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THERMAL EFFLUENT AND THE EPIZOOTIOLOGY OF THE CILIATE *EPISTYLIS* AND THE BACTERIUM *AEROMONAS* IN ASSOCIATION WITH CENTRARCHID FISH[1]

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ESCH, G. W., HAZEN, T. C., DIMOCK, R. V., JR. & GIBBONS, J. W. 1976. Thermal effluent and the epizootiology of the ciliate *Epistylis* and the bacterium *Aeromonas* in association with centrarchid fish. *Trans. Amer. Micros. Soc.*, 95: 687–693. *Epistylis* sp. (Ciliophora: Peritricha) is a facultative ectosymbiont of fresh-water fishes in streams and lakes throughout the southeastern U.S. In combination with the gram-negative bacteria *Aeromonas hydrophila*, epizootic outbreaks of *Epistylis* have been implicated in the death of many thousands of game fish, primarily striped bass and several species of Centrarchidae (sunfish), in several reservoirs in North Carolina. While these epizootics have been correlated with high levels of organic loading, recent studies suggest that thermal loading from a nuclear production facility may influence the level of infection by *A. hydrophila* and *Epistylis* sp. (A-E complex) on centrarchid sunfish in Par Pond, Savannah River Plant, Aiken, S.C. Utilizing electro-fishing techniques, in excess of 11,500 sunfish, representing six different species, were collected and examined for external lesions associated with the A-E complex. Largemouth bass (*Micropterus salmoides*) were most heavily infected, followed in incidence of infection by bluegill (*Lepomis macrochirus*), warmouth (*Lepomis gulosus*) and redbreast sunfish (*Lepomis auritus*); lesions were not observed on the surface of black crappie (*Pomoxis nigromaculatus*). The body conditions (or K-factor) of more than 2,000 largemouth bass were calculated; direct correlations were found between thermal loading, body condition, and incidence of infection. These data are discussed in terms of thermal effluent and the epizootiology of *Epistylis* sp. and *Aeromonas hydrophila*.

A recent series of fish kills in several North Carolina reservoirs has been attributed to a combined epizootic outbreak of the gram-negative bacterium *Aeromonas hydrophila* and the peritrich ciliate *Epistylis* sp. (Plumb, 1973). Gamefish, including striped bass, *Roccus saxatilis*, and largemouth bass, *Micropterus salmoides*, appeared to be among the primary targets for infection.

[1] In order to collect and process 11,500 fish, it is necessary to have a great deal of assistance. Accordingly, we want to thank the technicians and students of the Savannah River Ecology Laboratory who gave so generously of their time in making this effort possible. We also want to thank Dr. Michael Smith, Director, S.R.E.L., for assisting in making the study possible. Mr. William Strack helped with the computer analyses and Mrs. Jean Coleman aided in preparing the figures. This research was supported by Contract AT(38-1)-819 between ERDA and the University of Georgia and Contract E(38-1)-900 between ERDA and Wake Forest University.

While *A. hydrophila* and *Epistylis* sp. are facultative ectosymbionts, they also ubiquitously occur in the water column and sediments of various lakes and ponds throughout the southeastern U.S. and elsewhere (Plumb, 1973; Shotts et al., 1972; Thorpe & Roberts, 1972; Trust & Sparrow, 1974; Vezina & Desrochers, 1971). Under appropriate conditions, each may become infective for fish. According to Rogers (1971), *Epistylis* sp. is the primary invader, producing scale erosion and pit-like lesions which may eventually cover up to 75% of the body surface of an infected fish. Secondary invasion, by *A. hydrophila*, may ultimately produce hemorrhagic septicemia and death. While several water quality characteristics have been associated with outbreaks of the *Aeromonas-Epistylis* (A-E) complex, the most frequently mentioned are increased organic loading and lowered dissolved oxygen (Meyer, 1970).

During the winter of 1973, a number of infected largemouth bass were observed in Par Pond, a cooling reservoir located on the Savannah River Plant (SRP) near Aiken, South Carolina. Preliminary observations suggested that thermal effluent may, in some way, have influenced the course of the disease among these fish.

There is a paucity of information regarding the epizootiological characteristics of these disease producing organisms. Further, there is virtually nothing known about the influence of thermal effluent on the disease. For these reasons, and because of the obvious economic impact of the disease in the southeastern United States, the present study was initiated in September of 1974.

The primary goals of the first year's study were: (1) to compare the incidence of infection by *Aeromonas* and *Epistylis* among centrarchid fish, (2) to follow seasonal changes of disease among centrarchid fish, and (3) to determine the influence of thermal effluent on the disease throughout a 12-month period.

