The impact of thermal loading and other
water quality parameters on the
epizootiology of Epistylis and Aeromonas
infections of centrarchids

Progress Report

September 1, 1976 - July 30, 1977

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ABSTRACT

During the past year, mounting evidence has been accumulated which indicates that the causative agent for red-sore disease among bass is the bacterium, Aeromonas hydrophila, and not the ciliate, Epistylis, as suggested in previous literature. Red-sore disease among bass in Par Pond continues to be highly seasonal with peak periods of infection occurring in spring and summer, with declining levels in fall and summer. The disease continues to be more prevalent among bass from thermally altered areas of the reservoir during all seasons except summer. Definite correlations were established between temperature, dissolved oxygen, pH and Aeromonas density in the water column. Furthermore, the level of Aeromonas cell counts in the water column and incidence of the disease in bass was clearly established. Body condition of bass and probability of acquiring red-sore disease were also related. Histopathology of tissues from bass suggested lesions were produced by acute, lytic toxicity such as would be produced by bacterial exotoxins. Based on these observations, a tentative hypothesis is proposed which attempts to link various physico-chemical parameters, body condition of bass, probability of infection by Aeromonas and stress.

I. Brief Description of Scope of Investigation and Significant Results

Red-sore disease has been shown to be a widespread problem among various species of fish within aquatic systems in the southeastern United States. Studies conducted in the Par Pond reservoir near Aiken, SC have shown that thermal effluent from a nuclear production reactor will increase the incidence of disease among largemouth bass, Micropterus salmoides. Results of studies over the first year indicated that there was: 1) a relationship between bass length and probability of infection; 2) bass were the most likely to be infected among five species of centrarchids studied; 3) seasonal highs of infection occurred during spring, followed by summer, with declining levels in fall and winter; 4) body condition of bass and infection probability were related; and 5) long-range movement of bass was limited.

Most of the lines of study initiated in 1974 were continued into the current contract year. These results are outlined in the following section.

II. Progress in Achieving Objectives

A. Tentative identification of causative agent of red-sore disease.

The literature is contradictory as to whether Epistylis or Aeromonas is the etiological agent for red-sore disease. Rogers (1971) stated that Epistylis induces scale erosion, permitting secondary invasion by Aeromonas hydrophila. Lom (1973), on the other hand, indicated there was no evidence to suggest that Epistylis could produce the histolytic enzymes required to cause scale erosion. Based on results of study during the current contract year, we now believe there is evidence to indicate that A. hydrophila is the primary etiological agent for red-sore disease and that Epistylis is a secondary, but innocuous, ectocommensal, which opportunistically becomes attached to regions of fish

surfaces wherein reside dense concentrations of bacteria. These assertions are based on three lines of study.

First, an examination by scanning electron microscopy of the site of attachment by Epistylis to a lesion on the surface of a largemouth bass (Figure 1) does not even slightly suggest that the protozoan is causing erosion of the mucus-epithelium layer of the scale. Bacteria could be seen adhering to the stalk, but not the zooids (or feeding bodies) of the protozoan (Figure 2). Sections of paraffin embedded tissues reveal a very loose association of the colonial ciliate with scale surfaces; beneath the same colonies, there is evidence of extensive inflammation of muscle tissue in association with infiltrating bacterial cells.

A second line of evidence was generated through a collaborative study on red-sore disease in alligators. During the fall and winter of 1976-77, Dr. Robert Gorden and his colleagues (see annual report for Contract EY-76-S-09-0965) were able to generate substantial experimental evidence to show that A. hydrophila was capable of inducing surface lesions on alligators and that both lesions and mortality could be induced without a primary infection by Epistylis. Indeed, the presence of Epistylis was not noted among any of some 50 alligators with experimentally-induced, red-sore disease.

Finally, in order for A. hydrophila to be the primary invader, it is essential that the bacteria be capable of producing an extracellular toxin with the ability to lyse tissue. Liu (1961), Wretling, et al. (1972) and Bernheimer and Avigad (1974) all provided evidence that Aeromonas produces at least two toxins which have potent lytic properties. Aerolysin, as one of the toxins is called, is reported to have an LD₅₀ of 0.062 ug per mouse.

It is also worth noting in this regard, that many of the tissue lesions observed in bass, resemble those which would be induced via acute toxicity caused by lytic toxins from bacteria.

In the absence of any evidence to suggest that Epistylis can produce surface damage at the site of attachment, and with knowledge that A. hydrophila alone can produce lytic toxins as well as induce surface lesions and death in alligators, it is concluded that the primary etiological agent for red-sore disease is not the protozoan Epistylis, but is the bacterium, A. hydrophila.

B. Water quality parameters for Par Pond (1975-76).

Par Pond is a warm, monomictic reservoir. This generalization can be supported by examining the profile for temperature, redox potential, conductivity, dissolved oxygen and pH during August, 1975 and in December, 1976 (see Table 1 and Table 2).

