SEASONAL PREVALENCE OF Chondrococcus columnaris INFECTION IN BLACK BULLHEADS FROM CLEAR LAKE, IOWA *

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SEASONAL PREVALENCE OF *Chondrococcus columnaris* INFECTION IN BLACK BULLHEADS FROM CLEAR LAKE, IOWA*

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Abstract: The prevalence of asymptomatic columnaris infection in black bullheads *(Ictalurus melas)* from Clear Lake, Iowa, during 1971 was the highest during the spring when the lake was warming, very low as summer approached and nil from July 28 through the autumn.

The mean condition factor of adult black bullheads decreased through the month of June and fluctuated at a lower level through the remainder of the summer and autumn. Mean hematocrit of adult black bullheads followed the same trend as that of condition factor. A positive correlation was evident between weekly mean condition factor and hematocrit ($r = 0.90$; $P = 0.01$).

INTRODUCTION

Spring mortalities of black bullheads, *Ictalurus melas* (Rafinesque), from Clear Lake, Iowa, have been attributed to columnaris disease, recently reviewed by Bullock, Conroy and Snieszko.[4] The disease is characterized by the appearance of grayish-white or yellow areas of erosion surrounded by a reddish border that marks the edge of the advancing infection. Damage to the gills, followed by respiratory distress, is the probable cause of death. My study was conducted in 1971 to determine the incidence of both symptomatic and asymptomatic columnaris among black bullheads in the lake.

Clear Lake is located in Cerro Gordo County in northcentral Iowa. The lake is eutrophic and measured 7.8 km long and 3.4 km in maximum width. Maximum depth of the lake is 6 m. Bailey and Harrison[2] described 23 species of fish found in the lake. The black bullhead has been the most abundant fish species in the lake since a mass mortality of yellow bass in 1968.

MATERIALS AND METHODS

Adult black bullheads from Clear Lake were collected during 1971 with experimental gillnets on a semimonthly basis in spring, weekly in summer and monthly in autumn. An effort was made to collect 30 fish per sample. Total length and weight of each fish were recorded. The coefficient of condition KTL which describes the condition or plumpness of a fish was later calculated using the formula:

$$K_{TL} = \frac{W10^5}{L^3}$$

where W = weight in grams

L = total body length in millimeters

A bacterial sample was taken with a cotton swab from the gills of each fish and from any external lesions present. The sample was cultured on Cytophaga agar,[1] a medium containing 0.05% tryptone (Difco), 0.05% yeast extract, 0.02% sodium acetate, 0.02% beef extract, and 0.9% agar (Difco), adjusted to pH 7.2 to 7.4 with 0.1N NaOH. To suppress the growth of bacteria other than *C. columnaris*, 5 μg/ml Neomycin and 10 IU/ml Polymyxin B were added to the medium.[5]

Identification of the isolated *C. columnaris* was made on the basis of characteristic rhizoid shape, the yellow color of

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the colonies and slide agglutination test.[1,8] Serum for the agglutination test was prepared by subcutaneously injecting female laboratory rabbits with *C. columnaris* plus a complete (Difco) Freund adjuvant.[1] The culture of *C. columnaris* used for the injection was obtained from the Western Fish Disease Laboratory of the Bureau of Sport Fisheries and Wildlife, Seattle, Washington. The serum was divided into 2-3 ml amounts and frozen without a preservative.

Two blood samples were taken in commercially heparinized capillary tubes from the dorsal aorta of each fish after severing the caudal peduncle.[11] Tubes were centrifuged for 3 min in a microhematocrit centrifuge before the hematocrit was recorded. Sex of the fish was then determined.

To determine seasonal nature of fish mortality in Clear Lake, the shoreline was divided into 10 segments. Within each segment, a 45 m sampling site was arbitrarily selected. This site was divided into three 15 m sections. The number, species, and size of dead fish in each segment were recorded at semi-monthly intervals from late April until the end of August and monthly during the autumn. Lake temperature data for comparison with incidence of disease were obtained from the Clear Lake City Water Treatment Plant.

