EFFECTS OF HIGH pH ON UNIONIZED AMMONIA AND THEIR COMBINED EFFECTS ON THE STRESS PHYSIOLOGY OF STEELHEAD TROUT, *Oncorhynchus mykiss*, REARED IN RECIRCULATING AQUACULTURE SYSTEMS

(short communication)

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**Abstract.** Experiments were conducted to determine the effects of pH on ammonia and their combined effects on the stress response of steelhead trout, *Oncorhynchus mykiss*. Stress levels were studied by monitoring both primary and secondary stress responses. It was found that fish subjected to higher levels of pH and ammonia exhibited increased levels of stress. This was demonstrated through primary physiological stress response, plasma cortisol, and secondary physiological stress response, plasma glucose and blood hematocrit. It was concluded that careful regulation of pH and ammonia in the recirculating aquaculture system could relieve excessive stress allowing the fish for better survival and growth.

**Key words:** ammonia, pH, re-circulating system, stress

**INTRODUCTION**

Recirculating water systems, by using less water and space than traditional aquaculture ponds, offer a feasible way to conduct both research and farming of fish. The recirculation of water in these systems, however, mandates constant maintenance to ensure optimal water quality for the fish. One of the more common water quality problems encountered in recirculating systems is high ammonia levels, which result from the release of fish metabolic waste within the system [Emerson et al. 1975, Redner and Stickney 1979, Randall and Tsui 2002]. In water, ammonia occurs in two forms, which together are called ‘Total Ammonia Nitrogen’, or TAN. Chemically, these two forms are represented as NH$_4^+$ and NH$_3$. NH$_4^+$ is called ‘Ionized Ammonia’ because it

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has a positive electrical charge, and NH₃ is called ‘Unionized Ammonia’ since it has no charge [Francis-Floyd and Watson 1990]. Particularly problematic is the unionized form of this ammonia, which is extremely toxic to the fish within the system [Russo 1985, Tomasso 1994]. Chronically high ammonia levels cause an increase in physiological stress responses, leading to decreased growth and survival of the fish [Mazeaud et al. 1977, Wedemeyer and McLeay 1981, Tomasso 1994]. Based on observations made in our lab over the past years and various comments from other peer-reviewed articles in the aquaculture field, it has been observed that lethal ammonia doses are strongly correlated with fluctuations in the pH of the system [Emerson et al. 1975, Randall and Tsui 2002] but no one has ever described their effects on the host stress physiology. This study looked at the combined effects of unionized ammonia and high pH on the stress response of steelhead trout, Oncorhynchus mykiss. Stress levels were studied by monitoring indicators of both the primary (plasma levels of cortisol) and secondary (plasma levels of glucose and blood hematocrit) physiological stress response.

It was found that fish subjected to higher levels of ammonia and higher pH exhibited increased levels of hormones indicative of stress response [Mustafa et al. 2000]. This was demonstrated through both the primary physiological stress response (increased plasma cortisol levels) and the secondary response to stress (increased plasma glucose, and increased hematocrit percentages). It was concluded that careful regulation of pH and ammonia levels in the system could relieve excessive stress, allowing for better growth and survival of the fish.

MATERIAL AND METHODS

160 steelhead trout, Oncorhynchus mykiss, 1+ year old) with a mean weight of 50 g were obtained from Bodine State Fish Hatchery, Indiana. The fish were divided into two groups: one group to be held at an approximately steady pH level (Group-1) and the other group at an increased pH level (Group-2) (pH was increased over time be using pH buffer (sodium bicarbonate). The fish groups were kept separated by using two complete recirculating systems, each with its own reservoir, chiller, sand filter, and biofilter). Fish from each group were randomly distributed between two tanks (410-litre each) within each system to eliminate any tank effects). The fish were allowed to acclimate to the laboratory environment for two weeks prior to the onset of the experiment and were then maintained at optimum aquatic conditions for this species (dissolved oxygen levels were monitored and maintained at ~8 ppm and the photoperiod was maintained at 14 h light: 10 h dark). In addition, the pH and ammonia levels in the tanks were monitored frequently, at least twice daily. Once the experiment began, both tanks of Group-1 were kept at a steady pH of approximately 7.75 without any manipulation but the pH in both tanks of Group-2 was increased slowly over time with the use of a HCl per NaHCO₃ buffer system. The fish were fed once daily to satiation with commercial trout pellets and cared for according to the guidelines of the Purdue Animal
Care Usage Committee. Sampling occurred once a week for a period of four weeks. At each sampling, the total ammonia nitrogen was analyzed again and was then converted into unionized ammonia, with consideration to pH and temperature [Emmerson et al. 1975]. These values at sampling are used in the table. Ten fish per group (5 fish per tank x 2 replicates) were randomly selected from each tank and were then placed into a smaller tank containing MS222 at ~100 mg·L\(^{-1}\) to facilitate immediate immobilization (it has been shown that when used in high dosages to cause immediate immobilization, anesthesia does not cause a spike in cortisol levels [Wedemeyer et al. 1990]. The fish was then measured and weighed and blood samples were obtained from the caudal vessel by heparinized vacutainers. Fish were placed in a recovery tank following sampling and were then returned to their respective holding tanks once the anesthesia had worn off. This blood was analyzed for plasma cortisol, blood hematocrit, and plasma glucose following Mustafa et al. [2000] and Bowers et al. [2000] to analyze the severity of the physiological stress response in the fish.

