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Aquaculture-related stress on rainbow trout (Oncorhynchus mykiss) and Arctic char (Salvelinus alpinus)

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Aquaculture-related Stress on Rainbow Trout (*Oncorhynchus mykiss*) and Arctic Char (*Salvelinus alpinus*)

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Abstract

Aquaculture-related Stress on Rainbow Trout (*Oncorhynchus mykiss*) and Arctic Char (*Salvelinus alpinus*)

Christopher D. Nelson

Recent expansions in aquaculture characterize it as one of the fastest growing segments of the U.S. agricultural sector. Expansion has been facilitated by increased production of many important aquatic species including catfish, salmon, trout, tilapia, shrimp, oysters, and crawfish. West Virginia aquaculture, also an expanding industry, is currently characterized by small-scale, widely dispersed farms. To facilitate future expansion of aquaculture in West Virginia, focuses on product quality as well as production efficiency, identification of limiting factors and investigation into new or alternative management techniques are needed. Objectives of this project were to determine the effects of nitrite and carbon dioxide on the survival and physiological responses of rainbow trout and to evaluate the effectiveness of fish transport with anesthesia on reducing the physiological responses of Arctic char. Results from this project indicate that nitrite toxicity is affected by elevations in environmental carbon dioxide, and conversely, carbon dioxide tolerance is affected by environmental nitrite concentrations. Results also indicate that the anesthetic treatments investigated in this project (AQUI-S^{TM}, ice-slurry, and carbon dioxide) were not effective in consistently reducing the physiological responses of Arctic char during a simulated transport. We feel that additional experiments examining the interacting effects of nitrite and carbon dioxide on fish are needed to identify the physiological mechanisms or effects that lead to increased nitrite and carbon dioxide toxicities in fish, and additional experiments evaluating efficient procedures of reducing physiological responses of fish during transport is also needed.

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Foreword

This thesis has been formatted for submission to the North American Journal of Aquaculture, according to the journal's manuscript preparation guidelines published

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Abstract

Effects of Nitrite and Carbon Dioxide in Rainbow Trout (*Oncorhynchus mykiss*): Survival and Physiological Responses

Christopher D. Nelson

West Virginia's Appalachian landscape encourages development of smaller, intensive aquaculture systems rather than the traditional, expansive earthen ponds found further south. Intensive aquaculture systems, especially recirculating and those using oxygen injection technologies, create new and unique water chemistry characteristics not found in traditional flow through and pond culture systems. Higher loading rates can enhance and increase production; however, they also can result in increased accumulation of metabolic by-products (i.e. carbon dioxide, ammonia, and nitrite) in the grow-out system. Expansion of aquaculture in West Virginia may create a unique challenge where both environmental carbon dioxide (CO_2) and nitrite (NO_2^-) are elevated to toxic levels due to the use of oxygen injection and recirculating aquaculture technologies. While there is some understanding of how each of these parameters independently affects fish, there is no information as to how these parameters interact in intensively cultured fish systems when elevated simultaneously. Therefore, the objective of the present study was to evaluate the survival and physiological responses of rainbow trout when exposed to CO_2 and NO_2^-. Three preliminary and two primary experiments were conducted. The two primary experiments were; 1) 96-h exposure of rainbow trout to NO_2^- and three levels of CO_2 and 2) 96-h exposure of rainbow trout to NO_2^-, chloride (Cl^-), and three levels of CO_2. During experiments, rainbow trout survival and their physiological responses (hematocrit, plasma nitrite, glucose, lactate, and chloride) were quantified. Results indicate that NO_2^- toxicity is affected by elevations in environmental CO_2, and conversely, CO_2 tolerance is affected by environmental NO_2^- concentrations. Additional experiments examining the interacting effects of NO_2^- and CO_2 on fish are needed to identify the physiological mechanisms or effects that lead to increased NO_2 and CO_2 toxicities in fish.

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Introduction

West Virginia Aquaculture Industry

Recent expansions in aquaculture characterize it as one of the fastest growing segments of the U.S. agricultural sector. Expansion has been facilitated by increased production of many important aquatic species including catfish, salmon, trout, tilapia, shrimp, oysters, and crawfish (NASS 1998). Through the 1980's, the U.S. aquaculture industry increased four fold to a farm value of $762 million by 1990. In 1998, the U.S. aquaculture industry was valued at nearly $1 billion; however, seafood imports still represent the country's second largest non-combustible trade deficit (NASS 1998; FAO 2002). Thus, despite rapid growth of the industry, there is still demand for even more seafood products.

West Virginia may provide an ideal opportunity for expansion of the U.S. aquaculture industry. West Virginia's economy has experienced major structural changes over the past two decades with declines in the traditional industrial base (e.g., coal, primary metals, and chemicals) leading to a reduction in economic growth and double-digit unemployment rates (WVBEP 2003). The role of agriculture in the future economic development of the state is unclear because West Virginia agriculture is dominated by small part-time farmers and the rough terrain is unsuitable for highly mechanized, intensive, and large-scale operations (Vanderpool et al. 1993). However, West Virginia has an abundance of land and water supplies suitable for commercial aquaculture production (Jenkins and Hankins 1996). Thus, aquaculture may provide an opportunity for farm and industry diversification and offer promise for strengthening the state's agricultural sector (Vanderpool et al. 1993). Abundant water supplies as well as the increased demand for seafood products favor expansion of the industry throughout the state.

The mountainous terrain of West Virginia's Appalachian landscape encourages development of smaller, intensive aquaculture systems rather than the traditional, expansive earthen ponds found further south. Intensive aquaculture systems, especially recirculating and those using oxygen injection technologies, create new and unique water chemistry characteristics not found in traditional flow through and pond culture systems. For example, use of oxygen injection eliminates dissolved oxygen as a limiting factor in fish culture, thereby allowing more fish to be stocked into the same volume of water, resulting in higher loading rates (Summerfelt et al. 2000). Higher loading rates can enhance and increase production; however, they also can result in increased accumulation of metabolic by-products (i.e. carbon dioxide, ammonia, and nitrite) in the grow-out system when combined with lower water exchange rates.

Carbon Dioxide Challenges in the WV Aquaculture Industry

Free carbon dioxide (CO_2) is one by-product produced by fish that may accumulate to toxic levels in intensive systems. Carbon dioxide is a gas that easily dissolves in water, and may accumulate even when concentrations in the surrounding air may be negligible (Boyd 1979). Once CO_2 is expired by fish into water, accumulation of CO_2 depends on a variety of factors including rate of production, water exchange rate, aeration technology, and buffering capacity of the water (Smart 1981; Summerfelt 2001).

In addition to production and release of CO_2 by fish, West Virginia also faces environmental challenges such as regionally elevated concentrations of free CO_2 in the water. Many watersheds in West Virginia are susceptible to high levels of CO_2 because of karst topography of the region (Kozar et al. 1991). Spring or bore-hole water supplies are those typically associated with high levels of free CO_2 (Smart 1981) and can routinely exceed 50 mg/L CO_2 in Appalachian spring waters (Kozar et al. 1991; Hainly and Looper 1995).

Elevated CO_2 levels can present potential physiological problems and harmful effects to fish. High CO_2 levels lower water pH and can limit the capacity of the fish's blood to carry oxygen by lowering blood pH at the fish's gills (Eddy et al. 1977; Wurts and Durborow 1992). Oxygen transport in salmonids can become impaired when environmental CO_2 concentrations reach 20 mg/L (Wedemeyer 1996). In addition, high concentrations of CO_2 can result in acidosis, hypercapnia, sedation, or death (Summerfelt 2000), as well as reduce fish growth, decrease food conversion ratios, lower condition factors, and reduce hematocrit and plasma chloride concentrations (Smart 1981; Danley 2001). Therefore, thoroughly understanding the effects of CO_2 on fish health and productivity is important to the development of the West Virginia aquaculture industry.

Nitrite Toxicity in the Aquaculture Industry

In intensive aquaculture systems, water quality issues are rarely regulated to an isolated chemical change. For example, with the production of CO_2 also comes the production of nitrogenous compounds such as ammonia and nitrite. Ammonia, the primary nitrogenous waste of fish, is oxidized into nitrite by the *Nitrosomonas* bacteria and further to the relative harmless nitrate ion by the *Nitrobacter* species (Rodriguez-Moreno and Tarazona 1994; Vedel et al. 1998). Nitrite can be problematic in recirculating systems because it is constantly generated and may accumulate when water is recycled (Russo et al. 1981; Timmons et al. 2001). Increased nitrite concentrations may cause harmful effects to fish such as decreased growth and survival (Perrone and Meade 1977; Colt et al. 1981; Tomasso 1994).

Unlike CO_2, which has received abundant research attention in both human medicine and aquatic animal production sectors, the mechanisms behind nitrite toxicity are complex and not thoroughly understood. Nitrite is thought to be transported across the gill membrane of the fish

by the mechanism that normally transports chloride ions (Perrone and Meade 1977; Tomasso 1994). In some species of fish, the transport process across the gills allows nitrite to concentrate in the blood relative to the concentration in the environment (Williams and Eddy 1988; Tomasso 1994). Accordingly, the toxicity of nitrite varies widely among species (Russo and Thurston 1977; Palachek and Tomasso 1984; Mazik et al. 1991; Atwood et al. 2001), with salmonids being most susceptible and eels being least susceptible (Williams and Eddy 1988).

The most recognized toxic effect of nitrite in the blood is the conversion of hemoglobin to methemoglobin. This process results in the oxidization of iron in the hemoglobin molecule from the ferrous (+2) to the ferric (+3) state (Tomasso 1994; Timmons et al. 2001), which is unable to bind or transport oxygen (Wedemeyer and Yasutake 1978; Tomasso 1994). Although methemoglobinemia is an obvious effect of nitrite, other possible toxic mechanisms may exist including gill and tissue damage, and liver hypoxemia (Crawford and Allen 1977; Arillo et al. 1984; Scarano and Saroglia 1984; Tomasso 1994).

Many studies have shown that the toxicity of nitrite can be ameliorated by specific environmental ions (Crawford and Allen 1977; Perrone and Meade 1977; Russo and Thurston 1977; Palachek and Tomasso 1984). Environmental chloride appears to be the most important ameliorating ion, although other ions such as calcium may also have beneficial effects (Tomasso 1994). Environmental chloride may reduce nitrite toxicity through a competitive interaction for the chloride transport mechanism at the gills (Perrone and Meade 1977; Tomasso 1994) given that nitrite and chloride are both monovalent anions and are similar in size and shape (Tomasso 1994).

The absorption of environmental nitrite by different fish species may reflect the inability of branchial chloride cells to discriminate chloride from nitrite ions (Tomasso 1994). Studies

have shown that the most susceptible species to nitrite toxicity also have the highest chloride uptake rates, while low chloride uptake rates are characteristic of more tolerant species (Williams and Eddy 1988). Furthermore, the presence of elevated environmental chloride has been shown to lower plasma nitrite concentrations during environmental nitrite exposure (Palchek and Tomasso 1984; Mazik et al. 1991; Eddy et al. 1983). Therefore, the greater the environmental chloride:nitrite ratio, the lower the plasma nitrite concentrations (Tomasso 1994). Recognition of this relationship has resulted in the use of salts as a means of alleviating nitrite toxicity in fish caused by high environmental nitrite concentrations.

Nitrite and CO_2 challenges in the West Virginia Aquaculture Industry

Expansion of aquaculture in West Virginia may create a unique challenge where both environmental CO_2 and nitrite are elevated to toxic levels due to the use of oxygen injection and recirculating aquaculture technologies (Summerfelt et al. 2000). While there is some understanding of how each of these parameters independently affects fish, there is no information as to how these parameters interact in intensively cultured fish systems when elevated simultaneously. Indirect evidence suggests nitrite toxicity may be affected by elevations in CO_2, since both nitrite and CO_2 influence chloride homeostasis (Cameron and Iwama 1989; Tomasso 1994). During hypercapnia, acid-base disturbances in the blood appear to be partially compensated by decreasing the influx of chloride, with an associated increase of bicarbonate levels (Cameron and Iwama 1989). Goss et al. (1994a, 1994b) has demonstrated that the reduction in chloride flux across the gill is the result of gill remodeling, where fewer chloride cells, and thus branchial Cl^-/HCO_3^- exchangers, are exposed to the water for ion exchange.

If nitrite competes with chloride for entrance at the branchial Cl^-/HCO_3^- exchanger in the gill, then the possibility exists that nitrite uptake could be reduced by hypercapnia since chloride uptake rates will be reduced. Jensen et al. (2000) observed this in freshwater crayfish (*Astacus astacus*). Alternatively, the combined effects of hypercapnia and elevated nitrite may be additive and negative, since they affect different aspects of the same physiological systems (respiratory and ionic balance). The two possible CO_2 x nitrite interactions hypothesized above (beneficial vs. toxic effects of CO_2 and nitrite) would be important and have very different management implications. To facilitate aquaculture expansion in West Virginia, it is important to understand which of the two possible CO_2 x nitrite interactions may occur. Considering the work of Jensen et al. (2000), it was hypothesized that the reduced nitrite uptake rates seen in crayfish would also occur in rainbow trout, thereby reducing plasma nitrite concentrations and increasing survival.

Therefore, the objective of the present study was to evaluate the survival and physiological responses of rainbow trout when exposed to CO_2 and nitrite. Three preliminary and two primary experiments were conducted. The two primary experiments were; 1) 96-h exposure of rainbow trout to nitrite and three levels of CO_2 and 2) 96-h exposure of rainbow trout to nitrite, chloride, and three levels of CO_2.

Materials and Methods

Fish Care and Maintenance

Rainbow trout were raised at the Conservation Fund's Freshwater Institute, Shepherdstown, WV. Fish were housed in 1200-L fiberglass tanks supplied with oxygen-injected spring water (temperature 13.2 ± 0.2°C; dissolved oxygen 13.3 ± 0.2 mg/L; pH 7.6 ± 0.1; total alkalinity 241.7 ± 4.6 mg/L; calcium hardness 237.4 ± 2.3 mg/L and 11.7 ± 0.7 mg/L free CO_2). Photoperiod was maintained on a 14 h:10 h, light:dark cycle. Fish were fed daily by

automatic feeders with commercial feed (Melick Aquafeed, Catawissa, PA), and they were fasted 24 h before stocking, and throughout the experiments, unless otherwise described. Water temperature, dissolved oxygen, and pH were measured daily, while total alkalinity, calcium hardness, free CO_2, and total ammonia-nitrogen (TAN) were measured weekly in the holding tanks using commercially available equipment and test kits described below.

