The impact of thermal loading and other
water quality parameters on the
epizootiology of red-sore disease
in centrarchids.

PROGRESS REPORT

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Gerald W. Esch

and

Terry C. Hazen

MASTER

Department of Biology
Wake Forest University
Winston-Salem, NC 27109

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I. Brief statement of problem

Epistylis sp., a stalked colonial ciliate, has been identified as the causative agent for red-sore disease which is known to affect a variety of fish species in various aquatic systems throughout the southeastern United States (Rogers, 1971). This protozoan has a motile telotroch stage which attaches to a given substrate, forms a stalk and produces feeding bodies, called zooids. When attached to the surface of a fish, it is said to cause scale erosion, producing pit-like lesions. Via these lesions, the gram-negative bacterium, Aeromonas hydrophila, enters the tissues and, in time, produces hemorrhagic septicemia and death.

Because of the heavy mortality produced by the disease, it has drawn considerable attention over the past few years. In 1972, Shotts, et al. reported that more than 100,000 fish were killed in Lake Apopka, Florida, as a consequence of red-sore disease. In excess of 37,500 fish were killed in the space of a few weeks in 1973 in Badin Lake, North Carolina (Miller and Chapman, 1976). During the fall of 1976, approximately 95% of the white perch population was killed in Albemarle Sound, NC, due to an epizootic of the disease (Mr. Grover Cook, Department of Natural and Economic Resources, State of North Carolina, Raleigh); during the same outbreak, 50% of the commercial catch of all fish species was discarded because of the presence of unsightly surface lesions associated with red-sore.

In spite of the widespread occurrence of red-sore disease in the southeast, its epizootiology has not been extensively studied. Indeed, much of the literature regarding the problem is confusing, if not actually contradictory. For example, Rogers (1971) stated that Epistylis sp. is the

primary invader, with A. hydrophila producing the secondary infection. On the other hand, Lom (1973) emphatically stated that Epistylis sp. is incapable of producing lesions and, that as a bacteriovore, it only secondarily associates with fish. Earlier, Lom (1966) reported that heavy infections of fish with Epistylis were seasonal (occurring mostly in winter) and were independent of the amount of organic solids and density of bacteria in the water, while Bullock and McLaughlin (1970) and Meyer (1970) reported the most severe outbreaks to occur in summer when temperature was high and dissolved oxygen was low. During the winter of 1973 (Esch and Gibbons, unpublished observations) noted the presence of red-sore disease among several species of centrarchids in a South Carolina cooling reservoir and tentatively concluded that it could be related to thermal effluent from a nuclear production reactor.

Based on this brief overview, it is clear that red-sore disease is a serious problem, that its epizootiology is not well understood and that, because of its impact on both commercial and sport fisheries, it deserves vigorous study. Toward these ends, the following objectives were either listed, or implied, as being critical for study in the original proposal submitted in 1974:

- A.) Determine the level, and seasonal changes, in the incidence of red-sore disease among largemouth bass in ambient and thermally-altered areas of Par Pond, a 1020 hectare cooling reservoir located on the Savannah River Plant near Aiken, SC.
- B.) Establish a long-term, mark-release-recapture program for largemouth bass in order to monitor movement within the reservoir as well

as determine if the disease, once acquired in the field, was always fatal.

- C.) Study the histopathology of red-sore disease in largemouth bass.
- D.) Study basic blood parameters among infected and non-infected bass from ambient and thermally-altered areas of the reservoir.
- E.) Measure anti-Aeromonas hydrophila antibody titers among infected and non-infected bass taken from ambient and thermally-altered areas of the reservoir.
- F.) Initiate comparative biochemical and serological studies on
 - A. hydrophila isolated from red-sore lesions, from the intestinal flora of bass and from the water column of Par Pond and other aquatic systems in the southeastern and other parts of the United States.
- G.) Initiate long-term studies on selected water quality characteristics in Par Pond and determine which, if any, is related to the abundance and distribution of A. hydrophila.
- H.) Identify the etiological agent for red-sore disease, i.e. whether the colonial ciliate, Epistylis sp., or the gram-negative bacterium, Aeromonas hydrophila.
- I.) Develop a descriptive model for the epizootiology of red-sore disease among largemouth bass within Par Pond.
- J.) Initiate laboratory studies on red-sore disease with the aim of testing hypotheses developed from investigations.

II. Progress in achieving results

- A. The etiological agent: Based, for the most part, on studies by Hazen,

Raker, Esch and Fliermans (1978), the etiological agent for red-sore disease among largemouth bass in Par Pond, has been identified as the gram-negative bacterium, Aeromonas hydrophila. They based their conclusions on the observation that Epistylis sp. could be isolated from only 35% of 114 lesions from 114 different bass, while A. hydrophila was confirmed in 96% of the same lesions. Transmission and electron microscopy of lesions associated with red-sore disease further revealed that neither the stalk, nor the attachment structure of Epistylis sp., possessed organelles capable of producing lytic enzymes necessary for producing scale erosion and localized hemorrhaging. Since A. hydrophila is known to produce lytic toxins (Bernheimer and Avigad, 1974; Liu, 1961; Wretling, Mollby and Wadstrom, 1971), and in absence of evidence to the contrary, it was concluded that Epistylis sp. is a benign ectocommensal and that the bacterium, A. hydrophila, is the primary etiologic agent for red-sore disease.

Support for these conclusions were provided by the studies of Huizinga, Hazen and Esch (in preparation; see also discussion of histopathology in this report). Investigations by Gorden, et al. (in press) also indicate that A. hydrophila, in the absence of Epistylis sp., is capable of producing red-sore disease in alligators. Furthermore, A. hydrophila alone (without Epistylis sp.) was found to be capable of producing lesions in largemouth bass, under laboratory conditions (Hazen and Esch, unpublished observations).

