EFFECTS OF OZONE AND WATER EXCHANGE RATES ON WATER QUALITY AND RAINBOW TROUT, ONCORHYNCHUS MYKISS, PERFORMANCE IN REPLICATED WATER REUSE AQUACULTURE SYSTEMS

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ABSTRACT Rainbow trout performance and water quality criteria were evaluated and compared within replicated 9.5 m³ water reuse aquaculture systems (WRAS) operated with: 1) low water exchange with and without ozone; 2) low water exchange with ozone versus high water exchange without ozone; and 3) near-zero water exchange with and without ozone. Ozone caused a significant increase in ultraviolet transmittance of the culture water and significantly reduced total suspended solids, color, and biochemical oxygen demand, as well as dissolved copper, zinc, and iron. Reduction of the aforementioned dissolved metals was important since each can be toxic to fish at elevated concentrations. The origin of dissolved copper, zinc, and iron in the WRAS was likely feed related since these metals are added in trace quantities within the vitamin pack, but copper was also found to leach from copper piping which supplied water to the systems. Ozone did not inhibit nitrate nitrogen concentration. Nitrate nitrogen accumulated to approximately 100 mg/L in WRAS operated at low exchange and >400 mg/L in WRAS operated at near-zero water exchange. Rainbow trout mortality was greater in WRAS with mean nitrate nitrogen of 400+ mg/L and fish exhibited erratic behaviour. Thus, nitrate nitrogen accumulation could represent a barrier to operating WRAS as closed or near-zero exchange, without the addition of unit processes capable of denitrification. Rainbow trout growth rates, feed conversion, and condition factor were generally greater within WRAS operated with ozone due to the improvements in water quality described in this paper.

Keywords: Rainbow Trout, Ozone, Water Reuse, Water Recirculating Aquaculture System, Aquaculture, Water Quality, Copper, Heavy Metal, Waste Metabolite Accumulation, Nitrate

INTRODUCTION

A series of studies are being conducted at the Freshwater Institute to identify water quality parameters that could limit rainbow trout performance (i.e. growth, health, and survival) within water reuse aquaculture systems (WRAS) operated at low and near-zero water exchange with high feed loading rates. These studies originated based on a previous unreplicated experiment during which rainbow trout mortality increased and fish
health declined within a commercial scale (150 m\(^3\)) WRAS as makeup water was reduced to 1% of the total flow with relatively high feed loading. The decline in fish health within the 150 m\(^3\) WRAS was unrelated to infectious disease and occurred when typical water quality concentrations were within safe limits (Davidson et al., 2009). Interestingly, mortality decreased and fish health improved when ozone was added or when the makeup water flow rate was increased to the 150 m\(^3\) WRAS.

Previous research from the water treatment and aquaculture industries has shown that ozone (O\(_3\)) has the potential to reduce and control a variety of water quality parameters that could be detrimental to optimal fish performance. Dissolved ozone has been proven effective in water treatment for the reduction of biochemical oxygen demand, chemical oxygen demand, dissolved organic carbon, color, nitrite, turbidity, total organic carbon, and total suspended solids (Rosenthal and Kruner, 1985; Hozalski et al., 1995; Summerfelt and Hochheimer, 1997; Summerfelt et al., 1997; Tango and Gagnon, 2003), algae control (Rice et al., 1981; Plummer and Edzwald, 2002), reduction of off-flavor producing compounds such as MIB and geosmin (Nerenberg et al., 2000; Park et al., 2007), improved micro-flocculation of fine particulates (Rice et al., 1981; Rueter and Johnson, 1995), increased unit process efficiency (Rosenthal and Otte, 1980; Paller and Lewis, 1988; Summerfelt et al., 1997), reduction of heavy metals such as iron and manganese (Rice et al., 1981), and significant reduction of bacterial populations depending on ozone dose, contact time, and operation with or without ultraviolet irradiation (Summerfelt, 2003; Sharrer and Summerfelt, 2007). Additionally, O\(_3\) reacts rapidly within water, produces few harmful by-products in freshwater, and forms dissolved oxygen as a reaction end product in freshwater (Summerfelt and Hochheimer, 1997; Summerfelt, 2003).