In August, the surface temperature at the thermally altered end of the lake was 35.5°C while at 8 m it was 23.5°C; there was not a clearly defined thermocline. There was, however, a well defined chemocline between 2 m and 6 m where the dissolved oxygen dropped from 6.4 mg/l to zero; there were also substantial shifts in pH, conductivity and redox potential at the same depths. If the same profiles are examined at a representative ambient location, a thermocline (though not sharply defined) can be seen to occur between 5 m and 7 m. A chemocline can be seen to occur at the same depths where dissolved oxygen drops from 6.4 ppm to virtually zero ppm; a similar shift can be seen in pH and conductivity.

An examination of the same physico-chemical profiles in December, 1976 (Table 2) suggests the monomictic character of the reservoir, i.e. there is no

stratification during winter. A comparison of these physico-chemical data for representative ambient and thermally-altered stations in Par Pond, suggests very little impact of thermal effluent during winter months. It should be noted that winter, 1977, was one of the coldest in recent times, and that in other years, temperature differences would normally range up to 5°C.

The data in Table 3 represent a composite for mean values of profiles taken at a representative site in ambient and thermally altered areas of the reservoir for each season, beginning in the summer, 1975 and extending through spring, 1977. Both the seasonal changes and the differences in the two locations are what might be expected. Thus, there are strong seasonal shifts, reflecting the various biological and physico-chemical processes such as would be occurring in any monomictic reservoir. The locational variations are obviously the result of thermal effluent release and can be seen in temperature differentials. The differences in dissolved oxygen during summer suggest that anoxia may be occurring (and it does) but it also indicates lower levels in ambient locations in spring, summer, and fall; these figures are misleading since the data actually reflect a hypolimnetic effect at the ambient location, i.e. 15 m depth at the ambient location and only 8 m total depth at the thermally altered location.

Organic loading (measured as mg total organic carbon per liter of water), is shown (Table 4) for each season beginning in summer, 1976 and extending through spring, 1977. As can be seen, the profiles vary seasonally, but there is little difference between location. Maximum TOC occurs during summer, drops off in fall and winter and appears to rise again in spring. The high TOC at the surface during the summer, 1976 at the ambient location and in the

winter, 1976 at the heated location could have been the result of sampling from an 'organic slick' of the kind mentioned by Lewis (1974). Generally speaking, TOC levels were highest at 1 m in all seasons, regardless of whether the lake was stratified or not; high TOC's also did not coincide with either the chemocline or thermocline. The single maximum peak at 1 m is at the present time inexplicable. It does not correspond with Aeromonas hydrophila density profiles. It should also be noted that TOC levels in the Savannah River, measured from samples taken on the same days as for those seen in Table 4, were comparable to those seen below 5 m at both ambient and thermally altered locations. While not shown, the data for other locations in both ambient and thermally altered areas of the reservoir are comparable to those shown in Table 4.

C. Aeromonas hydrophila in Par Pond and other locations.

A. hydrophila was measured seasonally, in profile, at several locations throughout Par Pond during part of 1975, all of 1976 and into 1977 (these measurements will continue through the remainder of 1977). A composite for the A. hydrophila densities in the water column for the reservoir are shown in Figure 3. These data indicate a striking seasonal shift with maximum densities occurring in March and June of 1976. The data in Figure 4 show A. hydrophila densities at a depth of one meter at ambient and thermally altered locations and again, there is a striking seasonal pattern with maximum densities in heated areas being recorded in mid-winter, 1975-76 and in spring, 1976; maximum density at one meter of A. hydrophila occurred in April, 1976 at the ambient location. These data do not, however, accurately reflect A. hydrophila densities since Fliermans, et al. (1977) have shown that densities will vary according to

reactor activity, i.e. densities are higher at all depths when the reactor is operating than at the same location when the reactor is not operating.

Furthermore, the densities of A. hydrophila are always higher in hypolimnetic water than in the epilimnion when the lake is stratified, i.e. in late spring, summer and early fall months.

A. hydrophila densities are also variable within the heated arm of Par Pond, depending on where the sample is taken and at what depth (Figures 5 and 6). Thus when measured in profile, it can be seen that A. hydrophila densities are, in general, higher near the bottom of the reservoir, but vary also with where the samples are taken. The high values in profile (Figure 5) near the east shore of the lake may actually represent the effect of effluent flow through this segment of the lake (the reactor effluent enters the heated arm at the center and then disperses laterally along the shoreline on each side of the lake). Figure 6 reflects the horizontal distribution of A. hydrophila along the first 200 m of the heated arm from the point of effluent entry into the reservoir. It can be seen that densities are considerably higher along the shoreline. This may reflect effluent flow from the point of entry of the effluent, or it may reflect greater decomposition of organic material in shallow water along the shoreline. It is also known that A. hydrophila densities may be as high as 1000 cells/ml in the organic surface slicks mentioned previously. The highest densities of A. hydrophila (>8,000 cells/ml) were measured, however, among floating mats of Myriophyllum spicatum; it is worth noting that bacterial densities were 1.0 cell/ml in open water less than 10 m distant from the M. spicatum mats.