Materials and Methods

The study site was the Par Pond reservoir located on the Energy Research and Development Administration's Savannah River Plant near Aiken, South Carolina. Par Pond is a 1,120 hectare reservoir which provides cooling water for a nuclear production facility. Water temperatures in the reservoir are variable, depending on the season of the year as well as on changes in reactor power levels. The average maximum temperatures at the heated end of Par Pond exceeded those in ambient areas by ca. 10 C (for a review of thermal conditions and a description of Par Pond, see Bennett & Gibbons, 1972 and Lewis, 1974). Ambient and thermally altered areas selected for study in Par Pond were separated by more than 4 km of open water.

During the first 12 months of study, in excess of 90% of the fish examined were taken by electro-fishing; the remaining fish were caught by hook and line. The total length of each fish was measured and individuals more than 15 cm in length were weighed, tagged, and released back into the reservoir. Each fish was carefully examined for lesions which, when found, were recorded on an infection-topography chart. Lesions were randomly checked for the presence of *Aeromonas* and *Epistylis*, the former by inoculation of scrapings onto agar plates of Rimler-Shotts media (Shotts & Rimler, 1973) and the latter by microscopic examination of scrapings from suspected lesions. In 100% of the lesions checked, the disease-producing organisms were isolated.

Results

From September 1974 through August 1975, ca. 11,500 centrarchid fish were checked for infection. Five species of centrarchids were taken with some degree

TABLE I
Percentage infection in four species of centrarchids from thermal and ambient locations

Location	Species			
	Lepomis macrochirus	*Micropterus salmoides*	*Lepomis auritus*	*Lepomis gulosus*
Ambient	4%	12%[1]	1%	5%
N =	3,586	1,466	1,954	318
Thermal	4%	18%[1]	1%	4%
N =	1,826	1,639	509	206

[1] Significant difference in ambient vs. thermal locations.

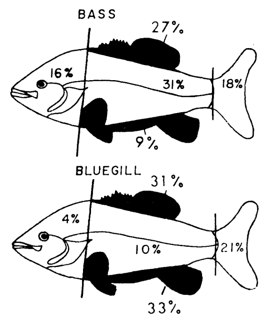
of frequency during the study period (Table I). The most commonly infected species (12%) was *Micropterus salmoides*, the largemouth bass. Approximately 4–5% of the bluegill, *Lepomis macrochirus*, and the warmouth, *Lepomis gulosus*, also were infected. The redbreast sunfish, *Lepomis auritus*, was rarely infected while the black crappie, *Pomoxis nigromaculatus*, was never found to be infected.

In addition to the differences in infection percentages observed among different species of fish, the location of lesions on the body surface of bass and bluegills was also quite different (Fig. 1). Examination of spatial distributions of lesions on bass showed that 65% of them were located in a zone along the body surface beginning at the anterior tip of the mandible, paralleling the lateral line, and continuing to the posterior end of the tail. For bluegills, on the other hand, only 36% of the lesions were situated in locations similar to those on bass while 64% were located on the dorsal, pectoral, pelvic, and anal fins (Fig. 1).

There was obvious variability among the four species of centrarchids as regards infection percentage and the location of lesions. However, only the largemouth bass appeared to be influenced by thermal effluent. A comparison of the infection percentages among largemouth bass on an annual basis revealed a definite pattern (Table II). The lowest infection percentages were in fall and winter, followed by a sharp rise in the spring and a subsequent decline in summer. In all seasons of the year, including summer, the levels of infection were higher among bass taken in thermally altered areas of Par Pond as opposed to those from ambient areas.

At least part of the seasonal variability can be attributed to changes in size structure of the bass population during the year. Large numbers of fingerling bass were taken in summer and early fall and a lower infection percentage among large numbers of fingerling bass would reduce the overall infection percentage for bass within these two seasons. Examination of bass by size class clearly showed that larger size classes of bass were more frequently infected than smaller ones (Fig. 2). Thus, infection percentages increased from 2% among bass less than 14 cm to 25% among bass greater than 44 cm in total length (Fig. 2).

The body condition, or K-factor, is indicative of the relative robustness of a fish (Bennett, 1972). Under certain conditions it may be considered a useful method for estimating the relative health or physical well-being of a fish. Our data indicate that body condition of bass from Par Pond changed from season to season (Fig. 3). The seasonal changes closely paralleled shifts in dissectable body fat (research in progress). Body condition was highest in winter, declined in spring and summer, and began to rise again in fall. While the differences in the magnitude of body condition were significant between bass taken in thermally altered and ambient areas, the annual pattern of change was the same within the collection areas.