Experimental Conditions

Rainbow trout were randomly selected from the holding tanks and stocked into 68-L polypropylene tanks at 48 h before the start of the experiments (5 fish per tank). The number of tanks used varied with experiments and are described below with the appropriate experiment. Temperature, dissolved oxygen, pH, TAN, and nitrite were measured every 24-h in each of the tanks throughout the experiments using handheld meters (YSI Models 58, 95, or 60/10 FT Yellow Springs, OH) and standard tests kits (Hach Company, Loveland, CO). Total alkalinity and calcium hardness were measured twice during each experiment (Hach Company, Loveland, CO). Free CO_2 and chloride concentrations also were measured when appropriate (Hach Company, Loveland, CO and digital chloridometer Labconco, Lenexa, KS). Mean ($\pm$ SEM) water quality measurements from each experiment are provided in Appendix A.

At the beginning of each experiment (0 h), the appropriate amount of nitrite as sodium nitrite ($NaNO_2$) was added into the experimental tanks to obtain treatment concentrations (see Appendix B for calculations). Fish were continuously exposed to treatment concentrations throughout each experiment via static water baths or peristaltic drip. Nitrite treatment concentrations were confirmed using standard water quality test kits (Hach Company, Loveland,

CO). Chloride as sodium chloride (NaCl) was added in a similar manner where applicable, and concentrations confirmed using a digital chloridometer (Labconco, Lenexa, KS).

After each 24-h exposure, dead fish were counted and removed from the tanks. Fish were considered deceased when no gill movement was visible. At the end of each experiment, all remaining live fish were anesthetized in a 0.02 mg/L solution of tricaine methanesulfonate (MS-222) and blood samples collected.

Blood was collected into lithium-heparinized syringes from vessels in the caudal peduncle (Houston 1990). Sampling was completed within 5 minutes of initial tank disturbance and each fish was bled only once. Blood was transferred into heparinized microhematocrit tubes and 3-ml glass tubes (Vacutainers^{TM}, Becton Dickinson & Co., Sparks, Maryland, USA). Microhematocrit samples (in duplicate) were centrifuged for 5 min at 11,500 rpm and the percent hematocrit determined using a microhematocrit reader (IEC, Needham Heights, MA).

The whole blood that was collected into 3-ml glass tubes was centrifuged for 10 min at 3500 rpm, and the resulting plasma was transferred into 1.5-ml polypropylene microcentrifuge tubes. Plasma samples were stored at -20 C until analyzed for nitrite, chloride, glucose, and lactate concentrations. Plasma NO_2^- concentrations were determined according to USEPA (1974) as modified by Palachek and Tomasso (1984). Overall percent recovery for the plasma nitrite assay was 95.64 % (range of means = 93.0 to 98.3 %, pooled SEM = 1.7, n = 2 per experiment). Plasma chloride was measured using a digital chloridometer (Labconco, Lenexa, KS) and glucose and lactate by a digital glucose/lactate analyzer (2300 Select, YSI Inc., Yellow Springs, OH).

Experimental Design

Preliminary experiments 1 through 3 were conducted under static conditions, using 68.1-L polypropylene tanks filled with 45 L of water. Experimental tanks were partially submerged in a larger flow-through water bath to maintain constant water temperature. Approximately 50% of the water in each experimental tank was exchanged daily to minimize accumulation of metabolic waste. Dissolved oxygen concentrations were maintained by supplemental aeration. The median lethal concentration (LC_{50}) was calculated for experiments 1 and 2 by the trimmed Spearman-Karber method (Hamilton et al. 1977), while a percent survival was determined for experiment 3. Experiments 1 through 3 served as preliminary experiments to obtain baseline data (e.g., nitrite and chloride concentrations, fish survival) necessary to conduct the primary experiments 4 and 5. Experiments 4 and 5 were conducted under flow-through conditions in the 68.1-L polypropylene tanks (containing 45-L of water). Nitrite treatments were maintained using a peristaltic pump which continuously dripped an appropriate concentrated stock solution into the flow-through experimental tanks.

Experiment 1: 24-h LC_{50} with NO_2^-

Seven concentrations of NO_2^- (3.6 ± 0.5, 7.1 ± 1.5, 13.2 ± 2.0, 20.9 ± 3.0, 28.6 ± 1.2, 59.4 ± 13.8, and 108.1 ± 16.4 mg/L NO_2-N), along with a control (0.4 ± 0.5 mg/L NO_2-N), were tested in triplicate for a total of 24 tanks. At the beginning of the experiment the appropriate amount of nitrite was pre-dissolved and added into each tank. Rainbow trout, 179.4 ± 3.7 g and 21.7 ± 0.2 cm long, were exposed for 24-h to the treatment concentrations. At the end of the experiment, blood samples were collected from all remaining live fish and the LC_{50} determined as previously described.

Experiment 2: 96-h LC_{50} with NO_2^-

Eight nitrite concentrations (1.4 ± 0.05, 1.6 ± 0.06, 2.1 ± 0.06, 3.2 ± 0.09, 4.7 ± 0.14, 6.5 ± 0.10, 9.3 ± 0.19, and 13.3 ± 0.18 mg/L NO_2-N), along with a control (0.5 ± 0.08 mg/L NO_2-N), were tested in triplicate for a total of 27 tanks. At the beginning of the experiment the appropriate amount of nitrite was pre-dissolved and added into each tank. Rainbow trout, 201.9 ± 5.6 g and 23.5 ± 0.24 cm long, were exposed for 96-h to the treatment concentrations. At the end of the experiment, blood samples were collected from remaining live fish, and the LC_{50} was determined as previously described.

Experiment 3: 96-h with $NO_2^- + Cl^-$

Eight concentrations of Cl^- (20.4 ± 0.18, 24.1 ± 0.17, 28.3 ± 0.25, 35.7 ± 0.21, 48.0 ± 0.33, 67.5 ± 0.46, 100.0 ± 0.48, and 165.2 ± 6.6 mg/L Cl^-) were tested in combination with a single, lethal concentration of nitrite (9.03 ± 0.04 mg/L NO_2-N). A freshwater control and a nitrite-only control also were tested. All treatment combinations were replicated three times for a total of 30 tanks. At the beginning of the experiment the appropriate amount of nitrite and Cl^- were pre-dissolved and added to each tank. Rainbow trout, 226.7 ± 4.9 g and 24.8 ± 0.21 cm long, were exposed for 96-h to the lethal nitrite and chloride concentrations. At the end of the experiment, blood samples were collected from remaining live fish as previously described, and percent survival was calculated.

Experiment 4: CO_2 x NO_2^-

Two concentrations of NO_2^- (0.84 ± 0.03 and 2.1 ± 0.03 mg/L NO_2-N), along with a control (0.08 ± 0.02 mg/L NO_2-N), were tested with three CO_2 levels (9.0 ± 0.27, 13.8 ± 0.76, and 23.4 ± 1.0 mg/L free CO_2) in duplicate for a total of 14 tanks. Rainbow trout, 270.1 ± 7.6 g and 25.7 ± 0.25 cm long, were initially stocked into 500-L flow-through tanks equipped with CO_2 diffusers, and allowed to acclimate to the respective CO_2 treatments for two weeks before the start of the experiment. The CO_2 concentrations were maintained using micro pore diffusers (MBD 75 s A grade, Point Four Systems Inc., Port Moody, British Columbia, Canada) and gas flow meters (Key Instruments, Trevose, PA). The entire system was connected by polyethylene tubing to a remote liquid CO_2 cylinder (Messler MG Industries, Malvern, PA). During CO_2 acclimation, fish were fed during week 1, but were fasted during week 2.

After the two week acclimation period, fish were stocked into experimental tanks. Experimental tanks were also equipped with CO_2 diffusers connected to a liquid CO_2 tank. Carbon dioxide treatments were randomly assigned among the experimental tanks, and water CO_2 concentrations were adjusted to match those of the CO_2 acclimation tanks before fish were stocked. Thus, when fish were moved from the CO_2 acclimation tanks into experimental tanks, CO_2 treatments were maintained. Fish were acclimated to the experimental tanks for 48 h before the experiment was started.

At the beginning of the experiment, the appropriate amount of nitrite was pre-dissolved and added into each tank to initially reach target concentrations. Treatment concentrations were thereafter maintained via the peristaltic pump and concentrated stock solutions. Free CO_2 treatments were checked daily via the CO_2 nomogram method (APHA 1998). In addition, the nomogram method was validated by titrating water samples from selected tanks at each CO_2 treatment level. Titrations were completed using a hand-held digital titrator (Hach Company)

and a bench top pH meter (Accumet Model 915, Fisher Scientific, Pittsburgh, PA) equipped with a G-P Gel combo pH electrode (Corning Incorporated, Corning, NY). Fish were exposed to the treatments for 96-h, with survival quantified every 24 h. At the end of the experiment, blood samples were collected from remaining live fish as previously described.

Experiment 5: CO_2 x NO_2^- x Cl^-

A single lethal nitrite concentration (9.6 ± 0.06 mg/L NO_2-N), along with controls, was tested in combination with three CO_2 levels (7.7 ± 0.43, 14.8 ± 0.74, and 29.3 ± 1.96 mg/L free CO_2) and two Cl^- levels (48.0 ± 1.75 and 109.7 ± 1.39 mg/L Cl^-) in duplicate for a total of 16 tanks. Acclimation and experimental conditions were similar to those described for experiment 4. Rainbow trout, 311.9 ± 11.3 g and 26.5 ± 0.34 cm long were exposed to tank conditions for 96-h. At the end of the experiment, blood samples were collected from remaining live fish as previously described.

Statistical Analyses

Treatments were compared using individual fish responses and multiple ANOVA (Proc GLM) procedures. Results are presented as treatment means ± standard error of the mean (SEM). Following ANOVA testing, significant differences were further analyzed by Duncan's multiple comparison procedures and correlation used to determine linear relationships. The alpha level (α) of significance for all tests was 0.05. Statistical analyses were completed using Microsoft Excel (Excel XP, Microsoft Co., Redmond, CA) and SAS (SAS 8.2, SAS Inst., Cary, NC).

Results

Experiment 1: 24-h LC_{50} with NO_2^-

Rainbow trout mortality showed a dose-dependent response to nitrite-N concentrations. Mortality was observed in 7.1 mg/L and higher nitrite-N treatments (Fig. 1). Total mortality (100 %) was observed in the 28.6 and higher mg/L nitrite-N treatments. No control fish died during the experiment. The 24-h LC_{50} was 8.68 mg/L nitrite-N (95 % CI = 7.30 to 12.85 mg/L nitrite-N).

Plasma nitrite increased significantly with increasing environmental nitrite and was linearly related ($r^2 = 0.96$; Fig. 2). Plasma nitrite was significantly different from the control at environmental nitrite-N concentrations of 3.6 mg/L and higher. Plasma chloride was inversely correlated to environmental nitrite-N concentrations ($r^2 = -0.85$) and decreased with increasing environmental nitrite (with the exception of the 7.1 mg/L nitrite-N concentration; Fig. 3). Plasma chloride differed significantly ($p < 0.005$) compared to controls at 20.9 mg/L environmental nitrite-N level. Percent hematocrit was significantly different ($p = 0.04$) in the 7.1 and 20.9 mg/L nitrite-N treatment groups compared to the control (Table 1). No linear relationship was detected between hematocrit and environmental nitrite-N. Plasma glucose or lactate differences were not detected among treatments (Table 1).

Experiment 2: 96-h LC_{50} with NO_2^-

Rainbow trout mortality showed a dose-dependent response to nitrite-N concentration. Mortality was observed at 2.1 mg/L and higher nitrite-N concentrations (Fig. 4). Total mortality (100 %) was observed in the 6.5 and higher mg/L nitrite-N treatments. No control fish died

during the exposure. The 96-h LC_{50} was 2.96 mg/L nitrite-N (95 % CI = 2.64 to 3.31 mg/L nitrite-N).

Plasma nitrite increased with increasing environmental nitrite concentrations, with the exception of the 3.2 nitrite-N level, and these responses were linearly related ($r^2 = 0.88$; Fig. 5). Plasma nitrite concentrations for NO_2^- treated fish, however, were not significantly different (with the exception of the 4.7 mg/L nitrite-N; $p = 0.01$) from those of the control. Overall glucose levels were significantly decreased in nitrite exposed fish compared to the control (Table 2). Plasma glucose was significantly different ($p= 0.02$) at 2.1 mg/L nitrite-N compared to all other treatments. Significant differences among treatments were not detected for hematocrit, plasma lactate, and chloride concentrations when compared to control values (Table 2).

Experiment 3: 96-h with $NO_2^- + Cl^-$

Supplemental environmental chloride increased survival of rainbow trout exposed 9.03 ± 0.04 mg/L nitrite-N (Fig. 6). Survival was > 0 % at 28.3 mg/L and higher chloride treatments. Survival reached 100 % at the 165.2 mg/L chloride treatment (Fig. 6).

Addition of environmental chloride significantly decreased plasma nitrite among nitrite-exposed fish in a dose-dependent manner (Fig. 7). Plasma lactate decreased with increasing environmental chloride (with the exception of the 35.7 mg/L NaCl level) and a significant difference ($p < 0.005$) among chloride treatments was detected (Fig. 8). Plasma glucose, plasma chloride, and hematocrit were not affected by treatments (Table 3).

Experiment 4: CO_2 x NO_2^-

Mortality was observed in all CO_2 x NO_2^- treatments. At 0.8 mg/L nitrite-N, mortality was significantly increased in the high CO_2 treatment compared to the low and medium CO_2 treatments. In the 2.1 mg/L nitrite-N, treatment mortality increased from the low to medium CO_2 treatment, and decreased from the medium to high CO_2 treatment (Fig. 9). Mortality was higher in all of the 2.1 mg/L nitrite-N treatments compared to the 0.8 mg/L nitrite-N treatments. No significant differences were detected in hematocrit, plasma nitrite, glucose, lactate, or chloride concentrations among treatments (Table 4).

Experiment 5: CO_2 x NO_2^- x Cl^-

Supplemental environmental chloride (as NaCl) increased survival of rainbow trout exposed to 9.60 ± 0.06 mg/L nitrite-N (Fig. 10). Mortality was observed in all of the CO_2 x low (48.0 mg/L) NaCl treatments. Mortality was also observed in the medium CO_2 x high (109.7 mg/L) NaCl treatment.

Increased environmental chloride significantly decreased plasma nitrite levels in fish exposed to the lethal level of 9.60 ± 0.06 mg/L nitrite-N (Fig. 11). A significant difference in plasma nitrite ($p = 0.04$) was detected among environmental chloride treatments. There were no significant differences, however, observed in plasma nitrite among CO_2 treatments or among chloride x CO_2 treatments. Plasma chloride was significantly different ($p < 0.005$) among environmental chloride treatments but not among CO_2 or chloride x CO_2 treatments (Fig. 12). There were no significant differences in hematocrit, plasma glucose and plasma lactate concentrations (Table 5).

