

**Effects of Ozone and Water Exchange Rates on Water Quality and Fish Performance within Replicated Water Reuse Aquaculture Systems
Culturing Rainbow Trout, *Oncorhynchus mykiss***

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Abstract

Rainbow trout performance and water quality were evaluated and compared within replicated 9.5 m³ water reuse aquaculture systems (WRAS) during three separate studies: 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 (backwash replacement only) 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 and iron. Zinc was consistently lower, but not significantly, in WRAS that used ozone. 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 because these metals are added in trace quantities within the vitamin pack, but copper was also found to leach from copper piping that supplied water to the systems. Ozone did not prevent nitrate nitrogen accumulation. Nitrate nitrogen accumulated to approximately 100 mg/L in WRAS operated at low exchange (6.7 day system HRT) and >400 mg/L in some WRAS operated at near-zero water exchange. Rainbow trout mortality was greater and fish exhibited erratic behavior in WRAS with mean nitrate nitrogen of 400+ mg/L. Thus, nitrate nitrogen accumulation could represent a barrier to operating WRAS as closed or near-zero exchange, without unit processes capable of denitrification. Rainbow trout growth rates were generally greater within WRAS operated with ozone. Rainbow trout survival was greater, but not significantly, within low exchange WRAS operated with ozone during Study 1. During Study 3 survival was lower in near-zero exchange WRAS operated with ozone, but the increased mortality was correlated with nitrate nitrogen concentration.

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 results from a previous unreplicated experiment during which rainbow trout mortality increased and fish health declined within a commercial scale (150 m³) WRAS as makeup water was reduced to 1% of the total flow with relatively high feed loading (Davidson et al., 2009). Interestingly, mortality increased and fish health improved when ozone was added or make-up water flow was increased to the 150 m³ WRAS (Davidson et al., 2009).

Previous research from the water treatment and aquaculture industries has shown that ozone (O₃) 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; Summerfelt et al., 2009), 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; Summerfelt et al., 2009). Additionally, O₃ 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, O₃ 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 O₃ within low and near-zero exchange WRAS are discussed. The primary objectives of these studies were: 1) to determine if O₃ creates a more favorable water quality environment for salmonids cultured within low and near-zero exchange WRAS; 2) by determining which water quality parameters are improved as a result of O₃ 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 and near-zero water exchange, or as completely closed systems for the future commercial production of rainbow trout and other salmonid species.