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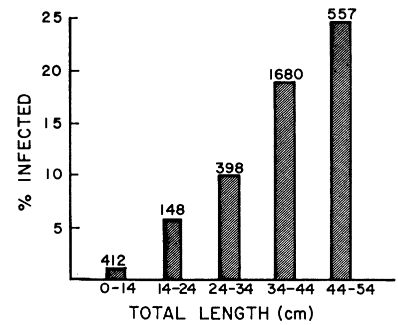

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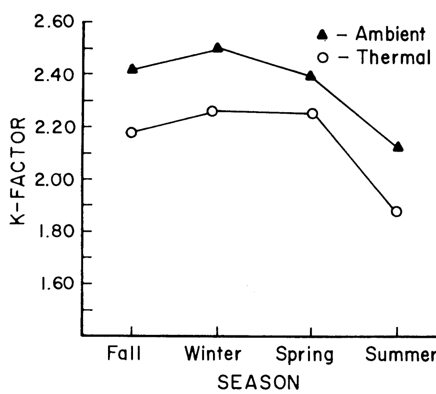

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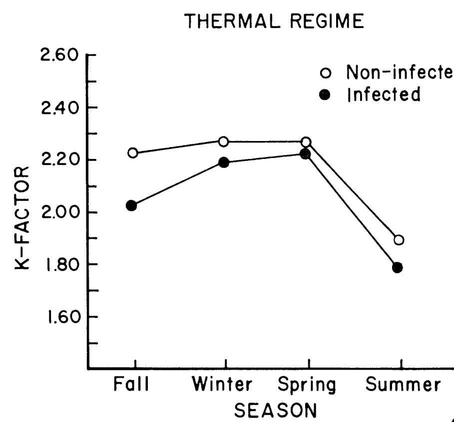

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Fig. 1. Comparison of spatial distribution of lesions on bluegill, *Lepomis macrochirus*, and largemouth bass, *Micropterus salmoides*. Fig. 2. Infection percentages by the *Aeromonas-Epistylis* complex in relation to the size class of largemouth bass. Note that the size classes range (in cm) from zero–14, 14–24, 24–34, 34–44, and 44–54. Fig. 3. Relationship between season and K-factor of largemouth bass taken in ambient and thermally altered areas of Par Pond. Fig. 4. Relationship between season and K-factor of infected and noninfected bass taken in thermally altered areas of Par Pond.

When K-factors of uninfected and infected bass, taken from thermally altered areas of Par Pond, are compared on a seasonal basis (Fig. 4), it is apparent that uninfected bass are in better body condition than infected individuals. Furthermore, the mean K-factors of uninfected and infected bass taken from ambient areas were significantly higher than those of both uninfected and infected bass taken in thermally altered areas of Par Pond (Table III).

Discussion

The levels of infection by *Epistylis* and *Aeromonas* among the five species of centrarchids examined from Par Pond were variable. This variation in infection cannot readily be attributed to any as yet identified environmental factor. It seems likely, however, it could be due to physiological or behavioral differences among the five species of centrarchids. Certainly, the differences in location of lesions on bluegills and largemouth bass would lend credence to this conclusion. Variability in infection levels among different species of fish has been previously

TABLE II
Per cent infected bass in ambient vs. thermal regimes by season

Temperature regime	Season				
	F	W	Sp	Su	Total
Ambient[1]	7% N = 134	6% 242	15% 499	14% 491	12% 1,466
Thermal[1]	11% N = 217	11% 426	29% 473	16% 523	18% 1,639

[1] Differences are significantly different between bass from thermal and ambient areas during all seasons of the year except summer; the totals are also significantly different.

reported by Plumb (1973), but the sample size of fish examined in that study was so low that conclusions regarding the occurrence of infections are questionable.

Among largemouth bass there is a strong correlation between size and the probability of infection, with a range from 2% infection among bass less than 14 cm in total length to 25% among fish between 44 and 53 cm in length. This observation could be related to the duration of exposure to infection or to an increase in surface area, both factors being a function of age; their increase would result in larger fish having a higher probability of infection. Additionally, infection could be related, in space or time, to the probability of contact by a fish with the infective agent. Information regarding the spatial distribution of the primary infective stage (either *Aeromonas* or *Epistylis*) will not be meaningful, however, until such time as the etiologic agent is determined. Rogers (1971) claimed that the primary invader is the telotroch of *Epistylis*. However, Lom (1966) indicated that the stalked form of the ciliate is incapable of producing a histolytic enzyme which could produce scale erosion which would ultimately lead to the characteristic pit-like lesions on the body surface of infected fish. Identification of the primary infective agent should eventually lead to a correct interpretation of the size frequency and infection correlation as well as to permit the development of a realistic approach in searching for the infective agent within the water column itself.