Discussion

The independent effects of nitrite and CO_2 on rainbow trout have been well documented (Russo and Thurston 1977; Eddy et al. 1983; Eddy et al. 1977; Iwama and Heisler 1991), but there is no information available on the interaction between nitrite and CO_2 in fish. Because of known changes in chloride transport under conditions of hypercapnia (Goss et al. 1994a), we hypothesized that nitrite toxicity (mortality and plasma nitrite concentrations) would be reduced during hypercapnia; however, our results did not support this hypothesis. Instead, our results suggest increased toxicity to elevated nitrite x hypercapnia compared to either nitrite or elevated CO_2 toxicity alone (Figs. 9 and 11).

Nitrite Toxicity During Normocapnia

Results from the preliminary (normocapnic) nitrite toxicity experiments (24-h LC_{50} = 8.68 mg/L and 96-h LC_{50} = 2.96 mg/L nitrite-N) were similar to those observed by Eddy et al. 1983 (9.7 mg/L nitrite-N) and Russo and Thurston 1977 (0.2 mg/L nitrite-N), respectively. Minor differences in environmental nitrite tolerance may have resulted from water quality disparities among studies, such as higher CO_2 concentrations in the water of the present study (average CO_2 = 12 mg/L), and/or different fish sizes (Tomasso 1994). The linear relationship detected in the present study between plasma nitrite and environmental nitrite has been well documented elsewhere (Crawford and Allen 1977; Perrone and Meade 1977; Eddy et al. 1983). Plasma nitrite levels in the current study consistently exceeded environmental concentrations, supporting results of previous studies that showed rainbow trout actively transport nitrite from the environment to the blood (Tomasso et al. 1979; Tomasso 1986). During experiment 1, plasma chloride also decreased with increasing environmental nitrite. Williams and Eddy (1988)

reported a similar inverse relationship between plasma chloride and environmental nitrite concentrations in fish.

Although significant hematocrit differences were detected among treatment groups in experiment one, all results were within the range reported as normal for rainbow trout (Barton 2000), and so it is not clear if the difference is meaningful. There was a slight decrease observed in plasma glucose at 2.1 mg/L nitrite-N during experiment two; however, plasma glucose levels were not significantly different compared to control levels in the higher environmental nitrite treatment groups. The reduction in plasma glucose levels may suggest that rainbow trout were experiencing stress-related toxicity responses.

Supplemental environmental chloride can alleviate the toxicity of nitrite in some fishes (Tomasso er al. 1979; Palachek and Tomasso 1984; Mazik et al. 1991), and results of experiment 3 (NO_2^- x Cl^-) are consistent with those of previous studies. Results of experiment 3 showed that rainbow trout simultaneously exposed to a lethal level of nitrite with increasing environmental chloride levels had correspondingly and significantly decreased plasma nitrite (Fig. 7). Percent survival also increased with increasing environmental chloride levels (Fig. 6). Russo et al. (1981) reported that the 96-h LC_{50} values of rainbow trout with chloride were an order of magnitude higher than those obtained without chloride. In experiment 3, approximately 165.2 mg/L NaCl was needed to observe 100% survival and 100 mg/L NaCl to reduce plasma nitrite levels below environmental levels (Fig. 6), and these observations are consistent with results reported by Russo et al. (1981).

Physiologically, a significant decrease was observed in plasma lactate levels among nitrite exposed fish with increasing environmental chloride concentrations; whereas, plasma lactate values in the higher chloride treatments were similar to control levels. Such a relationship

has not been documented previously. Results may indicate that trout at higher chloride treatments were experiencing less nitrite-induced stress (i.e. less burst-type swimming or escape behavior) than those at the lower chloride levels (Thomas et al. 1999). Decreased stress-related responses would be expected with decreased nitrite toxicity.

Toxicity Responses to CO_2 Only (No Nitrite)

As expected, free CO_2 concentrations used in experiments 4 and 5 did not cause mortality among control fish exposed only to CO_2 (no nitrite). Other CO_2 studies conducted at the Freshwater Institute also have demonstrated 100% survival among rainbow trout chronically exposed to concentrations as high as 48 mg/L (or double the concentrations used in the current study; Danley 2001). Thus, all CO_2 concentrations used in the current study were within a tolerable (100% survival) range for rainbow trout.

Although not significant, plasma chloride concentrations also showed an expected decrease with increasing CO_2 concentration at low or zero nitrite concentrations (Tables 4 and 5). Cameron and Iwama (1987) have documented a similar decrease in plasma chloride in channel catfish (*Ictalurus punctatus*) with increasing CO_2.

Toxicity Responses to NO_2^- x CO_2

Increasing CO_2 concentrations resulted in increasing mortality for both NO_2 treatment groups of experiment 4 (with the exception of the 2.1 NO_2 x 23 CO_2 treatment). Assuming LC_{50}'s are generally stable under normocapnic conditions, our results suggest that increasing both environmental nitrite and carbon dioxide simultaneously increases the toxicity of both water quality variables in fish. During experiment 2, mortality was first observed at a nitrite treatment dose of 7.1 mg/L nitrite-N; however, during hypercapnia in experiment 4, mortality was

observed in nitrite treatments as low as 0.8 mg/L nitrite-N, with mortality increasing in a dose-dependent manner. Thus, fish appear to be less tolerant to environmental nitrite during hypercapnia than they otherwise might be.

Conversely, fish also appear to be less tolerant to environmental hypercapnia during exposure to elevated environmental nitrite (Figs. 9 and 11). As discussed previously, survival to environmental hypercapnia is usually high (Fivelstad et al. 1998; Danley 2000), with survival of approximately 100% at free CO_2 levels of up to 48 mg/L. During experiment 4, the presence of 0.8 mg/L environmental nitrite resulted in a dose-dependent decrease in survival with increasing CO_2 at concentrations that are usually otherwise tolerable (i.e. 0 vs. 30% mortality at 23.0 mg/L CO_2). Plasma nitrite and chloride values indicated no consistent response to the NO_2^- x CO_2 treatments, although it is not clear why this occurred. The CO_2 x NO_2 mortality trend was also observed in experiment 5, for the low Cl^- treatment group. High environmental chloride levels appeared to ameliorate the toxic effects of NO_2, regardless of the CO_2 concentration (survival was between 80 and 100%) thus indicating that the nitrite x CO_2 toxicity may be ameliorated by salt treatment. Overall, it appears there is a negative and additive effect of NO_2 and CO_2 on rainbow trout survival, though it is yet unclear how these two variables interact physiologically within the fish to increase mortality.

The conclusions discussed above appear to be in direct contrast with results from the only other results available for CO_2 x NO_2^- toxicity in aquatic organisms; Jensen et al. (2000) conducting a similar study in freshwater crayfish, observed a reduction in nitrite uptake during hypercapnia leading to the conclusion of reduced nitrite toxicity during hypercapnia. However, differences in experimental design and sampling method may explain the seemingly conflicting results. For example, one notable difference between the present study and Jensen et al. (2000)

was that Jensen and colleagues did not use a CO_2 acclimation period before exposing the crayfish to nitrite. According to Eddy et al. (1977), an acclimation period of approximately 24 h is needed to restore physiological conditions to a near steady-state level after acute exposure to a hypercapnic environment. Crayfish used by Jensen et al. (2000) may have been suffering from acute hypercapnic blood acidosis and other physiological changes in addition to the toxic effects of nitrite, a scenario less likely to occur in a relatively stable culture environment. Another important difference between the two studies was the times in which fish sampling occurred. Jensen et al. (2000) sampled crayfish at 0, 3, 24 and 48 h, whereas the present study only sampled at 96 h. The sampling times of Jensen et al. (2000) could have possibly been too short to allow the blood of crayfish to reach a near steady state condition. From their results, prominent differences in plasma nitrite were observed at 24 and 48 h between the nitrite-only treatment and the nitrite with hypercapnia treatment; however, the differences between the treatments seem to decrease as time increases. If their experiment would have continued past 48 h, the differences observed in plasma nitrite might have been minimal between the two treatments. In the present study, fish sampling occurred only at 96 h. This time period could have produced results in which some level of equilibrium inside the fish had already been reached.

Conclusions

Intensive aquaculture systems, particularly recirculating and those using oxygen injections, have the potential for increased accumulation of metabolic by-products, especially nitrite and CO_2. Results of the present study indicate nitrite toxicity is affected by elevations in environmental CO_2, and conversely, CO_2 tolerance is affected by environmental nitrite

concentrations. Additional experiments examining the interacting effects of nitrite and CO_2 on fish are needed to identify the physiological mechanisms or effects that lead to increased NO_2 x CO_2 toxicities in fish.

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Table 1. Blood and plasma parameters (mean ± SEM) of rainbow trout (*Oncorhynchus mykiss*) from experiment 1. Same lower case letters indicate no significant difference

Environmental (mg/L nitrite-N)	n	Hematocrit (%)	Plasma Glucose (mg/dL)	Plasma Lactate (mg/dL)
0.4	15	42.8 ± 1.7 a	80.4 ± 16.7 ab	28.2 ± 2.5 a
3.6	15	35.0 ± 1.6 ab	70.5 ± 8.2 b	38.0 ± 4.7 a
7.1	11	31.6 ± 1.4 b	66.0 ± 4.8 b	76.8 ± 21.5 a
13.2	1	34.0 ± 0 ab	117.0 ± 0 a	74.0 ± 0 a
20.9	2	31.4± 2.5 b	83.3 ± 6.3 b	72.0 ± 36.5 a

Table 2. Blood and plasma parameters (mean ± SEM) of rainbow trout (*Oncorhynchus mykiss*) from experiment 2. Same lower case letters indicate no significant difference

Environmental (mg/L nitrite-N)	n	Hematocrit (%)	Plasma Glucose (mg/dL)	Plasma Lactate (mg/dL)	Plasma Chloride (mEq/L)
0.5	15	42.5 ± 1.5 a	62.8 ± 1.8 a	26.0 ± 1.4 ab	126.0 ± 1.8 a
1.4	15	44.1 ± 1.5 a	58.7 ± 2.1 a	23.0 ± 1.3 ab	119.4 ± 1.9 a
1.6	15	38.4 ± 1.5 a	55.7 ± 3.9 a	17.8 ± 2.0 b	124.8 ± 1.6 a
2.1	13	38.5 ± 1.6 a	41.1 ± 3.0 ab	20.9 ± 1.9 b	116.4 ± 2.5 a
3.2	6	41.5 ± 4.6 a	69.2 ± 8.0 a	29.7 ± 4.9 ab	115.9 ± 10.0 a
4.7	2	40.1 ± 0.9 a	56.3 ± 0.1 a	40.4 ± 18.6 a	123.5 ± 10.0 a

Table 3. Blood and plasma parameters (mean ± SEM) of rainbow trout (*Oncorhynchus mykiss*) from experiment 3. Same lower case letters indicate no significant difference

Environmental (mg/L NaCl)	n	Hematocrit (%)	Plasma Glucose (mg/dL)	Plasma Chloride (mEq/L)
28.3	1	35.0 ± 0 a	71.9 ± 0 a	120.5 ± 0 a
35.7	5	34.7 ± 2.8 a	45.1 ± 5.8 b	114.4 ± 2.2 a
48.0	11	36.8 ± 1.1 a	50.9 ± 2.9 b	121.5 ± 2.3 a
67.5	14	37.7 ± 1.5 a	57.3 ± 3.4 ab	117.0 ± 1.8 a
100.0	13	41.5 ± 1.6 a	54.0 ± 3.2 b	122.6 ± 2.2 a
165.2	15	39.8 ± 1.4 a	49.9 ± 1.9 b	124.2 ± 1.5 a

Table 4. Blood and plasma parameters (mean ± SEM) of rainbow trout (Oncorhynchus mykiss) from experiment 4. Same lower case letters indicate no significant difference.

Environmental (mg/L nitrite-N)	Environmental (mg/L CO_2)	n	Hematocrit (%)	Plasma Nitrite (mg/L nitrite-N)	Plasma Glucose (mg/dL)	Plasma Lactate (mg/dL)	Plasma Chloride (mEq/L)
0.08 ± 0.02	9.1 ± 0.5	10	37.6 ± 1.9 a	0.24 ± 0.02 a	62.6 ± 1.7 a	21.0 ± 1.5 a	109.9 ± 1.8 a
0.8 ± 0.03	9.0 ± 0.3	9	34.1 ± 1.3 a	5.6 ± 1.7 a	61.5 ± 5.7 a	21.8 ± 1.1 a	107.0 ± 1.9 a
0.8 ± 0.03	13.8 ± 0.8	9	28.0 ± 2.0 a	22.4 ± 4.0 a	44.8 ± 3.7 a	16.5 ± 2.1 a	104.4 ± 1.3 a
0.8 ± 0.03	23.4 ± 1.0	7	30.1 ± 2.1 a	14.9 ± 3.4 a	45.3 ± 4.8 a	12.2 ± 1.0 a	93.2 ± 3.4 a
2.1 ± 0.03	9.0 ± 0.3	6	27.9 ± 1.2 a	24.7 ± 8.4 a	51.9 ± 10.0 a	18.1 ± 3.6 a	101.2 ± 4.1 a
2.1 ± 0.03	13.8 ± 0.8	3	37.1 ± 5.8 a	8.3 ± 5.3 a	77.1 ± 9.5 a	21.9 ± 5.5 a	97.8 ± 3.6 a
2.1 ± 0.03	23.4 ± 1.0	6	31.5 ± 1.1 a	14.9 ± 9.0 a	56.5 ± 3.5 a	24.1 ± 4.7 a	100.8 ± 1.5 a

Table 5. Blood and plasma parameters (mean ± SEM) of rainbow trout (*Oncorhynchus mykiss*) from experiment 5. Same lower case letters indicate no significant difference.

