BLACK TUMOR OF THE CATFISH[1]

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A conspicuous and very unsightly disease of the common bullhead, or horn-pout (*Ameiurus nebulosus* LeSueur), appeared a few years ago in a pond at Wauquoit, near Falmouth, Mass. The tumorous excrescences produced by the disease are so striking that it seems almost impossible that no mention of it has been made in the scientific literature. Ichthyologists and fish-culturists consulted have professed their ignorance of the existence of the disease, and examination of the literature of fish pathology proved futile.

The keen-eyed Thoreau, however, did observe the bullheads of the Concord River to be affected with this trouble more than 70 years ago. I should have overlooked this reference to it but for the kindness of Henry W. Henshaw of the United States Biological Survey, who called my attention to certain statements in Thoreau's "Journal." Under date of July 10, 1852, that writer states: "One of these large pouts had a large velvet-black spot, which included the right pectoral fin; a kind of disease which I have often observed on them." The location was on the Concord River. Later (July, 1858) Thoreau again wrote in his "Journal": "I see a pout this afternoon in the Assobet" (a tributary of the Concord) "lying on the bottom near the shore, evidently diseased. * * *. Nearly half the head, from the snout backward diagonally, is covered with an inky-black kind of leprosy, like a crustaceous lichen." There can be no doubt that Thoreau observed the disease which forms the subject of the present paper, but it seems strange that so long a period should elapse before such a striking malady should again come to the attention of a naturalist.

In the summer of 1917, while I was spending a short time at the Marine Biological Laboratory at Woods Hole, Mass., the late Vinal Edwards, collector for the Bureau of Fisheries, brought me a specimen of the diseased fish and asked my opinion of the nature of the growth. I took the opportunity to visit the pond where the disease occurred and collected some more material for preservation for sectioning and made some photographs. The best I could do at the time was to pronounce the disease some form of a tumor, and this opinion was concurred in, without any further information, by several pathologists to whom material was submitted.

In the summer of 1919 the Bureau of Fisheries made possible my further study of this disease, and I spent six weeks at the Woods Hole station. My results, though not complete in every detail, have been satisfactory.

[1] A very brief account of this disease has already been published in Bureau of Fisheries Doc. 896, Progress in Biological Inquiries, 1920, p. 17.

So far as known, the disease at present occurs in only one pond, a small natural lake of some 10 acres, situated on the property of Dr. L. C. Jones, of Falmouth, Mass. Doctor Jones first observed the tumors and submitted specimens to Mr. Edwards. I owe him many thanks for assistance in obtaining material and for various observations.

GROSS DESCRIPTION OF THE TUMOR

In the younger stages the tumors appear as intensely black areas of variable size and form in the skin of the fish (figs. 1, 2, 3, and 4). With later development these areas become thick and warty looking, with a very irregular surface, which sometimes, especially on the lips, may be fimbriated (fig. 4). Though at first the skin is only slightly thickened (figs. 1 and 7), the tumors may eventually rise 6 or 8 millimeters, or even more, above the level of the surrounding skin (fig. 3). They are soft to the touch and, in older tumors, the slightest pressure causes the extrusion of any inky black fluid. This color, as will be seen later, is due to the presence of enormous numbers of a minute black coccoid bacterium.

Any part of the skin may be affected, but the tumors are more commonly found on the fins and lips, parts which are especially subject to abrasion and are thus exposed to direct infection. In the majority of cases the skin on the outside of the body only is affected, but not infrequently tumors on the lips spread to the inside of the mouth (fig. 4, tumor on upper jaw). Vinal Edwards told me that he had seen one specimen in which the tumor had extended across the roof of the mouth and down the esophagus into the stomach. I observed one case in which the tumor, originating on the side of the body near the pectoral fin, had spread forward through the branchial chamber, involving part of the gills, and extended across the floor of the mouth to the lower jaw. In one case a tumor originated on the roof of the mouth.

Development proceeds so slowly that the rate of growth has not been determined. The tumors have been observed in all stages of development, from small spots less than 3 millimeters in diameter to areas involving more than one-third of the skin of the fish, and from cases in which the skin was scarcely thickened to those in which the tumor projected 8 or 10 millimeters above the natural surface. As a rule the larger tumors are more mature and much thickened; but this does not necessarily follow, for I have seen cases in which a tumor covering several square inches was scarcely raised above the surface (fig. 1) and others in which the tumor was as high as its diameter.

