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

Progress Report

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ABSTRACT

During the current contract year, the following results were obtained: (1) Data from field-generated studies have shown that hematocrit, hemoglobin, total red blood cell counts, total white blood cell counts and cortisol levels are significantly affected in largemouth bass with body conditions < 2.0, suggesting that stress, body condition and the probability of bass acquiring red-sore disease are related. Laboratory studies appear to at least partially confirm these results. (2) Chemotaxis studies show that Aeromonas hydrophila is attracted to specific sugars and amino acids and that there appears to be a strain-related affect of fish mucous on A. hydrophila isolated from red-sore lesions. (3) Immunologic and serologic tests suggest that A. hydrophila isolated from red-sore lesions on bass from one lake system will not cross-react with antibodies in sera isolated from bass in another lake system, suggesting the existence of different strains in different lakes.

I. Brief Description of Scope of Investigation and Significant Results

Red-sore disease has been shown to be a significant problem for the sport and commercial fishing industries in the southeastern United States (Rogers, 1971; Shotts, et al., 1972; Miller and Chapman, 1976; Esch and Hazen, 1978). Evidence generated over the past four years has strongly indicated that the epizootiology of red-sore disease in a South Carolina cooling reservoir is affected by thermal effluent from a nuclear production reactor (Esch, et al., 1976; Esch and Hazen, 1978). Several lines of study pursued during the same period of time have conclusively shown that the gram-negative bacterium, Aeromonas hydrophila, is the primary etiologic agent for the disease (Hazen, et al., 1978; Huizinga, et al., 1979). A stress hypothesis, formulated by Esch and Hazen (1978) appears to have substantial field and laboratory evidence for support.

Results of studies during the current contract year are presented in manuscript form and in reprints comprising the next section of the report.

II. Progress in Achieving Objectives

Rather than repeating verbatim information contained within manuscripts and reprints submitted or published during the current year, an abstract and/or summary of each is presented. These abstracts and/or summaries will comprise the annual report for the current contract year.

A.) Hazen, T. C., M. L. Raker, G. W. Esch and C. B. Fliermans, 1978. Ultrastructure of red-sore lesions on largemouth bass (Micropterus salmoides): the association of the peritrich Epistylis sp. and the bacterium Aeromonas hydrophila. J. Protozool. 25: 351-355.

ABSTRACT: Epizootic outbreaks of red-sore disease in several reservoirs in

the southeastern United States have been reported to cause heavy mortality among several species of fish having sport and commercial value. The etiologic agent is said to be the peritrich ciliate Epistylis sp.; secondary infection by the gram-negative bacterium Aeromonas hydrophila produces hemorrhagic septicemia which results in death. However, in recent studies on the largemouth bass Micropterus salmoides, Epistylis sp. could be isolated from only 35% of 114 lesions from 114 fish, while A. hydrophila was found in 96% of the same lesions. Transmission and scanning electron microscopy of lesions associated with red-sore disease indicate that neither the stalk nor the attachment structure of Epistylis sp. have organelles capable of producing lytic enzymes. Since other investigators have shown that A. hydrophila produces strong lytic toxins, and in absence of evidence to the contrary, it is concluded that Epistylis sp. is a benign ectocommensal and that A. hydrophila is the primary etiologic agent of red-sore disease.

B.) Hazen, T. C., C. B. Fliermans, R. P. Hirsch and G. W. Esch. 1978. The prevalence and distribution of Aeromonas hydrophila in the United States. Appl. Environ. Microbiol. 36: 731-738.

ABSTRACT: The abundance of Aeromonas hydrophila was measured in 147 natural aquatic habitats in 30 states and Puerto Rico. Viable cell counts were used to estimate density at all sites by using Rimler-Shotts medium, a differential presumptive medium for A. hydrophila. Temperature, pH, conductivity, salinity, and turbidity were measured simultaneously with water sample collection. The density of A. hydrophila was higher in lotic than in lentic systems. Saline systems had higher densities of A. hydrophila than did freshwater systems. A. hydrophila could not be isolated from extremely saline, thermal,

or polluted waters, even though it was found over wide ranges of salinity, conductivity, temperature, pH, and turbidity. Of the water quality parameters measured, only conductivity was significantly regressed with density of A. hydrophila.

