# INFLUENCE OF AMMONIA ON AEROMONAD SUSCEPTIBILITY IN CHANNEL CATFISH

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*Abstract*: The effects of un-ionized ammonia on Channel catfish (*Ictalurus punctatus*) resistance to aeromonad invasion were tested. Host susceptibility to *Aeromonas hydrophila* was related to ammonia concentration and time of exposure. Numbers of bacteria recovered from host livers increased as concentrations of un-ionized ammonia were increased in the range of 0.02-0.04 mg/l $NH_3$. The effect of longer exposure time at these concentrations also proved to be significant ($P < 0.01$) in lowering host resistance.

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Unfavorable environmental conditions have been propounded as the trigger mechanism for many fish diseases (Meyer 1970; Snieszko 1937; Wedemeyer et al. 1976). The realization that the health of a fish population is a delicate balance between the environment, parasite, and host physiological state, has shifted the emphasis of disease control from treatment to prevention. Prevention requires that the complex interactions in an aquatic system be delineated so that the fish culturist is provided guidelines within which to work.

Poor water quality is usually a prelude to epizootics caused by the facultative pathogen *Aeromonas hydrophila* (Meyer 1970). The basis for such a relationship is the physiological stress imposed by various physicochemical factors (Wedemeyer 1970). Most often low oxygen levels are cited as the debilitating agent (Fry 1969; Haley et al. 1967) but Meyer (1970) reported that excretory and decomposition products may also be instrumental factors. Collins (1970) demonstrated that the potential for aeromonad epizootics is greatly influenced by the organic load of the basin. The most obvious effect of eutrophication is the increase in the biochemical oxygen demand resulting in lower oxygen levels. In addition to a high biochemical oxygen demand, eutrophication contributes to the build-up of decomposition products resulting from the increased bacterial load. The most potentially harmful of the decomposition products is ammonia and this compound is also released by most freshwater fishes as their primary excretory product.

Hutchinson (1957) stated that a greater portion of the inorganic nitrogen in a water basin will be in the form of ammonia when oxygen levels are low and that the same chemical and biological conditions that are conducive to low oxygen may result in a build-up of ammonia. Snieszko (1974) noted the greater incidence of fish bacterial diseases under conditions of ammonia accumulation and high oxygen demand. Aeromonad epizootics are probably the result of the cumulative effect of these 2 stressors upon the fish metabolism but they could possibly act independently to the same end.

Most studies of the effects of ammonia have been directed to define toxicity levels rather than the effect on host resistance to disease. The un-ionized form of ammonia nitrogen has been considered toxic to fish and the ionized form less harmful (Downing and Merkens 1955). Un-ionized ammonia levels of only 0.02 mg/l $NH_3$ have been suggested as the upper limit for optimum health in many fishes (EPA 1976). The purpose of this study was to determine what relationship exists between un-ionized ammonia and aeromonad incidence.

## MATERIALS AND METHODS

Channel catfish fingerlings used in this experiment were secured from the Arkansas State University Experimental Farm near Walcott, Greene County, AR. All fish were recovered from the same spawn pond and were approximately 6 to 7 months old with a mean total length of 10.7 cm. Fish were collected by means of seining and were chosen by

random selection. During collection and transportation of fish, care was taken to avoid any undue stress. Fish were randomly stocked in the experimental tanks at a constant density ratio of 1 fish per 10 l of water.

All fish were acclimatized for 1 week to experimental conditions with the exception of the un-ionized ammonia concentration which was held to less than 0.001 mg/ l $NH_3$. During this week, fish were fed commercial feed and excess food was removed. Feeding was stopped when the tests commenced.

Twenty fish were used for each ammonia concentration and exposure time. Soapstone tanks of 100 and 200 l volumes were of recirculation design with granular activated carbon filtration. Filters were changed at 5 day intervals to reduce the possibility of nitrification. Sodium hydroxide was used to adjust the pH of the source tap water from 6.9 to 7.6 and sodium bicarbonate was added to increase the total alkalinity to at least 100 mg/ l $CaCO_3$. Oxygenation of water was accomplished by means of air stones attached to air pumps. To more closely simulate pond conditions a natural buildup of ammonia from fish excretion and natural decomposition taking place in the tanks was employed. The desired ammonia concentration was obtained and maintained by adjusting the filtration rate. Previous experience with the filtration system dictated the degree of adjustment necessary. The aquatic plants *Elodea* and *Lemna* were introduced into the tanks to keep free carbon dioxide levels low and prevent the build-up of nitrates and nitrites. Artificial illumination of a 12 hour cycle served as the light source for these plants.