B. Seasonal changes in the incidence of red-sore disease among largemouth bass in Par Pond: Since the fall of 1974, in excess of 8,000 largemouth bass have been captured from ambient and thermal locations of Par Pond. All of these bass were weighed, measured and the presence or absence of red-sore

lesions noted. For each bass, the body condition, or K-factor was calculated (body condition is a reflection of physical well-being, or "fitness"). Esch, Hazen, Dimock and Gibbons (1976), Esch and Hazen (1978), and Gibbons, Bennett, Esch and Hazen (1978) have reported on various facets of these studies and are summarized as follows.

Among five species of Centrarchidae in Par Pond, largemouth bass are 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 black crappie (Pomoxis nigromaculatus).

The body conditions of bass in Par Pond change seasonally (Figure 1). Highest K-factors are observed in winter, and lowest in summer. Based on data from >10,000 bass collected from 1967 to the present, body conditions of bass from thermal areas, in most seasons, are significantly lower than those during the same season from ambient areas (Table 1; Fig. 2).

Red-sore disease follows a distinct seasonal pattern, with highest levels occurring in spring and then with declining levels in summer and fall and lowest incidence in winter. Infection with red-sore disease closely followed seasonal changes in temperature during the period from 1974-78 (Figure 3). During the winters of 1975-1977, the fall of 1974 and the spring of 1975 and 1976, the levels of red-sore disease were significantly higher in bass from thermal locations (Figure 4).

Initially, these observations suggested that red-sore disease and body condition were, independently, related to temperature. It was then speculated that, perhaps, body condition and probability of acquiring

Figure 1. Seasonal changes in body conditions of largemouth bass from
Par Pond.

Table 1

Mean body conditions of largemouth
bass from heated and ambient locations
of Par Pond.

Year	Winter		Spring		Summer		Fall	
	H	A	H	A	H	A	H	A
1967	-	-	-	-	1.99	2.17	-	-
1968	-	-	-	-	-	-	-	-
1969	2.32	2.41	2.12	2.27	1.97	2.31	2.00	2.21
1970	2.28	2.35	2.12	2.17	2.12	2.32	2.19	2.14
1971	2.22	2.14	-	-	1.85	1.94	2.01	-
1972	2.54	2.46	-	-	2.10	2.32	2.09	2.20
1973	2.30	2.38	2.08	2.14	1.91	2.19	1.97	2.32
1974	-	-	-	-	-	-	2.14	2.28
1975	2.26	2.50	2.25	2.38	1.87	2.12	2.41	2.41
1976	2.37	2.44	2.30	2.33	2.02	2.20	2.05	2.16
1977	2.29	2.26	2.16	2.27	1.86	2.06	2.00	2.24
1978	2.31	2.28	2.23	2.33				

N > 10.000

Figure 2. Comparison of seasonal changes in body conditions of bass taken from ambient and thermal locations.

Figure 3. Seasonal infection percentages and temperature changes during the period 1974-1978.

N = 172 594 904 994 265 262 472 408 406 459 645 517 270 247 336

Figure 4. Comparison of seasonal infection percentages among bass taken from ambient and thermal locations of Par Pond.

red-sore disease might be directly related. To test this hypothesis, the infection percentages for each 0.2 unit K-factor subclass between 1.0 and 3.0 were compared; it can be clearly seen (Figure 5) that bass with the lowest body condition are, indeed, the most likely to be infected. A decline in infection percentage begins between K-factors of 1.8 and 2.0 and continues to decrease as body conditions improve. Infection percentages were then compared in each 0.2 unit K-factor subclass for bass from ambient and thermal locations (Figure 6). For bass from thermally-altered areas, infection percentages were higher when body conditions were <1.8 ; where K-factors were >1.8 , infection percentages were lower, but variably so. The pattern for bass in ambient areas was clear, with a decline in infection percentage beginning at 1.8 and continuing to decrease as body conditions improved. When all bass are separated into two groups, e.g. >1.8 and <1.8 , and the data for each season pooled, e.g. spring 1975-77, the patterns reveal a clear and potentially significant relationship between temperature, body condition and the probability of bass acquiring red-sore disease (Figure 7).

Based on these, and other observations to be subsequently detailed, several hypotheses were generated regarding the epizootiology of red-sore disease among bass in Par Pond. These will be discussed in detail in the section concerning the descriptive model.

C. Studies on bass movement in Par Pond: Based on observations regarding the epizootiology of red-sore disease in bass from ambient and thermally-altered areas of Par Pond, a need was evident to be certain that there was not extensive interchange of bass between ambient and thermally-altered locations. Quinn, Esch, Hazen and Gibbons (1978) conclusively showed that few bass moved long

Figure 5. Comparison of infection percentages for bass within 0.2 unit
K-factor subclasses.

Figure 6. Infection percentages of bass in 0.2 unit K-factor subclasses taken in ambient and thermal locations.

**Pooled 2.8 and 3.0
due to small sample sizes.*

Figure 7. Comparison of seasonal changes in infection percentages of bass with body conditions >1.8 and <1.8 .

distances (> 1 km) and that less than 10% moved between ambient and thermal areas. These studies confirm those of Gibbons and Bennett (1971) and strongly suggest that long-range movement of bass in Par Pond does not impact on the epizootiology of red-sore disease in various locations in the reservoir.

A collateral study by Hazen and Esch (1978) on the incidence of parasitism by the digenetic trematode Clinostomum marginatum in largemouth bass is instructive about bass movement in Par Pond. Results showed that infection percentages by this parasite varied from location^a to location within the reservoir. A significant rank correlation was found between infection percentage and the amount of littoral zone present in the locality from which the bass were taken. The authors suggested that the local "bay effect" are the result of limited home and foraging ranges of the bass in relation to the amounts of littoral zone present in various locations of the reservoir.

D. Histopathology associated with red-sore disease in largemouth bass:

A manuscript, describing the histopathology of red-sore disease in largemouth bass, is in preparation (Huizinga, Hazen and Esch, in preparation) and will be briefly summarized here.

In external lesions, the epidermis which normally covers bass scales, is reduced to a thin membrane, or is completely eliminated. The adjacent epidermis becomes hyperplastic (thickened, with an 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. Frequently, there is severe hemorrhagic necrosis of epidermis overlying the fins, to such an extent that it is denuded down to the bony substructure. In many cases, the functional integrity appears destroyed

beyond repair.

