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The effects of strain and ploidy on the physiological responses of rainbow trout (*Oncorhynchus mykiss*) to pH 9.5 exposure

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ABSTRACT

We characterized the physiological effects of exposure to pH 9.5 on one domesticated and four wild strains of diploid and triploid juvenile rainbow trout (*Oncorhynchus mykiss)* over two consecutive years. In the first year, 35–70% of the individuals from the wild strains showed a loss of equilibrium (LOE) at 12 h exposure to pH 9.5, with all fish from wild strains experiencing a LOE by 48 h. In contrast, <20% of the domesticated strain showed LOE over the 48 h exposure to pH 9.5. In our second experiment, similar strain effects were observed, but far fewer fish showed LOE (≤50% in all strains) over 72 h at pH 9.5. In both experiments, there was no effect of ploidy on time to LOE. In the fish that did not show LOE, high pH exposure resulted in significant increases in plasma, brain and muscle ammonia, with no effect of strain or ploidy on the extent of ammonia accumulation. Glutamine accumulated in the brain during high pH exposure, with a stoichiometric decrease in glutamate, but no differences were noted among strains or ploidies. Lactate also accumulated in the plasma to a similar extent in all trout strains and ploidies. Plasma chloride decreased at 24 h exposure in all trout strains and ploidies, but recovered by 72 h. No change was observed in plasma sodium. Overall, our data suggest that the domesticated strain of trout is more tolerant of pH 9.5 than the wild strains, but these differences in tolerance cannot be explained by our sub-lethal assessment of ammonia balance or ion regulation.

1. Introduction

Increases in water pH are known to have profound physiological effects on fish. In rainbow trout (*Oncorhynchus mykiss*), exposure to pH 9.5 or above can impair ionoregulation and result in an accumulation of ammonia in blood and tissues, which if uncorrected, can result in death (Wilkie and Wood, 1991; Yesaki and Iwama, 1992). Disturbances in ionoregulation occur primarily because high pH causes gill damage by hypertrophy of mucous secreting cells and the degradation of connective tissue between epithelial cells (Daye and Garside, 1976). High pH studies have demonstrated an increase in ion efflux across the gill in freshwater (Wilkie and Wood, 1994; Wilkie et al., 1996), which when coupled with a direct inhibition of ion influx (Wilkie et al., 1999), results in a loss of ions from the plasma.

High pH is also known to disrupt the acidic boundary layer at the gill (Wilson et al., 1998), which impairs the excretion of ammonia (the sum of NH_3 and NH_4^+; Wilkie and Wood, 1994). In freshwater fish, branchial ammonia excretion is maintained through a Na^+/NH_4^+ exchange complex that consists of several membrane transporters (i.e. Rhesus glycoproteins, H^+-ATPases, Na^+/H^+ exchangers, Na^+ channels) working together as a metabolon to create an acid trapping mechanism at the gill boundary layer (reviewed by Wright and Wood, 2009, 2012). As NH_3 diffuses across the gill and boundary layer, it is trapped as NH_4^+. Therefore, any disruption in the acidified microenvironment of the gill may lead to an impairment of excretion mechanisms and result in a detrimental internal accumulation of ammonia. Ammonia is lethal at high concentrations in mammals (Marcaida et al., 1992; Hermenegildo et al., 1996, 2000; Kosenko et al., 2004), caused by an over activation of N-methyl-D-aspartate (NMDA) receptors (Hermenegildo et al., 2000; Kosenko et al., 2004), with evidence suggesting that increased NMDA receptor current amplitude may also cause death in fish (Wilkie et al., 2011). These physiological effects combined with ion loss are thought to be the primary mechanisms associated with mortality in fish exposed to high environmental pH (Yesaki and Iwama, 1992; McGeer and Eddy, 1998).