With so many advantages, O\(_3\) application could be the key to optimal fish performance within low and near-zero exchange WRAS operated with high feed loading. Three studies evaluating the use of O\(_3\) within low and near-zero exchange WRAS are discussed. The primary objectives of these studies were: 1) to determine if O\(_3\) creates a more favorable water quality environment for salmonids cultured within low and near-zero exchange WRAS, 2) to determine which water quality parameters are improved as a result of O\(_3\) addition, and 3) to expand on the findings of Davidson et al. (2009) relative to accumulating water quality constituents and their potential toxicity to rainbow trout within low and near-zero exchange WRAS. Results will provide important information regarding the feasibility of operating WRAS at low water exchange, near-zero exchange, or as completely closed systems for the future commercial production of rainbow trout and other salmonid species.

**METHODS**

Three studies were conducted: 1) WRAS operated with low water exchange rates with and without O\(_3\); 2) WRAS operated with low water exchange rates with O\(_3\) vs. high exchange without O\(_3\); and 3) WRAS operated at near-zero exchange with and without O\(_3\). WRAS described as operating at “low” and “high” water flushing rates continuously
Six identical 9.5 m$^3$ WRAS were used. Each WRAS recirculated 380 L/min of water through a 5.3 m$^3$ dual drain culture tank, a radial flow settler, a microscreen drum filter, a fluidized sand biofilter, a heat exchanger, a carbon dioxide stripping column, and a low head oxygenator (LHO). Three WRAS were equipped with ozone generators (Model G22, Pacific Ozone Technology, Benecia, CA). Ozone was injected within the LHO and was monitored and controlled via oxidation reduction potential (ORP), an indirect measure of ozone residual. During Studies 1 and 2 an ORP setpoint of 250 mV was targeted and during Study 3 ORP ranged from 270-290 mV. The resulting ozone dose, which ranged from 20-25 g ozone/ kg feed, was not expected to disinfect the water (i.e. reduce bacterial loads) but was expected to improve general water quality.

**Study 1**- Rainbow trout (1000/tank), 74 ± 2 g, were stocked within the six systems at a density of approximately 15 kg/m$^3$ and allowed 8 weeks for biofilter acclimation prior to ozone startup. WRAS were then randomly divided into two treatments: 1) three WRAS operated at low exchange with ozone, and 2) three WRAS operated at low exchange without ozone (control). Rainbow trout were 196 ± 2 g in systems operated with ozone and 198 ± 0 g in systems without ozone to begin the study.

**Study 2**- Rainbow trout (1000/tank), 151 ± 3 g, were stocked within the six systems at a density of approximately 30 kg/m$^3$. WRAS were divided into two treatments: 1) three WRAS operated at low exchange with ozone and 2) three WRAS operated at high exchange without ozone. The primary purpose of this study was to determine if ozone could create similar water quality in low exchange WRAS as compared to high exchange WRAS, despite operating at an exchange rate that was ten times lower.

**Study 3** - Rainbow trout (approximately 3600/tank), 18 ± 0 g, were stocked within the six systems at a density of approximately 12 kg/m$^3$. WRAS were divided into two treatments: 1) three WRAS operated at near-zero exchange with ozone and 2) three WRAS operated at near-zero exchange without ozone.

Fish were fed equal rations with feed events occurring every two hours, around the clock, using automated feeders (T-drum 2000CE, Arvo-Tec, Finland). Feeding was estimated based on standardized feeding charts and observations of feeding activity and wasted feed. Feeding rates ranged from 1.5 - 2.0 % relative to fish body weight. Mean feed loading rates for WRAS operated at high, low, and near-zero exchange were 0.40, 3.98, and 45.1 kg feed/ m$^3$ make-up water per day, respectively. A standard slow-sinking trout diet (Zeigler Brothers, Inc., Gardners, PA, USA) with a protein: fat ratio of 42:16 was used throughout each. A constant 24-h photoperiod was provided.