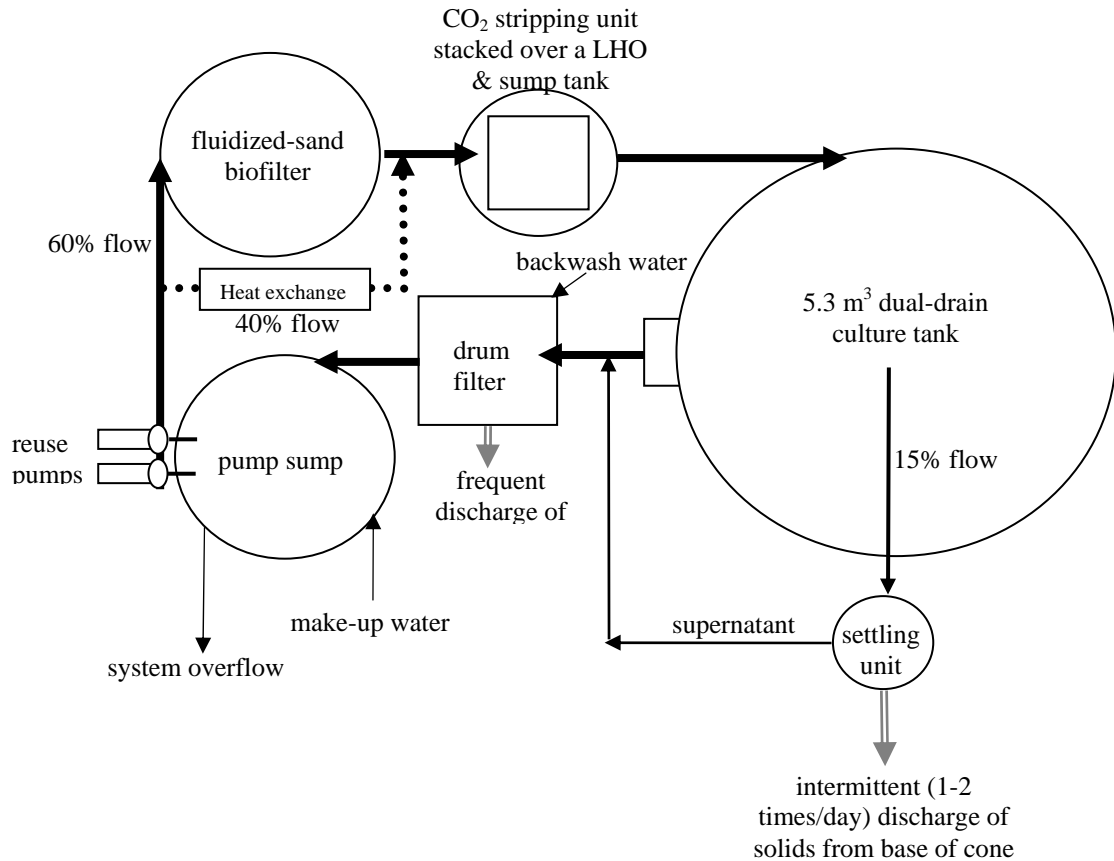
Methods

Experimental conditions - Three studies were conducted: 1) WRAS operated with low water exchange rates with and without O₃; 2) WRAS operated with low water exchange rates with O₃ vs. high exchange without O₃; and 3) WRAS operated at near-zero exchange with and without O₃. WRAS described as operating at “low” and “high” water flushing rates continuously exchanged 0.26 and 2.6% of the total recirculating flow, respectively, while WRAS operated at “near-zero” exchange only replaced water lost as backwash. During Study 3 periodic drum filter failures occurred in four of the six replicated systems which resulted in increased and variable dilution amongst WRAS. Mean system hydraulic retention times for the high, low, and near-zero exchange WRAS were approximately 0.67, 6.7 and 61 days, respectively.

System description - Six identical 9.5 m³ WRAS were used. Each WRAS recirculated 380 L/min of water through a 5.3 m³ 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) (Fig. 1). Three WRAS were equipped with ozone generators (Model G22, Pacific Ozone Technology, Benecia, CA). Ozone was injected within the oxygen feed gas of 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 in the culture tank; and

during Study 3 ORP setpoints 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.

Figure 1. Process flow drawing of an individual 9.5 m³ water recirculating system used for the present studies.



Culture - 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. To begin the study rainbow trout were 196 ± 2 g in systems operated with ozone and 198 ± 0 g in systems without ozone. **Study 2-** Rainbow trout (1000/tank), 151 ± 3 g, were stocked within the six systems at a density of approximately 30 kg/m^3 . 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 without ozone, 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 . During each study, fish were sampled for length and weights on a monthly basis and mortalities were removed and recorded daily to assess cumulative survival.

Feeding - 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/ m³ 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 study. A constant 24-h photoperiod was provided.

Water Sampling/Analysis - 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 near maximum feed loading and fish density (80 kg/m³). The majority of tests were carried out at the Freshwater Institute with the exception of dissolved metals which were analyzed by the Cornell Nutrient Analysis Laboratory (Ithaca, NY, USA).

Statistical Analysis - 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 sparsity of data, i.e. a relatively low n-value (3 WRAS per treatment). Statistical analyses were carried out using SYSTAT 11 software (2004).

Results and Discussion

Significant differences were expected between treatments during Study 2 due to the ten-fold difference in flushing rate between treatments. Therefore, discussion of Study 2 results will be limited. Ultimately, ozone produced water quality in low exchange WRAS that was similar to that of a high exchange WRAS operated without ozone despite the 10-fold difference in water exchange during Study 2.

Metals/Elements – Of the detectable metals/elements all were within safe recommended limits as reported by the literature (Table 1) with the exception of copper and possibly potassium. All metals/elements that were detected within the culture water during the present studies are listed in Table 2. During Study 1 copper was significantly greater within WRAS without O₃ and sulfur was significantly greater in WRAS with O₃ (Table 2). 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 2).

Table 1. Minimum detection limits for each metal/element analysis and upper recommended concentrations for each metal/element for salmonid culture as reported in the literature.