C.) Huizinga, H., G. W. Esch and T. C. Hazen. 1979. Histopathology of red-sore disease (Aeromonas hydrophila) in naturally and experimentally infected largemouth bass (Micropterus salmoides). J. Fish Dis. 2: 310-321.

ABSTRACT: The histopathology of red-sore disease, caused by the gram-negative bacterium, Aeromonas hydrophila, is described for largemouth bass, Micropterus salmoides. Externally, lesions range from those affecting a few scales (pin-point), to those associated with extensive chronic ulcerations; there is focal hemorrhage, oedema and dermal necrosis which exposes underlying muscles producing infiltration of mononuclear and granulocytic inflammatory cells. Internally, the liver and kidneys are foci for toxic products produced by A. hydrophila with, in the most severe cases, complete destruction of the structural integrity of both organs. Pathological changes were not serious in either the spleen or heart, even in cases with massive damage in the liver and kidney. Internal and external lesions were similar in both natural and experimentally induced infections. The pathobiology of red-sore disease in bass was postulated to be linked to elevated water temperature stimulating increased metabolism, decreased body condition and stress, leading to the increased production of corticosteroids and the concommitant rise in susceptibility to infection.

D.) Hazen, T. C. and C. B. Fliermans. 1979. The distribution of Aeromonas

hydrophila in natural and man-made thermal effluents. Appl. Environ. Microbiol. 38: (In press).

ABSTRACT: Densities of Aeromonas hydrophila showed distinct thermal optima (25-35°C) and thermal maxima (45°C) when measured along thermal gradients created by geothermal and nuclear reactor effluents. Survival of A. hydrophila never exceeded 48 hr at temperatures > 45°C. Thermophilic strains could not be isolated at any site.

E.) Hazen, T. C. 1979. The ecology of Aeromonas hydrophila in a South Carolina cooling reservoir. Microb. Ecol. (In press).

ABSTRACT: Densities of Aeromonas hydrophila were determined monthly from December 1975 to December 1977 in a South Carolina cooling reservoir which receives heated effluent from a single nuclear production reactor. Selected water quality parameters and prevalence of red-sore disease among largemouth bass were monitored simultaneously.

Higher densities of A. hydrophila were observed in areas of the reservoir receiving effluent from the reactor. Densities of A. hydrophila generally were heterogeneous in the water column. The sediments had lower densities of A. hydrophila than water immediately above. A. hydrophila could not be isolated from sediments greater than 1 cm from the water interface. Temperature, redox potential, pH and conductivity were all significantly correlated with densities of A. hydrophila in the water column. The temporal and spatial distribution and abundance of A. hydrophila in water were not related to total organic carbon, dissolved organic carbon, particulate organic carbon, inorganic carbon or dissolved oxygen. High densities of A. hydrophila were observed in mats of decomposing Myriophyllum spicatum and, enterically, in largemouth bass,

several other species of fish, turtles, alligators, and snails. The greatest densities of A. hydrophila in water occurred during the months of March and June with a second peak in October. The mean monthly densities of A. hydrophila were positively correlated with the incidence of infection in largemouth bass. Largemouth bass from thermally altered parts of the reservoir had a significantly higher incidence of infection. It is concluded that thermal effluent significantly effects the ecology of A. hydrophila and the epizootiology of red-sore disease within Par Pond.

F.) Fliermans, C. B. and T. C. Hazen. Submitted to Can. J. Microbiol.

Serological specificity of Aeromonas hydrophila as measured by immunofluorescence photometric microscopy.