Environmental (mg/L nitrite-N)	Environmental (mg/L CO_2)	n	Hematocrit (%)	Plasma Glucose (mg/dL)	Plasma Lactate (mg/dL)	Plasma Chloride (mEq/L)
0.1 ± 0.04	8.3 ± 0.7	10	43.4 ± 1.7 a	116.9 ± 18.5 a	36.3 ± 8.8 a	102.5 ± 2.5 a
9.6 ± 0.06	7.7 ± 0.4	5	32.1 ± 1.4 a	59.5 ± 15.8 b	25.5 ± 8.8 a	100.0 ± 3.0 a
9.6 ± 0.06	14.8 ± 0.7	4	30.2 ± 3.5 a	67.7 ± 8.7 b	16.1 ± 4.9 a	102.9 ± 3.6 a
9.6 ± 0.06	29.7 ± 1.9	2	29.9 ± 0.4 a	50.7 ± 3.4 b	20.7 ± 8.1 a	95.8 ± 3.3 a
9.6 ± 0.06	7.7 ± 0.4	10	38.4 ± 2.2 a	62.8 ± 6.5 b	31.6 ± 9.5 a	115.5 ± 2.7 a
9.6 ± 0.06	14.8 ± 0.7	8	33.7 ± 3.6 a	49.0 ± 2.8 b	10.5 ± 1.4 a	109.5 ± 1.5 a
9.6 ± 0.06	29.7 ± 1.9	10	36.0 ± 1.6 a	50.6 ± 3.b	13.8 ± 1.5 a	88.3 ± 1.6 a

Figure 1. Percent mortality of rainbow trout exposed for 24 h to environmental nitrite-N

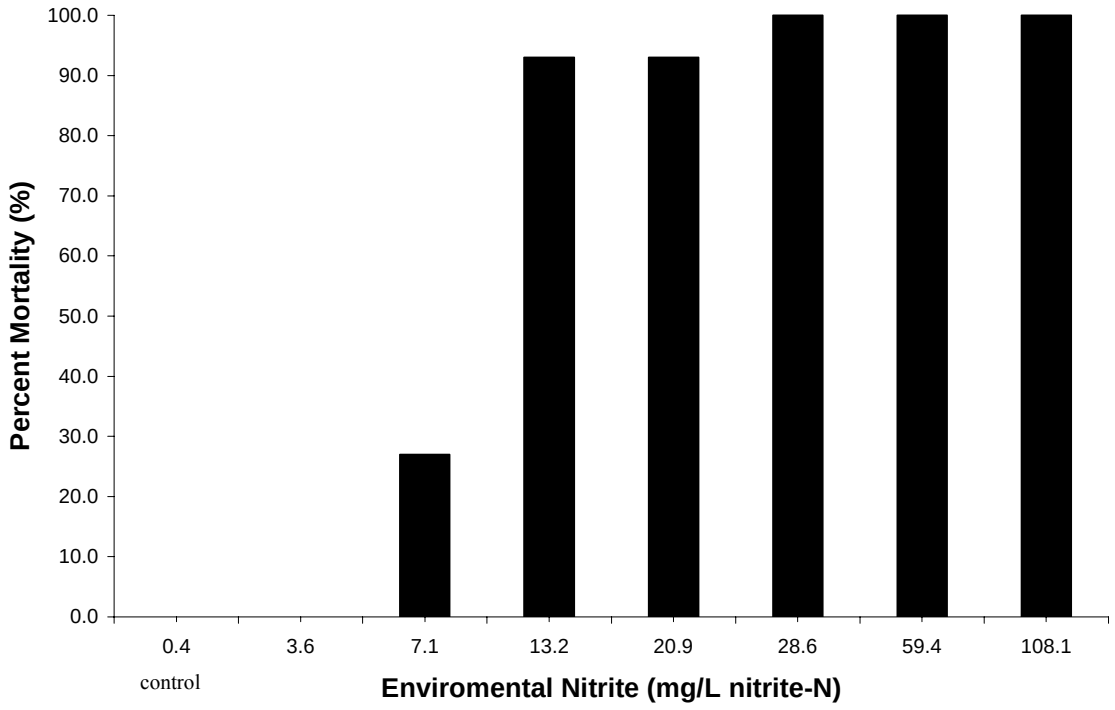


Figure 2. Plasma nitrite (mean ± SEM) in rainbow trout exposed for 24 h to increasing concentrations of environmental nitrite-N

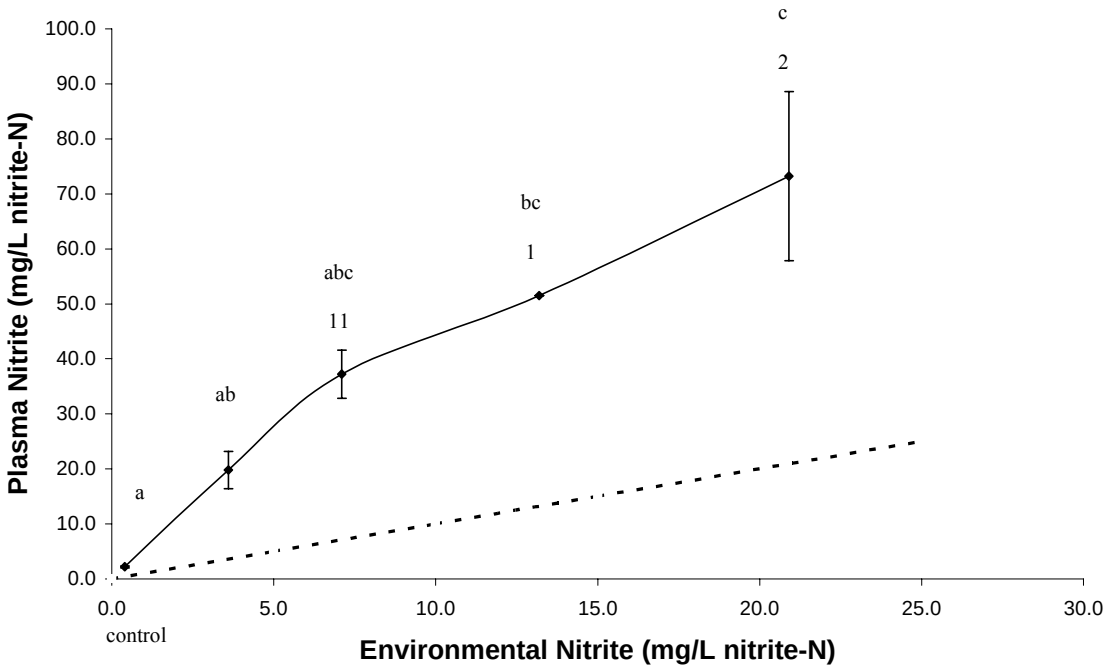


Figure 3. Plasma chloride (mean ± SEM) in rainbow trout exposed for 24 h to increasing concentrations of environmental nitrite-N

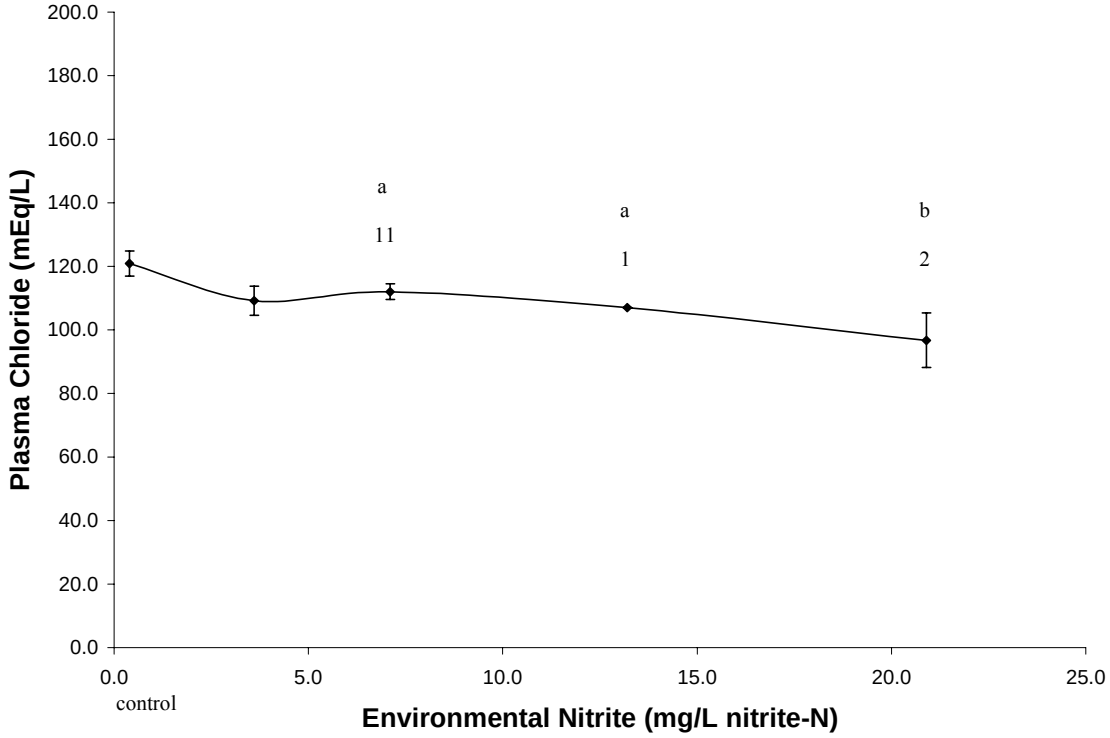


Figure 4. Percent mortality of rainbow trout exposed for 96 h to environmental nitrite-N

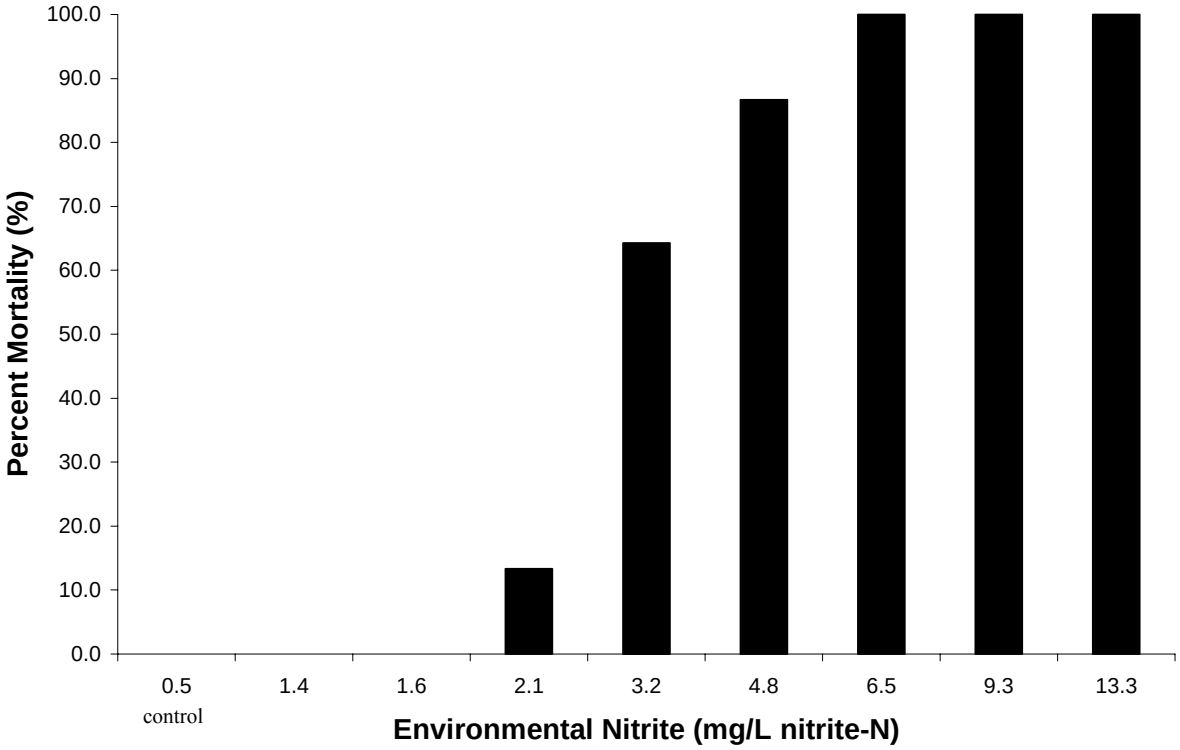


Figure 5. Plasma nitrite (mean ± SEM) in rainbow trout exposed for 96 h to increasing concentrations of environmental nitrite-N

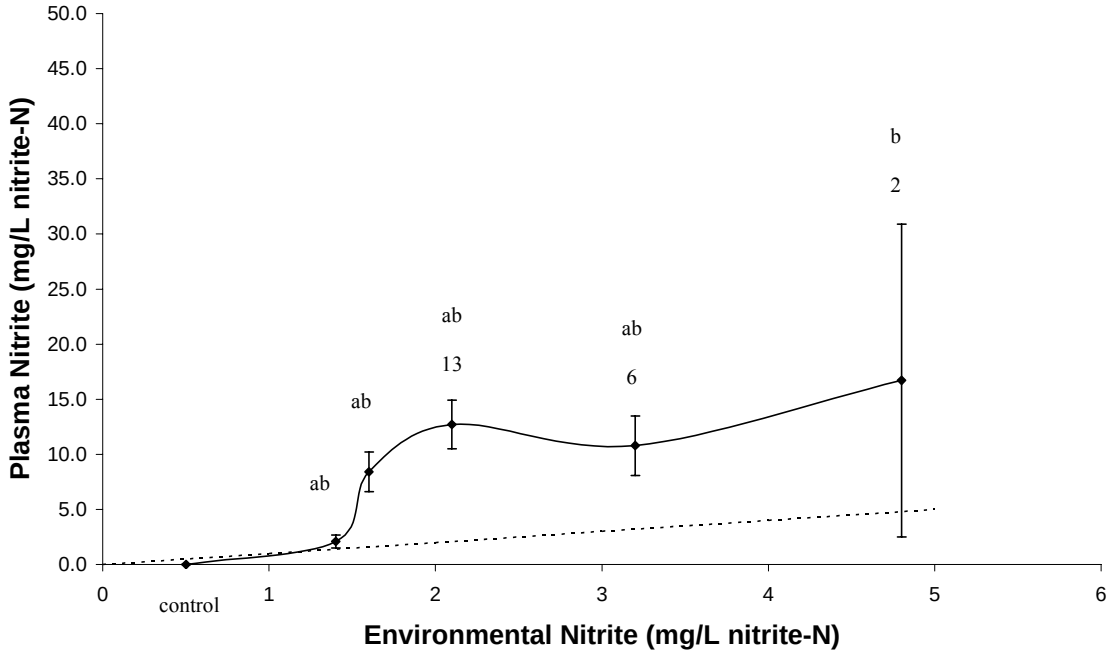


Figure 6. Percent survival of rainbow trout exposed for 96 h to a lethal level of nitrite-N to increasing concentrations of environmental chloride