Parameters	Minimum Detection Limit (mg/L)	Recommended Upper Limit (mg/L)
Aluminum	0.130	0.01 - 1.00
Arsenic	0.019	0.05 - 0.40
Barium	0.002	5
Beryllium	0.002	0.01- 1.10
Boron	0.200	5
Cadmium	0.004	0.0003 - 0.0700
Calcium	0.495	4 -160+
Chromium	0.008	0.03 - 0.10
Cobalt	0.009	0.010 - 0.05
Copper	0.005	0.006 - 0.070
Iron ^(Total)	0.200	0.1 - 1.1
Lead	0.200	0.01 - 4.0
Magnesium	0.031	15 - 28+
Manganese	0.002	0.05 - 1.00
Mercury	0.350	0.0001 - 0.0020
Molybdenum	0.011	8+
Nickel	0.016	0.01 - 0.40
Phosphorous ^(Total)	0.019	3+
Potassium	0.332	5 -10+
Selenium	0.075	0.005 - 0.020
Silicon	0.286	NA
Sodium	0.097	600 - 1500+
Strontium	0.002	NA
Sulfur	0.130	NA
Titanium	0.013	NA
Vanadium	0.018	0.1
Zinc	0.016	0.005 - 0.269

The toxicity of many parameters is dependant upon alkalinity, hardness, and other variables. Limits are cited from: Piper et al. (1982), Meade (1989), Heinen et al. (1996), Wedemeyer et al. (1996), EPA (2002; 2007), Boyd (2009).

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) provided a literature review regarding the toxicity of copper to salmonids. No obvious signs of acute or chronic copper toxicity were observed during these studies. In addition, copper concentrations measured in the livers of trout were within normal safe levels and were not significantly different between treatments. 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 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, we determined that the majority of the backwash spray was flushed with the captured biosolids. Therefore, the feed was determined to be the primary source of copper accumulation within the WRAS because trace amounts of copper are included within the vitamin pack.

Very little information exists regarding the toxicity of potassium to fish. Buhse (1974) reported that potassium > 200 mg/L was toxic to fish. Additionally, Heinen et al. (1996) suggested that ≥ 10 mg/L potassium is acceptable for culture water with hardness > 100 mg/L. Timmons and Ebeling (2007) provided a recommendation of < 5 mg/L, but did not include an associated alkalinity. With such a wide range of recommendations, conclusions could not be made regarding potential potassium toxicity at the concentrations measured during these studies. Ozone did not appear to have an impact on potassium, as it did with other metals/elements, thus potassium accumulated within the low and near-zero exchange WRAS. In order to rule out potassium as a potentially problematic element within low and near-zero exchange WRAS, future controlled studies would certainly be necessary.

Table 2. Mean dissolved metal and nutrient concentrations (mg/L) at the tank side drain outlets when WRAS were operated near maximum feed loading and fish density during study 1, 2, and 3.

Element	Study 1		Study 2		Study 3	
	Low Exchange No Ozone	Low Exchange Ozone	High Exchange No Ozone	Low Exchange Ozone	Near-Zero Exchange No Ozone	Near-Zero Exchange Ozone
Barium ^{*2}	0.036 ± 0.005	0.038 ± 0.004	0.055 ± 0.001	0.043 ± 0.001	0.367 ± 0.066	0.233 ± 0.008
Boron	<MDL	<MDL	<MDL	<MDL	0.061 ± 0.011	0.065 ± 0.014
Calcium ^{*2}	94 ± 5	100 ± 2	108 ± 0	104 ± 1	99 ± 2	84 ± 12
Copper ^{*1, *2, *3}	0.064 ± 0.001	0.021 ± 0.008	0.014 ± 0.002	0.038 ± 0.004	0.119 ± 0.008	0.041 ± 0.001
Iron ^{*3}	<MDL	<MDL	<MDL	<MDL	0.041 ± 0.013	<MDL
Magnesium ^{*2}	12.8 ± 0.7	13.7 ± 0.3	12.1 ± 0.1	14.8 ± 0.4	19.8 ± 0.4	23.7 ± 2.6
Manganese	<MDL	<MDL	<MDL	<MDL	0.008 ± 0.004	<MDL
Phosphorous ^{*2}	2.4 ± 0.1	2.2 ± 0.1	0.5 ± 0.1	2.7 ± 0.2	7.0 ± 1.6	11.8 ± 2.7
Potassium ^{*2}	17 ± 1	18 ± 0	5 ± 0	25 ± 3	44 ± 7	85 ± 27
Silicon	32 ± 2	36 ± 1	48 ± 0	43 ± 2	44 ± 1	43 ± 2
Sodium ^{*2}	125 ± 12	138 ± 2	5 ± 0	164 ± 20	346 ± 86	568 ± 189
Strontium ^{*2}	0.95 ± 0.06	0.90 ± 0.03	0.90 ± 0.00	0.83 ± 0.01	0.89 ± 0.02	0.80 ± 0.08
Sulfur ^{*1, *2}	15.7 ± 0.2	17.1 ± 0.4	9.5 ± 0.2	18.4 ± 1.1	26.7 ± 2.0	39.8 ± 8.6
Zinc	0.005 ± 0.003	0.001 ± 0.001	0.011 ± 0.003	0.007 ± 0.002	0.128 ± 0.023	0.078 ± 0.003