RESULTS AND DISCUSSION

Fish in Group-2 were exposed to increasing levels of total ammonia nitrogen concentrations and considerably higher unionized ammonia as the pH slowly increased over the period of the experiment. Ammonia nitrogen in this group showed a drastic increase from 0.13 mg·l\(^{-1}\) to 0.99 mg·l\(^{-1}\) over the four week period (Table 1). Fish that were maintained at a lower, steady pH (Group-1), however, did not see the drastic increase in ammonia levels in the tank. The fish in Group-2 with higher pH and ammonia were also shown to have higher (not statistically significant in all cases) levels of plasma cortisol, blood hematocrit, and plasma glucose as the experiment progressed (Table 1). The differences between the levels of both plasma cortisol and glucose in Groups-1 and -2 became significant at weeks 3 and 4, when the difference in the pH and ammonia levels of the two groups became the greatest. When comparing the effects of the pH on the ammonia and the stress levels, a comparison was made between the stress response seen at the ammonia level of 0.15 mg·l\(^{-1}\), observed at week 1 in Group-2 at a pH of 8.22 and at week 4 in Group-1 at a pH of 7.69. The cortisol levels for Group-1 at this lower pH were much lower (126.5 ± 6.0 nmol·l\(^{-1}\)) than that of Group-2 at the higher pH (219.0 ± 14.3 nmol·l\(^{-1}\)). In addition, the amount of unionized ammonia was much lower (0.001 mg·l\(^{-1}\)) at the lower pH than when the pH was increased in Group-2 (0.006 mg·l\(^{-1}\)).

Comparison of the two groups throughout the four weeks indicates that fish subjected to higher levels of ammonia and elevated pH exhibit increased levels of physiological stress response. The increase in cortisol points to a primary stress response by the fish and is followed by an increase in glucose and hematocrit as the heightened demands on the fish lead to higher demand for oxygen and increased metabolism. As shown in numerous other studies, increased stress levels lead to lower fish weight [Mazeaud et al. 1977, Wedemeyer and McLeay 1981, Barton and Iwama 1991, Tomasso 1994].
Interestingly, the comparison between the two groups at the same ammonia level indicates another correlation between pH and stress response. The smaller concentration of unionized ammonia at the lower pH indicates that lower pH helps regulate the toxicity of the ammonia in the tank at any given time when the temperature is kept constant. The difference in the cortisol concentration also indicates that the lower pH may help modulate the primary stress response in the fish contained in these systems. Since the comparison involves the first week of the experiment, there was not enough time elapsed for a difference in the secondary stress response to be observed. Both comparisons indicate that a correlation exists between the pH, total ammonia nitrogen, unionized ammonia, and stress levels of fish in these tanks.

CONCLUSIONS

While increased pH is associated with increased ammonia and causes increased stress responses, the same total ammonia nitrogen concentration found at a lower pH can have a less severe physiological stress impact. We suggest that careful regulation of pH can lead to avoidance of excessive stress and better growth and survival of farmed fish.

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REFERENCES

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WPŁYW WYSOKIEGO pH NA POZIOM NIEZJONIZOWANEGO AMONIAKU ORAZ ICH ŁĄCZNY WPŁYW NA FIZJOLOGIĘ STRESU U PSTRĄGA TĘCZOWEGO *Oncorhynchus mykiss* HODOWANEGO W SYSTEMIE O OBIEGU ZAMKNIĘTYM

**Streszczenie.** Prezentowane badania zostały przeprowadzone w celu określenia wpływu pH na poziom amoniaku oraz łącznego wpływu tych czynników na reakcję stresową pstrąga tęczowego *Oncorhynchus mykiss*. Poziom stresu był analizowany w oparciu o zapis zarówno pierwotnej jak i wtórnej reakcji na stres. Zaobserwowano, że ryby poddane działaniu wyższego poziomu pH i amoniaku wykazywały podwyższony poziom stresu. Zostało to wykazane poprzez pierwotną fizjologiczną reakcję na stres, tj. zawartość korytułu w osoczu, oraz wtórną fizjologiczną reakcją na stres, tj. zawartość glukozy w osoczu oraz hematokrytu. Stwierdzono, że pieczułowita kontrola pH i stężenia amoniaku w systemach hodowlanych ryb w obiekcie zamkniętym może doprowadzić do redukcji nadmiaru stresu, co z kolei może przyczynić się do poprawy przeżywalności i tempa wzrostu ryb.

**Słowa kluczowe:** amoniak, pH, stres, system w obiekcie zamkniętym

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