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THE EFFECTS OF TEMPERATURE AND CROWDING ON THE PATHOGENICITY OF *EDWARDSIELLA ICTALURI* IN CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

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ABSTRACT

Channel catfish were injected with *Edwardsiella ictaluri* and stocked at increasing temperatures and densities. Bacteriological examination of kidney, liver and spleen revealed the greatest numbers of organisms in fish from the highest temperature and stocking density tested. Survival time was the shortest for fish held at the highest temperature and stocking density. Increased temperature and crowding were directly proportional to the number of organisms recovered from the organs and inversely proportional to fish survival time.

INTRODUCTION

The commercial catfish farming industry as an important facet of American agriculture has grown into multimillion dollar proportions. Fish farming tends to be more profitable when fish are raised at high densities. These intensive culture practices serve to continually expose fish to environmental stressors that are chemical, biological, and physical in nature. Wedemeyer (1970) stated that stress requiring an adjustment that exceeds a fish's ability to accommodate will be lethal; however, less severe stress will predispose to physiological disorders or to infectious diseases if fish pathogens are present.

Bacterial diseases occur in nature. Some fish diseases are caused by obligate bacterial pathogens while others are due to facultative opportunistic organisms that produce infections only when fish are crowded, injured, or suffer from environmental stresses (Wedemeyer et al., 1976). Physical trauma or stress is not essential for the production of disease by obligate pathogens but would simply increase the chances for infection. In their natural wild habitat fish are able, in most cases, to seek the best living conditions available (Warren, 1981). In hatcheries or commercial aquaculture ponds, they must live under conditions imposed upon them.

Fish diseases have been associated with management stress. This type of stress includes temperature fluctuations of 10 C or more, drug treatment, hauling, handling, and stocking. An additional management stress is the high population density required in intensive fish culture (Wedemeyer and Wood, 1974).

As fish culture becomes more intensive, we can expect to encounter additional organisms which previously may have been overlooked in mortalities that occurred in natural waters (Meyer, 1966). Also, as population densities increase with intensive culture, the effects of crowding, poor water conditions, inadequate nutrition, or other stress conditions may induce pathogenic effects from otherwise saprophytic bacteria (Meyer, 1966). Data are available that suggest environmental stresses may provide a major cause for the development of certain diseases under pond conditions.

Edwardsiella ictaluri, is the causative agent of enteric septicemia of catfish (ESC). ESC is a newly discovered bacterial disease of cultured channel catfish. Although the range of this disease is unknown, it constitutes an economic threat to the catfish industry. In this present study, the effects of temperature and crowding on the pathogenicity of the organism were examined.

METHODS AND MATERIALS

Throughout the experiment one 760 L plastic tank (Living Stream, Frigid Units, Inc., Toledo, Ohio) was used as a holding tank. The

research aquaria included three 60 L plexiglass tanks and one 180 L soapstone tank. Each tank was equipped with one or two aquarium heaters for temperature maintenance as well as charcoal filters and air stones which were attached to aeration pumps.

In order to minimize the chlorine content, warm tap water was used to fill each tank. The tanks were allowed to set for 48 hours after filling to allow stabilization and permit further loss of chlorine. The pH was adjusted to 7.5 with sodium bicarbonate since tap water was found to be acidic.

Fish for the entire experiment were donated by Mr. Tommy Keuter of Keuter's Lake, Paragould, Arkansas. The fish were placed in the 760 L holding tank where they were held for 48 hours prior to injection and transfer to research aquaria. The size of fish averaged 95.37 g in weight and 19.18 cm in length, with weight and length ranges from 89.18 to 118.95 g and 16.56 to 20.29 cm respectively (Cooper, 1983).

The bacterium used for this study was *E. ictaluri* strain #571. The culture was obtained from Thomas Schwedler, Mississippi Cooperative Extension Service, Stoneville, Mississippi. Upon receipt, the organisms were transferred to brain heart infusion (BHI) agar and incubated for 48 hours at 25 C. Washings were made with BHI broth and aliquots were then lyophilized. Lyophilized cells were kept at 4 C. Fresh cultures for infecting fish were prepared by inoculating BHI broth with lyophilized cells and incubating at 25 C for 24 hours.