*Indicates statistically significant between treatments ($p < 0.10$), ^{1, 2, or 3} following * indicates study 1, 2, or 3,

<MDL = less than minimum detection limit of the test

Notes: The following elements were below the minimum detection limit within the culture water, makeup water, and drum filter high pressure spray (backwash) for all treatments within all studies: aluminum, arsenic, beryllium, cadmium, chromium, cobalt, lead, mercury, molybdenum, nickel, and selenium.

Nitrogen – The WRAS maintained relatively low total ammonia nitrogen (TAN) and nitrite nitrogen (NO₂-N) concentrations for each treatment over the duration of Studies 1 and 3, i.e. 0.31-0.92 mg/L and 0.05-0.21 mg/L, respectively (Table 3). Significant differences were not detected for TAN, NO₂-N, or nitrate nitrogen (NO₃-N) during Studies 1 and 3 during near maximum feed loading and density (Table 4). During Studies 1 and 3 TAN and NO₂-N levels were generally lower within WRAS operated with O₃, but not significantly (Table 3, 4). NO₃-N was significantly greater within the low exchange WRAS with ozone over the duration of Study 1 (Table 3). The greater NO₃-N levels within the ozonated WRAS for the study duration were attributed to slight differences in feeding between treatments. At one point during Study 3 an ozone generator failed and nitrite nitrogen increased, indicating that that the biofilter was not acclimated to the suddenly present (NO₂-N) previously oxidized by O₃. Removal of NO₂-N 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-N) accumulated to ≥100 mg/L, rainbow trout swimming speed dramatically increased (Fig. 2), possibly indicating agitation; and when (NO₃-N) exceeded 400 mg/L erratic behavior, as well as low level mortality resulted (Fig. 3).

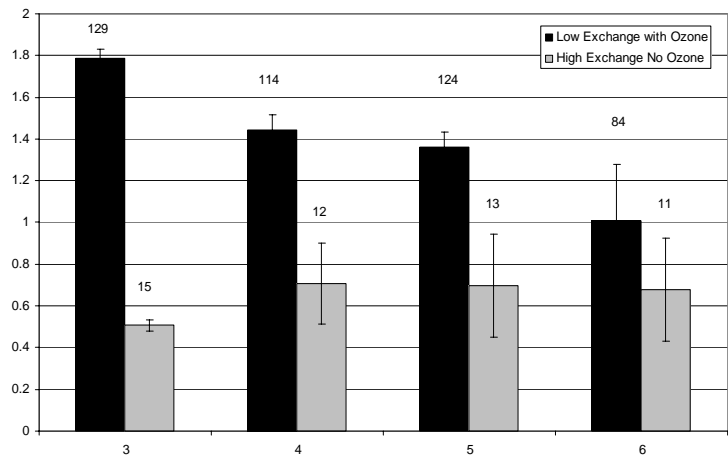


Figure 2. Rainbow trout swimming speed within WRAS operated with high exchange no ozone vs. low exchange ozone (Study 2).

*Numeric notations above each bar represent nitrate nitrogen levels measured for each treatment during each week.