Internally, three organs appear to be affected by red-sore disease. In the 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 of different fish, ranging from mild (paranecrotic), to severe, necrosis. In the kidneys, there is acute tubular and glomerular necrosis. In many cases, there was significant destruction of kidney tubules and loss of functional integrity; pathogenesis was variable between bass, ranging from mild (paranecrotic), to severe, necrosis (tissue unrecognizable). In the spleen, pathogenesis was variable, 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.

Histopathology indicates that red-sore disease is acute. It is marked by: 1) hemorrhagic necrosis of the body surface, including the fins, and 2) septicemia and severe necrosis of internal organs due, undoubtedly, to toxic metabolic products released by A. hydrophila. Death of fish is probably induced by failure of major organs (liver, kidney and spleen). These observations are consistent with those reported by Huizinga, Hazen and Esch (manuscript in preparation) for red-sore disease in the American alligator.

E. Studies on water quality and Aeromonas hydrophila distribution and density in Par Pond: The distribution and abundance of Aeromonas hydrophila, in relation to various water quality characteristics in Par Pond, have been studied and reported by Hazen (1978). Among the water quality parameters

measured were temperature, dissolved oxygen, pH, conductivity, redox potential, total organic carbon (TOC) particulate organic carbon (POC), dissolved organic carbon (DOC) and inorganic carbon. Measurements of these parameters were made seasonally, or bi-weekly, in vertical profile at various ambient and thermal locations in Par Pond, within reactor canals leading to Par Pond, in Pond C and Pond B on the SRP, and in the Savannah River at a point near the intake source for Par Pond 'make-up' water. Because of the extensive nature of this study, only the more significant results will be highlighted here (a complete copy of Dr. Hazen's doctoral dissertation is appended to this document).

Densities of A. hydrophila were higher in areas of the reservoir receiving thermal effluent from the reactor. Densities of A. hydrophila were generally heterogeneous in the water column in Par Pond, with the highest levels observed in the surface microlayer (usually in association with organic slicks described and discussed by Lewis in 1974). The highest densities occurred in the spring months of each year (March, April, May), with a second peak in fall, corresponding with the time of fall turnover.

Temperature, redox potential and pH were all significantly correlated with densities of A. hydrophila. High densities of A. hydrophila corresponded with reduced redox potential and pH. It was speculated that the reduction in pH and redox potential were caused by increased densities of A. hydrophila, or in combination with other reducing bacteria such as described by Kuznetzov (1970). TOC, POC, DOC and IC were all measured quarterly, in profile, and in conjunction with the other water quality parameters at selected thermal and ambient locations. There was no correlation between TOC, POC, DOC and density of A. hydrophila at any point in the water column or at any time during the

year. Other investigators (Rogers, 1971; Vezina and Desrochers, 1971; Shotts, et al. 1972; Haley, Davis and Hyde, 1967; Snieszko, 1974) had previously suggested a relationship between dissolved oxygen, organic loading and outbreaks of red-sore disease. The study by Hazen (1978), clearly suggests that neither dissolved oxygen nor organic loading, were related to A. hydrophila distribution and abundance in Par Pond. From the studies by Esch, et al. (1976) and Esch and Hazen (1978), it was also shown that dissolved oxygen and organic loading were not related to red-sore disease in Par Pond. Of all the water quality parameters measured in Par Pond (Hazen, 1978) the only one which was found to affect the density of A. hydrophila was temperature. Thus, within the reservoir at any given point in time, higher temperatures in thermally-altered areas were correlated with higher densities of A. hydrophila. Over a period of time in thermal locations, however, A. hydrophila densities and temperature were negatively correlated. It was suggested by Hazen (1978) that higher temperatures in thermal areas were responsible for decreasing generation and survival times of A. hydrophila, leading to lower densities but higher turnover rates.

The seasonal incidence of red-sore disease and the density of A. hydrophila in Par Pond show a significant positive correlation (Figure 8). While the studies by Hazen (1978) are thorough, and revealing, it must be emphasized that red-sore disease occurs in a variety of habitats within the southeastern U. S., many of which do not receive thermal effluent (Rogers, 1971; Meyer, 1974; Esch and Hazen, 1978). Efforts are currently underway to measure the spatial and temporal distribution and abundance of A. hydrophila in relation to a much wider array of water quality parameters in two North Carolina systems (Albemarle Sound

Figure 8. Relationship between density of A. hydrophila and monthly changes in incidence of red-sore disease among largemouth bass.

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and Badin Lake), both of which have had serious fish kills due to red-sore disease in the past few years.

It can be stated, with confidence, that the epizootiology of red-sore disease is affected by temperature, that temperature clearly affects the density of A. hydrophila, and that temperature also affects body condition of largemouth bass in Par Pond. These relationships have been outlined in Figure 9 and will be discussed more thoroughly in Section 2I of this report.

F. Antibody studies on Par Pond bass: Antibody titers in infected and non-infected largemouth bass were assayed using the ^{51}Cr -labeling technique developed by Kuhn and Iannuzzi (1975). Briefly, the procedure calls for incubation of ^{51}Cr -labeled Aeromonas hydrophila cells with various dilutions of bass serum and then measurement of the number of cells which did not agglutinate.

The highest antibody titers (antibody concentration) were seven (this number represents the Log_2 reciprocal of the last dilution where 50% agglutination occurred). By comparison, Bullock and Stuckey (1976) reported that any titer in fish greater than three should be considered as significant. In our study, 50% of the infected fish were found to have significant titers (>3), while less than 10% of the uninfected fish had significant titers for A. hydrophila antibody. The latter observation suggests that these fish may have had an infection and then recovered, a definite likelihood since the mark-release-recapture study (previously discussed) also indicates that bass with red-sore disease are capable of recovery.

