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The apparent regularity of spring and early summer fish kills, which occur in varying intensities throughout a wide variety of Minnesota lakes, has been the subject of great interest to our biologists and general public. A large number of physical, chemical, ecological and biological causes of these mortalities have been established. Factors such as availability of dissolved oxygen, presence of pollutants, density of the fish population, parasites, pathogenic microorganisms, etc., must all be considered in the process of ascertaining the cause of a mortality.

A sizeable proportion of the fish kills, which were investigated between January and June of 1957, yielded fish specimens that were almost without exception infested or infected with the pathogenic myxobacterium, *Chondrococcus columnaris*. Out of this observation arose the present study, one of a series of investigations into the nature of bacterial diseases of Minnesota fish.

Columnaris disease, which is a malady of several genera of North American fishes, is caused by the myxobacterium *Chondrococcus columnaris*. The disease is commonly associated with a progressive necrosis of the fins, gill filaments and musculature, and is characterized by the presence of the specific pathogen in the lesions and the absence of any tissue hyperplasia.

Columnaris disease has been recognized as a pathological entity since Davis (1922) reported on a case of severe fish mortalities from the Mississippi River near Fairport, Iowa. He named the microorganism which he found in the necrotic tissues *Bacillus columnaris*. The descriptive species name assigned to this organism, which alludes

to the column-like configuration of the fruiting cells, was given at a time when the myxobacteria group was imperfectly known. The gathering together and heaping up of vegetative cells that begins the period of fructifaction or encystment, which Davis considered to be a monospecific characteristic of *B. columnaris,* is now recognized as part of the complex colonial or communal existance shared by many species of the myxobacteria.

Davis' nomenclature was changed after an extensive study of Columnaris disease was made in 1942 by Ordal and Rucker. While investigating an epizootic among salmon fingerlings at Leavenworth, Wash., these investigators found the causative agent was pathologically and morphologically indistinguishable from *B. columnaris.* Upon recognizing the myxobacterial nature of Columnaris disease, the organism was identified as a new species of *Chondrococcus.* Ordal and Rucker (1944), acknowledged the account of *B. columnaris* by Davis (1923) as a former observation, and retained the epithet "columnaris" in renaming the organism *Chondrococcus columnaris.*

The causative organism of Columnaris disease possesses a striking resemblance to certain of the protozoa (*Mycetozoa,* or "slime animals"). Similar to the myxamoebae which produces a fructifaction stage, *C. columnaris* gives rise to columnar and branching fruiting cells out of which small coccoidal or oval resting cells (microcysts) are formed. A gelatinous slime hardens over the microcyst thereby protecting it from drying and other unfavorable conditions. These cells measure 0.7 to 1.2 microns in diameter. The vegetative phase of the myxobacterium is rod shaped, varying in length from 4.0 to 8.0 microns, and in breadth from 0.5 to 0.7 micron. The cells lack typical bacilli ridgidity and appear in a variety of shapes such as loops, commas and s-shaped forms, and in this respect bear a resemblance to the Spirochetes. The cells do not possess flagella or cilia but move by means of a twisting-gliding action, secreting slime as they advance and possibly deriving some motility from this secretory process. The organism is gram-negative.

In active lesions the organisms are very numerous, and they show a marked tendency to occur in compact masses with their sides roughly adjacent to each other and perpendicular to the attachment surface. The cell aggregates held together by the gelatinous material

which they have secreted, can be readily demonstrated in a wet mount of lesion material examined with the 43 mm. microscope objective. *In vitro* studies have shown that these cells are in the primary encystment stage (Rucker, Earp, and Ordal 1954).

A number of different culture media for the cultivation of *C. columnaris* have been used, but by far the most satisfactory is a semi-solid tryptone agar. The colonies have a yellow color and are flat and spreading. In nutrient broth inoculated with diseased tissue, the growth of typical flexing vegetative cells and columnar and branched fruiting bodies is enhanced. Resting cells or microcysts develop in about a weeks time from the infusion when incubated at 22 C.

As in many other infections, there is evidence that there are different strains of *C. columnaris* which vary in their virulence to fish. Rucker and Ordal (unpublished data), isolated a strain of this organism from Columbia River salmon which proved highly pathogenic to young salmon with death resulting 12-24 hours after exposure by contact or ingestion. A fatty substance, believed to be present in in the bacterial cell membrane, has been found in comparatively greater amounts in virulent strains than in less virulent strains.

