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MYXOSPORIDIAN LESIONS OF THE SHEEPSHEAD MINNOW (*CYPRINODON VARIEGATUS*)

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ABSTRACT: An infection of sheepshead minnows (*Cyprinodon variegatus*) by myxosporidia (*Myxobolus lintoni*) was discovered in a brackish pond on Assateague Island, Virginia. Twenty-seven of the 550 (5%) captured sheepshead minnows exhibited epidermal lesions: white, raised, irregular tissue masses. There were no epidermal lesions noted in any of the four other fish species occurring in the pond. The apparent outcome in this episode of myxosporidian infection was death of the host fish.

Key words: *Myxobolus lintoni*, myxosporidia, sheepshead minnow, *Cyprinodon variegatus*, pathology, epidermal lesion, spontaneous neoplasm.

INTRODUCTION

Epidermal lesions resembling spontaneous neoplasms, but actually caused by protozoans, are occasionally found in fishes. Correct diagnosis of such lesions is important because spontaneous neoplasm is considered by many to be indicative of water pollution and a potential human health problem. However, lesions arising from protozoan infections may be only a consequence of normal but extreme environmental conditions, not important to human health, but adversely affecting the health and/or salability of fish. The present study documents the prevalence and pathology of lesions caused by *Myxobolus lintoni* in a naturally occurring population of the sheepshead minnow (*Cyprinodon variegatus*) from a brackish pond in Virginia.

MATERIALS AND METHODS

A survey of brackish water fauna of Assateague Island, Virginia, was conducted each July from 1981 through 1986. In 1983, three sheepshead minnows with epidermal, tumor-like lesions were taken in turtle traps from Carr's Marsh Pond (37°95'N, 75°25'W); no other pond contained similarly affected fish. To estimate the prevalence of lesions, a minnow seine (5 mm mesh; 1 × 6 m) was used to capture fish. Six seine hauls were made, each haul (different pond areas) covered approximately 25 m. The catch was tallied; all fish with epidermal lesions were removed and preserved for later examination.

Five lesions were subsequently processed for light microscopy using standard histological techniques; tissues were dehydrated in graded ethanol, cleared in xylene, embedded in paraffin, sectioned at 8 μm thickness and stained with hematoxylin and eosin.

Representative formalin-fixed fish were deposited with the Registry of Comparative Pathology (Armed Forces Institute of Pathology, Pathology Branch 33, Washington, D.C. 20306, USA; Accession No. 2088726). Monthly temperature records for the period of study were obtained from the National Oceanic and Atmospheric Administration (Wallops Station, Virginia 23337, USA) and impoundment water level records were provided by the Chincoteague National Wildlife Refuge (Chincoteague, Virginia 23336, USA).

RESULTS

Each year five species of fish were captured in Carr's Marsh Pond: *C. variegatus* (sheepshead minnow), *Fundulus heteroclitus* (mummichog), *F. luciae* (spotfin killifish), *F. majalis* (striped killifish), and *Menidia beryllina* (tidewater silverside). In 1983, 27 of the 550 (5%) captured *C. variegatus* had epidermal lesions. Lesions were not found on any of the other 1,620 fish examined.

During the period of study, mean July temperatures were 30.6 C ± 0.53 (range 28.7–32.3 C); mean water depths were 0.96 m ± 0.16 (range 0.56–1.59 m). The highest monthly temperature and the least water occurred in 1983.



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FIGURES 1, 2. 1. Flat myxosporidian lesion on *Cyprinodon variegatus*. Standard length 40 mm. 2. Pendant myxosporidian lesion on *C. variegatus*. Standard length 37 mm.

As many as six lesions were found in a fish, but usually only one occurred. Two types of lesions were seen. In flat lesions (Fig. 1), the overlying integument was pigmented, slightly raised and roughened, but the scale pattern remained intact. Pendant lesions (Fig. 2) extended above the surface as white, irregular masses of varying size; the largest measured $10 \times 6 \times 6$ mm. These pendant masses resembled spontaneous neoplasm. They were firm to the touch and did not deform easily; cut surfaces exhibited a homogeneous greyish-white color. The overlying integument was cracked and often sloughed giving the surface an uneven appearance. However, the gross appearance of these lesions gave no indication of their etiology.

Sections of a lesion revealed the presence of the myxosporidian *M. lintoni*. Thus, only in retrospect could the pathology be properly categorized: an integumental displacement lesion produced by a proliferating parasitic mass. In the flattened lesions, islands of spores and trophozoites were separated by cords of connective tissue. These connective tissue elements appeared to have been pushed apart; hypertrophied cells were not seen

nor was evidence of edema. Spores were seen most frequently toward the periphery of the lesion; trophozoites occurred medially. The spores measured $13.5 \times 10 \times 8 \mu\text{m}$. They were elliptical in front view, lenticular in side view and with an obvious sutural ridge. The two anterior polar capsules were convergent. The sporoplasm contained a large vacuole and two nuclei were seen usually. The trophozoites were oval and averaged about $20 \times 50 \mu\text{m}$.