Serum from bass with high antibody titers was fractionated using gel filtration (Sephadex 200) and ion chromatography (DEAE cellulose). Both

methods of fractionation revealed that anti-A. hydrophila antibody is a high molecular weight protein and possesses properties similar to IgM antibodies in mammals. If the bass antibody is functionally similar to the IgM class of antibodies in mammals, and there appears to be good reason for believing that it is, then anti-A. hydrophila antibody in bass is probably short-lived and is not incorporated into the immune memory of the fish. Thus, once a fish becomes infected, it would be able to initiate an immune response to A. hydrophila with the titer remaining high for brief periods after recovery from the disease. This would explain why some apparently uninfected bass had significant titers of anti-A. hydrophila antibody.

The highest titers were observed during the spring and summer months. Spring, as previously noted, corresponds to the period of highest infection in bass. Persistent high titers in summer could be explained by a large portion of the population acquiring the disease in spring, with some recovering during summer. If the antibody is protective, this may also explain why the infection percentage declines during summer, contrary to the decrease in body condition and the high densities of A. hydrophila in Par Pond.

G. Biochemical and serological studies on A. hydrophila: Results of biochemical and serological studies on A. hydrophila are, as yet, preliminary, but they are promising and appear to warrant further effort. A total of 365 A. hydrophila isolates from red-sore lesions, fish intestines, the water column and sediments were tested for similarities and differences, using 42 biochemical tests. It should be noted that these isolates were not only taken from Par Pond and Par Pond bass, but from fish, sediments and water throughout the United States.

The results of the biochemical tests on the 365 isolates were examined by cluster analysis. All 365 isolates were similar for at least 75% of the characters tested; at a similarity index of 0.95, there were two clusters, one containing 357 isolates from the various sources. The second cluster of eight isolates included seven from fish and one from Par Pond water. Of the seven fish isolates, five were from red-sore lesions. Using fluorescent antibody procedures, anti-water isolates infrequently reacted with A. hydrophila from red-sore lesions.

The data from biochemical tests on A. hydrophila isolates from various sources in Par Pond and throughout the United States, indicate a remarkable similarity. However, there is sufficient justification from the same biochemical tests, and from serological procedures, to warrant further examination. It would be of obvious significance in understanding the epizootiology of red-sore disease, if we could establish that there are strain differences between isolates from red-sore lesions and those from other sources in aquatic systems. It would also be important if we were to find strains with variable virulence within different geographic areas of the United States (for additional comments relating to this problem, see section III, entitled Concluding Remarks).

H. Blood parameters among infected and non-infected bass in Par Pond:
Early in this study, it became apparent that useful information regarding the epizootiology of red-sore disease might be obtained from knowledge of changes in selected blood parameters in bass from ambient and thermal locations in Par Pond. Results of such a study were presented in Hazen, Esch, Glassman and Gibbons (1978). They measured hematocrits, hemoglobin,

total iron-binding capacity, iron, sodium, potassium and thyroxine levels in bass from ambient and thermal locations taken during July and December 1975 and 1976. Differences in blood parameters in bass from ambient and thermal locations, and seasonally, not surprisingly suggested that temperature was a significant factor in altering the basic blood physiology of bass. Perhaps most significant was the finding of elevated thyroxine levels in bass during the summer months, for this would then be of importance in contributing to changes in body condition of bass, both spatially and temporally.

The above study suggested that metabolic rates, which changed seasonally, might also be increased by thermal effluent, resulting in decreases in body condition. Since body condition and probability of acquiring red-sore disease were known to be related, it was further speculated that stress, induced by elevated temperature, could be involved in the epizootiology of red-sore disease. This hypothesis, if confirmed, would have far-reaching consequences since it would identify not only a key element for red-sore disease in Par Pond bass, but might also become important in identifying the key element for red-sore epizootics in other reservoirs not affected by thermal effluent. In other words, stress caused by a number of etiologies, would then become the "bottom line" for red-sore epizootiology and not such isolated factors as elevated temperature, organic loading, dissolved oxygen, or other individual water quality parameters.

In order to develop the present report in a logical pattern, it is necessary to hold off on further discussion of blood parameters until the next section, dealing with the descriptive model for red-sore disease in Par

Pond bass.

I. Descriptive model for red-sore disease: Based on the results of studies presented to this point, Esch and Hazen (1978) developed a descriptive model for red-sore disease in Par Pond bass (Figure 9).

The core of the schematic rests with the concept of stress, discussed by Esch and Hazen (1978), but based on an amalgamation of ideas previously presented by Selye (1950; 1956) Brett (1958), Slobodkin (1967), Odum (1969), Cairns (1976) and Gibbons (1976). Basically, the concept of stress is described as a three stage series of events in an individual organism, beginning with the alarm response, followed by a stage of resistance and culminating in a stage of exhaustion which leads to death. A significant component in this series of reactions is homeostatic imbalance generated by prolonged and/or excessive quantities of corticosteroids produced during the so-called resistance stage. While these hormones are said to mitigate cellular damage, a serious consequence of their presence in excessive quantities is the simultaneous reduction in natural and/or acquired resistance to pathogenic agents (Esch, Bourque and Gibbons, 1974; Esch and Hazen, 1978). It is this specific phenomenon that requires special consideration in the epizootiology of red-sore disease in Par Pond bass.

Esch and Hazen (1978) proposed that elevated temperature in Par Pond increased metabolism in bass (Figure 9). Increased metabolism was accompanied by increased fat and protein catabolism, resulting in reduced "fitness" or body condition. Hazen, et al. (1978) have shown that thyroxine levels in bass are elevated at summer temperatures, indicating elevated metabolic rates during this period. Reduction in dissectable body fat, in conjunction with

Figure 9. Proposed relationships between red-sore disease, temperature, body condition and stress among largemouth bass in Par Pond.

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reduced body condition of bass in thermally-altered areas of the reservoir, has been reported by Gibbons, et al. (1978). Reduced foraging success in summer and early fall, in combination with increased expenditure of energy while foraging during this period were suggested to exacerbate the effects of increased metabolism of bass in thermally-altered locations. Contributing to the foraging problems during this period are the extensive stands of the rooted, aquatic, macrophyte, Myriophyllum spicatum (Grace and Tilly, 1976; personal observation), which provide refuge for potential forage species.