ABSTRACT: Strain specific fluorescent antibodies were prepared for three isolates of Aeromonas hydrophila. These antisera were reacted with 276 other A. hydrophila isolates and their immunofluorescence was measured quantitatively by photometric fluorescence microscopy. Statistical analyses indicated that the A. hydrophila isolated from aquatic habitats were serologically distinct from the other isolates as a serogroup.

G.) Esch, G. W. and T. C. Hazen. Submitted to Trans. Amer. Fish. Soc.

Relationship of body condition and various hematological parameters of largemouth bass (Micropterus salmoides) in a South Carolina cooling reservoir.

ABSTRACT: A total of 465 largemouth bass (Micropterus salmoides) were collected in Par Pond, a South Carolina cooling reservoir; bass were captured quarterly, beginning in summer 1976 and continuing through winter 1978. Hematocrit, hemoglobin, total red blood cells (TRBC), total white blood cells

(TWBC), cortisol and body condition (K-factor) were measured for each bass; the presence or absence of red-sore disease was confirmed by visual observation and subsequent isolation of the causative agent, Aeromonas hydrophila, on R-S medium. Results show that hematocrit, hemoglobin, TRBC and TWBC were significantly lower in bass with K-factors < 2.0, while cortisol levels and prevalence of red-sore disease were significantly higher in bass with body conditions < 2.0. Multiple regression analysis of the same data for the entire population confirmed that bass in relatively poor body condition (< 2.0) were more likely to have leucopenia and elevated cortisol levels. Since these bass were also more heavily infected with red-sore disease, it is suggested that the disease process in this reservoir system is related to stress, probably chronic, induced by elevated temperature from a nuclear production reactor.

III. Statement of Compliance

This is to indicate that all aspects of Contract EY-76-S-09-0900 have been complied with by the Contractor.

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

The Principal Investigator has devoted approximately 40% of his time to the project during the regular academic year and 100% during the summer months.

V. Papers and books published, or in press 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. Am. 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. Am. 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. Am. 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. Am. Midl. Nat. 98: 213-219.

7) Hazen, T. C. and G. W. Esch. 1978. Observations on the ecology of Clinostomum marginatum in largemouth bass (Micropterus salmoides). J. Fish Biol. 12: 411-420.

8) 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: 491-498.

9) 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: 542-545.

*10) 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: 351-355.

*11) 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). Pp. 331-363.

*12) Gibbons, J. W., G. W. Esch, D. E. Bennett and T. C. Hazen. 1978. A long-term study of the body condition in largemouth bass in a thermally altered reservoir. Nature (London) 274: 470-471.

*13) Hazen, T. C., C. B. Fliermans, R. P. Hirsch and G. W. Esch. 1978. The prevalence and distribution of Aeromonas hydrophila in the United States. Appl. Environ. Microbiol. 36: 731-738.

*14) Huizinga, H., G. W. Esch and T. C. Hazen. 1979. Histopathology of red-sore disease in largemouth bass (Micropterus salmoides). J. Fish Dis. 2: 310-321.

*Denotes papers published during current contract year.

B. In press

1) Hazen, T. C. The ecology of Aeromonas hydrophila in a South Carolina cooling reservoir. Microb. Ecol.

2) Hazen, T. C. and C. B. Fliermans. The distribution of Aeromonas hydrophila in natural and man-made thermal effluents. Appl. Environ. Microbiol.

C. Manuscripts submitted

1) Fliermans, C. B. and T. C. Hazen. Serological specificity of Aeromonas hydrophila as measured by immunofluorescence photometric microscopy.

2) Esch, G. W. and T. C. Hazen. Relationship of body condition and various hematological parameters of largemouth bass in a South Carolina cooling reservoir.

3) Esch, G. W., J. W. Gibbons and J. E. Bourque. Incidence and abundance of parasites in Pseudemys s. scripta from various habitats in South Carolina.

4) Esch, G. W., J. W. Gibbons and J. E. Bourque. Species diversity of endoparasitic helminths in Pseudemys. s. scripta in South Carolina.

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.