A. hydrophila is a ubiquitous gram-negative bacterium having been

previously reported in Utah, Virginia, Georgia and Florida. During the course of this study, attempts have been made to document it from as many other states as possible. To date, it has been identified from seventeen additional states (Figure 7), ranging from Montana to Arizona in the west, Michigan in the north and Texas, Louisiana and Alabama in the south. The importance of such a distribution is apparent when it is noted that red-sore disease appears to be restricted to the southeastern United States.

D. Seasonal periodicity of disease among bass.

Beginning in the fall of 1974 and continuing through the spring of 1977, a total of 5,581 bass were taken from Par Pond. The results show a very striking seasonal periodicity with peaks always occurring in spring and followed by declines in summer and fall to lowest levels in winter (exceptions to this pattern are due to: (1) variable reactor activity or (2) variable sample sizes in thermal versus ambient locations).

As can be seen in the Figure 8, the pattern of change in infection percentage is very comparable to that seen for temperature change except that peak temperatures were highest in summer. This would suggest that while there is a relationship between temperature and disease, another variable may be of significance in affecting the epizootiology of red-sore among bass. This variable will be discussed subsequently in sections F and I of this report.

When bass from thermal and ambient locations of Par Pond are compared, another distinct pattern emerges (Figure 9). First, the seasonal changes in the two locations remain quite similar although there are a couple of times that the levels of infection switch. Second, in general, bass in thermally-altered locations have higher levels of infection than in ambient locations.

Third, the most persistent differences are seen during the winter months, as should be expected since in other seasons the difference in temperatures are less.

E. Comparison of Aeromonas density and red-sore disease.

When the density of Aeromonas in the water column and incidence of red-sore disease are compared from August, 1975 through May, 1977, there appears to be a very striking correlation (Figure 10). In several cases, where there is a rise in infection with red-sore (shown with v's in Figure 10) there is a corresponding, but preceding, rise in Aeromonas density in the water column (shown with v's in Figure 10). The lag period between each of these parallelisms is about one month. While there are exceptions to this pattern, the relationship is too strong to pass off as spurious. It will definitely require monitoring over the next 12-18 months in order to confirm it.

F. Body condition studies.

Throughout the red-sore study (fall, 1974 through spring, 1977), body condition, or K-factor, for each bass has been calculated. This measurement provides a good estimate of the well-being or 'fitness' of an individual fish and, taken at the population level, can be a very useful parameter (Carlander, 1944).

Body condition, as would be expected, changes seasonally. Maximum seasonal K-factors occur in winter with minimum levels in summer. These data are nicely related with corresponding seasonal changes in temperature.

When K-factors of bass from ambient and thermally-altered locations are compared seasonally (Figure 11), it can be seen that mean values in each

location show similar patterns of change. Moreover, it can be seen that bass from thermal locations generally have lower body conditions than their ambient counterparts. There is some variability in this pattern, e.g. fall, 1976 and winter, 1977, but these exceptions may be attributed to either small sample sizes or to variability in reactor activity.

An attempt was made to show a relationship between body condition subclasses in 0.2 units and infection percentage of red-sore disease in each subclass. As can be seen in Figure 12, there is a clear relationship between K-factor and probability of infection with red-sore disease. There is a clear break-point between bass having a K-factor of > 1.8 and those with a K-factor < 1.8 . The data in Table 5 brings this relationship into sharper focus. Thus, for bass from thermal locations, the infection percentages of the group with a K-factor less than 1.8, are consistently higher than 23%, ranging up to 33% in spring. The pattern is somewhat muted in bass from ambient areas. Thus for bass with K-factors less than 1.8, infection percentages are highest in spring, matching at 33%, bass from thermal locations. However, in summer and fall, levels of infection drop to 19% and 17%, respectively, while those for bass in thermal locations are 24% and 28%, respectively. Another area of interest is the infection percentage of bass with K-factors greater than 1.8. As can be seen, levels are generally higher in thermal locations with a major exception being during summer months when infection percentages are higher among bass from ambient locations. However, if the data are carefully analyzed, it will be noted that there is a significant drop in the percentage of bass from thermal areas in the greater than 1.8 category. While there is a similar shift in the same category of bass in ambient locations, it is not nearly so striking.

We believe that a large number of bass in thermal areas which would have otherwise been infected, and in the > 1.8 category, were shifted down into the < 1.8 category. This is substantiated when it is noted that the overall infection percentage for bass from thermal locations in the summer time is 13% while it is 18% for bass from ambient locations.