By whatever mechanism, and during any given period of time, there are substantial data indicating that bass in the thermally-altered areas of Par Pond are in poorer body condition than those taken in other areas of the reservoir (Gibbons, et al., 1978; Esch, et al., 1976). Furthermore, there is also abundant evidence indicating that the probability of acquiring red-sore disease is directly related to body condition (Esch and Hazen, 1978). Contributing to the increased potential for red-sore disease in Par Pond bass is the correlation between elevated water temperature and increased density of Aeromonas hydrophila. It seems reasonable to conclude that increased levels of the etiological agent for red-sore disease, especially in combination with bass in poor body condition, will lead to increasing probability of bass acquiring the disease.

Up to the winter of 1978, direct evidence for the relationship of stress and the probability of acquiring red-sore disease was circumstantial at best. Likewise, the relationship between body condition and stress was also problematical. However, as suggested previously, elevated thyroxine levels suggested increased metabolic rates in bass, and furthermore, elevated

thyroxine levels in mammals is sometimes known to be related to stress. If stress were to be involved in the epizootiology of red-sore disease among bass in Par Pond, then it would be necessary to first demonstrate a correlation between body condition and elevated levels of circulating corticosteroids and then between corticosteroids and changes in natural or acquired resistance in bass. Toward these ends, more than 400 bass from ambient and thermally-altered areas of Par Pond were systematically captured and bled by cardiac puncture throughout the period from 1975 to 1978. The mean values for body condition, hematocrit, hemoglobin, total red blood cell count, total white blood cell count and cortisol were compared according to season, site of capture (ambient versus thermal), sex, presence or absence of red-sore disease and body condition.

Season (disregarding sex, disease and body condition): An examination of mean blood parameters (Table 2) does not reveal a seasonal pattern over the two year period of study. Body conditions did, however, follow the pattern reported previously by a number of investigators for Par Pond bass (Esch, et al., 1976; Esch and Hazen, 1978; Gibbons, et al., 1978). In spite of the apparent inconsistency of seasonal variability among these blood parameters, there is a suggestion that depressed levels of several blood parameters may be related to low body conditions; this relationship will be considered subsequently.

These and other blood parameters are known to vary in different fish species according to diet, age, sex, season, method of capture and state of sexual maturity (McCarthy, Stevenson and Roberts, 1973). It is suggested that the absence of seasonal periodicity for the blood parameters measured

Table 2

Seasonal comparisons of
blood parameters in bass from
ambient and thermal locations
in Par Pond.

Blood factor	Spring 1976		Summer 1976		Fall 1976		Winter 1977	
	HD	CD	HD	CD	HD	CD	HD	CD
K-factor	2.26	2.26	1.87	2.33	2.18	2.21	2.42	2.06
Hmtct	40.77	42.67	37.74	42.24	38.46	36.38	38.22	31.02
Hmglbn	8.58	9.30	4.66	5.96	8.58	8.14	8.64	8.94
WBC	4476.0	3147.0	1893.0	2845.0	1252.0	1367.0	2582.0	1646.0
RBC	1968.0	2313.0	1503.0	1617.0	1156.0	1664.0	1775.0	1399.0
Cortisol	----	13.15	10.10	13.13	16.57	14.85	13.59	11.01

Blood factor	Spring 1977		Summer 1977		Fall 1977		Winter 1978	
	HD	CD	HD	CD	HD	CD	HD	CD
K-factor	2.21	2.23	1.74	1.98	1.83	2.32	2.21	2.33
Hmtct	40.56	36.46	33.35	32.97	32.22	35.32	37.15	36.72
Hmglbn	9.74	9.82	8.93	9.01	7.52	9.39	9.40	9.60
WBC	2034.0	2046.0	2247.0	2298.0	1538.0	2335.0	2260.0	2764.0
RBC	1092.0	1257.0	1078.0	915.0	563.0	836.0	543.0	776.0
Cortisol	12.78	8.28	12.94	17.59	12.99	13.12	12.21	17.94

Values for blood parameters are as follows:

Hematocrit = % cell volume

Hemoglobin = gm%

RBC = $N \times 10^3$ cells/mm³

WBC = cells/mm³

Cortisol = ug/100 ml

in the present study is primarily due to inadequate quarterly sample sizes and variability in reactor activity over the two year study period, i.e. reactor shut-down for long periods during a given quarter of one year would create differences during the same quarter of the second year if the reactor were operational during the latter year.

Location (disregarding infection, sex and season): Data for blood parameters and body conditions of all bass from ambient locations were pooled and compared with those from thermal areas; several trends are apparent (Table 3). First, body conditions of bass from heated areas were significantly lower than those from ambient locations; this observation conforms to those previously reported by Esch, et al. (1976), Esch and Hazen, 1978 and Gibbons, et al. (1978). Second, hematocrits, hemoglobins, total white blood cell counts and cortisol levels were not significantly different in bass from the two areas. Finally, the only blood parameter consistently different in bass from ambient and thermal locations was total red blood cell count. This difference may be related to levels of dissolved oxygen and/or percent oxygen saturation which were significantly higher throughout the water column during periods of mixis and down to 3 m in depth during periods of stratification (Hazen, 1978). Such an explanation should be cautiously considered, however, since depressed levels of oxygen have been shown to stimulate increases in total red blood cell counts among some teleost species (Phillips, 1974) as well as depress total red blood cell counts in other species (Westphall, 1943).

Sex (disregarding location, season, infection and body condition): Data for blood parameters and body conditions were pooled and compared according to sex. There was not a significant difference in mean values between female

Table 3

A comparison of different blood parameters from bass taken in ambient and thermal locations.