It seems possible that many of the puzzling variations in the pathogenicity of *C. columnaris,* as well as the sporadic nature of Columnaris disease outbreaks in Minnesota waters, might be explainable on the basis of a genetic recombination in the highly specialized life history of the myxobacterium.

The mode of transmission of the pathogen is not yet known although direct contact with active cases or carriers of the disease is a tenable hypotheses. The writer has found *C. columnaris* in the body slime of fish in the absence of any symptoms, and even some time after symptoms have subsided. Water destratification, in stirring up quiescent forms of the pathogen from a lake bottom, might also be considered as a possible means of transmission.

The following epidemiological data was obtained from fish kill investigations made on 13 different Minnesota lakes between April 1 and July 31, 1957. The total number of lakes which experienced a notable fish mortality during this period is understandably greater, but on the basis of past experience, there were probably only a few

major fish kills which were not reported at all. Several others were in the terminal stages when investigated and did not allow for meaningful interpretation, while the rest of the outbreaks did not permit on the spot inspection and so although duly noted, were not used in this study. Data obtained during the approximate peak of mortality are used whenever available and consistent with the method employed for securing fish specimens.

Dead and moribund fish specimens were examined along 100 foot intervals of a randomly selected stretch of 500 feet of leeward shoreline. Species were individually tallied for each 100 foot interval and noted for any gross pathological processes. The total number of identical species found along the 500 foot stretch were each divided by 5 to obtain an average number of each species per 100 feet of shoreline. Fresh specimens with demonstrable lesions were brought to the laboratory for pathological examination.

This study showed that the greatest period of fish kill activity took place during the month of June in lakes of relatively high water fertility. The fish kills seemed to follow a geographical pattern. The first seven outbreaks occurred in comparatively small southern lakes such as Gervais, Phalen, and Johanna in Ramsey County. Subsequent outbreaks occurred in most instances progressively further North, while the last two mortalities investigated were at Sand Lake, Pine County and Sullivan Lake in Morrison County. If this apparent northerly migration or progression of fish kills has any basis in fact, it might be explained in the future on a temperature lag phenomenon. Columnaris disease is known to attack fishes only at comparatively high water temperatures (Davis, 1946 and 1953).

The predominant species of fish involved in these mortalities were in the order of their occurrence; the northern black bullhead (*Ameiurus melas*), black crappie (*P. nigro-maculatus*), bluegill (*Lepomis machrochirus*) and the perch (*Perca flavescens*). Various year classes were represented and mortalities were much higher among the stunted populations. Both sexes were found with equal frequency.

Bacteriological examinations demonstrated that the greater proportion of fish kill specimens were infected with Columnaris disease.

Necrotic lesions in the gills, fins, skin and skeletal muscles occurred with a high degree of frequency. The infection of the gills appeared to begin on the tips of the gill filaments and then progress towards the gill arch, producing a pasty grayish-green liquefactive necrosis of the filament tissue as it advanced. Spreading of the infection to adjacent filaments anywhere along the course of the primary lesion was noted. Multiple lesions in various stages of morbidity and complete involvement of the gills in severe cases were found. The membranous tissue of the fins and later the more resistant fin rays were attacked by the myxobacterium. The infection of the skeletal muscles seemed to arise as a result of an abrasion or puncture of the epidermis. Extensive muscle tissue necrosis associated with hyperemia and hemorrhaging was produced. All lesions conspicuously lacked any evidence of hyperplasia or effective leukocytolytic activity.

Diagnosis of Columnaris disease was made on the basis of gross pathology and confirmed by demonstrating the readily identifiable *C. columnaris* by microscopic and cultural techniques.

Pyridymercuric acetate has been found effective in reducing morbidity in hatchery fish which have become infected with Columnaris disease. (Burrows and Palmer, 1949 and Snieszko, 1954). The method of treatment consists of exposing the fish to a concentration of 2 p.p.m. of P. M. A. in the water for one hour. Several treatments may be instituted without fear of harmful side reactions to the fish. The prognosis in subacute infections is excellent.

The eradication of Columnaris disease in fish from lakes and streams is a separate problem which is under study at this time. However, there is little indication on the basis of our knowledge of the magnitude of fish loss due to this disease, that a fish population can be substantially reduced as a result of this periodic mortality.

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