Water samples were collected from each tank and tested for a variety of parameters according to methods described in APHA (2005) and HACH (2003). Water quality was assessed weekly over the duration of each study as well as a period that coincided with maximum feed loading and fish density (80 kg/m$^3$). The majority of tests were carried out...
at the Freshwater Institute with the exception of dissolved metals which were conducted by the Cornell Nutrient Analysis Laboratory (Ithaca, NY, USA). Fish were sampled for length and weights on a monthly basis and mortalities were removed and recorded daily to assess cumulative survival. Feed conversion ratios and fish condition factor were also calculated for each study and compared between treatments.

All parameters that were sampled during multiple events over time from the same location were analyzed using multivariate repeated measures analysis of variance (MANOVA). Mean water quality data for the duration of each study, as well as metals data was compared using a Student’s t-test or Mann Whitney U-test depending on normality. For Study 3 most variables were analyzed for differences between treatments using analysis of covariance (ANCOVA) due to the unexpected differences in flushing rates measured amongst WRAS. A probability value (α) of 0.10 was used to determine significance for each statistical test as opposed to the traditional 0.05 due to a relatively low n-value (3 WRAS per treatment). Statistical analyses were carried out using SYSTAT 11 software (2004).

RESULTS AND DISCUSSION

Metals/Elements - During Study 1 copper was significantly greater within WRAS without O₃ and sulfur was significantly greater in WRAS with O₃ (Table 1). During Study 3, copper and iron were statistically greater within the near-zero exchange WRAS without O₃. Although significant differences weren’t detected between treatments for zinc during any study, it is worth noting that this potentially harmful metal was always lower within WRAS operated with O₃ (Table 1). Of the detectable metals/elements all were within safe recommended limits as reported by the literature with the exception of copper and possibly potassium. The most noteworthy metal/element detected was dissolved copper. Results indicate that the use of O₃ caused a 3-4 fold reduction of dissolved copper within low and near-zero exchange WRAS during Studies 1 and 3.

The differences in dissolved copper noted between treatments during Studies 1 and 3 are important when considering copper’s toxicity to salmonids. Dissolved copper concentrations measured within low and near-zero exchange WRAS without O₃ during Studies 1 and 3 exceeded limits established for salmonids (Alabaster and Lloyd, 1982; U.S. EPA, 2007). Davidson et al. (2009) provide a literature review regarding the toxicity of copper to salmonids. No obvious signs of acute or chronic copper toxicity were observed during these studies. Hardness, alkalinity, pH, temperature, dissolved organic carbon (DOC), total suspended solids, and silicates can alter copper toxicity (Alabaster and Lloyd, 1982; Spear and Pierce, 1979; Sprague, 1985; U.S. EPA, 2002; 2007); therefore, a toxic effect of copper was likely buffered. Without the buffering effect of other water quality parameters, mortality would likely have resulted in WRAS operated without O₃, while fish cultured within ozonated WRAS would likely have been safe.

Since accumulation of copper could be problematic in low and near-zero exchange WRAS, it was important to gain an understanding of the origin of dissolved copper. Copper was typically undetectable (0.001 ± 0.001 mg/L) within makeup water entering each WRAS through a PVC pipe, but was consistently detected within the water entering
as backwash spray through a copper pipe (0.013 ± 0.002 mg/L). However, the feed was determined to be the primary source of copper accumulation within the WRAS since trace amounts of copper are included within the vitamin pack.

**Nitrogen** – No significant differences were detected for total ammonia nitrogen (TAN), nitrite nitrogen (NO₂⁻), or nitrate nitrogen (NO₃⁻) during Studies 1 and 3 (Table 1). During Studies 1 and 3 TAN and NO₂⁻ were generally lower within WRAS operated with O₃ (Table 1). At one point during Study 3 an ozone generator failed and nitrite nitrogen increased, indicating that the biofilter was not acclimated to the suddenly present (NO₂⁻) previously oxidized by O₃. Removal of TAN and NO₂⁻ by O₃ has been documented, but O₃ is not known to remove nitrate nitrogen, which was confirmed during the present studies. It was noted that when (NO₃⁻) accumulated to ≥100 mg/L, rainbow trout swimming speed dramatically increased, possibly indicating agitation; and when (NO₃⁻) exceeded 400 mg/L erratic behaviour, as well as low level mortality resulted.