Location	K-factor*	Hmtct*	Hmglbn*	WBC* °	RBC*	Cortisol**
HD	2.08	39.95	8.50	2647.0	1213.0	13.06
CD	2.22	39.68	8.96	2694.0	1348.0	13.22
F =	15.918	.146	4.766	.115	6.514	.039
P =	<.0001	>.5	.05>p>.1	>.5	.05>p>.02	>.5

*N = HD, 234; CD, 239

**N = HD, 184; CD, 211

Values for blood parameters are as follows:

Hematocrit = % cell volume

Hemoglobin = gm%

RBC = $N \times 10^3$ cells/mm³

WBC = cells/mm³

Cortisol = ug/100 ml

and male bass for any blood factor or body condition.

Infection (disregarding location and season): Of all the blood factors and body condition, only total red blood cell counts were different in infected and non-infected bass (Table 4). This observation was unexpected since red-sore disease is known to cause hemorrhagic septicemia which should result in decreased red blood cell counts and corresponding changes in both hematocrit and hemoglobin. Based on this knowledge, we have tentatively concluded that differences in total red blood cell counts in infected and non-infected bass may be spurious. Contributing to this assessment is the observation that K-factors for infected and non-infected bass were not significantly different. Based on substantially larger sample sizes taken since 1975, we know that bass with red-sore disease have significantly lower K-factors than non-infected individuals. We have concluded, therefore, that the sample size for the present study is as yet too small to permit any general statement regarding potential relationships between red-sore disease and various blood parameters in Par Pond bass.

Body condition (disregarding location, infection, season and sex): A consistent feature of the Par Pond bass population is the presence of large numbers of individuals with relatively poor body conditions. Further, a strong correlation has been demonstrated between body condition and the probability of a given bass having red-sore disease (Esch, et al., 1976; Esch and Hazen, 1978). We have speculated that these bass (those in poor body condition) have been subjected to stress, probably induced by thermal effluent from the nuclear production reactor, and, that as a consequence of stress, there should be a relationship between body condition and various blood

Table 4

A comparison of blood parameters
and body conditions from infected
and non-infected largemouth bass.

	K-factor	Hmtct	Hmglbn	WBC	RBC	Cortisol
Non-infected	2.16	39.57	8.70	2602.0	1217.0	13.04
Infected	2.09	40.97	8.84	2991.0	1573.0	13.84
F =	2.152	2.384	0.239	4.572	27.151	.493
p =	.5>p>.2	>.5 p>.2	>.5	.1>p>.05	<.0001	>.5

The N values for cortisol levels in non-infected and infected bass respectively were 341 and 54; for all other parameters, they were, respectively, 382 and 81.

Values for blood parameters are as follows:

Hematocrit = % cell volume

Hemoglobin = gm%

RBC = $N \times 10^3$ cells/mm³

WBC = cells/mm³

Cortisol = ug/100 ml

factors. Accordingly, bass were separated into four, 0.5 unit K-factor subclasses and the mean value for each blood factor in each group computed and compared with the others (Table 5.)

The data clearly show striking trends for each blood factor in relation to body condition. For each blood factor (except cortisol), the lowest mean value was for bass in the poorest body condition. Mean values were next highest in bass with body conditions ranging from 1.5-2.0; mean values for the two subclasses above 2.0 were highest and generally quite similar. These data suggest that bass with K-factors above 2.0 are relatively "fit" while those below 2.0 are, in some manner, indisposed. It is interesting to note the parallel for the relationship between body condition and blood factors as compared with body condition and the probability of bass acquiring red-sore disease (see section II B of this report).

Cortisol was the only parameter which did not fit the relationship established for body condition and hematocrit, hemoglobin, total red blood cell count and total white blood cell count. However, when cortisol and body condition were compared using simple multiple regression analysis, a significant negative relationship was found to exist. Thus, as body condition declined, cortisol levels increased. When all the data were examined by multiple regression analysis, each variable was found to be significantly related to K-factor (the dependent variable); hematocrit contributed most to variability in the regression equation ($r^2 = 27\%$) and total white blood cell count the least ($r^2 = 2\%$).

In general, the data for blood factors and body conditions in the Par Pond bass tend to confirm, at least in part, the stress hypothesis. In

Table 5
Comparisons of various blood
factors for bass within 0.5 unit
K-factor subclasses.

K-factor subclass	Hmtct	Hmgln	Blood factors		Cortisol
			WBC	RBC	
<1.5 ¹	30.23	6.50	1678.0	871.0	12.08
1.5-2.0 ²	35.96	7.88	2325.0	1169.0	15.33
2.0-2.5 ³	41.83	9.18	2896.0	1347.0	12.38
>2.5 ⁴	42.75	9.36	2818.0	1381.0	12.71
F =	45.69*	22.11*	8.74*	8.42*	3.52*

*Values within each blood factor group are significantly different.

¹N = 28 for all blood parameters except cortisol which = 22

²N = 114 for all blood parameters except cortisol which = 98

³N = 256 for all blood parameters except cortisol which = 216

⁴N = 65 for all blood parameters except cortisol which = 59

Values for blood parameters are as follows:

Hematocrit = % cell volume

Hemoglobin = gm%

RBC = $N \times 10^3$ cells/mm³

WBC = cells/mm³

Cortisol = ug/100 ml

terms of bass acquiring red-sore disease, the most critical of the blood factors are the rise in cortisol accompanied by the decline in total white blood cell counts (= leucopenia). As pointed out previously, however, the same data set does not reveal a relationship between white blood cell count, cortisol and bass infected with A. hydrophila. The absence of a significant relationship could have been due to (1) small sample size, or (2) stress-induced variability in cortisol or white blood cell counts produced by crude collecting or handling procedures, both of which are known to increase, by significant amounts, the levels of circulating catecholamines and corticosteroids. The only way in which either of these problems can be overcome is to design laboratory experiments so that more exacting control can be exerted over various procedures and protocols involved in handling and bleeding of bass. Efforts are proceeding at the present time to achieve this goal.