High pH lakes occur in locations worldwide (Galat et al., 1981; Wood et al., 1989; Danulat and Kempe, 1992; Duckworth et al., 1996; Hincks and Mackie, 1997), and in British Columbia (BC) there are unpublished reports of a gradual increase in pH of some freshwater lakes over the past several decades, where several lakes are nearly pH 9.5 (Yesaki and Tsumura, 1991; Toth and Tsumura, 1993; Godin et al., 1994; Mathias et al., 1995). The primary cause of the increasing pH is unknown, but bedrock geology and low flushing rates are thought to be the primary reason (Krueger and Waters, 1983; Jones et al., 1998). British Columbia has an active lake-stocking program to support recreational fishing with more than 4-million rainbow trout (*O. mykiss*) stocked annually into 837 lakes (G.S. Gislason and Associates Ltd, 2009), several

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of which are at pH 9 or above. This lake-stocking program uses trout from at least 6 different strains of which roughly half are stocked as triploids. Triploidy is a condition that is produced by hatcheries in an attempt to induce sterility and promote growth, but triploid fish tend to be more sensitive to environmental challenge (Ojolick et al., 1995; Cotter et al., 2002; Scott et al., 2014). The increasing pH in many of BC lakes, particularly popular fishing lakes such as Green Lake, which has already reached a pH of ~9.3, is of great concern for fisheries biologists and puts continued stocking efforts in jeopardy. In order to continue a successful lake-stocking program in BC it is essential to characterize the effects of high pH exposure on the various strains and ploidies of trout used by the Freshwater Fishery Society of British Columbia (FFSBC), in hopes of identifying the most high pH tolerant trout strain for future stocking efforts.

The objective of this study was to compare the physiological responses of juvenile trout to pH 9.5 exposure among five strains, four wild and one domesticated, as diploid and triploid, all of which are currently used by the FFSBC for stocking. In this study we exposed rainbow trout to pH 9.5 in soft water and sampled blood and tissues for up to 72 h and analyzed aspects of ion regulation and ammonia balance.

2. Materials and methods

2.1. Experimental animals

This study used four wild strains and one domesticated strain of rainbow trout. The fish were obtained from the Fraser Valley freshwater trout hatchery in Abbottsford, BC. Wild strains were named after their site of origin: Blackwater River, Tzenzaicut Lake, Pennask Lake, and Carp Lake. Each spring, FFSBC staff collected breeding pairs of each strain from their native environment and bred three families at the Fraser Valley trout hatchery. The progeny of each family were divided, and half were allowed to develop as diploids and the fertilized eggs of the other half underwent hydrostatic pressure shock to induce triploidy creating full siblings that differed only by ploidy. Three families of the Fraser Valley domesticated strain were also bred and diploid and triploid siblings were generated using the same strategy as used for the four wild strains. The Fraser Valley strain originates from the McCleary strain from the Trout Lodge Hatchery in Washington, USA. Strains used each year were based on availability. The wild strains of trout were reared at the Fraser Valley trout hatchery until December of each year when they were tagged with visible implant elastomere tags to distinguish among strains, ploidies, and families. In January, the wild strains were transferred to the University of British Columbia (UBC) and held in flow-through systems, and fish were separated by strain into two to three replicate tanks. Diploid and triploid fish from a strain were pooled. The Fraser Valley domesticated strain grows faster than the wild strains; therefore they were bred later and tagged in March of each year, transferred to the UBC in early April, and subsequently housed in the same system in replicate tanks. We kept the strains separated during holding to accommodate the later arrival of the Fraser Valley domesticated strain. At UBC, the fish were reared for 6 months in flow through de-chlorinated Vancouver city tap water (pH ~6.7, hardness as [$CaCO_3$] <17.9 mg/L). Temperature varied between years of study (see experimental protocol below for details on experimental design); in 2011 temperature ranged between 10 °C and 15 °C and in 2012 temperature ranged between 8 °C and 12 °C. These temperature differences may have accounted for the differences in body mass between years of analysis (see Table 1). Hardness was measured with a colormetric assay (API General hardness test kit). Fish were fed twice daily with 2% bodyweight of Bitovita fry food. All holding and experimental procedures were conducted according to the guidelines of the Canadian Council on Animal Care and the UBC Animal Care Committee (AUP A13-0024).

Table 1
Body mass of trout sampled for physiological analysis in experiment 1 and experiment 2.