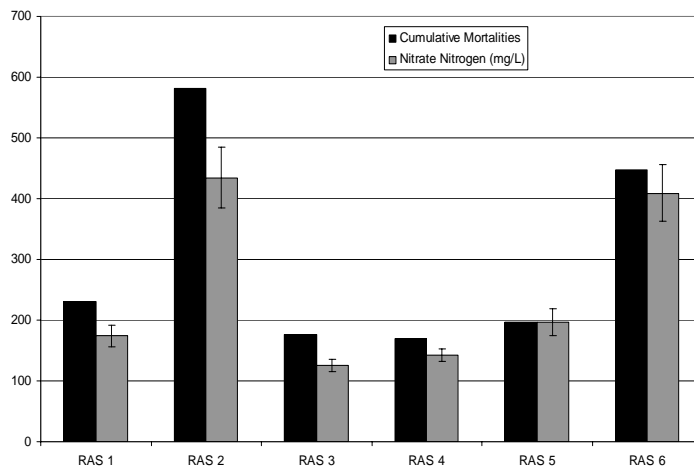
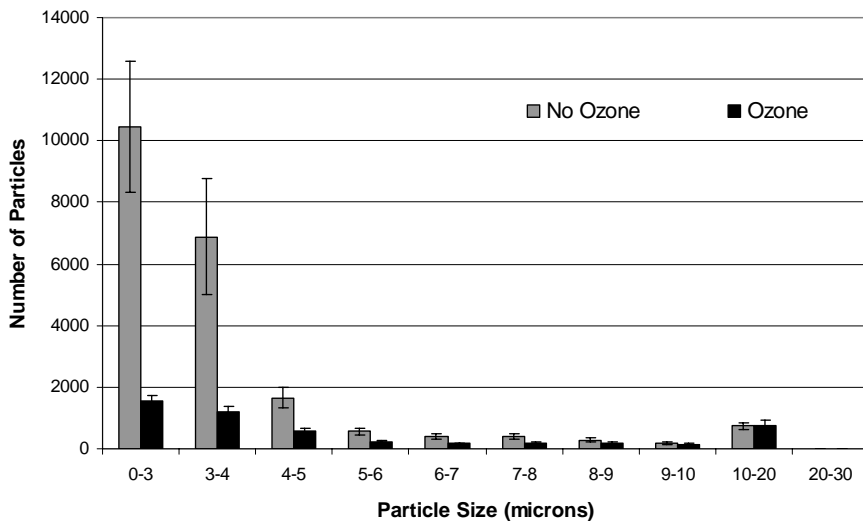


Figure 3. Cumulative rainbow trout mortality and mean nitrate nitrogen within each WRAS during when WRAS were operated at near-zero exchange with and without ozone (Study 3).

Total suspended solids/ particles – Mean total suspended solids (TSS) concentrations were significantly greater over the durations of Study 1 and 3 within WRAS operated without ozone, i.e. 8.7 and 18.9 mg/L, respectively versus 3.4 and 3.5 mg/L, respectively within WRAS operated with ozone (Table 3). TSS levels measured within WRAS operated with O₃ were always within safe limits (< 20 mg/L) as recommended by literature (Table 3, 4). 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.

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 microfloculation of solids caused by O₃. Analysis of particle counts and particle size distributions supports the hypothesis of solids microfloculation and removal (Fig. 4). 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 (Fig. 4).

Figure 4. Particle size distribution of the culture water from RAS operated with and without ozone at low water exchange during Study 1.



Carbonaceous biochemical oxygen demand – During Study 1 and 3 BOD was significantly greater within WRAS operated without O₃, i.e., 3.6 and 11.8 mg/L, respectively versus 1.7 and 3.9 mg/L, respectively within WRAS operated with ozone (Table 3). BOD alone generally would not be expected to impact fish health, but it does provide a substrate for opportunistic pathogens such as flavobacteria, aeromonas hydrophila, pseudomonas, and streptococcus, as well as heterotrophic bacteria and protozoa that can inhibit nitrification efficiency of biofilters (Zhu and Chen, 2001). The BOD concentrations measured during the present studies negatively impacted biofilter

performance, as TAN removal efficiency tended to drop in the presence of relatively high BOD. 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 at least 1 log₁₀ lower within WRAS operated with O₃ during Study 1 and Study 3 (Table 3). Additionally, coliform bacteria counts measured during Study 1 were approximately 1 log₁₀ lower within WRAS operated with ozone. 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 refractory organic compounds via ozonation visually resulted in a very clear water quality environment as evidenced by values for true color that were orders of magnitude lower, as well as significantly greater ultraviolet (UV) transmittance during Studies 1 and 3 (Table 3, 4). 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, behavior, 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 were generally equal between treatments including oxygen, carbon dioxide, and temperature. ORP and alkalinity were also controlled, as discussed in the methods.

Table 3. Mean water quality values at the culture tank side drains for studies 1-3 over the duration of each study.