Another problem regarding the cortisol data deserves comment. While simple regression analysis reveals a significant relationship between cortisol and body condition, intuitively we should not expect the bass with poorest body conditions to possess mean cortisol values approximating those of bass in relatively good body condition, i.e. >2.0. A possible explanation for this apparent inconsistency rests with an analysis of the stress concept itself. According to Selye (1950; 1956), the final stage of the General Adaptation Syndrome is one of adrenal exhaustion which leads to a cessation in production of adrenocorticosteroids. If bass in the poorest body condition are the most highly stressed, and if adrenal exhaustion in these bass has occurred (or is occurring), then it should follow that cortisol levels in these bass would, likewise, be in a process of decline from high levels reached

during the resistance stage of the General Adaptation Syndrome. Such an hypothesis would account for the relatively low cortisol levels in bass having the lowest K-factors. This hypothesis could be confirmed by careful examination of cortical cells in the head kidneys of bass representing a broad spectrum of K-factors. Unfortunately, histological and cytological examination was not made of the head kidneys from the 400 bass used in the present study. However, such an investigation is planned for the next contract period.

While we acknowledge the existence of some problems yet to be resolved, we nonetheless believe that the data from the blood study on Par Pond bass tend to support the stress hypothesis and its relationship to thermal loading and body condition. Data are, as yet, insufficient to permit an analysis of the relationship between cortisol, leucopenia and a reduction in natural and/or acquired resistance to infection by A. hydrophila. However, intense efforts, both in the field and in the laboratory, are now underway so that this step in the descriptive model can be completed.

III. Concluding remarks: The implications from these studies are varied, sometimes clear and sometimes less so, for many of the results have raised new and even more critical questions. Thus, for example, our data clearly show that A. hydrophila is the etiological agent for red-sore disease. Furthermore, they suggest that the effects of temperature are twofold, first in increasing the density of the pathogen in the water column and then in affecting the physiology of the host organism to such an extent as to increase the probability of acquiring the pathogen. On the other hand, organic loading, suggested by other investigators as being important in

red-sore disease, was not identified as being significant in the present study. However, if organic loading, or any of its consequences, can be shown to induce stress, then it may be as important in other systems as temperature is in Par Pond. Thus it is quite conceivable that it (organic loading), or some other water quality parameter, may create conditions conducive to increasing densities of *A. hydrophila* while simultaneously producing water quality characteristics which would lead to stress in fish, and then to increasing the probability of fish acquiring red-sore disease.

An enigmatic observation (see Hazen, 1978, for details), is that *A. hydrophila* has been recovered from a variety of habitats throughout the U. S., yet red-sore disease is known to occur only in the southeast. This peculiar distribution pattern raises several important questions regarding the epizootiology of red-sore, not the least of which is the possibility of there existing differentially virulent strains of *A. hydrophila* and/or more or less susceptible populations of potential hosts in various parts of the country. Other significant questions are related to the variability in amplitude of red-sore disease from one year to the next among bass in Par Pond, the mode of entry of the pathogen into largemouth bass, and the basic, cellular mechanisms of stress in largemouth bass.

In North Carolina alone, it is estimated that commercially valuable fish worth \$25.6 million (at the dock) are caught annually and that recreational fishermen generate revenues of up to \$300 million annually through their activities. If all of the southeastern states are included, it is conceivable that sport and commercial fishing together constitute a multi-billion dollar industry each year. The spector of red-sore disease is

thus a serious one for those in the southeastern United States who are dependent, in any manner, on commercial or sport fishing industries. In the absence of a clear understanding of the epizootiology of the disease and with the prospect of increasing numbers of epizootic outbreaks of the disease in the future, it appears clear to us that the studies begun in 1974, and reported herein, should be continued.

IV. Papers and books published, in press, or in preparation and papers presented at national meetings (supported completely, or in part, by Contract EY-76-S-09-0900 between DOE and Wake Forest University):

A. Published (papers)

- 1) Hazen, T.C., G. Smith and R.V. Dimock, Jr. 1976. A method for fixing and staining peritrich ciliates. Trans. Amer. Micros. Soc. 95: 693-695.
- 2) Esch, G.W., T.C. Hazen, R.V. Dimock, Jr. and J. W. Gibbons. 1976. Thermal effluent and epizootiology of the ciliate Epistylis and the bacterium: Aeromonas in association with centrarchid fish. Trans. Amer. Micros. Soc. 95: 687-693.
- 3) Esch, G.W., T.C. Hazen and J.M. Aho. 1977. Parasitism and r and K selection. In: Regulation of Parasite Populations. G.W. Esch ed. Academic Press. Pp. 9-62.
- 4) Fliermans, C.B., R.W. Gorden, T.C. Hazen and G.W. Esch. 1977. Aeromonas distribution and survival in a thermally altered lake. Appl. Environ. Microbiol. 33: 114-122.
- 5) Esch, G.W., G.M. Campbell 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.

- 6) Hazen, T.C. and G.W. Esch. 1977. Studies on the population biology of two larval trematodes in the amphipod, Hyalella azteca. Amer. Midl. Nat. 98: 213-219.

B. In press (papers)

- 1) Hazen, T.C. and G. W. Esch. 1978. Observations on the ecology of Clinostomum marginatum in largemouth bass (Micropterus salmoides). J. Fish Biol. 12: (In Press).
- 2) Hazen, T.C., G.W. Esch, A.B. Glassman and J.W. Gibbons. 1978. Relationship of season, thermal loading and red-sore disease with various hematological parameters in largemouth bass (Micropterus salmoides). J. Fish Biol. 12: (In Press).
- 3) Quinn, T., G.W. Esch, T.C. Hazen and J.W. Gibbons. 1978. Long range movement and homing by largemouth bass (Micropterus salmoides) in a thermally altered reservoir. Copeia 1978: (In Press).
- 4) Gorden, R.W., T.C. Hazen and C.B. Fliermans. 1978. The presence of Aeromonas hydrophila, a potential pathogen, in and on Alligator mississippiensis. J. Wildlife Dis. 14: (In Press).
- 5) Hazen, T.C., M.L. Raker, G.W. Esch and C.B. Fliermans. 1978. Ultrastructure of red-sore disease lesions on largemouth bass (Micropterus salmoides): the association of the peritrich Epistylis sp. and the bacterium Aeromonas hydrophila. J. Protozool. 25: (In Press).
- 6) Esch, G.W. and T.C. Hazen. 1978. Thermal ecology and stress: A case history for red-sore disease in largemouth bass

(Micropterus salmoides). In: Energy and Environmental Stress in Aquatic Systems. J.J. Thorpe and J.W. Gibbons, eds. DOE Symposium Series (CONF 771114). (In Press).

