Heavy metal and waste metabolite accumulation and their potential effect on rainbow trout performance in a replicated water reuse system operated at low or high system flushing rates

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1. Introduction

Anecdotal evidence from previous un-replicated experiments at the Freshwater Institute suggests that rainbow trout mortality increases and fish health declines as make-up water exchange rate in a water reuse aquaculture systems (WRAS) is reduced below a feed loading of 1.3–2.0 kg/day per m³/day of make-up water flow (Fig. 1). The decline in fish health was unrelated to infectious disease, but occurred when typical water quality concentrations (Table 1) were within safe limits. Fish health improved when ozone was added to the system or when make-up water flow rate was increased. Similar observations have been reported for hybrid striped bass and tilapia cultured in WRAS, but not necessarily at the same feed loading rate (Brazil, 1996). These observations...
suggest a need to examine a wide range of water quality variables in WRAS operated at low water exchange and a high feeding rate to determine their potential to negatively impact rainbow trout.

Metals toxicity (cadmium, copper, zinc, lead, selenium, and others) is a potential consideration in WRAS operated at a low flushing rate. These metals could enter the WRAS with the feed as part of the vitamin/mineral premix (i.e., copper and zinc), could leach from pipes and fittings, or could be carried into the system with the make-up water (Wedemeyer, 1996; Colt, 2006). Metals could potentially accumulate by operating WRAS at a reduced water flushing rate. Wedemeyer (1996) reports that the toxic effects of zinc and copper often result in sudden mortalities that occur for no apparent reason, with typical water chemistry analysis indicating normal background concentrations. Wedemeyer’s observations relative to metals toxicity are consistent with the anecdotal observations within the commercial scale WRAS, i.e., mortality for no apparent reason (pathology) and normal background water chemistry.

Additionally, populations of obligate or opportunistic pathogens can increase in WRAS and create fish health problems. Fish health problems could also result from the accumulation of high concentrations of fine suspended solids, fish excretory products, and by-products of bacterial or fungal metabolism, including components of dissolved organic carbon or dissolved inorganic compounds (Bullock et al., 1994, 1997; Noble and Summerfelt, 1996; Wedemeyer, 1996). However, little information is available to clearly define the effects of non-microbiological components on fish health. A more detailed understanding of the interaction between fish health/performance and the culture environment in WRAS, as well as insight into how to control the WRAS environment, would clearly help to overcome obstacles associated with large-scale culture tanks operated at high feed loading at low exchange rates.

This study was designed to determine the effects of high and low make-up water flushing rates on rainbow trout performance and water quality in replicated water reuse aquaculture systems. The primary objective of this study was to determine if specific water quality constituents accumulate to unsafe levels for fish health within WRAS, as measured by fish growth rate and survival. Rainbow trout histopathology and blood chemistry results from this study are reported elsewhere (Good et al., in press).

2. Methods

2.1. System description

Six identical 9.5 m³ recirculating systems (Fig. 2), three WRAS per treatment, were used for this study. In summary, each system recirculated 380 L/min (100 gpm) of water through the culture tank, a microscreen drum filter installed with 60 μm sieves, a forced-ventilated cascade aeration column, and a low head oxygenation (LHO) unit where pure oxygen feed gas was added to maintain dissolved oxygen concentration near saturation within the culture tank. Approximately 60% of the total recirculated flow in each system was pumped through a CycloBio™ fluidized-sand biofilter (FSB), while the remaining 40% of the recirculating flow was pumped through a stainless steel flat plate heat exchanger. Flows through each heat exchanger were counter-current, but not directly contacting ground water, to cool the recycle systems. The recirculating flow exchanged the culture tank water volume once every 15 min. Approximately 15% of the total flow through the culture tank (57 L/min) was carried from the bottom drain to a radial flow settler with a 60° cone-bottom to concentrate settled solids for daily flushing. Supernatant from the radial flow settler was combined with relatively clean water flowing from the culture tank’s side drain (approximately 85% of the culture tank flow) before being piped to a microscreen drum filter. Make-up water was added to the system within the pump sump. Make-up flows were measured and calibrated several times per week.

![Fig. 2. Process flow drawing of the replicated 9.5 m³ water recirculating system used in the present study.](image-url)
2.2. Experimental conditions

Rainbow trout (133 ± 1 g) were stocked at a density of approximately 25 kg/m³ (1000 fish per tank). High and low water exchange rates (i.e., 2.6 and 0.26% of total recirculating flow) were evaluated using three replicated recirculating systems per treatment. These make-up rates provided total system hydraulic retention times of 0.67 days for the high exchange WRAS and once every 6.7 days for the low exchange WRAS. A constant 24-h photoperiod was provided. All tanks were fed equal portions, every other hour, around the clock using automated feeders (T-drum 2000CE, Arvo-Tec, Finland). Feeding was estimated based on standardized feeding charts, as well as observations of feeding activity and wasted feed. Feeding rates ranged from 1.5 to 2.0% relative to fish body weight. During the first two months of the study, a slow-sinking, relatively “high energy” trout diet (Zeigler Brothers, Inc., Gardners, PA, USA) with a 45:20 protein–fat ratio was fed. Thereafter, a standard slow-sinking trout diet (Zeigler Brothers, Inc., Gardners, PA, USA) with a protein:fat ratio of 42:16 was used due to unavailability of the previous diet. The latter diet was in use when the fish reached maximum feed loading and water samples were collected to characterize treatment process performance. Mean feed loadings of 0.39 and 4.1 kg/m³ make-up water flow were maintained for the high and low exchange treatments, respectively. The feed loading in the high exchange WRAS was not exactly 10-fold different from the low exchange WRAS, due to slight variations in the actual daily make-up water flow rate vs. the target flow rate in each WRAS over the six-month study.