The un-ionized ammonia concentration was the only variable for the test groups. All groups were held under the following conditions: temperature 22 C (±0.5); dissolved oxygen level near saturation (8.8 mg/ l $O_2$) and never below 8.0 mg/ l $O_2$; pH of 7.6 (± 0.03); and free carbon dioxide less than 4.0 mg/ l $CO_2$. Control fish were kept at an un-ionized ammonia concentration of less than 0.001 mg/ l $NH_3$. Test groups A, B, and C were held for 17 days at 0.02 mg/ l $NH_3$ (±0.001), 0.03 mg/ l $NH_3$ (±0.002), and 0.04 mg/ l $NH_3$ (± 0.002) respectively while groups D and E were held for 28 days at 0.02 mg/ l $NH_3$ (±0.001) and 0.03 mg/ l $NH_3$ (±0.002) respectively.

Daily determinations were performed to measure dissolved oxygen, pH, temperature, and free carbon dioxide. A Precision Galvanic Oxygen Analyzer standardized with aquarium water at 22 C was used to measure dissolved oxygen while the pH was determined with a Beckman Expandomatic pH meter. Water temperature was verified daily with a mercury Celsius thermometer. Free carbon dioxide and ammonia nitrogen determinations were conducted according to APHA (1971). Ammonia determinations were carried out every 2 days by direct nesslerization using a Bausch and Lomb Spectronic 20 at 425 nm.

*Aeromonas hydrophila*, ATCC **N**19570, was used as the infectious agent. This was a stock culture from American Type Culture Collection and virulency was insured by passage through channel catfish before use in the experiment. *Aeromonas hydrophila* was introduced into tanks on the third day of the test at a rate of $1.75 \times 10^8$ viable cells/ l of tank. At the termination of the test period, the left lobe of the liver was homogenized in a Ten Broeck tissue homogenizer and plated out for standard plate counts using trypticase soy agar as the medium. Plates were incubated at 30 C for 48 hours.

The identification of *Aeromonas* was confirmed using phenol red indicator agar. The characteristic colony of *Aeromonas* was found to be almost in pure culture in the livers and only colonies resembling those of *Aeromonas* were counted. Bacterial counts were computed in terms of number of cells per gram of liver tissue.

Individual bacterial counts were converted to the logarithm of base ten for comparison. The arithmetic mean for each group was calculated using $\log_{10}$ of the individual counts. Data were treated with single classification analysis of variance according to Sokal and Rohlf (1969). A priori test for mean comparisons was employed for significance between different ammonia concentrations and exposure times.

## RESULTS AND DISCUSSION

As un-ionized ammonia concentrations increased, there was a corresponding increase in the number of *Aeromonas* recovered from host livers (Table 1). This effect was most pronounced in the 17 day tests and a near linear relationship between un-ionized ammonia concentration and $\log_{10}$ of the number of bacteria per gram of liver resulted (Fig. 1). The overall significance of increased un-ionized ammonia concentrations resulting in greater numbers of *Aeromonas* in host livers was proven statistically using analysis of variance (Table 2). A priori test of mean comparisons between control and test groups demonstrated a definite relationship between un-ionized ammonia and host resistance. Differences between groups held at 0.02 mg/l $NH_3$ (A and D) and groups at 0.03 mg/l $NH_3$ (B and E) were highly significant ($P < 0.01$). A priori test of exposure time and bacterial numbers in host livers demonstrated a highly significant ($P < 0.01$) relationship between duration and aeromonad invasion.