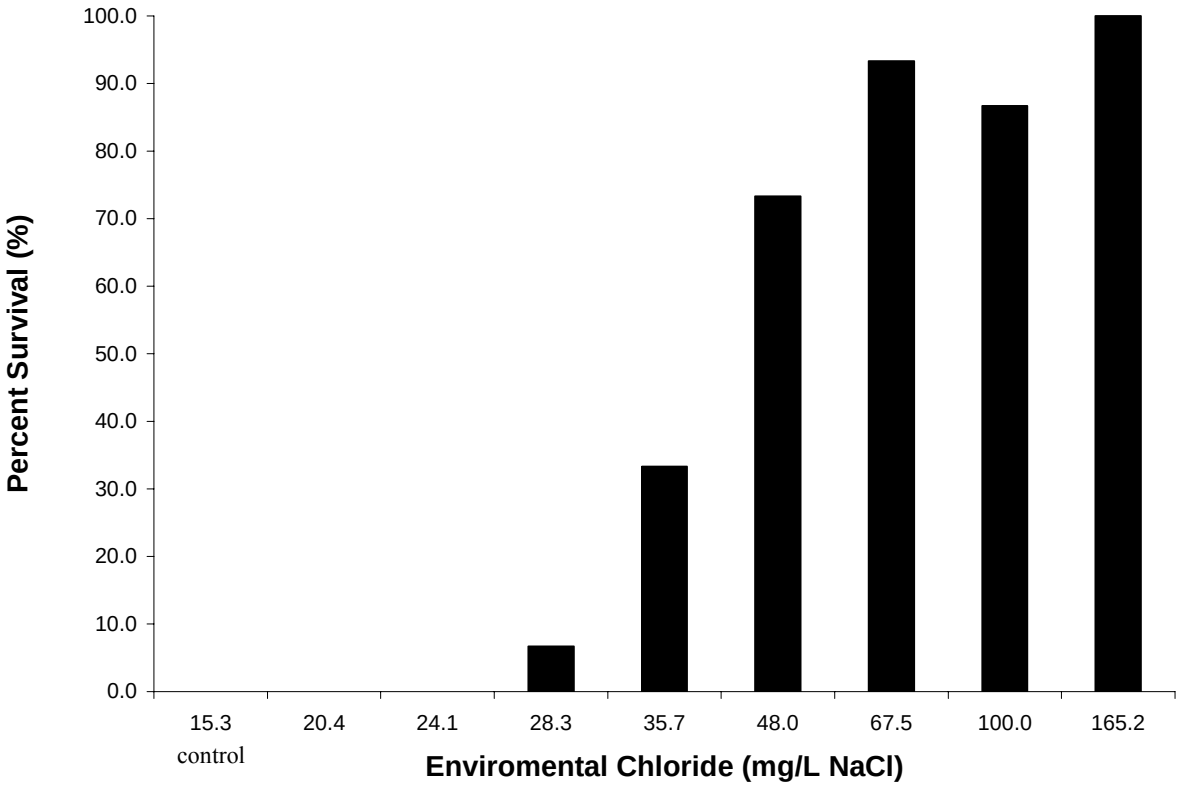


Figure 7. Plasma nitrite (mean ± SEM) in rainbow trout exposed for 96 h to a lethal level of nitrite-N in increasing concentrations of environmental chloride

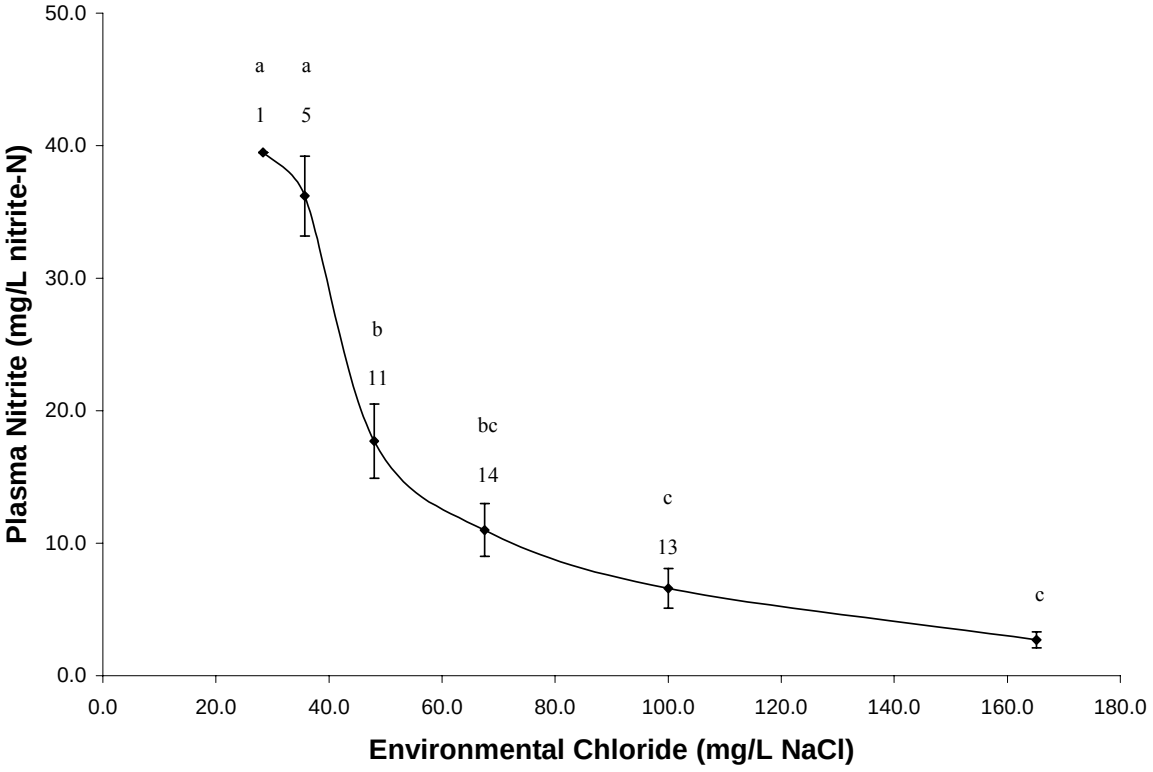


Figure 8. Plasma lactate (mean ± SEM) in rainbow trout exposed for 96 h to a lethal level of nitrite-N in increasing concentrations of environmental chloride

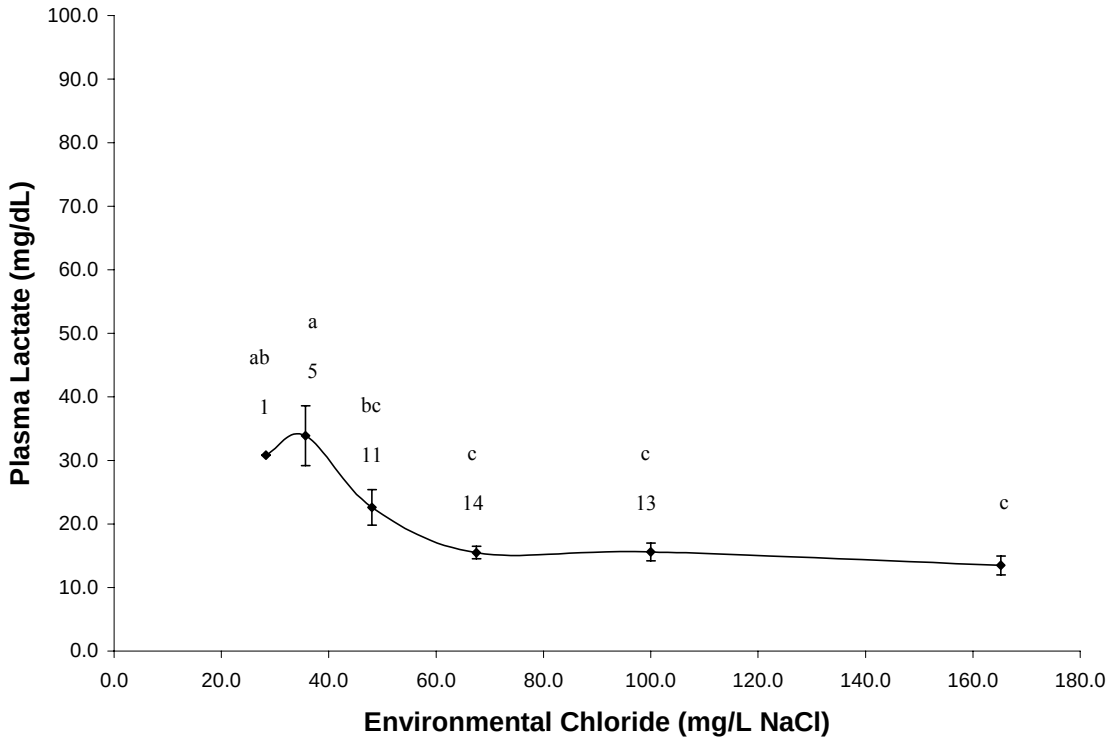


Figure 9. Percent mortality of rainbow trout exposed for 96 h to increasing concentrations of environmental nitrite-N and carbon dioxide

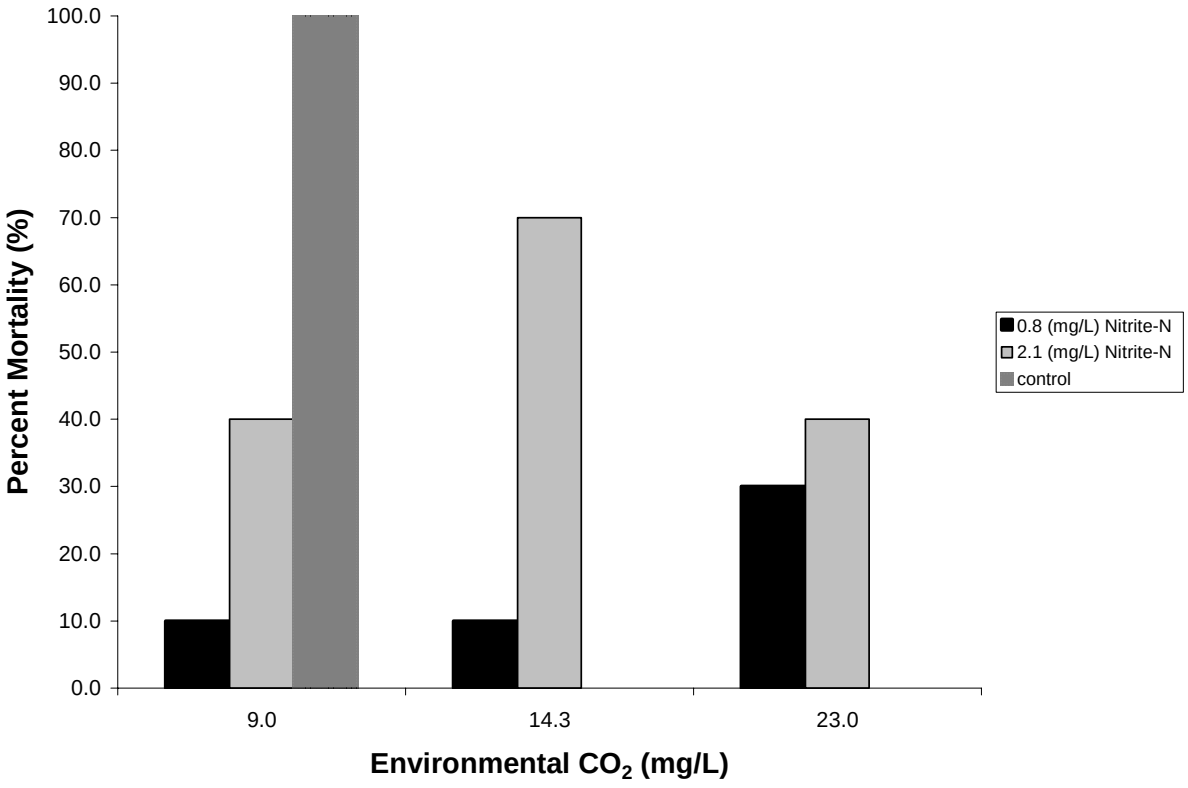


Figure 10. Percent survival of rainbow trout exposed for 96 h to a lethal level of nitrite-N to increasing concentrations of environmental chloride and carbon dioxide

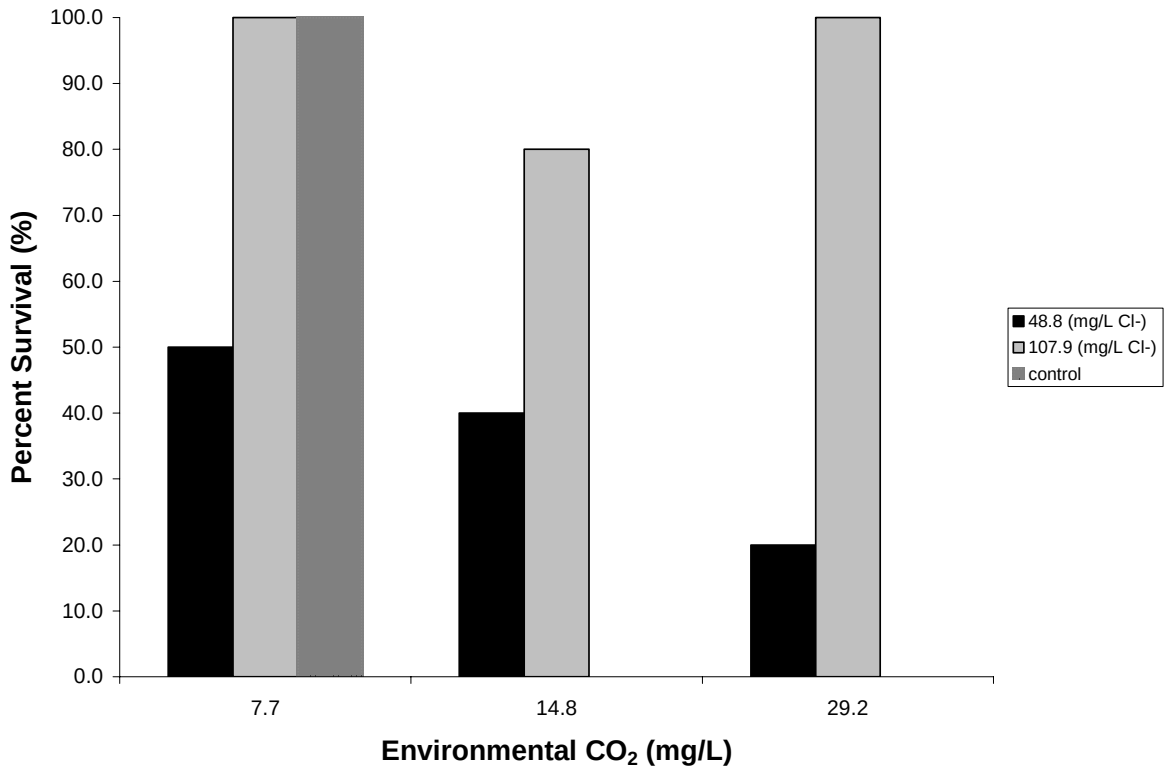


Figure 11. Plasma nitrite (mean ± SEM) in rainbow trout exposed for 96 h to a lethal of nitrite-N in increasing concentrations of environmental chloride and carbon dioxide