Treatment	Study 1		Study 2		Study 3	
	Low Exchange No Ozone	Low Exchange Ozone	High Exchange No Ozone	Low Exchange Ozone	Near Zero Exchange No Ozone	Near Zero Exchange Ozone
TAN ^{*2}	0.47 ± 0.01	0.45 ± 0.02	0.31 ± 0.02	0.45 ± 0.01	0.92 ± 0.09	0.72 ± 0.05
NH ₃	0.006 ± 0.000	0.005 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.008 ± 0.001	0.005 ± 0.000
Nitrite Nitrogen	0.05 ± 0.00	0.04 ± 0.01	0.11 ± 0.04	0.08 ± 0.00	0.13 ± 0.01	0.12 ± 0.05
Nitrate Nitrogen ^{*1, *2}	71 ± 1	84 ± 3	13 ± 0	99 ± 7	171 ± 16	323 ± 87
Alkalinity ^{*1, *2}	205 ± 1	196 ± 1	224 ± 3	200 ± 1	216 ± 3	208 ± 3
pH	7.66 ± 0.01	7.60 ± 0.02	7.61 ± 0.01	7.47 ± 0.01	7.54 ± 0.02	7.46 ± 0.02
CO ₂	10 ± 0	11 ± 1	10 ± 1	11 ± 0	14 ± 1	16 ± 0
cBOD ₅ ^{*1, *3}	3.6 ± 0.5	1.7 ± 0.1	2.5 ± 0.1	3.0 ± 0.2	11.8 ± 2.7	3.9 ± 0.2
TOC	15.9 ± 1.6	13.0 ± 1.3	11.2 ± 2.1	17.9 ± 2.8	-	-
DOC	15.3 ± 1.5	13.7 ± 1.4	9.0 ± 1.2	16.1 ± 1.6	-	-
True Color ^{*1, *2}	53 ± 2	4 ± 0	12 ± 0	5 ± 1	157 ± 25	5 ± 1
UV Transmitt. (%) ^{*1, *2}	60 ± 1	82 ± 0	89 ± 0	77 ± 2	30 ± 2	66 ± 4
Phosphorous ^{*2}	2.9 ± 0.0	3.0 ± 0.1	0.8 ± 0.0	3.9 ± 1.0	5.2 ± 0.1	7.4 ± 2.0
TSS ^{*1, *2, *3}	8.7 ± 1.8	3.4 ± 0.4	3.4 ± 0.1	4.6 ± 0.5	18.9 ± 1.1	3.5 ± 0.6
Coliform Bacteria	1.2 x 10 ⁴	3.3 x 10 ³	6.2 x 10 ³	7.2 x 10 ³	-	-
Heterotrophic Bacteria	2.0 x 10 ⁵	92	117 ± 23	114 ± 19	825 ± 407	77 ± 17
Temperature (°C)	15.1 ± 0.0	15.2 ± 0.0	12.9 ± 0.0	13.0 ± 0.1	15.6 ± 0.1	15.6 ± 0.0
DO ^{*2}	9.9 ± 0.0	9.8 ± 0.0	10.4 ± 0.0	10.6 ± 0.0	9.7 ± 0.0	11.0 ± 0.01
ORP ^{*1}	155 ± 1	248 ± 1	195 ± 8	238 ± 2	158 ± 12	269 ± 3

* Indicates statistically significant between treatments ($p < 0.10$), ^{1, 2, or 3} following * indicates study 1, 2, or 3

Table 4. Mean water quality values (mg/L) at the tank side drain outlets for studies 1-3 when WRAS were operated near maximum feed loading and fish density.