Table 1. Mean number of *Aeromonas* in host livers at various un-ionized ammonia concentrations.

| *mg/l $NH_3$* | *$\log_{10}$ cells/g liver* | |
|---|---|---|
| | *17 day* | *28 day* |
| Control | 0.796 | 0.796 |
| 0.02 (±.001) | 2.11 (A) | 3.35 (D) |
| 0.03 (±.002) | 3.38 (B) | 3.44 (E) |
| 0.04 (±.002) | 4.12 (C) | --- |

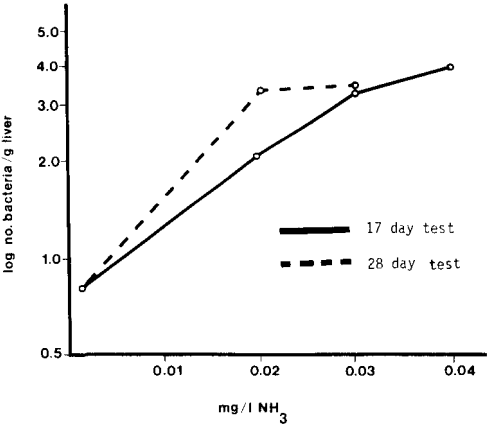


Fig. 1. The influence of un-ionized ammonia on aeromonad susceptibility.

Table 2. Analysis of variance of the mean number of *Aeromonas* in host livers at various un-ionized ammonia concentrations.

| *Interactions* | *SS* | *MS* | *df* | *F* | *Critical F*[a] |
|---|---|---|---|---|---|
| All groups | 145.3 | 29.1 | 5 | 44.4 | 3.32 |
| Control x Test | 9784.2 | 9784.2 | 1 | 14960.6 | 7.06 |
| 17 days x 28 days (A+B) x (D+E) | 8.4 | 8.4 | 1 | 12.8 | 7.06 |
| 0.02 x 0.03 mg/l $NH_3$ (A+D) x (B+E) | 9.2 | 9.2 | 1 | 14.1 | 7.06 |

[a]F values significant at 0.01 level.

Fromm and Gillette (1968) found a direct linear correlation between ambient ammonia and blood ammonia in rainbow trout (*Salmo gairdneri*). Excretion of ammonia was inhibited as there was an increase in ambient ammonia. As ammonia increases in the water, fishes will retain more ammonia in the blood and subsequently in the tissues. Since blood ammonia is directly proportional to ambient ammonia, an explanation emerges for the susceptibility to aeromonads being proportionate to ammonia levels. The quantity of 17-hydroxycorticosteroids and catecholamines released during stress is dependent on the intensity of the stressor in salmonid fishes (Hane et al. 1966; Hill and Fromm 1968; Wedemeyer 1969). These stress hormones tend to lower host resistance due to their effects on host defenses. Therefore, the host immunological response would be more-or-less inversely proportional to the quantity of stress hormones released.

Wedemeyer et al. (1976) suggested that duration, as well as intensity, of the stress is related to the host response. The difference in the slopes of the curves in Fig. 1 indicates that a relationship between stress duration and susceptibility does occur. This appears to be a linear relationship until the host is completely compromised. After a total host defense collapse, an increase in the stress may not proportionately increase susceptibility. A stressor can only continue to increase susceptibility until all host defenses are paralyzed, after this point a greater magnitude of the stressor may result in host death. The inflections on both curves are not necessarily the points at which the host defenses had collapsed. This was probably the threshold where nutrient availability became a limiting factor for the bacterium since this occurs at approximately the same pathogen population in the tissues.

Meyer (1970) reported that the summer peak incidence of aeromonad infections corresponded to periods of low oxygen levels in farm ponds. The spring incidence was attributed to winter starvation, overwintering under suboptimal conditions, and temperature stress. It is interesting to note that ammonia levels would be the greatest during these periods. Low oxygen levels in the summer are usually associated with heavy algal blooms. The result of an algal bloom is the death of many lower lying aquatic organisms and a subsequent high biochemical oxygen demand. This situation contributes to high ammonia levels due to decomposition and the higher proportion of inorganic nitrogen being in the form of ammonia. Hutchinson (1957) reported that a build-up of ammonia occurs in February to May. The accumulation and decomposition of dead organisms over the winter and the reduced assimilation by plants were cited as the cause of such increases. It seems likely that ammonia may be a common factor in the 2 peak periods of aeromonad infections. Evidence presented in this paper indicates that un-ionized ammonia can act as a stressing agent and lower the resistance of channel catfish to *Aeromonas*.

The role of ammonia in aeromonad infections in nature has yet to be established. It would be difficult to determine the relationship of ammonia and aeromonad infections in a natural pond environment. However, if fish culturists would consider ammonia determinations when checking water quality, data could be accumulated and the role of ammonia stress could be more thoroughly assessed.

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