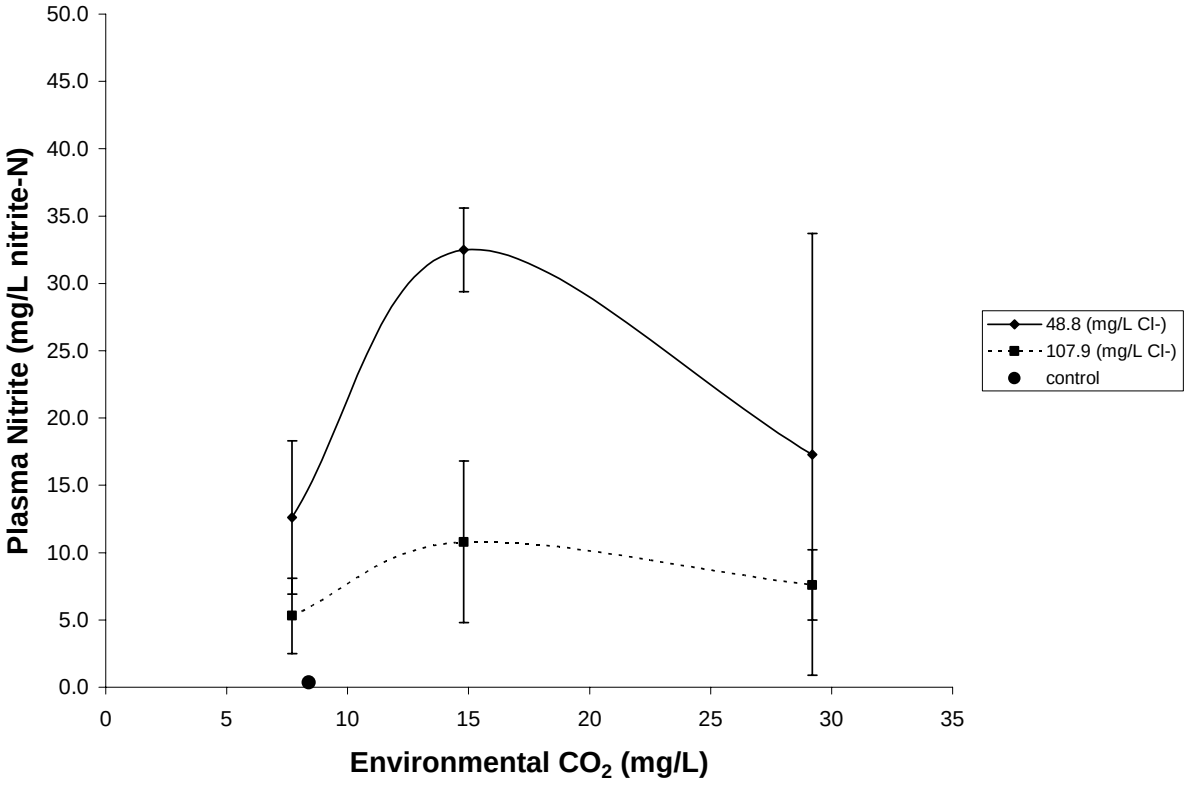
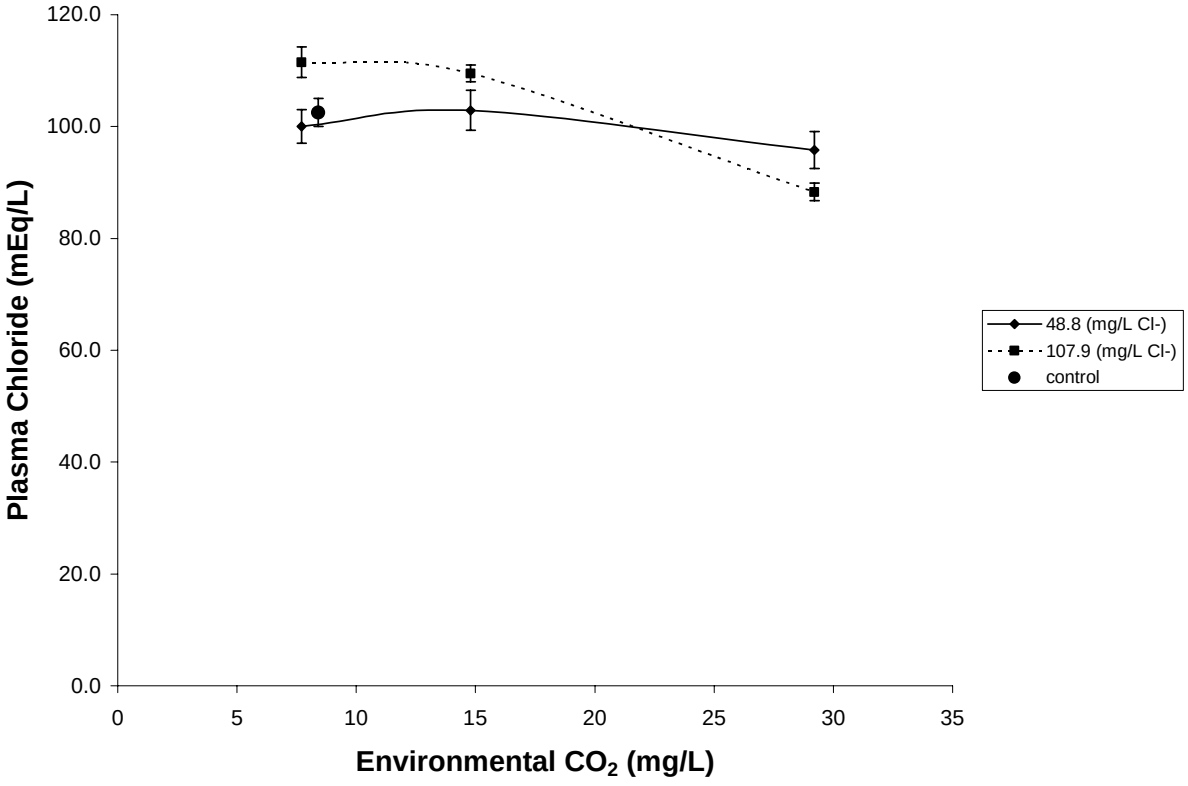


Figure 12. Plasma chloride (mean ± SEM) in rainbow trout exposed for 96 h to a lethal of nitrite-N in increasing concentrations of environmental chloride and carbon dioxide



Appendix A. Mean ± SEM of water quality values from experiments one through five.

Experiment	Temperature (°C)	Dissolved oxygen (mg/L)	pH	TAN (mg/L)	Alkalinity (mg/L)	Ca++ Hardness (mg/L)	Carbon dioxide (mg/L)
1	13.7 ± 0.4	8.75 ± 1.0	8.05 ± 0.13	1.8 ± 0.5	234.0 ± 1.4	238.0 ± 1.4	13.0 ± 1.4
2	13.7 ± 0.3	9.43 ± 0.7	8.12 ± 0.13	1.2 ± 0.4	250.0 ± 2.8	234.0 ± 2.1	11.5 ± 2.1
3	14.6 ± 0.1	8.99 ± 0.1	8.09 ± 0.01	0.8 ± 0.1	241.0 ± 1.0	244.0 ± 1.5	10.5 ± 0.5
4	14.0 ± 0.01	12.13 ± 0.2	7.82 ± 0.01 7.65 ± 0.01 7.42 ± 0.03	0.02 ± 0.002	201.0 ± 2.3	230.0 ± 5.2	9.0 ± 0.3 14.3 ± 0.8 22.9 ± 1.3
5	14.06 ± 0.01	12.54 ± 0.2	7.81 ± 0.02 7.60 ± 0.02 7.31 ± 0.04	0.04 ± 0.004	256.0 ± 26.1	240.0 ± 7.2	7.7 ± 0.4 14.8 ± 0.7 29.7 ± 1.9

Appendix B. Formulas for calculating the amounts of sodium nitrite and sodium chloride to add to reach target concentrations in the water.

Molecular weights

Sodium (Na^+) = 22.99
Nitrogen (N) = 14.01
Oxygen (O) = 16.00
Chloride (Cl^-) = 35.45
Nitrite (NO_2^-) = 46.01
Sodium Nitrite ($NaNO_2$) = 69.00
Sodium Chloride (NaCl) = 58.44

Percentages

NO_2^- = 66.7 % of $NaNO_2$
(46.01/69.00 = 0.667 x 100)

Cl- = 60.7 % of NaCl
(35.45/58.44 = 0.607 x 100)

Calculations

Step 1. target concentration x 45 liters (L) of water **{A}**
Step 2. divide **{A}** by 1000 to change milligrams (mg) to grams (g) **{B}**
Step 3. divide **{B}** by either 0.667 (for nitrite) or 0.607 (for chloride) to determine the amount of $NaNO_2$ or NaCl to add to the water **{C}**

Chapter 2: Physiological Responses of Arctic Char (*Salvelinus alpinus*) During a 5.5 Hour Transport with Ice-Slurry, AQUI-S^{TM}, and Carbon Dioxide

Abstract

Physiological Responses of Arctic Char (*Salvelinus alpinus*) During a 5.5 Hour Transport with Ice-Slurry, AQUI-S™, and Carbon Dioxide

Christopher D. Nelson

Transporting fish is a fish culture process that produces physiological alterations in fish. Transport-related stimuli, such as netting, loading, unloading, and en route transport, are usually unavoidable and cause detectable physiological responses in food fish that can be deleterious to product quality and value. Minimizing such disturbances, therefore, may be beneficial in maintaining a high quality product. The objectives of this study were to determine the physiological responses of Arctic char to transport, and to determine if anesthesia (CO_2 or AQUI-S™) or ice exposure during transport could help minimize the anticipated physiological responses. Four transport treatments (water-only, CO_2, AQUI-S™, and ice-slurry) were tested in triplicate. Insulated seafood totes, equipped with aerators and supplemental oxygen diffusers, served as the live-haul chambers. Each treatment tote was stocked with 50 Arctic char (mean 1.2 kg per fish) at the start of the study (0 h), and fish were exposed to transport treatments for 5.5 h. Physiological responses (hematocrit, plasma cortisol, glucose, lactate and chloride) to the treatments were determined from blood samples taken from fish. Sampled fish were removed from the transport totes at 1.0, 2.5, 4.0, and 5.5 h during transport. Results indicate that the transport of char under the anesthetics tested did not alleviate transport-related physiological disturbances. Physiological responses were detected among all treatments during the 5.5 h study. Results also show that AQUI-S™ had no clear advantage over the water-only treatment, CO_2 resulted in a more severe stress response (higher hematocrit and cortisol) compared to the water-only treatment, and the ice-slurry treatment also resulted in a more severe stress response (higher cortisol, glucose and lactate) when compared to the water-only treatment. Overall, use of anesthetics during transport of food fish provided no clear advantage, and in the case of ice-slurry and CO_2, may have resulted in additional loss of product quality because of a more severe stress response.

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Introduction

Ensuring consistent and optimal product quality is a constant challenge to fish producers and processors. Under ideal conditions, fresh fish products such as rainbow trout fillets have a relatively short shelf life (Rasmussen 2001). Short shelf life relative to other meat products results partly from increased amounts of unsaturated fatty acids, which are more susceptible to oxidation (Haard 1992). Oxidation of fatty acids can lead to off-flavor and faster development of rancid fillets (Haard 1992). In addition, the weak collagen structure of fish muscle makes it very sensitive to muscle pH fluctuations, especially during harvest (Robb 2001; Rasmussen 2001). Struggle of fish during harvest and transport has been shown to result in rapid decreases in muscle pH post-mortem, affecting fillet quality through loss of fillet firmness and increased fillet gaping (Berg et al. 1997; Robb 2001).

Physical disturbances during harvest and transport of fish are routine in aquaculture and are unavoidable. Studies have shown that transport-related disturbances, such as netting, capture, loading and unloading, and en route transport, cause detectable physiological stress responses in fish (Robertson et al. 1988; Erikson et al. 1997; Barton 2000a). Poor water quality and high fish densities during transport also can serve as additional stressors (Robertson et al. 1988; Erikson et al. 1997). Although such disturbances are usually unavoidable, there may be ways to minimize the disturbances so that physiological responses in the fish are minimized and subsequent product quality may be maximized.

Anesthetics have been used widely during the transport of fish and may help alleviate physiological stress (Morales et al. 1990; Harrell 1992; Sandodden et al. 2001). Wedemeyer (1992) reported that using anesthetics during transport of fish could be helpful in reducing physical activity and overall metabolic rates, thereby leading to reduced physiological stress.

Anesthetics that have been used in transporting fish include tricaine methanesulfanate (MS-222), benzocaine, etomidate, metomidate, clove oil and carbon dioxide (Amend et al. 1982; Gilderhus and Marking 1987; Mattson and Riple 1989; Gilderhus et al. 1991; Sandodden et al. 2001). Currently, carbon dioxide (CO_2) is the only anesthetic approved for use on U.S. food fish without a withdrawal period. However, use of CO_2 on food fish during transport or harvest has several disadvantages. Fish exposed to elevated CO_2 show increased aversive activity and reduced blood pH, both of which may be deleterious to product quality (Robb 2001). AQUI-S^{TM} (AQUI-S Ltd., New Zealand), a proprietary form of clove oil, is an alternative anesthetic that has not yet been approved but is currently under review by the US Food and Drug Administration. Physiological and product quality studies on fish exposed to AQUI-S^{TM} are limited, so its potential is yet unknown. Other additives, such as ice-slurries (ice + water) also have been used to help minimize stress during transport by inducing hypothermia (Wedemeyer 1992), and it is currently the most widely used anesthetic for food fish in West Virginia (B. Kenney personal comm.). Despite the possible shortcomings of anesthetics, they may still prove useful if they can decrease the stress responses of fish in comparison to harvest or transport without anesthesia.

In 2000, research conducted by West Virginia University as part of the West Virginia Aquaculture Research and Development Project, revealed significant variation in fillet yield and quality on farms sampled throughout West Virginia and neighboring Appalachian states. It was noted that harvest practices among farms was highly variable, ranging from on-site slaughter via asphyxiation to live-haul to the processing plant (B. Kenney personal comm.). The objective of the present study was to evaluate the effectiveness of transport under anesthesia (AQUI-S^{TM}, ice-slurry, or CO_2) in reducing the physiological stress responses in Arctic char (*Salvelinus alpinus*) in comparison to transport without anesthesia (aerated water only). The present study was part

of a broader research project that investigated physiological responses during transport of live char and the effects of transport conditions on fillet quality. We hypothesized that fish transported under anesthesia would have reduced stress responses (hematocrit, plasma cortisol, chloride, glucose and lactate) compared to fish transported in water.