RESULTS

The prevalence of columnaris infection in live Clear Lake bullheads was found to be seasonal, occurring primarily during the spring. Prevalence rose sharply during mid-May to 60% and then decreased to 0% as summer approached (Fig. 1). The trend in percentage carrier rate was related to water temperature. Prevalence of columnaris infection was highest as water temperature was increasing during the spring and reached a peak at the time when the water warmed to 15 C. As temperature continued to rise, prevalence dropped. Two small prevalence peaks

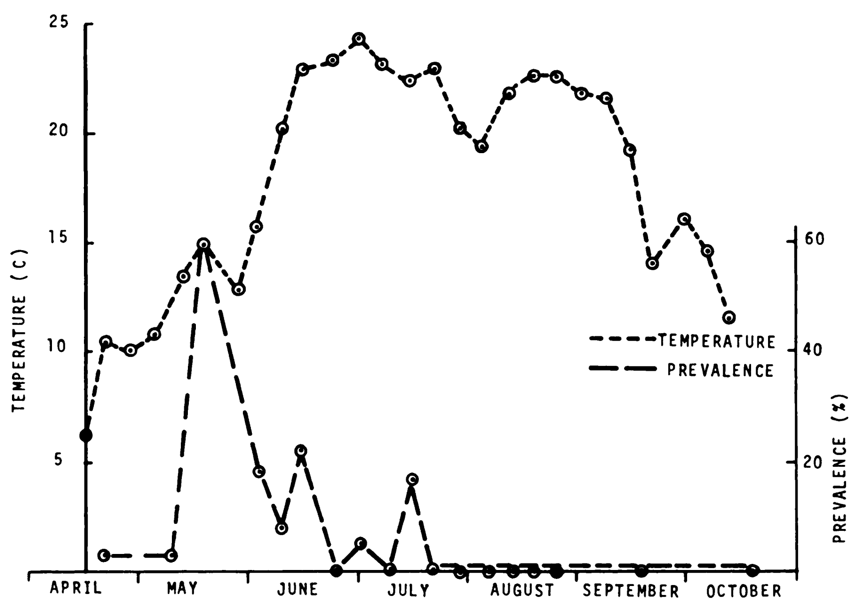

FIGURE 1. Prevalence of **C. columnaris** in Clear Lake bullheads, 1971, in relation to mean weekly water temperature. Lines connect data points.

occurred when the water temperature was approximately 23 C. At other times during the summer prevalence was very low or zero.

Mortality of bullheads was observed in mid-May during the period of highest columnaris carrier rate. Bacteria cultured from surface lesions of live bullheads collected while mortality was occurring were gram-negative, aerobic, motile rods, presumptively identified as *Pseudomonas* sp.[3] A flagellated protozoan was abundant in wet mounts of body slime. *C. columnaris* was found in later stages of the epizootic.

Shoreline counts of dead bullheads showed the same general trend as columnaris prevalence in live fish sampled (Fig. 2). The largest number of dead fish was found in the sampling areas during the mid-May die-off. Expansion of the sampling data gave an estimate of 2,081 dead bullheads on the entire Clear Lake shoreline at that time. Estimates on other dates were much lower. Shoreline counts decreased as summer approached.

Mean hematocrit levels in black bullheads decreased through the month of June from 44.9% on 4 June to 24.1% on 1 July and then fluctuated slightly during the summer (Table 1). Analysis of variance indicated significant differences between the sample means ($p=.05$; $df=13, 384$).

Body condition, KTL, followed the same trend as that of mean hematocrit (Table 1). The correlation coefficient between mean weekly KTL and mean weekly hematocrit was 0.90, significant at the 0.01 probability level ($df = 13$). A large decrease in KTL from 1.53 to 1.32 occurred during the month of June. During the remainder of the summer KTL values fluctuated. Analysis of variance showed significant differences between sample means ($p = .05$; $df = 16, 485$).

DISCUSSION

The prevalence of asymptomatic *Chondrococcus columnaris* infection in bullheads taken from Clear Lake was highest during the warming of the lake in the spring. The point of highest prevalence (60%) corresponded to the water warming to 15 C. Snieszko and Ross[13] described 15 C as the temperature at which high-virulence *C. columnaris* becomes a problem. Two smaller prevalence peaks could have been due to the low-virulence strain that usually causes outbreaks near 21 C.

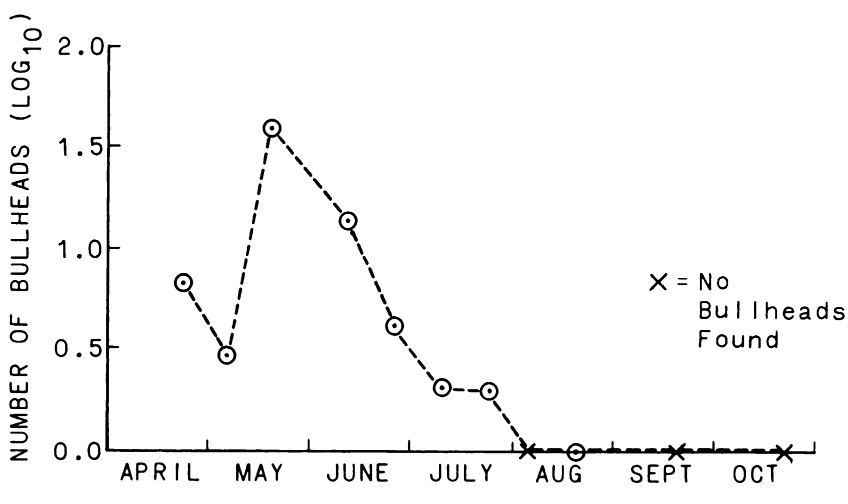

FIGURE 2. Numbers of dead bullheads found at selected shoreline sampling sites, 1971, Clear Lake, Iowa.

