

**LIFE AND DEATH IN A TOILET BOWL: THE EFFECTS OF  
CHRONIC SUB-LETHAL AMMONIA EXPOSURE  
ON IMMUNOPHYSIOLOGY OF RECENTLY SMOLTED  
CHINOOK SALMON**

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**EXTENDED ABSTRACT ONLY- DO NOT CITE**

Ammonia is an unusual toxicant in that it is produced naturally by animals. It is released into the environment through production of industrial fertilizers and biological wastes. It is a compound that is toxic to animals if accumulated in the

body tissues and fish rely mainly on diffusion down the concentration gradient between body and water to eliminate ammonia waste. The current North American saltwater standards are based on a limited marine database and the toxicity tests follow standard guidelines using static water conditions, unfed, unstressed, resting animals. It is under these unrealistic conditions that internal ammonia production is minimal. In addition, the available literature generally expresses concern primarily with growth, survival, and reproduction in fish. We were interested in the applicability of these standards to more realistic conditions (fed fish in culture situations and in nature) and asked the question... “...is the health status of these animals impacted?” The data we accumulated points to significant effects on health parameters and a reduction in disease resistance in fish exposed to acceptable environmental ammonia levels.

This study was designed to consider the effects of a sub-lethal chronic ammonia exposure on physiological and cellular stress responses of recently smolted chinook salmon and to determine if such a low-grade ammonia exposure affected disease susceptibility. Unvaccinated chinook salmon juveniles weighing approximately 20g obtained from Big Tree Creek Hatchery were acclimated and smolted at the Bamfield Marine Station in outdoor tanks containing seawater at 11.10C, pH 7.8, salinity 31 ppt and fed a daily ration of approximately 2% body weight per day. Ammonia (as NH<sub>4</sub>Cl) was introduced into the tanks at two concentrations, 2.5 and 10 mg/L (2.5 ± 1.1 and 12.1 ± 1.9 mg/L N actual dose as determined from water samples (indophenol blue)). Both test levels are below the acute standard (2.5 mg/L is also below the chronic standard) and resulted in increased internal levels of ammonia in the fish. Neither treatment level affected feeding rates and, during the course of the exposure, there was no mortality in any of the tanks. At four time periods (6 hrs, 48 hrs, 96 hrs, and 244 hrs) fish were terminally sampled from each of five tanks (two tanks per treatment, one tank controls).

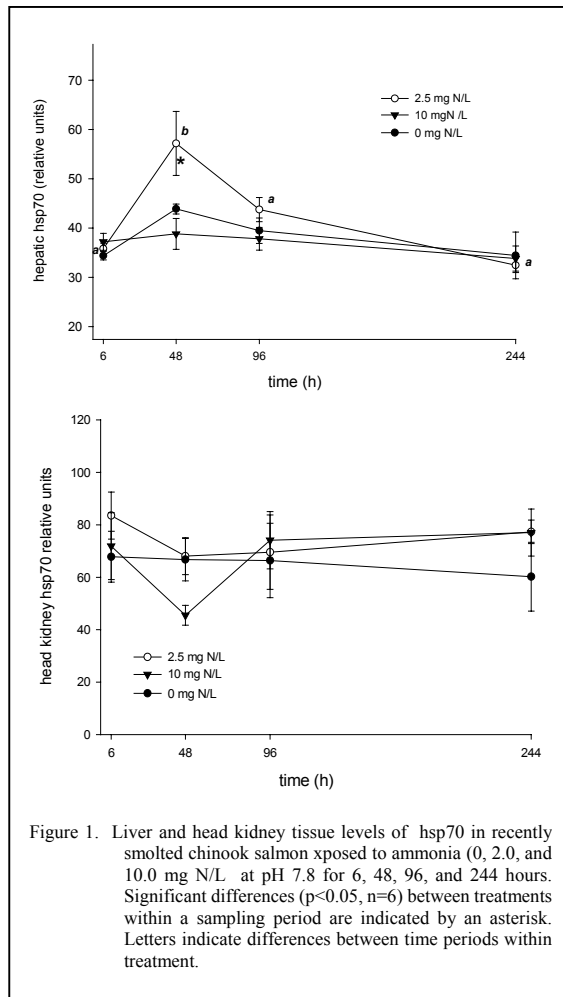
Lysozyme is a bacteriolytic enzyme produced by phagocytic cells that is involved in the destruction of invading pathogens and has been shown to increase in the plasma following an acute stress (Demers and Bayne, 1997). Lysozyme activity in plasma was determined by a modification of Litwack (1955) according to (Maule et al. 1996). Plasma lysozyme activity increased significantly ( $p < 0.05$ ,  $n = 12-16$ ) in both treatment groups compared to control fish. At 96 h post exposure both treatment groups had significantly higher levels than controls (mean value  $2.33 \pm 0.42$  µg HEWL Eq) and fish exposed to 10mg N/L (mean value  $4.05 \pm 0.29$  µg HEWL Eq) had significantly higher activity than did fish exposed to 2.5 mg N/L (mean value  $3.18 \pm 0.20$  µg HEWL Eq). By