These observations regarding body condition and infection with Aeromonas are significant for several reasons. First, they show that one must be careful in comparing seasonal changes in incidence of disease since variations in sample sizes in one location versus another may significantly alter whatever conclusions may be drawn - and do so in an incorrect manner. Second, the data clearly indicate that body condition and incidence of red-sore disease are dependent variables. Third, and as important, the data show that temperature is also a dependent variable in affecting both body condition and the probability of infection with red-sore disease.

G. Histopathology associated with red-sore disease.

During the past year and a half, numerous tissues have been removed from moribund bass, fixed in Bouin's, embedded in paraffin, sectioned at 6-8 u and stained with hematoxylin and eosin. These tissues have been examined by Dr. Harry Huizinga, Illinois State University, Normal, Illinois. A report, complete with photomicrographs, has been prepared. Because of the technical problems in reproducing several copies of each micrograph, only a summary of the report is presented at the present time. The report is to form a basis for a manuscript describing the histopathology of red-sore disease, and will be submitted as a reprint in the next annual report.

1. External lesions.

Body surface: In lesions associated with Epistylis and A. hydrophila,

the epidermis which normally covers bass scales is reduced to a thin membrane or is completely eliminated. The adjacent epidermis becomes hyperplastic (thickened with increase in cell numbers). There is intense mononuclear inflammation, hemorrhage and necrosis of dermal tissues. Underlying muscles become edematous, with inflammation and necrosis also apparent.

Gills: In experimental infectious (induced by addition of A. hydrophila being added to aquarium water), there is severe hemorrhagic necrosis of gill filaments and lamellae. Gill epithelium is denuded to the level of underlying cartilaginous supporting structures with necrotic blood and fibrinous exudate surrounding the lesion. There is mononuclear inflammation with hemorrhage and edema of supporting cartilage and muscle. Underlying muscles are edematous, inflamed and in various stages of necrosis.

Fins: There is severe hemorrhagic necrosis of epidermis overlying the fins to the extent that it is frequently denuded down to the bony substructure. Frequently, the functional integrity is destroyed beyond repair.

2. Internal lesions.

Liver: There is acute, diffuse necrosis of hepatocytes which exhibit severely damaged cell membranes and foamy cytoplasm. Adjacent to sinusoids, hepatocytes are conspicuously lysed, suggesting an acute toxic effect. There is variable pathogenesis between livers in different fish, ranging from mild (paranecrotic) to severe necrosis. Numerous encapsulated strigeid parasites, both living and dead, were noted.

Kidney: Bass kidneys exhibited acute, diffuse tubular and glomerular necrosis. There was major destruction of kidney tubules and loss of functional integrity in many bass. Pathogenesis was variable between bass, ranging from mild (paranecrotic) to severe necrosis (tissue unrecognizable).

Spleen: There was variable pathogenesis ranging from mild to severe necrosis of red and white pulp. There was a drastic reduction in numbers of blood cells, causing the white pulp and connective tissue stroma to stand out conspicuously.

Heart: Most are normal even in bass with severe kidney and liver necrosis. There was occasional slight inflammation, edema and minor focal necrosis.

General conclusions: Red-sore disease is an acute and highly pathogenic disease, characterized in bass by: 1) hemorrhagic necrosis of skin, fins and gills, 2) septicemia and severe necrosis of internal organs due to toxic metabolic products released by A. hydrophila. Death of fish is probably induced by failure of major organs (liver, kidney, spleen). It is also possible that anemia resulting from hemorrhage and hemolysis of blood, in combination with respiratory insufficiency due to gill necrosis, are major factors that contribute to mortality; it should be noted that gill involvement in natural infections has not been observed among Par Pond bass although it cannot be ruled out as a central factor in causing major fish kills in other reservoirs.

The role of Epistylis in the pathogenesis of red-sore disease is still unclear. The stalked ciliate was associated with some surface lesions, but not others. Since the ciliate does not penetrate deeper tissues, it seems likely that it is only an opportunistic ectocommensal which feeds upon surface bacteria.

H. Biochemical analysis of Aeromonas isolates

Preliminary biochemical and antibiotic susceptibility assays, using 43 different tests on more than 400 Aeromonas isolates from fish, alligators,

water, and sediments, indicate that the predominant strain of A. hydrophila in the water column is significantly different from the predominant strain isolated from fish and alligator lesions. Moreover, the predominant strains in fish and alligators are not significantly different. The predominant strains in other lakes in the southeast U.S., and in other areas as well (see Figure 7), are not significantly different from the predominant strain isolated in the Par Pond column. It should also be noted that the predominant strain in Par Pond water is not significantly different from A. hydrophila from the American type culture collection.

It must be emphasized that these observations are based on incomplete analysis of a substantial data base and that a complete analysis may provide more insight into the 'strain' problem. Hopefully, these data will be prepared for conclusion in the next annual report.

