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A POST-EPIZOOTIC SEARCH FOR FURUNCULOSIS IN A WARM-WATER FISH POPULATION[1]

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Abstract: Specimens were taken from the kidneys of 777 fish of 10 species from Clear Lake, Iowa, in 1970 and 1971 to determine the carrier rate of *Aeromonas salmonicida* after a 1968 furunculosis epizootic that destroyed most of the yellow bass population. *A. salmonicida* was not detected in any fish examined. Serum from 5 of 46 yellow bass old enough to have been present during the 1968 epizootic contained agglutinins to *A. salmonicida.*

INTRODUCTION

In May 1968, a furunculosis epizootic occurred in Clear Lake, Iowa yellow bass, *Morone mississippiensis,* and destroyed most of the population. Bulkley[3] attributed inadequate nutrition resulting from overpopulation as a causal factor. An earlier die-off in 1965 was also attributed to furunculosis.[1] These two epizootics were the first documented infection of yellow bass with *Aeromonas salmonicida,* the organism causing furunculosis in fish. The purpose of this study was to determine the carrier rate of furunculosis in surviving yellow bass and in other fish species in Clear Lake.

McGraw[14] and Herman[8] reviewed the many studies carried out on *Aeramonas salmonicida.* Furunculosis was so widespread in the United States and Canada in the early 1930's that the entire system of artificial propagation of salmon and trout was threatened. The disease also occurs in natural bodies of water and is being detected in additional species of nonsalmonid fishes as time goes on.[12]

Clear Lake is a natural eutrophic lake of 1,474 hectares located in northcentral Iowa in western Cerro Gordo County. Bailey and Harrison[2] listed 23 species of fish present in the lake. Yellow bass were introduced in 1932 and have fluctuated widely in abundance since that time.

MATERIALS AND METHODS

Biweekly samples of fish were collected by gillnet June 1 to Sept. 1, and bimonthly samples from Sept. 1 to June 1 during the ice-free period. Sampling started July 18, 1970, and ended Aug. 21, 1971. Scale samples were taken from each fish for age determinations. A piece of kidney tissue was taken from each fish and streaked on a "Bacto" furunculosis agar (Difco) slant. The slants were incubated at room temperature for 24 to 48 hr. Tubes containing no bacterial growth were then discarded.

Isolates from fish were identified presumptively by using the schematic outline suggested by Bullock.[5] Motility was determined by the hanging-drop method and also with Motility Test Medium (Difco). Production of brown pigmentation after prolonged incubation was used to presumptively identify *A. salmonicida.*

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Blood was taken from yellow bass by cardiac puncture, allowed to clot, and stored for 1 to 3 days under refrigeration. Presence of serum antibodies capable of agglutinating *A. salmonicida* was determined by using a slide agglutination test. A loopful of the undiluted serum and heavy suspension of viable bacteria was used (the suspension was not quantitatively controlled). The slide was rocked by hand for 3 minutes and flocculation observed under a 10x dissecting microscope. A control containing sterile saline instead of serum was provided for each sample. The strain of *A. salmonicida* used for antibody agglutination tests was obtained from the Eastern Fish Disease Laboratory, Bureau of Sport Fisheries and Wildlife, Leetown, West Virginia.

In an attempt to create conditions under which any fish carrying *A. salmonicida,* either in the kidney or other organs, would develop a generalized infection such that the probability of isolating the pathogen would be increased, yellow bass were captured and held in the laboratory under conditions of high temperature and low oxygen.

In April and May 1971, 66 yellow bass were transported live to the laboratory. These fish were all of sufficient age to have been exposed to *A. salmonicida* in the 1968 epizootic. The fish were in two groups, 10 fish on April 25 and 56 fish on May 8. Fish in the first group were held individually in aerated aquaria for 36 hr at 15 C (approximately lake temperature) for acclimitization. The water temperature was then raised 3 C each day until 100% mortality occurred. Aeration was stopped for 12 hr when the temperature was 33 C. Tanks were checked twice daily, dead or dying fish were removed, and specimens were taken from the kidneys. The second group of 56 fish was held under the same conditions, except that four fish were placed in each tank and the water aerated continuously.

RESULTS

Specimens were taken from the kidneys of 777 fish of 10 species in 1970 and 1971. A total of 369 yellow bass were sampled during the 2 years. Other species tested included 57 white bass *(Morone chrysops),* 87 walleye *(Stizostedion vitreum),* 54 yellow perch *(Perca flavescens),* 80 crappie *(Pomoxis* sp.), 76 black bullheads *(Ictalurus melas),* 47 northern pike *(Esox lucius),* 3 white suckers *(Catostomus commersoni),* 2 muskellunge *(Esox masquinongy),* and 2 channel catfish *(Ictalurus punctatus). A. salmonicida* was not detected in any fish tested, although several known bacterial fish pathogens commonly were found.[16]

A pigment-producing bacterium, presumptively identified as a *Pseudomonas* sp., was isolated from four fish during the 2 years. This rod-shaped organism was gram-negative, motile, cytochrome-oxidase positive, and did not oxidize or ferment glucose, but produced red-brown pigment after 10 to 14 days incubation similar to that produced by *A. salmonicida.* Colonial morphology also was similar to *A. salmonicida,* except that the colonies were a pinkish-orange rather than whitish-gray. Ross[15] isolated a strain of *A. liquefaciens,* from juvenile silver salmon, which also produced a red-brown pigment.