**Total suspended solids/ particles** – Total suspended solids (TSS) were significantly greater within WRAS operated without O₃ during Study 1 (Table 1). During Study 3 TSS was lower within near-zero exchange WRAS operated with O₃ vs. near-zero exchange WRAS operated without O₃ (Table 1), but not significantly due to variation of results. Removal efficiencies across solids removal devices (radial flow settler and drum filter) were greater within WRAS operated with O₃. The enhanced removal efficiency was likely due to microflocculation of solids caused by O₃. Analysis of particle counts and particle size distributions supports the hypothesis of solids microflocculation and removal. Mean total particle counts (2-60 µm) of samples collected for the duration of Study 1 were four times greater within WRAS without O₃ (19,749 counts/mL) as compared to WRAS with O₃ (4,786 counts/mL). WRAS operated with O₃ had substantially less fine particles for all size ranges. TSS levels measured within WRAS operated with O₃ were always within safe limits (< 20 mg/L) as recommended by literature. During Study 3 TSS reached maximum levels of 60 mg/L in WRAS operated at near-zero exchange without O₃, but rainbow trout mortality did not increase.

**Carbonaceous biochemical oxygen demand** – During Study 1 and 3 BOD was significantly greater within WRAS operated without O₃ (Table 1). BOD alone generally would not be expected to impact fish health, but it does provide a substrate for heterotrophic bacteria and protozoa, which in turn can inhibit nitrification efficiency of biofilters (Zhu and Chen, 2001). The BOD concentrations measured during the present studies did not appear to negatively impact biofilter performance. The results from these studies indicate that O₃ can substantially reduce BOD levels within WRAS, thus aiding in the optimization of the nitrification process.

**Bacteria** – Heterotrophic bacteria counts from the tank water were much greater within WRAS operated without O₃ during Study 1 and Study 3 and were similar within low exchange WRAS operated with O₃ as compared to high exchange WRAS without O₃ (Table 1). The O₃ dose applied during these studies was not high enough for disinfection, but other improvements in water quality initiated by ozonation that were previously mentioned, such as reduction of TSS and BOD, created an environment that was not as conducive to bacterial proliferation.
**Water Clarity** – The reduction of TSS, BOD, bacteria, and possibly other compounds via ozonation visually resulted in a very clear water quality environment as evidenced by significantly lower values for true color and ultraviolet (UV) transmittance in WRAS operated with O$_3$ during Studies 1 and 3 (Table 1). A clear water quality environment enhances the ability of the fish to see, feed optimally, and grow (Sigler et al., 1984). The clear culture water also allows the farmer to better observe fish (Christensen et al., 2000), including fish health, behaviour, and feeding activity and make appropriate adjustments to optimize productivity and profitability.

**Other Water Quality Parameters and Water Quality Notes** – Several water quality parameters listed in Table 1 were controlled during the study and equal between treatments including oxygen, carbon dioxide, and temperature. ORP and alkalinity were controlled, as discussed in the methods. Additionally, it is important to note that differences in water quality observed during Study 2 were expected due to a ten-fold difference in flushing/dilution rate. During Study 2 O$_3$ ultimately created water quality within low exchange WRAS that was similar to water quality of a high exchange WRAS.

**Rainbow Trout Growth Performance** – Rainbow trout growth was significantly greater within low exchange WRAS operated with O$_3$ at the conclusion of Study 1 (Fig. 1). Mean final weights for low exchange WRAS operated with and without O$_3$ were 1161 ± 12 and 993 ± 11 g, respectively. Rainbow trout within the ozonated WRAS became significantly larger than trout in WRAS without O$_3$ only one month after treatments were initiated despite being stocked at the same size (Fig. 1). During Study 2 rainbow trout growth was similar within WRAS operated at high water exchange and WRAS operated with low water exchange with O$_3$. At the conclusion of Study 2 mean rainbow trout weights in WRAS operated at high exchange without ozone were 1379 ± 38 g versus 1348 ± 72 g in WRAS operated at low exchange with ozone. During Study 3 rainbow trout cultured within near-zero exchange WRAS operated with O$_3$ were larger at the conclusion of the study, but not significantly. At the conclusion of Study 3 rainbow trout within the near-zero exchange WRAS with and without ozone were 206 ± 14 and 180 ± 10 g, respectively. Rainbow trout feed conversion and condition factor were generally better within WRAS operated with ozone.