In spite of the tendency to spread laterally, there is no evidence of metastasis, and the deeper tissues beneath the tumor do not seem to be invaded; at least, in none of the material sectioned is there any evidence of such an invasion below the lower layer of the dermis, except that in older tumors the connective tissues between the muscles may be involved to some extent. Even in these cases the disease does not appear to penetrate to any considerable depth. On the fins the tumor frequently affects the entire substance, and a tumor originating on one side will penetrate to the opposite side. Perhaps this is to be expected, since the fins consist almost entirely of connective tissues.

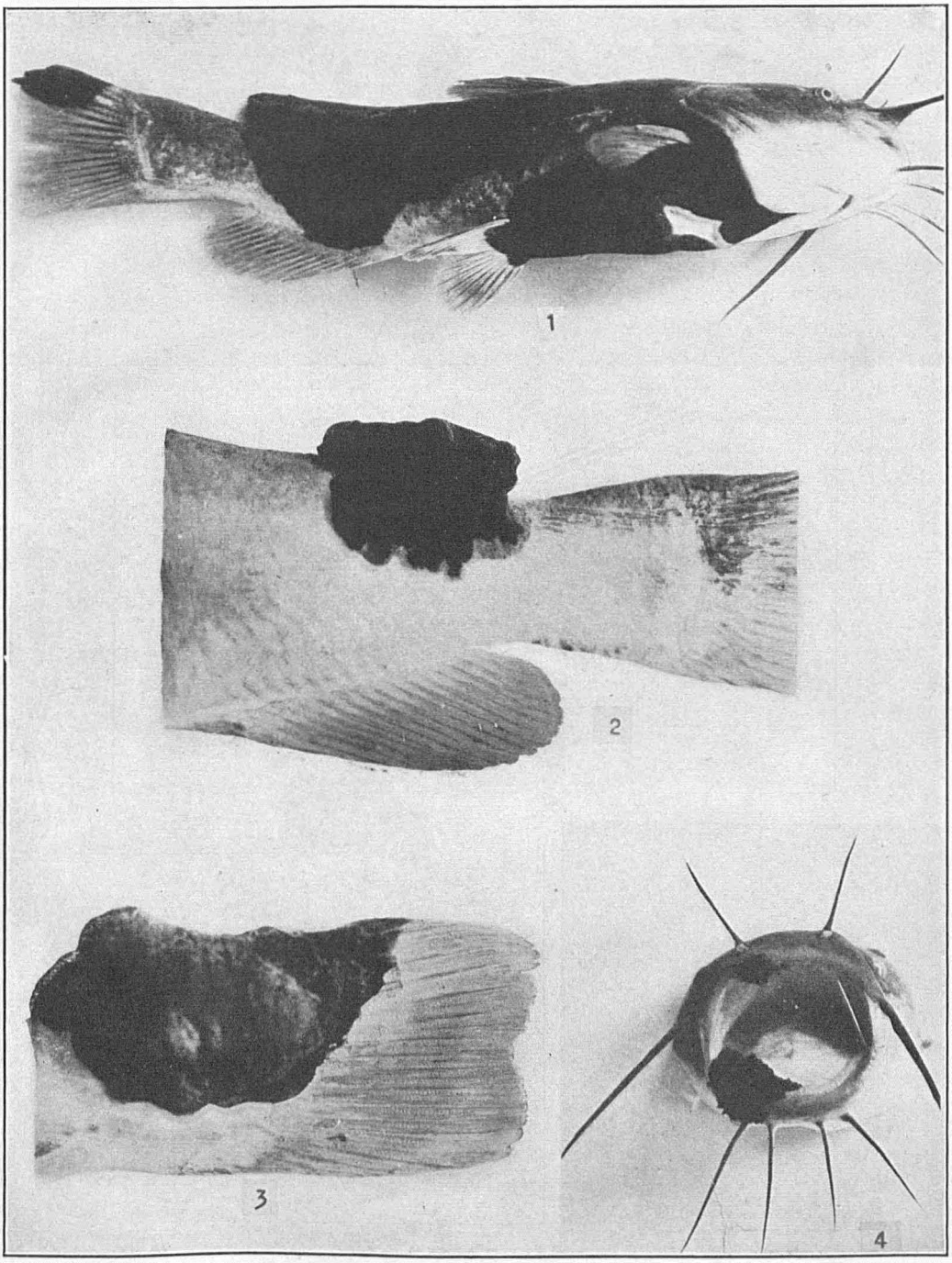

FIG. 1.—Extensive infection of a 9-inch fish. The tumors are still in the "young" stage, scarcely raised above the surface. The areas of dense black seem to indicate four centers of infection.