In microscopic sections of pendant lesions, traumatization and loss of epithelium was obvious. The external boundary consisted of a row of elongated corial melanophores supported by a layer of loosely arranged fibroblasts. Fibroblasts and attendant fibers also formed septae which separated and compartmentalized the spore and vegetative masses of the parasite. The proliferating parasitic mass was responsible for the size of the lesion. There was no evidence of neoplasia in either integumentary tissues or body musculature. Encapsulation of the lesions had not occurred. There was no evidence of any attempt to encapsulate the lesion from the underlying tissue. Granulation tissue was absent. Blood vessels and blood cells were

visible in the septae as well as at the integumental and somatic surfaces of the lesions. However, areas of hemorrhage were not observed nor was a leucocytic inflammatory reaction observed.

DISCUSSION

Myxosporidian lesions of superficial muscles and subcutaneous tissue of *C. variegatus* were first described by Linton (1889) from a fish caught in August 1889, at Woods Hole, Massachusetts. Gurley (1891) described the parasite causing the lesions as *M. lintoni*. Later, Gurley (1894) provided a more detailed description of the species. Similar parasites and lesions were observed by Hahn (1915) in a *C. variegatus* captured in August at Woods Hole. Nigrelli and Smith (1938) examined lesions of *C. variegatus* taken from Sandy Hook Bay, New Jersey, and the mouth of the Connecticut River during August and early September 1937, and found them to be caused by *M. lintoni*. Rigdon and Hendricks (1955) illustrated myxosporidian lesions in *C. variegatus* from the Galveston Ship Channel, Texas; this fish was caught between 20 May and 19 June 1954. Overstreet and Howse (1977) collected infected *C. variegatus* from Davis Bayou, Mississippi on 10 June 1976. This study is the first report of *M. lintoni* from Virginia and fish with *M. lintoni* lesions were seen only during July 1983. It appears that the range of this myxosporozoan is at least from Texas to Cape Cod, but probably it is concurrent with the range of *C. variegatus* (Massachusetts to Yucatan, Mexico). The appearance of recognizable lesions appears to be seasonal and the infection is perhaps more common than suggested by published reports.

Apparently, *M. lintoni* is only reported from *C. variegatus* and there is only one report of another species of *Myxobolus* from the sheepshead minnow. *Myxobolus capsulatus* was reported from visceral connective tissue of *C. variegatus* from Beaufort, North Carolina by Davis (1917), but

this myxosporozoan was present in a diffuse infiltrative lesion and did not form tumor-like masses. Hahn (1917) was able to infect *C. variegatus* with *Myxobolus musculi*, a common myxosporidian parasite of *Fundulus majalis*, *F. heteroclitus* and *F. diaphanus*, and all closely related to *C. variegatus*, but tumor-like lesions were never produced. The lack of reports of other fish species infected by *M. lintoni*, the lack of tumor-like lesions in experimentally infected fish, and the absence of infection in the co-occurring species captured in this study suggest a definitive host specificity for *M. lintoni*.

Myxosporidian lesions develop rapidly and cause death to the host in probably less than a week (Hahn, 1915). Fish with tumor-like lesions from Carr's Marsh Pond were noted only over a 4-wk period; death apparently occurred rapidly, since leucocytic infiltration and granulation tissue were absent. Hahn (1917) reported the incidence of infection in *Fundulus* spp. by *M. musculi* to be >4% in July and 0.1% in August. The incidence of infection in this study was <5%. An estimate of mortality was not attempted because Carr's Marsh Pond is a feeding area for herons and I made no attempt to maintain infected fish in the laboratory.

The occurrence of myxosporidiasis is thought to be related to stress (Davis, 1917; Hahn, 1917; Overstreet and Howse, 1977; Overstreet, 1978). Heat stress may be a factor but, other than this study, temperature data are available only from the study by Overstreet and Howse (1977). Davis Bayou was 26 C when fish with lesions of *M. lintoni* were collected. On the Texas coast, average daily high air temperatures approaching 30 C first occur in May. Corresponding temperatures in Mississippi occur in June and in Massachusetts they occur in July (Ruffner and Bair, 1974). In this study, fish with epidermal lesions were seen only in 1983. This outbreak occurred during the highest July temperatures of the survey years and during a period of

low water. Since Carr's Marsh Pond is within a wildlife refuge, water pollution was apparently not a factor.

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