	Body mass (g)	
Strain/ploidy	Experiment 1 (2011)	Experiment 2 (2012)
Blackwater/2n	15.49 ± 1.1 (n = 29)	6.49 ± 0.51(n = 36)
Blackwater/3n	15.62 ± 1.28 (n = 25)	5.92 ± 0.41 (n = 36)
Tzenzaicut/2n	24.48 ± 1.72 (n = 26)	4.91 ± 0.26 (n = 36)
Tzenzaicut/3n	19.25 ± 1.09 (n = 22)	4.41 ± 0.12 (n = 35)
Pennask/2n	16.72 ± 2.89 (n = 26)	3.78 ± 0.2 (n = 35)
Pennask/3n	10.97 ± 1.19 (n = 26)	3.47 ± 0.19 (n = 34)
Fraser Valley/2n	20.02 ± 0.79 (n = 40)	5.31 ± 0.32 (n = 36)
Fraser Valley/3n	18.05 ± 0.65 (n = 40)	4.49 ± 0.13 (n = 36)
Carp Lake/2n	N/A	5.37 ± 0.37 (n = 36)
Carp Lake/3n	N/A	3.99 ± 0.18 (n = 36)

Mean ± SEM and n = total number of fish sampled. Two-way ANOVA and post-hoc analysis of main effects showed a significant difference between strains ($p < 0.0001$), ploidy for experiment 1 ($p < 0.0001$), and ploidy for experiment 2 ($p < 0.001$). N/A = not available.

2.2. Experimental protocol

To examine the effects of pH 9.5 exposure on diploid and triploid trout strains, two separate experiments were conducted, with minor differences in protocol and the number of strains used. For experiment 1, which was conducted in June 2011, we exposed diploid and triploid Blackwater, Tzenzaicut, Pennask and Fraser Valley trout to pH 9.5 for 48 h. Briefly, all trout were fasted for 24 h in their stock tanks before 12 individuals (sized matched to within 3 g of the average body mass of a strain and consisting of 4 individuals/family, with three families for a total of 12 fish) of each strain and ploidy were transferred to a 160 L closed circuit exposure tank consisting of 8 compartments composed of nylon mesh with dimensions of 20 × 25 × 50 cm. Each strain and ploidy was housed in a separate compartment within the exposure tank. Trout were allowed to recover from the transfer for 24 h, during which the exposure system was supplied with a continuous flow of freshwater that circulated through the exposure tank and then went to drain. Water was circulated within the exposure tank using two submersible pumps that withdrew water from the bottom of the exposure tank and fed into a header tank that then flowed into each compartment containing fish. After the 24 h recovery period, two fish of each ploidy from each strain were removed, and body weight was recorded. Body weight of fish used for physiological analysis is presented in Table 1. Fish were selected randomly throughout the exposure yielding an uneven family distribution at sampling points, and results pertaining to family will not be presented here. The two fish were sampled for plasma, muscle, gill, and brain and this sampling served as the control (see tissue sampling below). Gill tissue was not analyzed in this experiment. Following the control sampling, the freshwater inflow was turned off and pH was adjusted from a pH of 6.7 to 9.5 over 6 h with the addition of 0.2 M NaOH directly to the header tank. Once pH 9.5 was achieved it was maintained using a pH electrode (Cole-Parmer) connected to an alpha-560 Eutech instruments pH regulator. This controlled a peristaltic pump that delivered 0.2 M NaOH when measured pH deviated from the set point by 0.01 pH units. The regulator was calibrated every 12 h and water pH was verified using a Fisher Scientific hand-held pH meter to ensure that pH remained at 9.5. Dissolved oxygen and water ammonia were measured hourly throughout the experiment using a handheld-oxygen probe (Oakton Acorn Series DO6) and colorimetric assay (API Ammonia test kit), respectively to ensure oxygen levels remained above 90% air saturation and total ammonia levels remained below 0.25 mg/L. The water within the exposure tank was replaced with water of the same chemical composition after each sampling and at 18, and 36 h. It was noted early on in the experiment that fish were showing a loss of equilibrium (LOE) during pH 9.5 exposure and we quantified this variable. Loss of equilibrium was defined as the point when a fish lost the ability to maintain normal dorso-ventral orientation and did not correct orientation when gently prodded with a fish net. If upon prodding the fish could not re-establish equilibrium, it was removed and euthanized, and the time was recorded. Fish were