FIG. 2.—"Old" tumor involving all of the adipose fin and extending downward on both sides. A slight margin of younger growth is seen about the base of the older, dense black portion.

FIG. 3.—An "old" tumor involving the caudal peduncle and the base of the caudal fin, extending on both sides. The highest portion of this tumor is nearly half an inch above the natural dorsal line.

FIG. 4.—Tumors on the lips. That on the lower jaw is "older" and somewhat fimbriated on the surface. That on the upper jaw involves a portion of the oral surface. The left maxillary barbel shows infection in a very young stage.

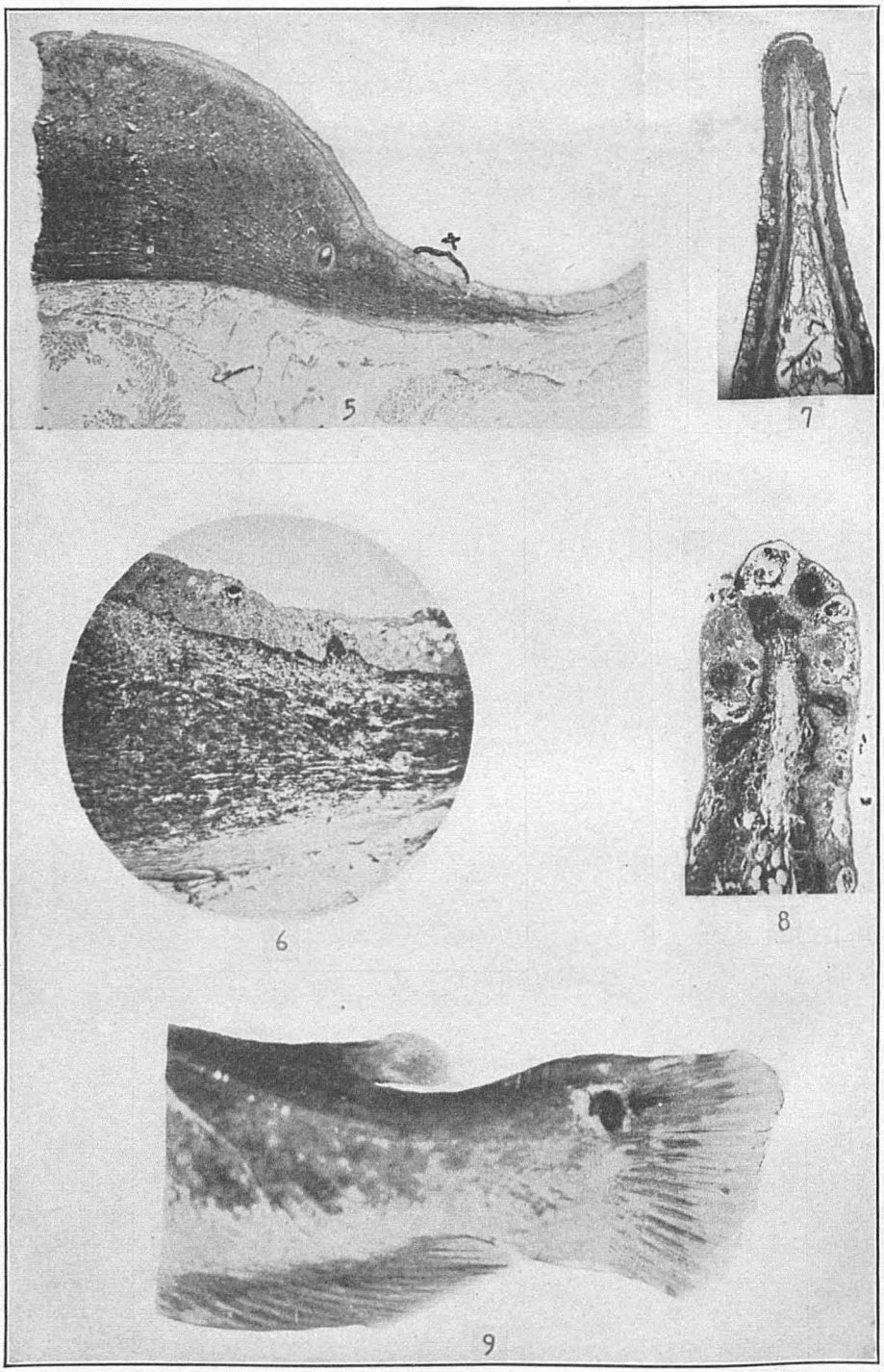

FIG. 5.—Microphotograph of a section through an older but as yet unbroken tumor. The epidermis is still continuous over the surface. The black area indicates the thickening of the dermis—5 millimeters in thickness at the thickest portion shown. The portion marked *X* is shown enlarged in Figure 6.