- 7) Gibbons, J.W., G.W. Esch, D.E. Bennett and T.C. Hazen. 1978. Effect of thermal effluent on the body conditions of largemouth bass (Micropterus salmoides). Nature (In Press).

C. In preparation (papers)

- 1) Esch, G.W. and T.C. Hazen. Cortisol and other blood factors in largemouth bass from a thermally-altered reservoir.
- 2) Hazen, T.C., M. Raker, G.W. Esch and C.B. Fliermans. Antibodies to Aeromonas hydrophila in largemouth bass (Micropterus salmoides).
- 3) Hazen, T.C., J. Matthews, C.B. Fliermans and G.W. Esch. The prevalence and abundance of Aeromonas hydrophila in various lakes and rivers across the United States.
- 4) Hazen, T.C., C.B. Fliermans and G.W. Esch. Isolates of Aeromonas hydrophila from water, sediment and fish and their relationship to isolates from red-sore lesions of largemouth bass (Micropterus salmoides).
- 5) Huizinga, H., T.C. Hazen and G.W. Esch. The pathology of red-sore disease in largemouth bass (Micropterus salmoides).
- 6) Hazen, T.C. The ecology of Aeromonas hydrophila in a South Carolina cooling reservoir.
- 7) Hazen, T.C. A comparison of Aeromonas hydrophila distribution and abundance in three South Carolina reservoirs.

D. Published (books)

"Regulation of Parasite Populations." Edited by G.W. Esch.

Academic Press, N.Y., 253 pp.

E. Papers presented at national meetings

- 1) Esch, G.W., T.C. Hazen and J.W. Gibbons. Thermal effluent and epizootiology of Epistylis (Ciliophora Peritricha). August, 1976, San Antonio, Texas. American Society of Parasitologists annual meeting.
- 2) Fliermans, C.B., R.W. Gorden, T.C. Hazen and G.W. Esch. Distribution and survival of Aeromonas in a thermally altered lake. May, 1976, Atlantic City, New Jersey. American Society for Microbiology annual meeting.
- 3) Hazen, T.C. and G.W. Esch. The distribution of Clinostomum marginatum metacercaria in the centrarchid fish of a South Carolina Cooling reservoir. August, 1976, San Antonio, Texas. American Society of Parasitologists annual meeting.
- 4) Hazen, T.C., G.W. Esch and M. L. Raker. Light and electron microscope studies on lesions associated with red-sore disease in largemouth bass. April, 1977, Raleigh, North Carolina. Southeastern Society of Parasitologists annual meeting.
- 5) Glassman, A.B., C.E. Bennett, T.C. Hazen, R.W. Gorden and C.B. Fliermans. Light and electron microscopy of the peripheral blood in Alligator mississippiensis. April, 1977, Savannah, Georgia. Georgia Academy of Science annual meeting. November, 1977, Augusta, Georgia. Symposium on Energy and Environmental stress

in Aquatic Systems.

- 6) Hazen, T.C., G.W. Esch and C.B. Fliermans. Distribution of Aeromonas hydrophila in a South Carolina cooling reservoir. May, 1977, New Orleans, Louisiana. American Society for Microbiology annual meeting.
- 7) Hazen, T.C., R.P. Hirsch and G.W. Esch. Hines and Nicholas revisited: a study of parasite distribution. August, 1977, Las Vegas, Nevada. American Society of Parasitologists annual meeting.
- 8) Fliermans, C.B., T.C. Hazen and M.R. Tansey. Distribution of Aeromonas hydrophila and other pathogens in a South Carolina reservoir. Microbiology of Power Plant Thermal Effluents.
- 9) Hazen, T.C., G.W. Esch and C.B. Fliermans. The distribution of the bacterium Aeromonas hydrophila in thermally altered environments. November, 1977, Augusta, Georgia. Symposium on Energy and Environmental Stress in Aquatic Systems.
- 10) Esch, G.W. and T.C. Hazen. Thermal ecology and stress: A case history for red-sore disease in largemouth bass (Micropterus salmoides). November, 1977, Augusta, Georgia. Symposium on Energy and Environmental Stress in Aquatic Systems.
- 11) Gibbons, J.W., D.H. Bennett, G.W. Esch and T.C. Hazen. Body condition of largemouth bass in a reservoir receiving heated effluent. November, 1977, Augusta, Georgia. Symposium on Energy and Environmental Stress in Aquatic Systems.
- 12) Hazen, T.C. The ecology of the bacterium Aeromonas hydrophila in

a South Carolina cooling reservoir. August, 1978, Athens, Georgia. Ecological Society of American annual meeting.

- 13) Esch, G.W. and T.C. Hazen. Cortisol and body condition of largemouth bass in a South Carolina cooling reservoir. August, 1978. Ft. Collins, Colorado. Wildlife Disease Association annual meeting.

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V. Statement of compliance

This is to indicate that all aspects of the Contract EY-76-S-09-900 have been complied with by the Contractor.

VI. Approximate percentage of time devoted to project and amount of time to be devoted during the remaining months. The principal investigator has

devoted approximately 50% of his time to the project during the current academic year (1977-78) and will do so again during the first three months of the next academic year. For two and one-half summer months, he will devote 100% of his time.

VII. Estimate of prob. events and occurrences during remainder of current contract period. The primary objectives during the remainder of this contract period will be as follows.

- A. Continue monitoring epizootiology of red-sore disease in Par Pond.
- B. Continue measuring physico-chemical water quality and density of A. hydrophila in Par Pond.
- C. Conduct laboratory experiments on thermally-induced stress in bass.
- D. Initiate studies on strain identification of A. hydrophila.
- E. Continue studies on various hematologic characteristics of Par Pond bass.
- F. Complete histopathology studies on red-sore disease in Par Pond bass.