Treatment	Study 1		Study 2		Study 3	
	Low Exchange No Ozone	Low Exchange Ozone	High Exchange No Ozone	Low Exchange Ozone	Near-Zero Exchange No Ozone	Near-Zero Exchange Ozone
TAN ^{*2}	0.59 ± 0.03	0.53 ± 0.02	0.55 ± 0.06	0.71 ± 0.02	1.23 ± 0.19	0.93 ± 0.01
NH ₃	0.006 ± 0.000	0.005 ± 0.000	0.005 ± 0.000	0.005 ± 0.000	-	-
Nitrite Nitrogen	0.06 ± 0.01	0.05 ± 0.01	0.17 ± 0.12	0.12 ± 0.04	0.21 ± 0.05	0.08 ± 0.03
Nitrate Nitrogen ^{*2}	91 ± 1	90 ± 1	17 ± 1	108 ± 18	191 ± 28	373 ± 66
Alkalinity ^{*1, *2}	233 ± 4	208 ± 3	217 ± 4	187 ± 3	176 ± 24	182 ± 14
cBOD ₅ ^{*1, *3}	4.7 ± 0.9	1.8 ± 0.2	4.1 ± 0.7	4.7 ± 0.9	18.9 ± 6.2	4.3 ± 0.5
TSS ^{*1, *2, *3}	9.7 ± 1.4	4.7 ± 0.6	2.8 ± 0.2	5.1 ± 0.3	18.2 ± 5.9	3.5 ± 0.5
TOC	-	-	-	-	35.4 ± 7.1	28.8 ± 6.4
DOC	-	-	-	-	21.0 ± 2.6	18.6 ± 1.6
CO ₂	12 ± 0	12 ± 0	15 ± 1	16 ± 0	21 ± 1	23 ± 1
O ₂ ^{*3}	9.7 ± 0.2	9.8 ± 0.2	10.6 ± 0.1	10.7 ± 0.1	8.4 ± 0.1	9.1 ± 0.0
Temperature (°C)	13.9 ± 0.1	14.0 ± 0.1	13.8 ± 0.0	14.0 ± 0.1	16.0 ± 0.2	16.3 ± 0.1

* Indicates statistically significant between treatments ($p < 0.10$), ^{1, 2, or 3} following * indicates study 1, 2, or 3

Rainbow Trout Growth Performance – Rainbow trout growth was significantly greater within low exchange WRAS operated with O₃ at the conclusion of Study 1 (Fig. 6). Mean final weights for low exchange WRAS operated with and without O₃ were 1161 ± 12 and 993 ± 11 g, respectively. Rainbow trout within the ozonated WRAS became significantly larger than trout in WRAS without O₃ only one month after treatments were initiated despite being stocked at the same size (Fig. 5).

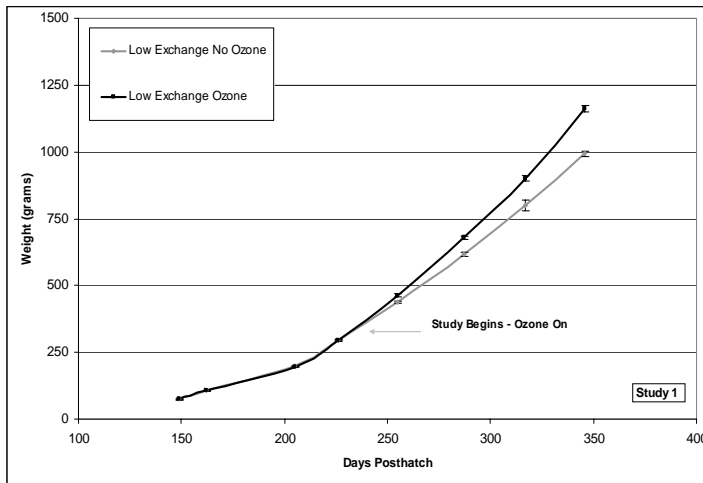


Figure 5. Rainbow trout growth rates in low exchange WRAS operated with and without ozone.

During Study 2 rainbow trout growth was similar within WRAS operated at high water exchange without O₃ and WRAS operated with low water exchange with O₃. 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 (Fig. 6). Thus, rainbow trout performance within the low exchange WRAS with ozone was similar to that of a high exchange system without ozone, despite the ten-fold difference in flushing rate.

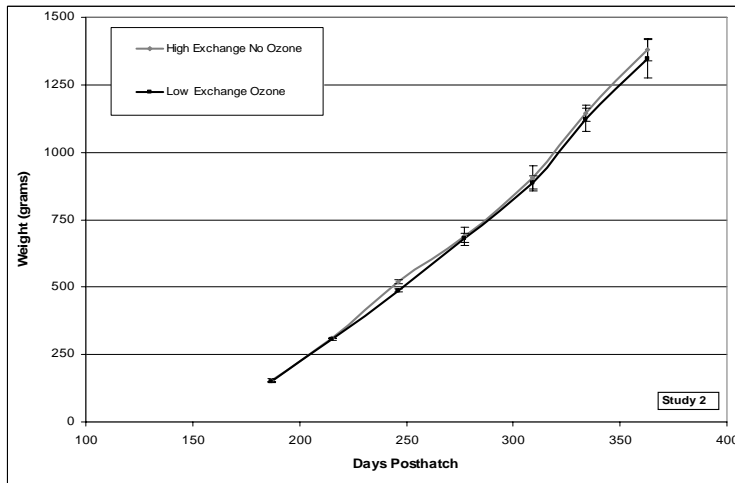


Figure 6. Rainbow trout growth rates in high exchange WRAS without ozone and low exchange WRAS with ozone.