I. Red-sore disease and stress: an hypothesis.

Based on evidence presented thus far, there appears to be a relationship between red-sore disease, bass body condition and water temperature (Figure 13). Since these relationships are clear and consistent from year to year, can an hypothesis be generated to provide an explanation? The answer to this question appears to be yes and, furthermore, it will incorporate a fourth variable, that of stress, into our thinking regarding the epizootiology of red-sore disease. Indeed, since red-sore disease is known to occur in aquatic systems which are not affected by thermal effluent, it is conceivable that stress may be the variable of greatest significance.

As water temperature increases in an aquatic system, the metabolic rates of bass will likewise increase. With an increase in metabolism, there is a

concomitant rise in catabolic processes, initially involving body fat, but ultimately involving body protein as well (Gibbons, Esch, Hazen and Bennett, manuscript in preparation). The body condition, or K-factor of bass affected by elevated temperature will, accordingly, decline in time (assuming, of course, that caloric intake is exceeded by metabolic demand). Contributing to a fall in body condition in summer is the exceedingly rapid and extensive growth of luxuriant stands of the submergent, rooted, vascular macrophyte, Myriophyllum spicatum. Large masses of this plant provide excellent refuge for certain species of forage fish. Reduced foraging success, coupled with increased energy expenditure by foraging bass would exacerbate those metabolic processes already conducive to reducing body condition. Lowered body conditions increase the probability of infection with red-sore disease. This explanation would certainly satisfy the situation as it occurs during part of the spring, throughout the summer and into the fall months before Myriophyllum dies back and disappears, but it does not explain the high infection percentages as they develop earlier in spring. During this period of time, sexually mature bass are involved with activities associated with spawning. It is also during this period that levels of circulating sex hormones (all steroids) are highest. While these hormones function primarily in the promotion of sexual behavior and development of secondary sexual characteristics, they are also known to enhance the establishment, maintenance and/or growth of numerous species of parasitic organisms (see Esch, et al., 1975 for review). It is thus conceivable that elevated levels of circulating sex hormones would also increase vulnerability to infection, e.g. red-sore disease.

Persistent, elevated temperature in thermally altered areas of the

reservoir during most seasons of the year and during summer in ambient locations, we believe, also promotes stress in bass. Stress, in the classical sense (see Esch, et al., 1975, for description of stress and the General Adaptation Syndrome), necessarily implies the production and release of excess levels of adrenocorticosteroids, many of which have a striking anti-inflammatory action which promotes increased susceptibility to invasion by pathogenic organisms. We must point out that we have no evidence for increased production of corticosteroids during periods when there are high levels of red-sore disease among Par Pond bass (this line of study will be pursued in the next contract year). However, we do know that thyroxine levels are highest in bass during the summer months (Hazen, Esch, Glassman and Gibbons, manuscript in preparation) and, that an indication of stress among mammals is an increase in the level of circulating thyroxine.

As previously noted, red-sore disease also occurs among fish species in aquatic systems which are not affected by thermal effluent. In these systems, the disease is associated with conditions such as lowered dissolved oxygen and increased organic loading (Meyer, 1970; Rogers, 1971; Dean, 1974). Under these circumstances, it is quite possible that bass are stressed, leading to increased circulation of corticosteroids and then to increased susceptibility to infection with red-sore disease.

This scenario, which describes the relationship between red-sore disease, body condition and temperature in Par Pond (see Figure 13) also includes the possibility that stress and the General Adaptation Syndrome (Selye, 1950) may be of significance in reducing innate or acquired resistance of bass to infection with A. hydrophila. While we have not yet generated data which might

indicate that corticosteroids are higher in bass from thermally altered areas, there is evidence to indicate that epinephrine and corticosteroids will vary in direct proportion to various types of stressors (Nakano and Tomlinson, 1967; Hill and Fromm, 1967).

The schematic outline shown in Figure 13, is the first effort to begin to tie together our observations regarding the epizootiology of red-sore disease among Par Pond bass. By manipulation of bass in the laboratory (as outlined in our renewal proposal), we are hopeful that some of the proposed relationships will become more sharply defined.

III. Statement of Compliance

This is to indicate that all aspects of Contract E(38-1)-900 have been complied with by the Contractor.

IV. Approximate Percentage of Time Devoted to Project and Amount of Time to be Devoted During Remaining Three Months

The principal and co-principal investigators have devoted approximately 50% of their time to the project during the academic year and 100% of their time during the summer months.

V. Technical Reports, Preprints and Articles Prepared for Publication

A. Published

1. Esch, G. W., Hazen, T. C., Dimock, R. V. and J. W. Gibbons, 1976.
Thermal effluent and the epizootiology of the ciliate Epistylis and the bacterium, Aeromonas in association with centrarchid fish. Trans. Amer. Microscop. Soc. 95: 687-693.
2. Hazen, T. C., Smith, G. and Dimock, R. V. 1976. A method for fixing and staining peritrich ciliates. Trans. Amer.