**Rainbow Trout Survival** - Survival calculated over the duration of Study 1 for WRAS with and without ozone was 99.3 ± 0.2 and 98.3 ± 0.5%, respectively. Thus, survival was better within ozonated WRAS, but not significantly. Rainbow trout survival was similar between treatments during Study 2. Survival within WRAS operated at low exchange with O$_3$ was 93.3 ± 1.6 % and 93.1 ± 0.5 % in WRAS operated at high exchange without ozone. Rainbow trout survival was similar between WRAS operated at near-zero exchange with and without O$_3$ during Study 3. Survival within WRAS operated at near-zero exchange with and without O$_3$ was 88.8 ± 3.3 % and 94.5 ± 0.5 %, respectively.

**CONCLUSIONS**

These studies indicated that the use of ozone within low and near-zero exchange WRAS significantly improved a variety of water quality conditions, many of which can cause toxic effects at elevated concentrations. Ozone effectively reduced total suspended solids,
color, biochemical oxygen demand, and resulted in a significant increase in ultraviolet transmittance. Heterotrophic bacteria populations were also much lower within ozonated WRAS. Most notably $O_3$ effectively reduced accumulating metals within low and near-zero exchange WRAS, particularly copper. Ozone created water quality within low exchange WRAS that was similar to that of high exchange WRAS operated with ten times more dilution and thus created an environment that generally resulted in improved rainbow trout growth rates. Overall, ozone proved to be a vital component for the future operation of low and near-zero exchange WRAS with clean water.

Based on the findings relative to copper origination in WRAS, copper piping and components should be avoided, and the water supply should be periodically tested for metals, which can be accumulate to toxic levels within WRAS. The current research also provided strong evidence that nitrate nitrogen can accumulate to concentrations that are detrimental to fish health within WRAS that are operated at low and near-zero exchange, indicating that sublethal effects of nitrate could occur at concentrations generally thought to be safe ($\geq 100$ mg/L). Thus, accumulation of nitrate nitrogen is also an important consideration within WRAS operated at low and near-zero water exchange.

Acknowledgements

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REFERENCES


FIGURES AND TABLES

Fig. 1. Rainbow trout growth rates within low exchange WRAS with and without ozone during Study 1.

Table 1. Mean water quality and metals/element concentrations within the fish culture tank.

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
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<th>Study 3</th>
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<tr>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Treatment Parameter</td>
<td>Low Exchange No Ozone</td>
<td>Low Exchange Ozone</td>
<td>High Exchange No Ozone</td>
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<td>---------------------</td>
<td>-----------------------</td>
<td>-------------------</td>
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<tr>
<td>TAN</td>
<td>0.47 ± 0.01</td>
<td>0.45 ± 0.02</td>
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<td>NH₃</td>
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<td>0.005 ± 0.000</td>
<td>0.003 ± 0.000</td>
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<td>NO₂⁻</td>
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<td>0.04 ± 0.01</td>
<td>0.11 ± 0.04</td>
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<td>NO₃⁻</td>
<td>71 ± 1</td>
<td>84 ± 3</td>
<td>13 ± 0</td>
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<tr>
<td>Alkalinity</td>
<td>205 ± 1</td>
<td>196 ± 1</td>
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<tr>
<td>pH</td>
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<td>CO₂</td>
<td>10 ± 0</td>
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<td>10 ± 1</td>
</tr>
<tr>
<td>cBOD₅</td>
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<td>1.7 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
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<td>True Color</td>
<td>53 ± 2</td>
<td>4 ± 0</td>
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<td>UV Transm(%)</td>
<td>60 ± 1</td>
<td>82 ± 0</td>
<td>89 ± 0</td>
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<td>TSS *1</td>
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<td>3.4 ± 0.4</td>
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<tr>
<td>H. Bacteria</td>
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<td>117 ± 23</td>
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<td>15.1 ± 0.0</td>
<td>15.2 ± 0.0</td>
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<td>DO</td>
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<td>9.8 ± 0.0</td>
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<td>ORP</td>
<td>155 ± 1</td>
<td>248 ± 1</td>
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<tr>
<td>Copper</td>
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<tr>
<td>Sulfur</td>
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