Materials and Methods

Fish Care and Maintenance

Arctic char were raised at the Conservation Fund's Freshwater Institute, Shepherdstown, WV. Fish were housed in 1200-L fiberglass tanks supplied with oxygen-injected spring water (temperature 13.3 ± 0.2°C; dissolved oxygen 13.1 ± 0.2 mg/L; pH 7.6 ± 0.1; total alkalinity 247.4 ± 4.3 mg/L; calcium hardness 236.4 ± 2.1 mg/L and 11.7 ± 0.7 mg/L free CO_2). Photoperiod was maintained on a 14 h L:10 h D cycle. Fish were fed daily with commercial trout feed (Melick Aquafeed, Catawissa, PA). Throughout the study the mean fish weight was 1228.2 ± 10.4 g. All fish were fasted for two days prior to experimention.

Experimental Design

Four transport treatments (water, AQUI-S^{TM}, CO_2 anesthesia, and ice-slurry) were tested in triplicate. During each simulated transport, the four transport treatments were randomly assigned among four cubical live-haul chambers. Each treatment was included during each of the three transport trips, for a total of three replicates per treatment. Four insulated seafood totes (1000-L capacity), equipped with aerators and supplemental oxygen diffusers, served as the live-haul chambers during the study. Totes were secured onto a trailer and filled with spring water (approximately 800 L). The anesthetics (AQUI-S^{TM}, CO_2, and ice) were added immediately

before the fish were stocked. For AQUI-S^{TM}, 1 ml of concentrated AQUI-S^{TM} was added below the surface of the treatment tote and thoroughly mixed. The CO_2 treatment was prepared and maintained using a micro pore diffuser (MBD 75 s A grade, Point Four Systems Inc., Port Moody, British Columbia, Canada) and a gas flow meter (Key Instruments, Trevose, PA). The CO_2 system was connected by polyethylene tubing to a remote liquid CO_2 cylinder (Messler MG Industries, Malvern, PA). The CO_2 treatment was checked via the CO_2 nomogram method (APHA 1998) and had a mean of 55.0 mg/L free CO_2. The ice-slurry treatment contained approximately 3 parts ice to 1 part water.

Five pre-transport control fish were sampled directly from the holding tanks as described below, before the start of each replicate experiment. Then, at the beginning of each transport (0 h), Arctic char were randomly netted from the holding tanks and 50 fish were placed into each replicate tote. Each simulated transport lasted 5.5 h, during which 5 fish were sampled from each tote at 1, 2.5, 4, and 5.5 h for blood analyses.

Sampled fish were individually dip-netted, given a quick blow to the head, and blood was collected into lithium-heparinized syringes from vessels in the caudal peduncle (Houston 1990). Sampling was completed within 5 minutes of initial disturbance and each fish was sampled only once. Blood was transferred into heparinized microhematocrit tubes (in duplicate) and 3-ml lithium-heparinized tubes. Microhematocrit samples were centrifuged for 5 min at 11,500 rpm, and the percent hematocrit was determined using a microhematocrit reader (IEC, Needham Heights, MA). Blood collected into the 3-ml tubes was centrifuged for 10 min at 3500 rpm, and the resulting plasma transferred into 1.5-ml microcentrifuge tubes. Plasma samples were stored at -20°C until analyzed for cortisol, glucose, lactate and chloride. All plasma parameters were analyzed in duplicate.

Plasma cortisol was analyzed by radioimmunoassay (RIA) using a commercially available kit (Coat-a-Count, Diagnostic Product Corp., Los Angeles, CA) and an automatic gamma counter (1470 Wizard Automatic Gamma Counter, Wallac Oy., Finland). The performance characteristics of the cortisol RIA were as followes: a 91.7% standard recovery; within-assay variability, defined as the coefficient of variation (CV), 4.3%; between-assay variability, defined as the CV, 4.1%; the minimum level of sensitivity was 2.0 ng and samples below the minimum level of sensitivity were assigned a value of zero (Barton et al. 1980). Plasma glucose and lactate were analyzed by an automatic glucose/lactate analyzer (YSI 2300 Select, Yellow Springs, OH), and plasma chloride was determined by a digital chloridometer (Labconco Co., Lenexa, KS).

During each replicated transport, temperature, dissolved oxygen, pH, and total ammonia-nitrogen (TAN) were measured. Temperature and dissolved oxygen were measured with a hand-held meter (YSI Model 58 or 95, Yellow Springs, OH). The water pH also was measured with a hand-held meter (YSI Model 60/10 FT, Yellow Springs, OH). Total ammonia-nitrogen was determined using a commercially available test kit (Hach, Co., Loveland, CO). Water quality parameters from each treatment are included in Appendix A.

Statistical Analyses

A repeated measure plus control (initial values) design was used in the present study. Data were analyzed with treatments (water, AQUI-S, ice-slurry and CO_2) and sampling times (1, 2.5, 4, and 5.5 h) as factors. When significant ($p< 0.05$), means were further separated using Fisher's Least Squared Difference. Results are presented as treatment means ± standard error of the mean (SEM). All data were analyzed using Microsoft Excel (Excel XP, Microsoft Co., Redmond, CA) and SAS (SAS 8.2, SAS Inst., Cary, NC).

Results

Physiological stress responses (hematocrit, plasma cortisol, glucose, lactate and chloride) showed significant time and treatment effects among transport treatments. Percent hematocrit increased in all treatments within the first hour of transport (Fig. 1). Water, AQUI-S^{TM}, and ice-slurry treatments recovered to near resting levels by 5.5 h; however, no recovery was observed for the CO_2 transport treatment. Mean hematocrit of fish from the CO_2 treatment was significantly different from all other transport treatments through the end of the study (5.5 h).

Plasma cortisol increased in all treatments within the first hour of transport (Fig. 2). The water and CO_2 treatments were significantly higher than the control (pre-transport group) at 0 h. By 2.5 h, all transport treatments were significantly higher than the control. All treatments (with the exception of AQUI-S at 5.5 h) remained significantly higher than the control throughout the 5.5 h transport (Fig. 2). Overall plasma cortisol concentrations were significantly different between AQUI-S^{TM} and ice-slurry, between AQUI-S^{TM} and CO_2 and between water and CO_2 treatments ($p = 0.04$, 0.01, and 0.03, respectively).

Plasma glucose showed treatment-dependent responses. The water treatment showed a trend of increasing plasma glucose with time, with the mean glucose concentration at 5.5 h significantly higher than 0, 1.0, or 2.5 h (Table 1). Glucose was significantly increased in the ice-slurry treatment at 1 h compared to 0 h, and remained elevated through the end of the study (5.5 h). No clear glucose trends were detected in the AQUI-S^{TM} or CO_2 treatments (Table 1). Overall plasma glucose concentrations were significantly different between AQUI-S^{TM} and ice-slurry, between CO_2 and ice-slurry, and between CO_2 and water treatments ($p = 0.03$, 0.01, and 0.04, respectively).

Plasma lactate levels increased in all treatments by the first hour of transport (Fig. 3). Water, AQUI-S^{TM}, and CO_2 treatments all recovered to near-resting levels by 5.5 h of transport; however, no recovery was observed for the ice-slurry treatment group which remained elevated through the end of the 5.5 h transport. Significant plasma chloride differences were detected among treatments, with mean plasma chloride of ice-treated fish being significantly higher than all other treatment groups (Table 2).

Discussion

Use of anesthetics during transport of non-food fish has been well documented (Strange and Schreck 1978; Robertson et al. 1988; Urbinati and Carreiro 2001; Sandodden et al. 2001), and adoption and adaptation of the use of anesthetics to the food fish industry may help minimize physiological stress and therefore, increase product quality. In the present study, we hypothesized that arctic char transported under various types of anesthesia would have reduced physiological stress responses (hematocrit, plasma cortisol, glucose, lactate, and chloride) compared to fish transported in water alone; however, the results did not support our hypothesis. Instead, the results suggest that different anesthetics used during the 5.5 h simulated transport were not effective in consistently reducing the stress responses of transported fish compared to fish transported in water alone.

Hematocrit is a useful indicator of stress in fish (Barton and Iwama 1991). Increases in percent hematocrit are common during stress responses, and functions to increase the oxygen carrying capacity of the blood in preparation for a fight or flight response (Soivio et al. 1980). Hematocrit usually returns to pre-stress levels within a few hours after the demand for oxygen returns to resting levels (Wendelaar Bonga 1997). In the present study, percent hematocrit was

30.6% before transport and increased among treatments to between 37.0 and 49.5% during transport, indicating that use of anesthesia during transport did not prevent or minimize the stress response. All treatments, with the exception of the CO_2 treatment, returned to near resting levels by 5.5 h indicating fish in these treatments were acclimating to the transport conditions.

Mean hematocrit of fish from the CO_2 treatment remained significantly higher than all other treatments at 4.0 and 5.5 h. Higher hematocrit in the CO_2 treatment may reflect a transient Bohr effect on the red blood cells of CO_2 anesthetized fish (Eddy 1977). A CO_2-induced Bohr effect has been shown to reduce the affinity of the red blood cells for oxygen (Eddy 1977), thereby necessitating the need for more red blood cells in circulation to meet the organism's oxygen demands. Thus, because CO_2 anesthetized fish showed a prolonged and elevated stress (hematocrit) response, use of CO_2 anesthesia may not be ideal for transport of fish compared to other potential transport treatments. While hematocrit is a useful physiological parameter to measure as an indicator of stress, other parameters such as plasma cortisol, glucose, lactate, and chloride need to be considered since they are indicative of different physiological systems of the fish and may relate to changes in end-product quality through other mechanisms.

Plasma cortisol is a primary stress response of fish, meaning its hormonal action directs other (secondary) physiological changes during a stress response (Strange and Schreck 1978; Barton et al.1980; Wendelaar Bonga 1997). Increases in plasma cortisol during transport of fish are routinely observed (Carmichael et al. 1984; Robertson et al. 1988; Urbinati and Carreiro 2001). In all transport treatments of the present study, plasma cortisol of fish in totes at 0 h was higher ($p < 0.05$) compared to pre-transport control fish, indicating that netting and moving fish into the transport totes had already initiated a stress response before the fish were even transported (Strange and Schreck 1978; Barton et al.1980).

Mean cortisol from all transport treatments continued to increase through 2.5 h transport, with the exception of the water-only treatment. The AQUI-S^{TM} and water treatments showed a decreasing or stabilizing trend towards resting or pre-transport levels by 5.5 h transport; fish in these treatments may have been recovering during transport to their resting pre-transport levels. Similar to hematocrit responses discussed above, transport with CO_2 anesthesia resulted in the highest cortisol responses of all treatments through 2.5 h transport, indicating use of CO_2 is not ideal for reducing the stress responses of fish during transport. Instead, use of CO_2 during transport may actually prolong or exacerbate the stress response in fish.

Although CO_2 resulted in significantly elevated cortisol responses through 2.5 h, the ice-slurry treatment exhibited the highest level of all treatments at 5.5 h transport. This response may be the result of a slower cortisol response resulting from the hypothermic transport conditions. Similar results were reported by Barton and Peter (1982) who detected significantly increased plasma cortisol responses in fingerling rainbow trout at 4 h immersion in chilled (1^{o}C) water. Thus, ice-slurry may not be ideal for transport of char for longer than 2-3 h, but may yet be useful for shorter transport times.

Blood metabolites, such as glucose and lactate, are characterized as secondary stress responses in fish and levels generally increased during and immediately following exposure to handling and transport (Morgan and Iwama 1997; Wendelaar Bonga 1997). Following a stressor, increases in plasma glucose are often observed in order to meet the elevated energy demand of the body (Morgan and Iwama 1997). Increased plasma lactate levels, however, reflect anaerobic metabolism due to increased activity or decreases in oxygen availability (Thomas et al. 1999) and can indicate a failure of homeostasis (Wardle 1978). In the present study, char plasma glucose showed a distinct treatment-dependent response with no single

treatment indicating an overall advantage when compared to the water-only treatment. In particular, glucose concentrations in the ice-slurry treatment were higher than all of the other treatments (with the exception of the water treatment at 5.5 h), indicating this treatment may be more deleterious to end-product quality than transport with water-only.

Plasma lactate responses also showed a significant increase among all treatments from < 4 mg/dL at 0 h to > 65 mg/dL at 1.0 h. All treatments responded with a gradual recovery to 0 h levels except for the ice-slurry treatment, which remained elevated throughout the 5.5 h transport, again indicating anesthesia did not provide any advantage over the water-only treatment in minimizing the stress response associated with transport of char.

Disturbance to osmoregulatory steady states are also characteristic of secondary stress responses in fish (Wedemeyer 1972; Mazeaud et al. 1977; Carmichael et al. 1984), and include disturbance such as handling and transport (Carmichael et al. 1984; Mazik et al. 1991). In the present study, a significant difference in plasma chloride was indicated, but all plasma values for all treatments were within normal ranges reported for fish (Barton 2000b). Thus, plasma chloride responses did not indicate any anesthetic treatment advantages during transport compared to the water-only treatment.

Conclusion

The anesthetic treatments investigated in the present study (AQUI-S^{TM}, ice-slurry, and CO_2) were not effective in consistently reducing the stress responses of Arctic char during the 5.5 h simulated transport. All treatment groups showed stress responses for each of the parameters measured. Use of AQUI-S during a 5.5 h transport indicated no clear advantage over water-only treatment and thus its use cannot be justified. Ice-slurry treatment actually indicated

more severe stress responses compared to the water-only treatment, as indicated by significantly higher cortisol, glucose, and lactate responses. Because stress responses immediately before and during harvest have been shown to affect product quality (Robb 2001), one can assume anything that increases the stress response before harvest will likely have a similar increased negative effect on the end product quality and therefore should be avoided if possible. Similar to results for the ice-slurry treatment, use of CO_2 resulted in more severe stress responses (hematocrit and cortisol) compared to the water-only control and likely indicates a deleterious rather then beneficial affect on end product quality. Overall, use of anesthetics during transport of food fish provided no clear advantage, and in the case of ice-slurry and CO_2, may have resulted in additional loss of product quality because of a more severe stress response.

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Table 1. Mean ± SEM values for plasma glucose from arctic char (*Salvelinus alpinus*) for each transport treatment and sampling time. Same lower case letter indicates no significant difference between control and other treatments. Values recorded in mg/dL.

Time (hr)	Water	AQUI-S	Ice-slurry	Carbon dioxide
0	79.5 ± 0.6 a	79.5 ± 0.6 a	79.5 ± 0.6 a	79.5 ± 0.6 ab
1.0	78.1 ± 3.6 a	85.3 ± 5.1 ab	97.5 ± 3.1 b	77.2 ± 1.0 a
2.5	84.5 ± 6.0 a	87.3 ± 5.2 ab	95.3 ± 4.0 b	77.8 ± 6.7 a
4.0	91.9 ± 7.3 ab	92.1 ± 3.0 b	98.9 ± 5.5 b	81.4 ± 2.5 ab
5.5	102.5 ± 4.5 b	88.2 ± 3.7 ab	97.4 ± 6.2 b	88.3 ± 0.6 b

Table 2. Mean ± SEM values for plasma chloride from arctic char (*Salvelinus alpinus*) for each transport treatment and sampling time. Same lower case letter indicates no significant difference between control and other treatments. Values recorded in mEq/L.