Microscop. Soc. 95: 693-695.

3. Esch, G. W., G. C. Campbel, R. E. Conners and J. R. Coggins. 1976. Recruitment of helminth parasites by bluegills (Lepomis macrochirus) using a modified live-box technique. Trans. Amer. Fish Soc. 105: 486-490.
4. Aho, J. M., J. W. Gibbons and G. W. Esch. 1976. Relationship between thermal loading and parasitism in the mosquitofish. In: Thermal Ecology II (eds.) G. W. Esch and R. W. McFarlane. ERDA Symposium Series (CONF. 750425), pp. 213-218.
5. Fliermans, C. B., R. W. Gorden, T. C. Hazen and G. W. Esch. 1976. Aeromonas distribution and survival in a thermally altered lake. Appl. Environ. Microbiol. 33: 114-122.
6. Esch, G. W., T. C. Hazen and J. M. Aho. 1977. Parasitism and r- and K-selection. In: Regulation of Parasite Populations, (ed.) G. W. Esch. Academic Press, N. Y. pp. 9-62.
7. Book: Thermal Ecology II. 1976. Edited by G. W. Esch and R. W. McFarlane. ERDA Symp. Series (CONF-750425), pp. 1-404.
8. Book: Regulation of Parasite Populations. Edited By G. W. Esch. Academic Press, N. Y. pp. 1-253.

B. In press

1. Hazen, T. C. and G. W. Esch. Population biology of two trematode parasites in the amphipod, Hyalella azteca. Amer. Midl. Nat.
2. Hazen, T. C. and G. W. Esch. Population biology of Clinostomum marginatum in a South Carolina cooling reservoir. J. Fish Biology.

3. Quinn, T., G. W. Esch, T. C. Hazen and J. W. Gibbons. Long range movement and homing by largemouth bass (Micropterus salmoides) in a thermally altered reservoir. Copeia.

C. Manuscripts in preparation

1. Gibbons, J. W., G. W. Esch, T. C. Hazen, and D. Bennett. A long-term study of body conditions of largemouth bass in a thermally altered reservoir.
2. Hazen, T. C., G. W. Esch, A. C. Glassman and J. W. Gibbons. Relationship of season, thermal loading and red-sore disease with various hematological parameters in largemouth bass (Micropterus salmoides).
3. Esch, G. W., T. C. Hazen and R. P. Hirsch. Thermal ecology and stress: A case history for red-sore disease in largemouth bass (Micropterus salmoides).
4. Huizinga, H., G. W. Esch and T. C. Hazen. Histopathology of red-sore disease in largemouth bass.
5. Hazen, T. C. and G. W. Esch. Scanning and transmission electron microscopy of lesions associated with red-sore disease among largemouth bass.

D. Papers presented (supported by Contract E(38-1)-900)

1. Observations on the ecology of Clinostomum marginatum in largemouth bass, Micropterus salmoides. Amer. Soc. Parasitol., Aug. 20-24, 1976. San Antonio, TX.
2. Further studies on the effects of thermal effluent on the population biology of parasites in the mosquitofish, Gambusia affinis.

Amer. Soc. Parasitol., Aug. 20-24, 1976. San Antonio, TX.

3. Distribution and abundance of A. hydrophila in a reservoir receiving thermal effluent from a nuclear production reactor. Amer. Soc. Microbiol., May 1-5, 1976. Atlantic City, NJ.
4. Ecology of Aeromonas hydrophila in a thermally altered reservoir. Amer. Soc. Microbiol., May 1-5, 1977. New Orleans, LA.
5. Light and electron microscopic studies of lesions associated with red-sore disease. Southeast. Soc. Parasitol., April 14-16, 1977. Raleigh, NC.
6. Population studies of two larval strigeids in the mosquitofish, Gambusia affinis. Amer. Soc. Parasitol. August 14-19, 1977. Las Vegas, NV.
7. Population biology of Crepidostomum cooperi (Trematoda: Allocreadidae) - An update. Amer. Soc. Parasitol., August 14-19, 1977. Las Vegas, NV.
8. Hynes and Nichols revisited. Amer. Soc. Parasitol., August 14-19, 1977. Las Vegas, NV.
9. Thermal ecology and stress: A case history for red-sore disease in largemouth bass (Micropterus salmoides). Thermal ecology and environmental stress symposium, Nov. 8-11, 1977. Augusta, GA.

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Table 1

Physico-chemical profile at a
representative ambient and thermally-altered
location during summer.