Blood samples removed from yellow bass in 1971 were tested to see if fish surviving the epizootic in 1968 still retained agglutinins to the bacterium, even though *A. salmonicida* could not be cultured from the kidneys. Krantz et al.[11] found hatchery trout with naturally acquired agglutinins to *A. salmonicida.* They concluded that the most probable cause for these antibodies was a subclinical infection of furunculosis. In mammals, recovery from infection often imparts immunity against re-infection. Fish produce antibodies in a manner similar to higher vertebrates, but production is slower, and the titer remains high for a longer time.[10] Of 64 fish tested for the presence of agglutinins, 40 were old enough to have been present as subadults or adults (4 years or older) during the 1968 epizootic. The period of incubation for yellow bass in Clear Lake is from 2 to 2¾ days,[1] and the spawning period in 1968 lasted from April 30 to May 14.[4] Therefore, fish 3 years old in 1971 would have been present as fry during the epizootic. Serum from 5 of the 64 fish tested contained antibodies that agglutinated *A. salmonicida* (Table 1). All positive tests

were from fish 4 years and older. Serum from one of the five fish strongly agglutinated the cell suspension, whereas four produced only weak agglutination. Hence, after 3 years, a small portion of the yellow bass population contained antibodies that agglutinated *A. salmonicida* suggesting previous exposure to the furunculosis-producing organism.

In April and May 1971, 66 yellow bass were transported live to the laboratory in an attempt to create conditions under which fish carrying *A. salmonicida* would develop a generalized infection. *A. salmonicida* could not be isolated from any fish held in this manner even though daily mortality suggested they were under constant stress.

TABLE 1. Age composition of yellow bass captured in 1971 and numbers possessing antibodies capable of agglutinating **A. salmonicida.**

	Age group		
	II	III	IV and older
Number of fish sampled	18	6	40
Number of fish possessing antibodies	0	0	5

DISCUSSION

Several explanations were considered for not detecting *A. salmonicida* during the course of this project. The first consideration was that the organism causing the previous epizootics was incorrectly identified. This possibility was discarded because verification by the Eastern Fish Disease Laboratory, based on serum agglutination tests, confirmed the original presumptive identification. Also, serum from 5 of 64 bass examined by me contained antibodies which agglutinated *A. salmonicida,* suggesting previous exposure to the organism.

The second possibility is that the bacterium may no longer be present in the lake. All carriers might have died, and remaining fish might have been resistant to the organism. This explanation is not likely. Fish resistant to the disease may act as carriers indefinitely.[17]

Another possible explanation is that the bacteria may have been distributed in organs other than the kidney and hence not detected. Although culture methods are adequate for detecting *A. salmonicida* in a population of salmonids that have just experienced an outbreak of furunculosis, they are not always adequate for reliable detection of the bacterium in carriers.[6] Klontz[9] using immuno-fluorescent techniques, found *A. salmonicida* in the gut wall of wild suckers, but he could not culture the organism. McDermott[13] could not culture *A. salmonicida* from the kidney, but did so from the other organs. In instances of latent infection, however, *A. salmonicida* usually is present in the posterior kidney.[17] Perhaps if immunofluorescent techniques had been used in my study, the organism would have been detected.

Klontz reported that suckers netted out of a hatchery water supply were negative to bacteriological culture, but were considered incubatory carriers of *A. salmonicida* because, 7 to 10 days after a severe physical or thermal stress, they would become clinically ill with furunculosis. *A. salmonicida* has not always been isolated from the kidney even though found in the gut, liver, spleen, reproductive organs, or the heart.[18] Bullock and McLaughlin[7] indicate that environmental stresses may trigger outbreaks of infectious diseases; however, the organism could not be isolated from yellow bass held by me under prolonged conditions of temperature and oxygen stress.

Yet another possibility for not detecting the organism is if larger numbers of yellow bass could have been sampled, the pathogen might have been detected. Carrier rate after an epizootic usually is

very low. McGraw,[14] for example, cited a carrier rate of only 2.7% after an experimental epizootic. In my study, only 120 of the 369 yellow bass sampled were present during the 1968 epizootic.

A small number of fish in my study showed presumptive serological evidence of past exposure to *A. salmonicida*. Krantz et al.[10] found that brown trout injected with formalin-killed cells had a low nonpersistent antibody response, which lasted only 3 months. Trout injected with cells, plus an adjuvant, maintained high titers for 24 months after injection. One yellow bass serum from Clear Lake caused strong agglutination, indicating that the fish may have been reexposed to *A. salmonicida* sometime following the 1968 epizootic. This suggests that the organism is presently in the lake even though not isolated in my study.

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