TABLE 1. Mean hematocrit and condition factor (KTL) of adult Clear Lake black bullheads (220-308 mm TL) collected 1971.

	Hematocrit				Condition factor			
Date	Sample size	Range	Mean	Standard deviation	Sample size	Range	Mean	Standard deviation
4 June	22	29-57	44.9	5.8	32	1.07-2.00	1.53	0.20
8 June	30	23-55	43.6	6.7	30	0.78-1.85	1.48	0.17
15 June	28	21-51	38.0	7.6	28	1.16-1.75	1.46	0.13
23 June	30	16-46	33.5	8.2	30	1.00-1.73	1.42	0.16
1 July	28	15-38	24.1	5.4	29	1.12-1.53	1.32	0.11
9 July	19	15-41	25.9	7.7	19	1.06-1.46	1.29	0.12
14 July	35	10-40	24.3	8.6	36	0.97-1.61	1.39	0.13
21 July	35	8-36	25.4	6.8	36	0.98-1.55	1.32	0.13
28 July	30	16-39	27.8	6.3	30	1.20-1.56	1.39	0.08
4 Aug.	29	14-39	25.4	5.7	30	0.95-1.58	1.32	0.13
11 Aug.	30	16-38	26.8	5.8	30	1.01-1.68	1.32	0.15
19 Aug.	30	19-39	30.2	5.6	30	1.12-1.56	1.35	0.13
24 Aug.	28	19-44	30.1	5.9	28	1.02-1.50	1.31	0.12
18 Sept.	24	15-41	27.0	6.9	24	0.94-1.61	1.33	0.15
16 Oct.	30	16-43	32.8	6.7	31	1.16-1.71	1.40	0.14

The high spring prevalence of the pathogen is not unexpected. After the winter fast, blood proteins in fish are at a low level and fish are likely to be anemic.[10,12] Also, when water temperature is low, antibodies are not produced by fish so that immunologically, fish in moderate climatic zones are at their weakest point in early spring.[12]

The hematocrit, or percentage of packed red blood cells in the blood, has been used to detect anemia in fish.[7] Hematocrit values can be used to indicate some types of malnutrition, chronic disease, or disturbances caused by environmental conditions.[11] When a fish becomes diseased, hematocrit values usually are lower. There are limitations to using the hematocrit as an indication of health, because fish may undergo rapid changes in hematocrit values due to stress.[11] Hence, readings should be taken as soon as possible after capture. If a waiting period is necessary, fish should be held under as near "natural" conditions as possible. Dilution of blood samples by external moisture on the fish also may affect hematocrit values if the fish is not blotted dry.[6] In my study, an effort was made to standardize the sampling procedure so that hematocrit readings would provide valid comparisons between samples.

Hematocrits of Clear Lake bullheads were high in early June just after high prevalence of columnaris. The values probably reflect the normal seasonal trend in hematocrit because channel catfish hematocrits tend to be higher during cold times of the year (F. P. Meyer, personal communication). Through the month of June, the hematocrit values dropped, as did the condition factor values. The drop was attributed to warming water temperatures. Spawning was at least a factor in lowering body condition. The summer fluctuations of hematocrit and condition factor were evidently due to changes in water temperature, food availability and other stress factors.

Death of bullheads periodically observed along the lake shoreline could not be attributed directly to columnaris disease, but close correlation between columnaris carrier rate in live bullheads and numbers of dead individuals suggested that columnaris was involved. The observed mortality in May was attributed to *Pseudomonas* sp. infection with only secondary involvement by *C. columnaris,* but in a concurrent study, Schwartz[9] detected no seasonal trend in *Pseudomonas* carrier rate among bullheads during 1970 and 1971. Mean bullhead carrier rate of *Pseudomonas* and *Aeromonas liquifaciens* combined was 19.8% during the warm months of the year.

Both columnaris and the pseudomonads are facultative agents of fish disease. That is, they are common residents of surface waters and usually become pathogenic only when natural resistance of the host fish is weakened by some stress. The high prevalence of these organisms in bullheads during 1971 suggest that whenever this fish species becomes seriously stressed in Clear Lake, infection by a pseudomonad or *C. columnaris* and ensuing disease is likely.

Acknowledgements

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