There is a definite seasonal periodicity of infection among largemouth bass in Par Pond, with peak levels occurring in spring and lowest levels in winter. Seasonal periodicity of infection of fish by *Aeromonas* has been reported previously (Meyer, 1970), with the highest infection levels occurring in midsummer and a lesser peak in April. Meyer's (1970) report also indicated a high degree of correlation between reduced dissolved oxygen and epizootic outbreaks of *Aeromonas* involving catfish and golden shiner in rearing ponds. In Par Pond, the highest levels of infection occurred in the spring when levels of dissolved oxygen were relatively high (Esch & Hazen, unpublished observations). Meyer (1970) further indicated that the April peak was correlated with the spawning activity of the golden shiner. In the Par Pond system, highest infections in bass

TABLE III
K-factors for noninfected and infected bass from ambient and thermal regimes

	Noninfected	Infected
Ambient	2.34 (1,092)[1]	2.18 (177)
Thermal	2.15 (1,028)	2.08 (279)

[1] Number in parentheses indicates sample size.

were also noted during late March and early April which coincides with spawning activities of the bass. While the seasonal periodicity of infection among bass is obvious, there is no clear causal relationship with any, as yet identified, environmental or host characteristic. Studies are now underway to characterize further several water quality parameters so that possible correlations can be established between environmental changes and the observed seasonal periodicity.

The results of this study clearly indicate that a bass in low body condition is more likely to be infected with the *Aeromonas-Epistylis* complex than one which is not. This observation holds for bass taken anywhere in Par Pond, regardless of thermal conditions. However, the data also show that bass with lower body condition are more likely to be taken in thermally altered areas of Par Pond than in ambient areas of the reservoir. The relationships between thermal regime, body condition, and susceptibility to infection are being further evaluated and will be subsequently reported.

There are at least two hypotheses which could be invoked to account for the strong correlation between body condition and infection among largemouth bass. First, it is possible that body condition declines when bass are infected with the *Aeromonas-Epistylis* complex. A problem with this explanation, however, is that there were many bass in poor body condition which did not also exhibit surface lesions, suggesting the absence of infection in these individuals. The second, and more plausible, hypothesis is that bass in lower body condition are more vulnerable to infection. High water temperature would produce higher metabolic rates which in turn would lead to reduced body condition and may, in the end, result in reduction in either natural or acquired immunity. The second hypothesis is contingent on more initial assumptions, but present evidence (Gibbons, unpublished observations) indicates that it, rather than the first explanation, may be more tenable. Whichever hypothesis is correct, there is, nonetheless, a strong correlation between thermal effluent and the occurrence of the *Aeromonas-Epistylis* complex among the centrarchid fish in Par Pond.

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A METHOD FOR FIXING AND STAINING PERITRICH CILIATES[1]

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HAZEN, T. C., SMITH, G. & DIMOCK, R. V., JR. 1976. A method for fixing and staining peritrich ciliates. *Trans. Amer. Micros. Soc.*, 95: 693–695. A simple technique for fixing and staining peritrich ciliates involving fixation in phosphate buffered formalin and staining with Semichon's Acetic-Carmine is presented. This procedure appears to be superior to conventional methods for achieving relaxation of zooids and for differentiation of the macronucleus.

Our ongoing investigation of the epizootiology of a disease involving the bacterium *Aeromonas hydrophila* and the peritrich ciliate *Epistylis* sp. among centrarchid fishes in a South Carolina cooling reservoir (Esch et al., 1976) necessitates fixing in the field the characteristic lesions produced by this disease. Several attempts with well established fixation methods have failed to produce sufficiently well relaxed and expanded *Epistylis* colonies to allow subsequent examination of the preserved specimens. A suitable method to achieve the desired relaxation and fixation is herein described.

Pennak (1953), Kudo (1971), and Humason (1972) suggest that either Bouin's or FAA (Formalin-Acetic Acid-Alcohol) can be used as a fixative for ciliated protozoa. However, we have found that the use of either of these fixatives resulted in such contraction of the zooids that poor stain penetration was obtained and it was impossible to observe the oral ciliature (Fig. 1). Neither heating nor cooling these fixatives prior to use significantly alleviated the contraction problem.

In contrast to the results obtained with either Bouin's or FAA, we have had considerable success in fixing several peritrichs with phosphate buffered formalin (pH 7.0) (Humason, 1972). This fixative resulted in good relaxation of the zooids which permits observation of the oral ciliature and effects better stain penetration (Fig. 2).

[1] This research was supported by Contract E(38-1)-900 between ERDA and Wake Forest University.