Time (hr)	Water	AQUI-S	Ice-slurry	Carbon dioxide
0	115.2 ± 2.8 a	115.2 ± 2.8 a	115.2 ± 2.8 a	115.2 ± 2.8 a
1.0	116.2 ± 4.3 a	113.6 ± 2.0 a	123.7 ± 3.6 ab	119.8 ± 4.0 a
2.5	118.2 ± 3.7 a	114.4 ± 3.4 a	126.1 ± 1.4 b	119.0 ± 3.2 a
4.0	117.6 ± 2.9 a	120.0 ± 2.5 a	121.1 ± 0.5 ab	116.0 ± 4.3 a
5.5	115.4 ± 2.9 a	117.6 ± 0.5 a	117.0 ± 4.1 a	113.7 ± 3.7 a

Figure 1. Percent hematocrit (mean ± SEM) in arctic char exposed for 5.5 h to four transport treatments

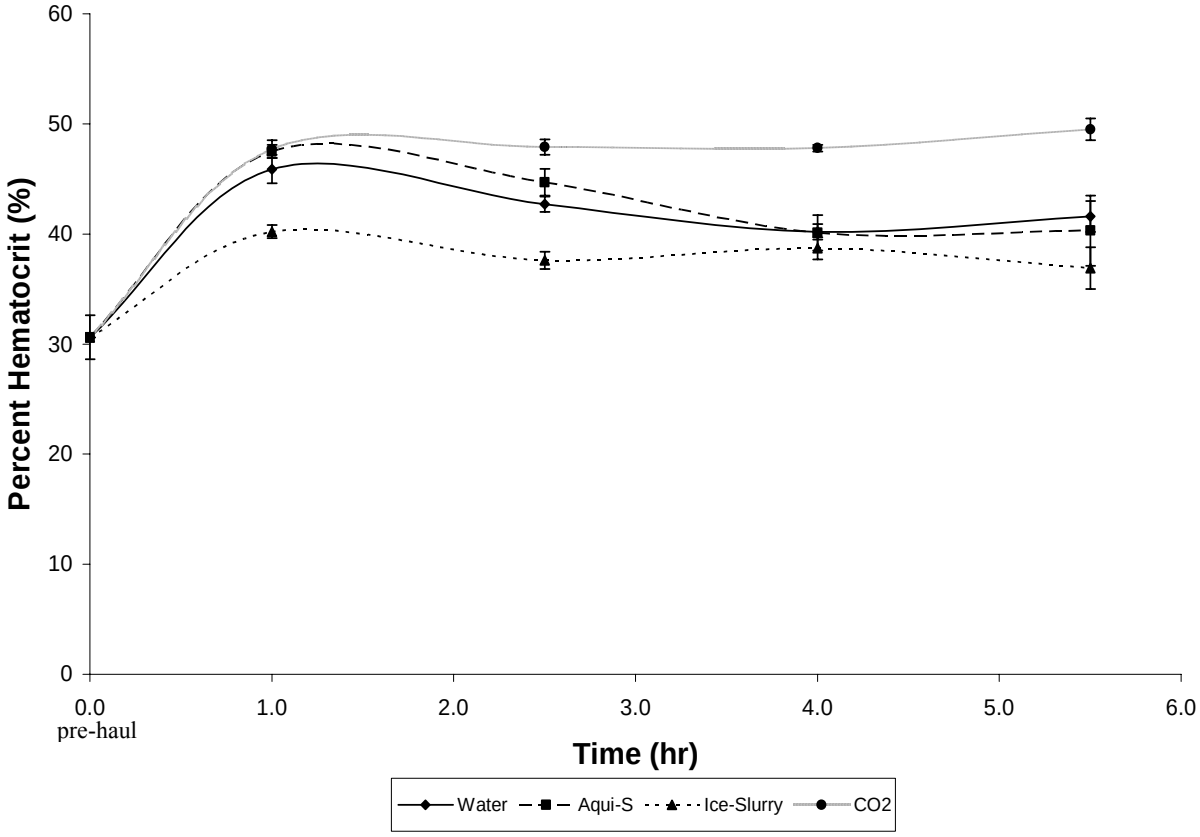


Figure 2. Plasma cortisol (mean ± SEM) in arctic char exposed for 5.5 h to four transport treatments

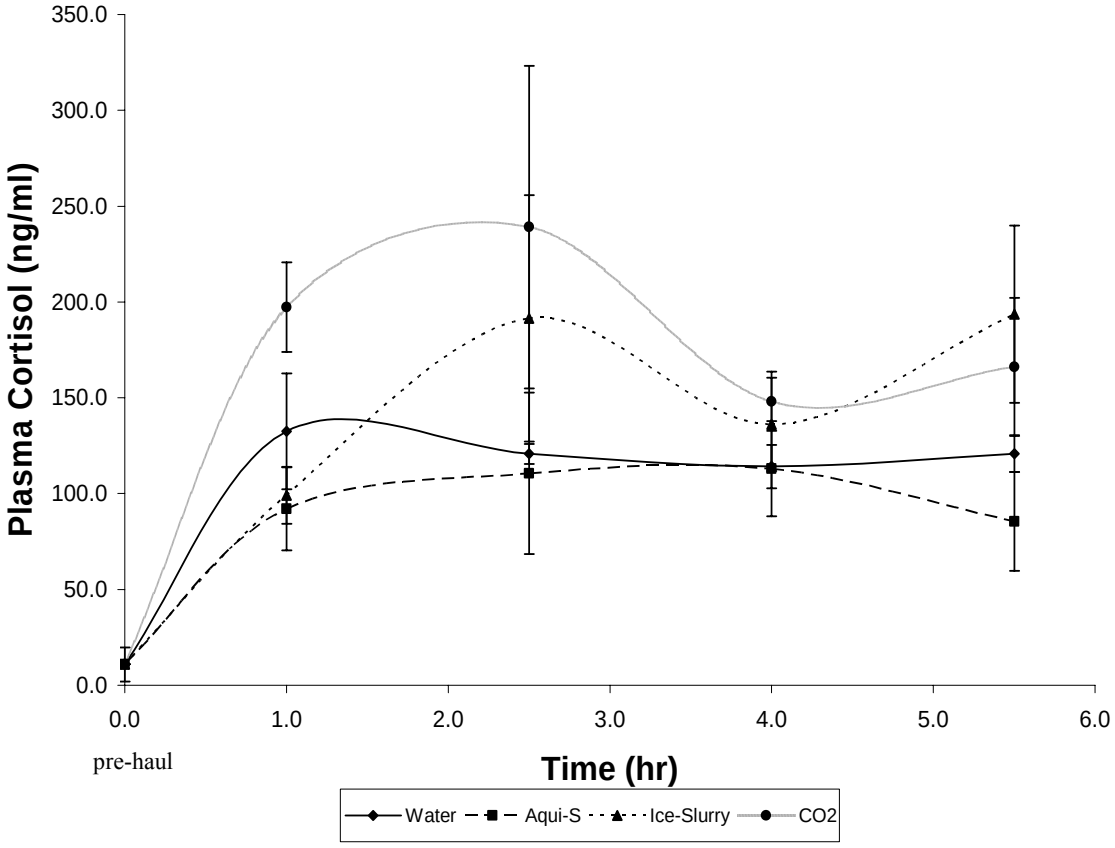
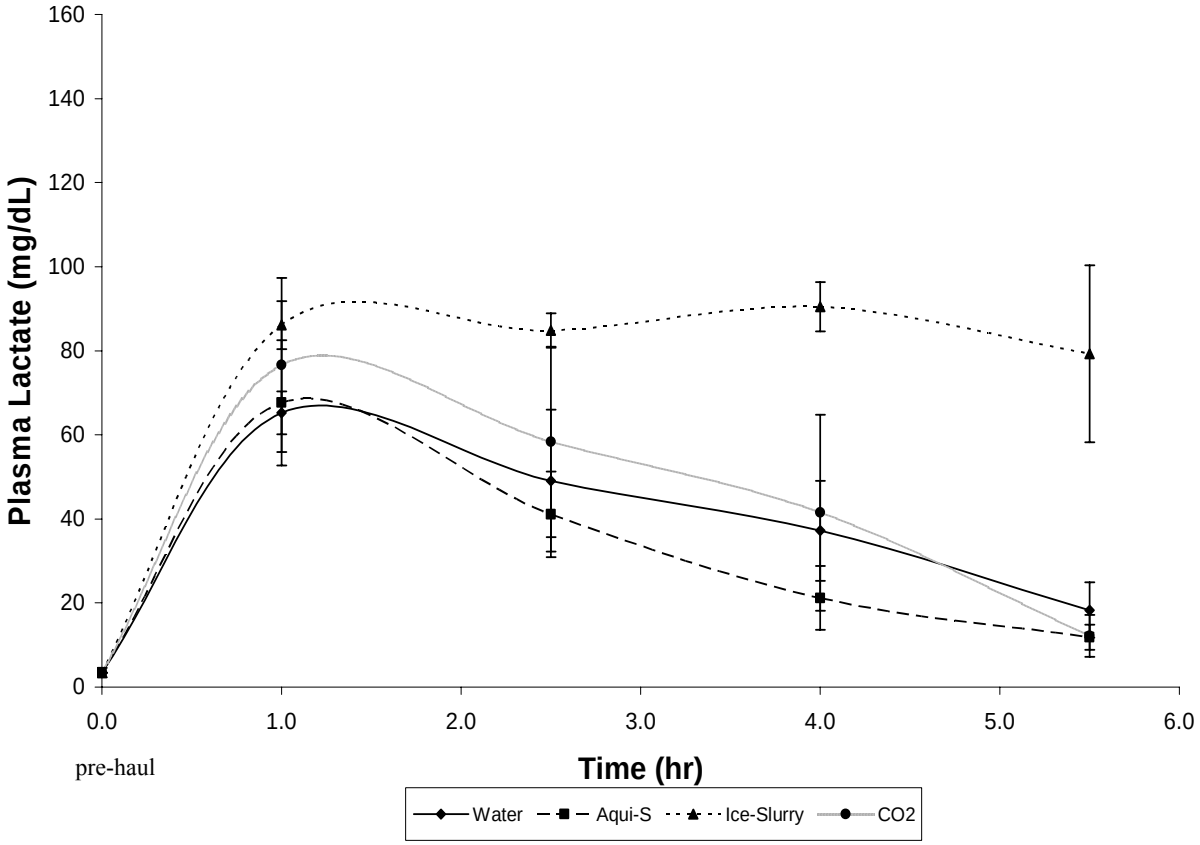


Figure 3. Plasma lactate (mean ± SEM) in arctic char exposed for 5.5 h to four transport treatments



Appendix A. Mean ± SEM of water quality values from each hour, with the exception of total ammonia-nitrogen (TAN) which was measured only at 5.5 h, and treatment for the 5.5 h transport experiment.

Transport Treatment	Hour	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	TAN (mg/L)
Water	1	14.4 ± 0.4	10.4 ± 0.4	7.78 ± 0.03	NA
Water	2.5	13.9 ± 0.8	11.2 ± 0.6	7.89 ± 0.06	NA
Water	4	12.9 ± 0.5	11.1 ± 0.7	8.01 ± 0.04	NA
Water	5.5	13.0 ± 0.4	11.3 ± 1.3	8.05 ± 0.05	0.9 ± 0.2
AQUI-S	1	14.5 ± 0.4	10.3 ± 0.5	7.77 ± 0.01	NA
AQUI-S	2.5	14.2 ± 0.5	12.7 ± 1.0	7.91 ± 0.02	NA
AQUI-S	4	13.5 ± 0.7	11.9 ± 1.2	8.02 ± 0.02	NA
AQUI-S	5.5	13.6 ± 0.7	10.6 ± 1.5	8.01 ± 0.04	1.1 ± 0.3
Ice-slurry	1	0.3 ± 0	14.2 ± 0.3	7.79 ± 0.02	NA
Ice-slurry	2.5	0.3 ± 0.2	16.8 ± 1.2	7.83 ± 0.06	NA
Ice-slurry	4	0.3 ± 0	15.9 ± 1.3	8.02 ± 0.03	NA
Ice-slurry	5.5	0.3 ± 0.2	15.6 ± 3.6	8.04 ± 0.04	0.6 ± 0.2
CO_2	1	13.9 ± 0.5	10.5 ± 0.8	7.04 ± 0.02	NA
CO_2	2.5	13.9 ± 0.6	10.8 ± 0.5	7.04 ± 0.05	NA
CO_2	4	14.0 ± 0.3	10.5 ± 0.7	7.05 ± 0.02	NA
CO_2	5.5	14.8 ± 0.05	10.3 ± 1.1	7.00 ± 0.06	0.8 ± 0.1

Curriculum Vitae

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Educational Background

M. S. *West Virginia University* (Aug. 2003)
Major: Fisheries Resources

B. S. *University of Georgia* (2000)
Major: Fisheries & Aquaculture

A. S. *Abraham Baldwin Agricultural College* (1999)
Major: Forest Resources

Research Experience

Position: G*raduate Research Assistant*
Place: West Virgnia University, Morgantown, West Virginia
Duties: Conducted research for Master's Thesis: 1) examined the effects of the interaction of nitrite and carbon dioxide on the survival and physiological stress responses of rainbow trout; and 2) examined the effects of transport-related stressors on arctic char. Assisted other graduate students in field research (e.g, boat and backpack electrofishing, water quality sampling) and lab research (e.g., bleeding fish, analyzing blood and plasma samples).
Dates: August 2001 – August 2003
Supervisor: Patricia M. Mazik, Ph.D.

Position: *Undergraduate Fisheries & Aquaculture Student*
Place: University of Georgia, Athens, Georgia
Duties: Examined the effects of hybrid striped bass on largemouth bass in a small wildlife management pond and investigated the amount of public usage in a wildlife management area.
Dates: August 2000 – December 2000
Supervisors: Robert Reinert, Ph.D. and James Shelton, Ph.D.

Relevant Skills and Courses

Computer skills: Experience using Microsoft Word, Excel, and PowerPoint, ArcGIS, SAS, and MARK.

Presentation skills: Training in oral and written communications.

Courses: Biology, Inorganic and Organic Chemistry, Statistics, Biometrics, Quantitative Ecology, Forest Ecology, Vertebrate and Invertebrate Zoology, Soils and Hydrology, Fisheries Management, Fish Physiology, Fish Culture, Water Quality Management, Icthyology, Limnology, Fish and Wildlife Management, Aquatic Toxicology, Fish Ecology

Certifications

First Aid/CPR
Backpack Electrofishing
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