FIG. 6.—Microphotograph under higher power of the portion marked *X* in Figure 5. The extent of the dermis is evident, and comparison with the right end of Figure 5 will indicate the amount of thickening above the normal. The extrusion of a lobe of the tumor into the epidermis is shown a little at the right of the center. The rounded black object in the epidermis at the left of the center is a section of another such lobe, and a third portion shows near the left border.

FIG. 7.—Microphotograph of section of adipose fin, fully infected, but in an only slightly advanced condition.

FIG. 8.—A similar section of an adipose fin at a more advanced stage of the disease. The connective tissue of the fin is entirely involved. The rounded cavities are large cystlike spaces filled with the bacteria, most of which were lost out in making the slide.

FIG. 9.—A "young" tumor at the base of the caudal fin, experimentally produced by inoculation, as indicated in the text. Two months after inoculation.

MICROSCOPIC STUDY

Under the lower powers of the microscope the sectioned material of the tumor appears as a mass of small black bodies, usually more or less rounded, so thickly distributed among the connective tissue fibers of the skin that they almost completely obscure everything else. These bodies appear to be cystlike masses of the cocci, and scattered individual cocci may also be observed everywhere in great numbers.

In smears made from fresh material the same cystlike bodies are present, along with scattered cocci, and under the oil immersion the cocci within the cyst may often be seen in active Brownian movement. It was at first supposed that this movement might be due to some activity on the part of the organism, but in smears made from material macerated in strong potassium hydroxide the same movement was evident. In fresh smears the cysts may be seen to break under slight pressure of the cover glass, liberating large numbers of the cocci. The cocci are densely black with an endogenous pigment, and they are so abundant that it is difficult to examine sectioned material with satisfaction, even when cut to only two or three microns.

From my observations it appears that the tumor begins its development in the outer layer of the dermis and later involves all of this layer, expanding it to many times its original thickness. The dermal blood vessels are enlarged, but I have seen no evidence of bleeding, even in old tumors. As the disease progresses small fingerlike projections of the tumor invade the epidermal layer and make their way to the surface (figs. 6 and 8). Eventually the epidermis sloughs off over the older portion of the tumor, leaving a ragged surface formed by the dermal connective tissue fibers and the substance of the tumor. A slight pressure of the tumor at this stage causes the extrusion of the inky-black fluid containing the cocci, as indicated above.

STUDIES AND EXPERIMENTS

As some form of fungus had been suggested as the cause of the disease, my first studies were directed toward this point. Ordinary fish fungus (*Saprolegnia* sp.) is not black, but some species of *Aspergillum* and some other fungi do produce black pigment. Some fishes were found with fungus on the tumors, but a little study revealed the fact that this fungus was not black and that it sometimes occurred elsewhere on the same fish where the skin had been abraded and where there was no evidence of the black tumor. Moreover, when growing in the tumor, the fungus was not found in the deeper portion. Careful search was made for any fungus in sectioned and fresh material and by macerating with strong potassium hydroxide, which breaks down the animal tissue but leaves fungi intact. Fungus as a cause of the tumors was thus thrown out of consideration.

The question of an animal organism as the causative factor was also considered, especially as the Myxosporidia often produce tumorlike growths, but no evidence of any such could be observed.

The hypertrophy of the pigment layer of the skin, due to stimulation by an organism or otherwise, at first seemed a possibility. The extension of the black tumors into unpigmented areas of the mouth, gill chamber, pharynx, and even

down into the stomach (according to Edwards), and the origin of the tumor in the mouth, as observed in one case, did not favor this view. The presence of the black granules throughout the tumor and the growth of black colonies in culture rendered the idea untenable.

That the minute black granules observed under the higher powers of the microscope could be bacteria did not at first seem likely. Bacteria which secrete any form of pigment intracellularly are rare, and the granules are so minute (averaging only one-third of a micron) that little could be made of them, even under the oil immersion, though diploid individuals were common enough. The conviction was finally forced upon me that we have to deal with a bacterial tumor of slow growth and of a rather benignant type, which, however, bids fair to destroy the catfish in this pond and which, if permitted to spread, may become a menace over a larger area.

