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The effects of high environmental ammonia on the structure of rainbow trout hierarchies and the physiology of the individuals therein

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ABSTRACT

Our goals were: (i) to determine whether sublethal concentrations of water-borne ammonia would prevent the formation of a dominance hierarchy, or alter its structure, in groups of 4 juvenile trout; (ii) to investigate the behavioral and physiological responses of individuals of different social rank exposed to a concentration of ammonia that still allowed hierarchy formation. Social hierarchies were created by using a technique in which a food delivery system that created competition also served to isolate individual fish for respirometry. Groups of 4 fish were exposed to elevated ammonia (NH_4HCO_3) 12 h before first feeding; aggression was recorded by video camera during morning feedings. Experimental ammonia concentrations were 700, 1200 and 1500 μmol L^{-1} at pH 7.3, 12 °C (9.8, 16.8, and 21.0 mg L^{-1} as total ammonia-N, or 0.0515, 0.0884, and 0.1105 mg L^{-1} as NH_3-N). Aggression was severely reduced by 1200 and abolished by 1500 μmol L^{-1} total ammonia, such that hierarchies did not form. However, groups exposed to 700 μmol L^{-1} total ammonia still formed stable hierarchies but displayed lower levels of aggression in comparison to control hierarchies. Exposure continued for 11 days. Physiological parameters were recorded on day 5 (end of period 1) and day 10 (end of period 2), while feeding and plasma cortisol were measured on day 11. In control hierarchies, dominant (rank 1) trout generally exhibited higher growth rates, greater increases in condition factor, higher food consumption, and lower cortisol levels than did fish of ranks 2, 3, and 4. In comparison to controls, the 700 μmol L^{-1} total ammonia hierarchies generally displayed lower growth, lower condition factor increases, lower O_2 consumption, lower cortisol levels, but similar feeding patterns, with smaller physiological differences amongst ranks during period 1. Effects attenuated during period 2, as aggression and physiological measures returned towards control levels, indicating both behavioral and physiological acclimation to ammonia. These disturbances in social behavior and associated physiology occurred at an ammonia concentration in the range of regulatory significance and relevance to aquaculture.

1. Introduction

In fish, stable dominance hierarchies may be beneficial to both subordinate and dominant individuals by reducing aggressive behavior. An additional benefit is that stable hierarchies may entrain different feeding strategies for fish of different rank (Gurney and Nisbet, 1979; Sneddon et al., 2006). There are also clear physiological differences in fish of different social rank, such as higher growth rates and lower cortisol levels in dominant individuals (Sloman et al., 2001a; Gilmour et al., 2005; Grobler and Wood, 2013). However, there is now increasing recognition that the social behavior of aquatic animals, especially the formation of dominance hierarchies, is a sensitive target for aquatic toxicants (for reviews, see Atchison et al., 1996; Scott and Sloman, 2004; Sloman and Wilson, 2006).

Ammonia (the sum of NH_3 and NH_4^+) is highly toxic to fish (for reviews, see Randall and Tsui, 2002; Eddy, 2005; Ip and Chew, 2010). Ammonia is also the most manufactured molecule in the world (N-fixation by the Haber-Bosch process), most of it being used for fertilizer manufacture, thereby supporting over 45% of the world's population (Erisman et al., 2008; Fowler et al., 2013). However, most of this is ultimately lost to the environment, resulting in increasing ammonification of natural waters by non-point source pollution. Ammonia is also generated by a wide variety of other anthropogenic processes. Indeed Ankley et al. (2011) identified ammonia as the key toxicant of concern in 37% of municipal discharges evaluated by Toxicity Identity Evaluation (TIE) tests in the US. Environmental Protection Agency's effluent testing program. Additionally, teleost fish produce ammonia as their major N-waste product (Wright, 1995), so ammonia build-up in

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aquaculture is of great concern (Handy and Poxton, 1993; Tomasso, 1994; Colt, 2006; Crab et al., 2007). Given this background, it is curious that, to our knowledge, there has been only one study to date on ammonia's effect on social interactions in fish (Tudorache et al., 2008), and none on the possible effects of ammonia on the physiology of fish within dominance hierarchies.

Other toxicants (e.g. organics, metals, complex effluents) at sublethal levels, as well as general environmental disturbances (e.g. lowered water levels, increased water turbulence, hypoxia) have been shown to disrupt or alter the formation of dominance hierarchies. Commonly reported effects include decreased stability of hierarchies or a failure to form hierarchies, either decreased or increased rates of aggression, and reductions in physiological differences among members of different rank (reviewed by Atchison et al., 1996; Scott and Sloman, 2004; Sloman and Wilson, 2006). Sublethal ammonia is well known to affect many of the processes thought to be important in hierarchy dynamics such as brain function (Walsh et al., 2007), swimming activity and muscle function (Shingles et al., 2001; Wicks et al., 2002; Tudorache et al., 2010), growth (Foss et al., 2003, 2004, 2009, Wood, 2004; Madison et al., 2009), appetite (Wicks and Randall, 2002a; Ortega et al., 2005), and cortisol regulation (Knoph and Olsen, 1994; Wicks and Randall, 2002b; Tsui et al., 2009).