Fish to be inoculated were first anesthetized by placing them in a solution of 80 ppm of tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Redmond, Washington) to minimize handling stress. Anesthetized fish were then injected intraperitoneally using a 5/8 inch 25 gauge needle. Each fish was given a 0.05 ml inoculum which contained approximately 1.3×10^7 cells.

Fish were sacrificed beginning 48 hours post injection. The length and weight of each fish were recorded to the nearest 0.01 unit.

The ventral area of each fish was disinfected using an iodine-alcohol solution and portions of the lower left ventral lobe of the liver, the entire spleen, and the center portion of the kidney was aseptically re-moved. Organs were weighed to the nearest 0.1 mg using an analytical balance.

Each organ was ground in a 7.0 ml Pyrex Tissue Grinder (Corning Glassworks, Corning, New York). The homogenate was then serially diluted using sterile water and plated onto BHI agar using the spread plate technique for making standard plate counts. Plates were incubated for 48 hours at 25 C. Only colonies that appeared identical to *E. ictaluri* were counted. Confirmation was made by randomly picking typical colonies and subjecting them to a series of biochemical cultivation tests as well as gram stains. The isolates were tested in triple sugar iron agar (TSI), methyl red-Voges Proskauer broth, indole test broth, nitrate broth, and citrate agar (Simmon's).

Temperature studies were done using a constant stocking density of

The Effects of Temperature and Crowding on the Pathogenicity of *Edwardsiella ictaluri* in Channel CatfishTable 1. Effect of temperature on *E. ictaluri* infection in channel catfish.

Temperature (°C)	Average log ₁₀ bacteria/g tissue/fish/day			No. of days of survival	No. of fish injected/examined	
	Kidney	Liver	Spleen			
21	0.86	0.85	0.90	9	15	12 ^a
25	1.45	1.77	1.91	5	15	9
29	4.40	4.42	4.72	2	15	8

^aOnly fish known to have been dead less than eight hours were examined.

1 fish/12 L of water at a pH of 7.5. Temperatures were maintained by aquarium heaters with an accuracy of ± 1.5 C. Temperatures of 21, 25, and 29 C were tested. The crowding studies were conducted using one fish per 8, 10, and 12 L of water. The same pH of 7.5 was maintained along with a constant temperature of 23 C.

Fish in both studies were sacrificed at a rate of two per day. Aquaria were checked at no less than eight hour intervals and only fish that had been dead less than eight hours when found were processed as well as those that were moribund. Control fish for both studies were also used. They were stocked at the same temperatures and stocking densities as infected fish. Control fish were processed in the same manner as the infected fish and held for the same period of time.

RESULTS

The results suggested a direct relationship between increasing temperatures and stocking densities and the number of bacteria recovered from the organs.

In all of the temperature studies the highest number of bacteria per g of tissue per fish per day was recovered from the spleen (Table 1). The number of bacteria found in each organ increased with an increase in temperature. The greatest number of organisms was found in the spleen at the 29 C temperature with the lowest numbers being present at 21 C (Table 1). At higher temperatures fish became ill in a shorter period of time.

The greatest numbers of bacteria per organ were recovered from fish held at a density of 1 fish/8 L of water. The lowest numbers were recovered from fish held at a density of 1 fish/12 L of water. In two of the three studies, the spleen was again the organ containing the greatest bacterial numbers. The study using 1 fish/10 L had the highest recovery from the liver with the spleen having the second highest numbers (Table 2). Fish held at higher densities developed symptoms faster than those at lower densities (Table 2).

The physical symptoms displayed by infected fish were similar in all cases. These included petechial hemorrhaging at various sites, gross discoloration of the skin, excess mucous, gastric bloating, thromboses of the mesentery and intestines, an enlarged and discolored spleen, and ascites.

DISCUSSION

Adverse temperatures and increased population density are considered physical and biological stressors respectively. These factors have a

negative impact on fish in intensive culture resulting in stress that predispose fish to disease.

Increased water temperature has been documented as a cause for the increase in outbreaks of bacterial infection in fish culture. This is explained by the fact that at higher temperatures certain bacterial species multiply faster resulting in an increase in the number of organisms present. This allows for the greater numbers of bacteria to infect the host and overwhelm the host's defenses.