Cultures and inoculations were made to determine whether the organism could be grown or transmitted. In this work I was fortunate to have the assistance and advice of Dr. W. W. Browne, of the College of the City of New York, and Miss Helen Mitchell, of Yale University, bacteriologists on the station staff for the summer of 1919.

It was found very difficult to grow the organism in cultures, but a measure of success was achieved by combining extract of catfish tissues with agar, according to a commonly approved procedure of bacteriological study. The growth was so slow that at the end of two weeks the largest colonies were only 4 or 5 millimeters in diameter. Contamination of the cultures was thus almost unavoidable, though a few of the many cultures made were successful to the extent above indicated. Incubation at higher temperature did not seem to hasten the growth, and cultures at air temperature grew about as well. The colonies in culture have the same intense black color as the tumors, and, on microscopic examination, the cocci showed the same size and appearance.

Inoculations of uninfected catfish of the same species from another pond were made by the following methods: (1) By hypodermic injection of the black fluid from a tumor; (2) by scraping the skin and rubbing in portions of a fresh tumor; and (3) by grafting portions of tumor under the skin. The catfish so inoculated were left at Woods Hole until the end of October, two months after inoculation, when they were preserved in formalin and forwarded to me. Some of these showed undoubted tumors of small size, indicating that the disease is directly transmissible (fig. 9). Unfortunately some of the fishes were allowed to become mixed up after I left the laboratory, so that it is impossible for me to state whether one method was more successful than another, though there are specimens of all three types that produced tumors.

The organism causing the tumor appears to be a very minute Coccus, and seems to have been hitherto undescribed. It is perfectly round; it averages about one-third of a micron in diameter, ranging from one-fourth to one-half a micron; and it secretes endogenously a black pigment. Unstained smears show the organism, because of the pigment, as well as stained preparations. In smears the individual cocci are not black when viewed under the oil immersion lens, but are distinctly dark. In mass they appear absolutely black. Diploid forms, indicating cell

division, may be observed commonly both in living and preserved material. Not being a bacteriologist, the writer will not attempt to describe or name the species.

As to the fatality of the disease, there is no definite information. Doctor Jones informs me that he has seen no dead fish, but the nature of the pond is such that they would not readily be observed, for the shores are brushy to the water's edge and there are no beaches. Moreover, there is much vegetation in the pond, especially near the shores, which would help to make the discovery of floating dead specimens difficult. Certainly the disease is not rapidly fatal, for fish with well-developed tumors have been kept under observation in aquaria in the laboratory for six weeks without showing any changes except for some slight growth in the area and thickness of the tumors. It would thus appear that there is no active systemic poison or toxin liberated, at least at the time of the year during which these studies were made. Fish with large tumors were taken with hook and line, even those with large tumors on the jaws accepting the bait readily. However, it was noted that old tumors, from which the epidermis had been lost, were frequently infected secondarily with *Saprolegnia*, and there seems little doubt that this fungus, which only enters abraded surfaces, would soon cause the death of the fish.

Doctor Jones believes that the disease has spread very rapidly since the summer of 1916, when its presence was first observed. In 1917 about half of the fish taken had tumors of various sizes and degrees of development. In the summer of 1919 it was a difficult matter to find any uninfected catfish in this pond. No doubt the habits of the bullhead in schooling together make the transmission of the disease an easy matter. It was not noted on other species of fish in this pond during my work there nor on the same species of catfish in neighboring ponds. That this disease is capable of attacking the bullheads in other ponds is evident from my inoculation experiments. Under date of February 2, 1921, Doctor Jones wrote me as follows:

> A man who has been fishing through the ice on this pond tells me that he caught a pickerel the other day which was covered with the same dark colored spots observed on the hornpout. Unfortunately he had disposed of the specimen, but so far as I know this is the only instance where the disease has been observed on any fish except the hornpouts.

As the evidence in this case is not complete, it may be well to suspend judgment as to whether the disease is capable of attacking other species than the bullhead.

In the course of my work no measures satisfactory for the control of the disease have been suggested. Salt and potassium permanganate solutions, both of which are useful in the control of *Saprolegnia*, were tried on individual specimens, but without any noticeable beneficial effect. Of course, it should hardly require mentioning that no species of fish from this pond, whether showing such infection or not, should be planted elsewhere.