244 h, both treatment groups had lower lysozyme activity levels than controls with fish exposed to 2.5 mgN/L ( $1.99 \pm 0.14 \mu\text{g HEWL Eq}$ ) having significantly lower activity levels than both control fish ( $2.74 \pm 0.31 \mu\text{g HEWL Eq}$ ) or fish exposed to 10 mg N/L ( $2.54 \pm 0.18 \mu\text{g HEWL Eq}$ ).

Preparation of tissues, and dilution and determination of hsp70 was carried out by ELISA according to Ackerman and Iwama (2001) as a modification of Forsyth et al (1997). There were significant differences in liver hsp70 levels between the treatment groups at 48 h ( $p < 0.05$ ,  $n=6$ ) (Figure 1). Fish exposed to 2.5 mg N/L had levels that were significantly higher than control fish or fish exposed to 10 mg N/L. Hsp70 levels in the head kidney tissue showed no significant differences over time or between treatments at  $p < 0.05$ .

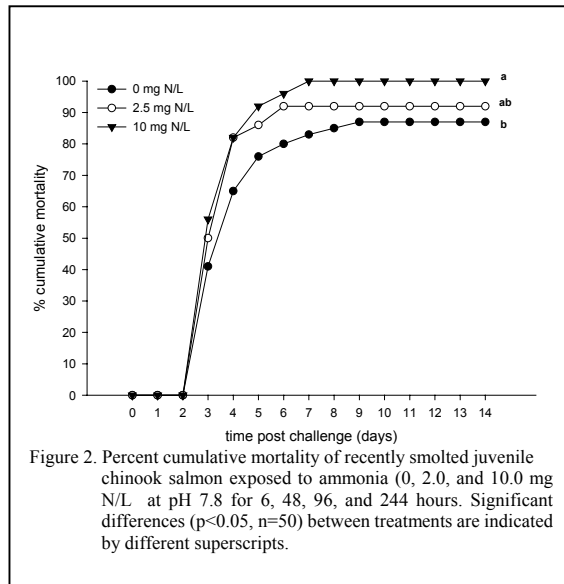
Following treatments, all fish were moved to clean water and challenged by i.p injection with the pathogen *Vibrio anguillarum* ( $1.7 \times 10^6$  cfu/mL). The challenge method and *V. anguillarum* isolate are outlined in Ackerman and Iwama (2001). Control fish had the lowest mortality at 87%.

Fish exposed to 2.5 mg N/L had a total cumulative mortality of 92% which was



not statistically different from control fish. Exposure to 10 mg N/L for 244 hours resulted in 100% cumulative mortality when pathogenically challenged. This was a statistically significant difference from control fish (Figure 2.)

In addition to the above data, we also saw significant effects on respiratory burst activity of glass adherent white blood cells, plasma cortisol and glucose concentrations, and differential blood cell numbers. The data we accumulated points to significant effects on health parameters and a reduction in disease resistance in fish exposed to currently acceptable environmental ammonia levels. We believe that we have indicated the need for the re-evaluation of the existing ammonia standards for the marine environment.



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