The behavior of a fish in response to a change in temperature may vary according to its biological condition (Love, 1970). The studies conducted with conditions of increasing temperatures revealed a decrease in the survival time of the fish and a more rapid onset of symptoms. More severe and rapidly developing infections were also observed in the crowding studies with the more densely populated fish. This suggested that increased temperatures and population density result in a more rapid onset of disease. Crowding, like other stresses, probably triggers the release of stress-related hormones that tend to act as immunosuppressives and the amount released is dependent upon the intensity of the stressor. The immune response is more or less inversely proportional to the quantity of stress hormones released (Flagg and Hinck, 1978).

In the fish injected with the bacterium and held at lower temperatures, the disease took longer to manifest itself. Umminger (1970) in his work with *Fundulus heteroclitus* found a decrease at low temperatures in those serum proteins which correspond to human gamma globulins. He also noted that disease erupts more rapidly in fish in warmer water and suggested that since pathogens grew more slowly at low temperatures there is less need for an immune response.

The most general response to stress, from whatever source, is a pronounced rise in blood sugar (Doudoroff, 1957). This phenomenon is thought to occur to provide extra energy to the stressed fish to allow it to escape a stressful situation (Thorpe and Ince, 1974). This increase in blood sugar could have been beneficial in providing a source of food for the bacteria and helping to maintain greater numbers.

Catecholamine levels in the blood become increased due to stresses such as muscular agitation, asphyxia, hemorrhage, and wounding as demonstrated by Muzeaud (1964), using *Cyprinus carpio*. Wedemeyer (1969) found that the quantity of 17-hydroxycorticosteroids and catecholamines released during stress is dependent on the intensity of the stressor. These hormones lower the resistance of the host thus affecting its immunological response. The catecholamine adrenaline also causes an increase in the blood glucose level. Adrenaline has been shown to be responsible for the blanching of skin color displayed by certain species of fish such as *Pterophyllum scalare* (Adler, 1975). When cer-

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Table 2. Effect of crowding on *E. ictaluri* infection in channel catfish.

Volume of Water (liter/fish)	Average log ₁₀ bacteria/g tissue/fish/day			No. of days of survival	No. of fish injected/examined
	Kidney	Liver	Spleen		
8	2.58	2.62	2.79	3	10 ^a
10	2.28	2.88	2.34	3	9
12	1.45	1.30	1.53	6	11

^aOnly fish known to have been dead less than eight hours were examined.

tain species of fish are frightened the dark particles of melanin in the pigment cells contract. In this study blanching was seen in nearly all injected fish.

Plasma cortisol levels have been noted to rise in response to stress in *Oncorhynchus kitsutch* and *Salmo gairdneri* (Wedemeyer, 1969). Wedemeyer (1969) also noted that the ascorbic acid content of the adrenals decreased during stress, with an accompanying decrease in an animal's resistance to infection.

Spleen enlargement was detected in all of the infected fish. Some forms of stress are known to have a significant effect on the hematological response. Capture stress, for example, in *Labeo umbratus* caused a decrease in the concentration of hemoglobin (Hattingh and van Peltzen, 1974). The reduction in blood constituents can result in splenomegaly. These changes in blood content occur as a result of infection. Splenomegaly might be a result of infection-induced hematological changes or it could be attributed to increased antibody production and/or increased phagocytic activity of the spleen as a consequence of increasing bacterial numbers.

Several stresses acting on *Cyprinus carpio* were found by Szokolcaizai (1969) to elicit the detachment of the mucous epithelium from the intestine, with consequent leakage of serum into the lumen. This could explain the serosanguineous fluid found in the peritoneal cavity of the fish.

The most characteristic external lesion is the presence of a raised or open ulcer on the frontal bone of the skull between the eyes (Plumb and Schwedler, 1982). In the current study, this lesion was found on one occasion. This is probably because fish were artificially injected with the bacterium by a method which caused the disease to manifest itself more quickly than it would have with a natural infection. The lesions very likely require a more prolonged exposure to the bacterium, therefore producing a slowly developing disease.

The results of this study demonstrated that both warm temperatures and crowding tend to favor the development of *E. ictaluri* infections in channel catfish. This information may be of value to fish farmers who generally practice high density culture methods. These data suggested that if *E. ictaluri* infections should appear in a farming operation it might be important to reduce the number of fish per pond. This would seem especially important during warmer weather.

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