Summer - Thermal location

	Temperature	Redox potential	Dissolved oxygen	pH	Conductivity
Surface	35.5	330	6.3	7.0	60
1	34.0	320	6.1	7.1	60
2	30.5	320	6.4	7.4	60
3	30.0	320	6.3	7.3	60
4	29.5	330	5.2	6.8	60
5	28.5	350	2.6	6.3	60
6	27.5	370	0.2	6.0	60
7	26.0	380	0.2	6.1	70
8	23.5	370	0.2	6.3	85
9	21.5	185	0.2	6.5	100
10	20.5	90	0.2	6.6	110

Summer - Ambient location

Surface	31.0	350	7.3	7.5	60
1	30.3	340	6.9	7.6	60
2	30.0	340	6.7	7.5	60
3	30.0	350	6.5	7.3	60
4	30.0	360	6.3	7.2	60
5	29.8	360	6.4	7.2	60
6	27.5	390	1.1	6.3	60
7	25.5	410	0.2	6.3	65
8	23.0	410	0.2	6.4	80
9	21.5	410	0.2	6.5	85
10	20.5	410	0.2	6.6	90
11	19.5	380	0.2	6.6	90
12	18.5	145	0.2	6.6	90
13	18.0	110	0.2	6.7	90
14	18.0	70	0.2	6.7	100
15	17.8	50	0.2	6.8	100

Temperature - °C

Redox potential - MV

Dissolved oxygen - MG/L

Conductivity - MH0

Table 2

Physico-chemical profile at a
representative ambient and thermally-altered
location during winter.

Winter - Thermal location

	Temperature	Redox potential	Dissolved oxygen	pH	Conductivity
Surface	12.8	385	10.2	6.2	50
1	12.8	380	9.9	6.4	50
2	12.8	380	9.7	6.4	50
3	-	-	-	-	-
4	-	-	-	-	-
5	12.8	380	9.5	6.4	50
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	12.5	385	9.1	6.4	50
10	12.5	385	9.0	6.3	50

Winter - Ambient location

Surface	12.5	350	10.6	7.0	60
1	13.0	350	10.2	7.2	60
2	-	-	-	-	-
3	12.8	350	10.1	7.3	60
4	-	-	-	-	-
5	-	-	-	-	-
6	12.8	350	10.1	7.2	60
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	12.5	350	10.1	7.2	60
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	12.5	350	10.0	7.2	60
15	12.5	355	10.0	7.1	60

Temperature - °C

Redox potential - MV

Dissolved oxygen - MG/L

Conductivity - MHO

Table 3

*Composite Physico-Chemical
Data for Thermally Altered and
Ambient Locations; Seasonally, 1975-77.

	Summer (75) (one month only)		Fall (75)		Winter (76)		Spring (76)	
	H.	A.	H.	A.	H.	A.	H.	A.
C ^o	28.1	23.2	22.4	21.2	16.7	13.4	21.4	18.5
D.O.	3.1	2.6	5.6	4.2	8.3	9.0	7.3	6.2
Redox	308.3	247.7	356.1	314.1	363.2	377.4	344.0	363.1
pH	6.8	6.9	7.1	6.9	6.8	7.0	7.2	6.9
Cond.	70.0	76.7	56.3	70.4	65.8	59.8	66.7	69.8
Cells/ml	0.23	0.4	6.1	2.7	34.7	2.4	90.7	97.4

	Summer (76)		Fall (76)		Winter (77)		Spring (77)	
	H.	A.	H.	A.	H.	A.	H.	A.
C ^o	29.2	23.4	21.2	20.3	11.9	10.7	17.7	15.3
D.O.	5.1	3.2	6.1	5.5	10.1	10.6	9.0	9.1
Redox	295.8	261.7	353.4	341.7	421.8	428.7	545.0	402.4
pH	6.7	6.8	6.6	6.7	6.5	6.8	6.9	7.1
Cond.	63.1	74.0	68.8	76.0	70.9	71.6	66.8	70.6
Cells/ml	21.1	67.2	10.9	3.8	6.0	4.5	3.2	1281.9

*These data represent a mean value for a profile at a given ambient and thermally altered location; N for each value was always > 14, and generally exceeded 80. Maximum N for a given value was 311.

Temperature - °C

Redox potential - MV

Dissolved oxygen - MG/L

Conductivity - MHO

Table 4

Depth Profile for
Total Organic Carbon (Seasonally)
(mg total organic carbon/L)

Depth(m)	Summer (76)		Fall (76)		Winter (76)		Spring (77)	
	H.*	A.**	H.	A.	H.	A.	H.	A.
0	5.6	19.5	2.2	1.2	34.3	2.1	0.8	1.5
1	66.4	76.6	25.1	10.6	18.6	25.1	33.6	37.0
3	7.7	3.5	2.8	1.8	1.2	6.4	4.7	15.3
5	2.7	3.0	2.1	2.1	1.7	1.9	2.0	4.0
7	2.1	2.9	1.6	1.2	1.2	1.1	0.9	1.7
9	-	3.0	-	2.1	-	1.5	-	1.4
11	-	3.1	-	2.7	-	1.9	-	1.6
13	-	3.2	-	2.5	-	1.1	-	1.8
15	-	3.8	-	2.2	-	1.1	-	2.0

***S.R. water=0.71

S.R. water=1.0

*H = thermally altered location

**A = ambient location

***S.R. water = water from Savannah River

Table 5

Infection percentage
and per cent of all bass
with K-factors < 1.8 and > 1.8
during each season of the year.