During Study 3 rainbow trout cultured within near-zero exchange WRAS operated with O₃ 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 (Fig. 7). Unfortunately, failure of the backwash controller of four of six drum filters created differences in water flushing rates amongst WRAS that likely masked potentially greater statistical growth for trout cultured within systems with ozone.

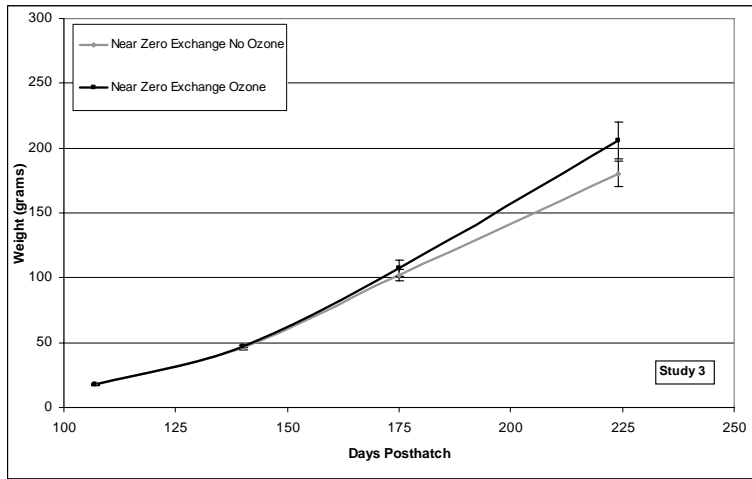


Figure 7. Rainbow trout growth rates in near-zero exchange WRAS with and without ozone.

Rainbow trout feed conversion and condition factor were generally better within WRAS operated with ozone during each study.

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 (Table 7). 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 O₃ was 93.3 ± 1.6 % and 93.1 ± 0.5 % in WRAS operated at high exchange without ozone (Table 7). Rainbow trout survival was similar between WRAS operated at near-zero exchange with and without O₃ during Study 3. Survival within WRAS operated at near-zero exchange with and without O₃ was 88.8 ± 3.3 % and 94.5 ± 0.5 %, respectively, but was not statistically different between treatments (Table 7). The lower survival for the WRAS operated with ozone during Study 3 was attributed to mean nitrate nitrogen concentrations that were >400 mg/L within two of three WRAS.

Table 7. Cumulative rainbow trout survival for each treatment over the duration of Studies 1-3.

Study	Treatment (Flushing Rate)	Treatment	Cumulative Survival (%)
1	Low Exchange	Ozone	99.3 ± 0.2
1	Low Exchange	No Ozone	98.3 ± 0.5
2	Low Exchange	Ozone	93.3 ± 1.6
2	High Exchange	No Ozone	93.1 ± 0.5
3	Near Zero Exchange	Ozone	88.8 ± 3.3
3	Near Zero Exchange	No Ozone	94.5 ± 0.5

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 O₃ effectively reduced accumulating metals within low and near-zero exchange WRAS, particularly copper. 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. Ozone did not prevent nitrate nitrogen concentration. Nitrate nitrogen accumulated to approximately 100 mg/L in WRAS operated at low exchange and >400 mg/L in WRAS that were maintained at near-zero water exchange without additional flushing caused by drum filter failure. Rainbow trout mortality was greater and fish exhibited erratic behavior in WRAS with mean nitrate nitrogen of 400+ mg/L. Nitrate nitrogen has generally been thought to be harmless to fish at levels below 1,000 mg/L; however, there has not been definitive research to prove this. The results of the present studies indicate that previous assumptions could be inaccurate and additional research is necessary to better define nitrate nitrogen concentrations that cause sublethal to chronic toxicity for salmonids and other cultured species. Controlled studies focusing specifically on accumulating nitrate nitrogen as a limiting factor will be conducted at the Freshwater Institute in the near future. If nitrate nitrogen is proven to be a limiting factor, as the present research suggests, then low and near-zero exchange WRAS operated at high feed loading will need to be equipped with unit processes capable of denitrification.

Overall, 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. We conclude that ozone is a vital component for the operation of low and near-zero exchange WRAS for the culture of salmonids and other species that require relatively clean water.

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