*14) Huizinga, H., G. W. Esch and T. C. Hazen. Histopathology of red-sore disease in largemouth bass. December, 1978. Richmond, Virginia. American Microscopical Society Annual Meeting.

*15) Hazen, T. C. and G. W. Esch. Chemotaxis studies on Aeromonas hydrophila. May, 1979. Los Angeles, California. American Society of Microbiologists Annual Meeting.

*16) Hazen, T. C. and G. W. Esch. Agglutinating antibodies in largemouth bass. May, 1979. Los Angeles, California. American Society of Microbiologists Annual Meeting.

*Denotes papers presented during current contract year.

VI. Literature Cited

Esch, G. W. and T. C. Hazen. 1978. Thermal ecology and stress: a case history for red-sore disease in largemouth bass. In Energy and Environmental Stress in Aquatic Systems, J. W. Tharp and J. W. Gibbons (eds.), ERDA Symposium Series (CONF-771114), p. 331-363.

Esch, G. W., T. C. Hazen, R. V. Dimock 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.

Hazen, T. C., M. L. Raker, G. W. Esch and C. B. Fliermans. 1978. Ultrastructure of red-sore lesions: association of the peritrich Epistylis sp. and the bacterium Aeromonas hydrophila. J. Protozool. 25: 351-355.

Huizinga, H., G. W. Esch and T. C. Hazen. 1979. Histopathology of red-sore disease (Aeromonas hydrophila) in natural and experimentally infected largemouth bass (Micropterus salmoides). J. Fish Dis. 2: 310-321.

Miller, R. M. and W. R. Chapman, 1976. Epistylis and Acromonas

hydrophila infections in fishes in North Carolina reservoirs. Prog. Fish-Cult. 38: 165-168.

Shotts, E. B., J. L. Gaines, L. Martin and A. K. Prestwood. 1972. Aeromonas-induced deaths among fish and reptiles in an eutrophic inland lake. J. Amer. Vet. Med. Assoc. 161: 603-607.

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

A. Continue laboratory experiments on thermally-induced stress in bass.

B. Continue chemotaxis studies on Aeromonas hydrophila.

C. Continue strain identification studies on Aeromonas hydrophila.

D. Continue studies on hematological characteristics of Par Pond bass.

VIII. Appendix

A. Reprint of:

Hazen, T. C., M. L. Raker, G. W. Esch and C. B. Fliermans. 1978. Ultrastructure of red-sore lesions on largemouth bass (Micropterus salmoides): the association of the peritrich Epistylis sp. and the bacterium Aeromonas hydrophila. J. Protozool. 25: 351-355.

B. Reprint of:

Hazen, T. C., C. B. Fliermans, R. P. Hirsch and G. W. Esch. 1978. The prevalence and distribution of Aeromonas hydrophila in the United States. Appl. Environ. Microbiol. 36: 731-738.

C. Xerox copy of (reprints not yet available):

Huizinga, H., G. W. Esch and T. C. Hazen. 1979. Histopathology of red-sore disease (Aeromonas hydrophila) in naturally and experimentally infected largemouth bass (Micropterus salmoides). J. Fish. Dis. 2: 310-321.

D. Copy of manuscript in press:

Hazen, T. C. and C. B. Fliermans. 1979. The distribution of Aeromonas hydrophila in natural and man-made thermal effluents. Appl. Environ. Microbiol. In press.

E. Copy of manuscript in press:

Hazen, T. C. 1979. The ecology of Aeromonas hydrophila in a South Carolina cooling reservoir. Microb. Ecol. In press.

F. Copy of manuscript submitted to Can. J. Microbiol.:

Fliermans, C. B. and T. C. Hazen. Serological specificity of Aeromonas hydrophila as measured by immunofluorescence photometric microscopy.

G. Copy of manuscript submitted to Trans. Am. Fish. Soc.:

Esch, G. W. and T. C. Hazen. Relationship of body condition and various hematological factors of largemouth bass (Micropterus salmoides) in a South Carolina cooling reservoir.