THERMAL			AMBIENT			X ² and proba- bility (compar- ing ambient and thermal locations)
<u>Winter</u>	% All bass	% Infected	<u>Winter</u>	% All bass	% Infected	
<u>K-factor</u>			<u>K-factor</u>			
< 1.8	10%	23%	< 1.8	9%	7%	4.29 (.05 > p > .025)
> 1.8	90%	12%	> 1.8	91%	6%	10.25 (.005 > p > .001)
	Total = 13%			Total = 7%		18.28 (p < .0005)
	N = 793			N = 522		
<u>Spring</u>			<u>Spring</u>			
< 1.8	18%	33%	< 1.8	11%	33%	N.S.
> 1.8	82%	23%	> 1.8	89%	17%	11.52 (.001 > p > .0005)
	Total = 25%			Total = 22%		N.S.
	N = 1006			N = 837		
<u>Summer</u>			<u>Summer</u>			
< 1.8	58%	24%	< 1.8	31%	19%	6.57 (.025 > p > .01)
> 1.8	42%	12%	> 1.8	69%	18%	N.S.
	Total = 13%			Total = 18%		N.S.
	N = 633			N = 769		
<u>Fall</u>			<u>Fall</u>			
< 1.8	24%	28%	< 1.8	13%	17%	18.69 (p < .0005)
> 1.8	76%	12%	> 1.8	87%	10%	N.S.
	Total = 16%			Total = 13%		N.S.
	N = 524			N = 13		
<u>Sum of all seasons</u>			<u>Sum of all seasons</u>			
< 1.8	27%	28%	< 1.8	18%	21%	87 (p < .0001)
> 1.8	73%	16%	> 1.8	82%	14%	N.S.
	Total = 19%			Total = 16%		N.S.
	N = 2956			N = 2431		

Legend - Figure 1

Scanning electron micrograph showing point of attachment between Epistylis and underlying host scale. As can be seen, there is no evidence that the ciliate has produced erosion of the underlying epithelium. S - stalk of Epistylis.

Legend - Figure 2

Transmission electron micrograph showing dense concentrations of bacteria (presumably A. hydrophila) in close association with stalks of Epistylis but not zoids or feeding bodies of the protozoan. B - bacteria; S - stalk of Epistylis; Z - zoid (feeding body) of Epistylis.

Legend - Figure 3

Monthly composite for mean densities of A. hydrophila in Par Pond beginning in August, 1975 and continuing through May, 1977.

Legend - Figure 4

Monthly composite for mean densities of A. hydrophila comparing thermally-altered and ambient locations in Par Pond beginning in August, 1975 and continuing through May, 1977.

Legend - Figure 5

Vertical profile for Aeromonas hydrophila densities in the hot arm of Par Pond.

HOT ARM VERTICAL PROFILE OF AEROMONAS

*All values in cells/ml

Legend - Figure 6

Horizontal distribution of Aeromonas hydrophila in the hot arm of Par Pond.

AEROMONAS DISTRIBUTION IN THE HOT ARM

Legend - Figure 7

Geographic distribution of Aeromonas hydrophila in the U. S. Closed circles reflect confirmed isolation; open circles indicate absence of the bacterium; x's indicate reported identification by other investigators.

Legend - Figure 8

Seasonal changes in mean infection percentages and mean surface temperatures
for all of Par Pond beginning in fall, 1974 and extending through spring, 1977.

Legend - Figure 9

Seasonal changes in mean infection percentages for bass from thermally-altered and ambient locations in Par Pond beginning in fall, 1974 and continuing through spring, 1977.

Legend - Figure 10

Relationship between mean monthly infection percentage (dotted line) and mean density of A. hydrophila in Par Pond beginning August, 1975 and continuing through May, 1977. The v's on the figure emphasize peaks in A. hydrophila densities which are followed the next month by a sharp rise in infection percentage.

Legend - Figure 11

Seasonal changes in mean body condition (K-factor) in bass taken from thermally-altered and ambient locations beginning fall, 1974 and continuing through spring, 1977.

Legend - Figure 12

Infection percentage for bass in 0.2 unit body condition subclasses ranging from 1.0 to 3.0. Bass less than 2.0 would be considered to be in poor, or relatively poor, body condition.

Legend - Figure 13

Proposed relationship between probability of acquiring red-sore disease and temperature, organic loading and stress.

Probable relationships between elevated temperature, increased organic loading, stress and red-sore disease in bass.