected in late spring. A. Low magnification showing location of the lesion in the epidermis. B. High magnification showing lymphocytic infiltration in the lesion. 3. Dermal sarcoma from a walleye from Oneida Lake, New York collected in early fall. A. Low magnification showing location of the lesion in the epidermis. B. High magnification showing absence of an inflammatory response within the lesion.

There appears to be an inverse relationship between water temperature and the prevalence of lesions. The data most strongly supports this relationship for lymphocystis disease and dermal sarcoma. Due to the relatively few discrete and diffuse epidermal hyperplastic lesions observed, a relationship between water temperature and these skin lesions could not be established. However, from the limited data available, a trend consistent with that for lymphocystis disease and dermal sarcoma was observed.

The nature of the seasonality of these lesions is not known at this time. The interaction of the immune system of fish with water temperature has been described in several recent reports (Corbel, 1975; Manning and Tatner, 1985). Low water temperatures can suppress the immune response of trout (Finn and Nielson, 1971; Etlinger et al., 1976) and channel catfish (Cuchens and Clem, 1977; Clem et al., 1981; Miller and Clem, 1984). It is not unreasonable to expect a similar temperature effect for the walleye. Thus, one mechanism of the temperature-associated tumor growth and regression could be an immune system that varies in its capability to evoke a host response against the tumor.

All four skin lesions described in this study have associated viruses. A casual relationship between virus and lesion has only been demonstrated for lymphocystis disease (Dunbar and Wolf, 1966). It is possible that a temperature-virus interaction exists, similar to that of the herpesvirus-induced tumors of frogs (Mizell et al., 1968). Other possibilities also exist. High tumor prevalence in the spring coincides with spawning. Spawning is a very stressful period physiologically for fish. Spawning-associated stress could play a role in providing a more susceptible host for tumor development. Appropriate laboratory protocols must be developed to determine which of these, or other factors, participate in the growth and regression of these lesions of the walleyes.

A trend of increasing skin lesion prev-

alence with increasing size of fish was observed. The increasing prevalence with size can be interpreted, within limits, to be an increasing prevalence with age. Growth and life history studies of walleyes on Oneida Lake have shown very little difference in total length between walleyes of different year classes measured at the same age (Forney, 1965, 1977). Although ages were not determined for fish in this study, from walleye growth information one could conclude that fish in excess of 351 mm total length, where tumor prevalence was greatest, were  $\geq 4$  yr old. Fish with total lengths of 300 mm or less, in which only one fish of 47 had a tumor, were  $\leq 3$  yr old. This trend is consistent with observations in other animals in which many tumors increase in prevalence with age.

The variation in prevalence between male and female walleyes must be examined carefully, with consideration given to the environment from which the fish were collected. Skin tumors in walleyes from Cren Lake, Saskatchewan, Canada were more prevalent in females than males in studies by Yamamoto et al. (1976) and Mathias et al. (1985). In both studies, walleyes were examined during spawning. Cren Lake has been characterized as a lake with a typical unexploited walleye population (Mathias et al., 1985). With female walleyes tending to be larger than males of the same age class, most of the largest fish in the population were female fish. Oneida Lake is a very heavily exploited lake. Sport fishing has been estimated to annually remove as much as 50% of the standing crop of walleyes. Our finding of a higher tumor prevalence in males may be due in part to the heavily exploited nature of the walleye population from which we sampled. Sport fishing could selectively harvest the larger fish, primarily the females. Of those fish remaining, more of the larger fish might be males that would be older than females of equivalent size. It also is possible that the prevalence rate varies with year class of walleyes. Mathias



et al. (1985) observed the prevalence of tumors on 5–8-yr-old walleyes in 1984 was 0% in contrast to a 20% prevalence for 5–6-yr-old walleyes in 1975. Future investigations of skin tumors on walleyes from Oneida Lake are planned and will include age determinations for all fish examined.

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