Therefore in the present study, we tested the overall hypotheses that sublethal exposure to high environmental ammonia would (i) disrupt the formation of dominance hierarchies in juvenile rainbow trout, (ii) alter aggression rates, and (iii) reduce physiological differences among members of different rank. We used a method developed by Grobler and Wood (2013) to establish dominance hierarchies in groups of four rainbow trout and to measure respirometric parameters (O_2 consumption, and ammonia excretion rates) of each member over an 11-day period. Social rank was assessed by measurements of aggression; additional determinations included growth, condition factor, feeding, and terminal plasma cortisol concentrations. Several levels of total ammonia were tested initially to assess concentration-dependency, and then detailed measurements were made at the lowest concentration.

2. Methods and materials

Procedures were approved by the McMaster University Animal Research Ethics Board (AUP 09–04–10), and complied with the regulations of the Canada Council for Animal Care.

2.1. Experimental animals and holding conditions

Juvenile rainbow trout (6–10 g) were obtained from Humber Spring Trout Hatchery in Orangeville, Ontario. At McMaster University, the fish were held for 3 weeks prior to experimentation under a 12.5 h light: 11.5 h dark photoperiod in batches of 50 individuals per 200-L aerated aquaria. The tanks were supplied with flowing (~ 1 L min^{-1}) dechlorinated Hamilton tap water (12 °C, pH ~ 7.3, Na^+ = 0.5, Cl^- = 0.7, Ca = 1.0 mmol L^{-1}, hardness ~140 ppm as $CaCO_3$). A 1% total tank weight ration of commercial dried pellet feed (1 point, Martin Mills Inc., Elmira, Ontario) was fed to the fish, three times per week. Water composition was: (in mmol L^{-1}) Na^+ = 0.5, Cl^- = 0.7, Ca = 1.0, hardness ~140 mg L^{-1} as $CaCO_3$. Access to food and space was sufficient during holding that aggression was minimal and social hierarchies did not form.

2.2. Control and experimental group preparation

After anaesthesia in neutralized MS – 222 (0.08 g tricaine methanesulfate L^{-1}), fish were weighed (0.01 g), measured for fork length (0.1 cm), uniquely freeze branded to allow for visual identification, as described by Grobler and Wood (2013), and assigned to groups, as described below. Normal behavior was re-established by 24 h, and food then was then provided on day 2, when the experiments were started.

Seven control groups, ten ammonium bicarbonate (NH_4HCO_3) groups, and four sodium bicarbonate ($NaHCO_3$) groups were formed, each containing 4 fish, using methods similar to those of Grobler and Wood (2013). The 4 fish in each group were size-matched in terms of both length and mass, and were sourced from the same batch of 50-fish in the holding aquaria. Each group was housed in a 30-L aerated tank (53 × 26.7 × 30 cm) fitted with a clear lid to facilitate observations and 5 pieces (1 floating) of PVC pipe (7 × 2.5 cm) for shelter. Tanks were supplied with aeration and flowing (~0.5 L min^{-1}), dechlorinated Hamilton tap water. Water quality was the same as in the holding conditions, and water pH was measured daily using a combination glass electrode (GK24O1C) and pHM 84 meter (Radiometer, Copenhagen, Denmark).

For the ammonia treatments, three different total ammonia concentrations (700, 1200 and 1500 μmol L^{-1}) were created by adding solutions of analytical grade ammonium bicarbonate (NH_4HCO_3, Sigma-Aldrich, St. Louis, Missouri), dispensed through drip bottles, to the tanks 12 h before first feeding. These correspond to 9.8, 16.8, and 21.0 mg L^{-1} as total ammonia-N, or 0.0515, 0.0884, and 0.1105 mg L^{-1} as NH_3-N (unionized ammonia-N) at a mean pH = 7.3and temperature = 12 °C, calculated using the online calculator accompanying the USEPA (2013) ammonia criteria document. These experimental groups were set up in the exact same way as the control groups. Two groups were exposed to 1200 μmol L^{-1} total ammonia for 5 days, two groups to 1500 μmol L^{-1} for 5 days, and six groups to 700 μmol L^{-1} for 11 days. Total ammonia concentrations were verified daily using a modified Verdouw et al. (1978) procedure with concentrations varying by no more than ± 10 μmol L^{-1} from the nominal concentration for each concentration. In tank measurements of total ammonia in control tanks were routinely 0–6 μmol L^{-1}, and pH was 7.2–7.4 in both control and elevated ammonia tanks. Ammonium bicarbonate (NH_4HCO_3) was chosen for the ammonia treatments as it did not appreciably alter the water pH.

To account for any effect that the elevated bicarbonate in the ammonia-exposed groups (dissociation of NH_4HCO_3) might have on hierarchy structure or individual physiology, four groups were exposed to 700 μmol L^{-1} $NaHCO_3$, (Sigma-Aldrich, St. Louis, Missouri) for 11 days using the exact same protocol as outlined for the 700 μmol L^{-1} NH_4HCO_3 exposures. All parameters except ammonia excretion rate, % feeding, and plasma cortisol were measured in the 700 μmol L^{-1} $NaHCO_3$ treatments.

2.3. Hierarchy creation and feeding regime

The experimental design comprised an 11-day exposure, divided into two 4-day periods (days 1–4, and 6–9) in which social interactions were observed, 2 days (days 5 and 10) in which physiological measurements (respirometry for oxygen consumption and ammonia excretion rate, growth indices) were made, and a final day 11 when feeding and plasma cortisol measurements were made. As days 5 and 10 involved disturbance to the fish (handling, anaesthesia), the two periods were treated separately for analysis.

The method of Grobler and Wood (2013) was used to create social hierarchies and record physiological differences among fish with a minimum of disturbance. The basic principle is that food delivery was restricted to a chamber, thereby creating competition. This chamber also served to isolate individual fish for respirometry. The technique