2.3. Sampling protocols

Water samples were collected weekly from the side drain of each tank and tested for alkalinity, carbon dioxide (CO₂), carbonaceous biochemical oxygen demand (cBOD₅), dissolved organic carbon (DOC), nitrite nitrogen, nitrate nitrogen, total phosphorous, true color, total ammonia nitrogen (TAN), total organic carbon (TOC), total suspended solids (TSS), and UV transmittance. Particle count and particle size distribution were measured weekly using a 2200 PCX Particle Counter (Hach Company, Loveland, CO, USA). All water quality parameters were measured according to methods described in APHA (2005) and HACH (2003). Dissolved oxygen, pH, and temperature were monitored continuously using a SC100 Universal Controller (Hach Company, Loveland, CO, USA) and kept equal between systems. Additionally, alkalinity was controlled within low exchange systems by adding sodium bicarbonate (NaHCO₃) at a rate of 0.15 kg NaHCO₃ per kg feed. During a one-week period when fish had reached maximum feed levels (7.3 kg/day/tank) and densities (80 kg/m³) near the middle of the study, water samples were collected across all unit processes to compare water quality and unit process removal efficiencies between treatments. Mean feed loadings for this week were 0.53 and 5.3 kg/m³ make-up water flow for high and low make-up conditions, respectively. Samples for total heterotrophic bacteria plate counts (Hydrochem Laboratories, Inc., Shenandoah Junction, WV, USA) and a suite of 28 metals/elements analyzed using the Inductively Coupled Plasma-Atomic Emission Spectrometry technique (Cornell Nutrient Analysis Laboratory, Ithaca, NY, USA) were collected near the end of the study when feed loading rates were just below maximum levels.

Fish were sampled for length and weights on a monthly basis and mortalities were removed and recorded daily. A 5% cumulative survival. Sample size ranged from 50 to 120 fish and was calculated as follows: \( n = \left( \frac{Z \times \text{stdev grams}}{ \text{accepted error grams}} \right)^2 \), where \( Z = 1.65 \) (relative to a 90% confidence interval) and accepted error was 5 g. Fish were reared to a maximum density of 80 kg/m³ and periodically thinned to approximately 50 kg/m³. The study was conducted for six months.

2.4. Statistical analyses

All water quality parameters that were sampled during multiple events over time at the same location were analyzed using multivariate repeated measures analysis of variance (MANOVA). Growth parameters were also analyzed using MANOVA. Metals and cBOD₅ sampling was conducted as a one-time event during the week at maximum feed loading and density; therefore, a Student’s t-test or Mann–Whitney U-test was used for statistical analysis of metals, depending upon normality. Linear regression analysis was used to analyze potential relationships between water quality parameters and mortality. A probability level (α) of 0.05 was used to determine significance for each statistical test. Statistical analyses were carried out using SYSTAT 11 software (2004).

3. Results and discussion

3.1. Culture tank water quality and potential effects on fish health

The constant 24-h photoperiod and uniformly dispersed feeding events produced a relatively constant biological respiration rate as indicated by the nearly constant mean 24-h O₂ demand across the culture tank (Fig. 3) measured over a 96-h period. The nearly constant mean 24-h O₂ demand across the culture tank produced a quasi-steady state, i.e., constant waste production rate that created relatively consistent water quality concentrations throughout the day. Consistent water quality through time was important since all samples could not be collected simultaneously.

3.1.1. Metals/element analysis

Of the 28 metals/elements analyzed, the following were below the minimum detection limit: aluminum, antimony, arsenic, beryllium, cadmium, iron, lead, manganese, mercury, molybdenum, nickel, selenium, and titanium. Of those detectable, there were no significant differences between the high and low exchange systems for chromium, cobalt, and vanadium (Table 2). The low exchange systems had significantly greater concentrations of barium, calcium, and strontium (Table 2). Suggested metal concentrations for optimal salmonid health are provided in
The resulting acute and chronic copper toxicity limits relative to a concentration that would indicate an acute toxic effect. However, mortality did not result to a degree of concern.

L) measured in the low exchange WRAS exceeded the calculated toxicity limits for copper by as much as fourfold (U.S. EPA, 2007). Therefore, the increased DOC levels likely prevented an acute toxic effect to the rainbow trout cultured within the low exchange WRAS. The safe copper levels derived with the BLM are similar to those recommended by Alabaster and Lloyd (1980) for rainbow trout, which define a safe level of copper at a hardness of 300 as 0.28 mg/L. However, we hypothesize that the highest copper levels measured within the low exchange WRAS could still have been chronically toxic, since chronic toxicity data for copper is not well defined or absent from the literature. Several studies have shown that copper levels similar to those measured within the low exchange WRAS (i.e., 0.040–0.225 mg/L Cu, hardness 100–448) have caused sublethal effects in rainbow trout such as ionic imbalance, increased oxygen consumption, reduced critical swimming speed, increased energetic costs, reduced feeding, short term growth inhibition, biochemical stress response, and low-level mortality (Lett et al., 1976; Dixon and Hilton, 1985; McGreer et al., 2000; Besser et al., 2007). In the majority of these studies rainbow trout acclimated to the increased copper levels and long term growth and survival rates were not negatively affected.

In the current study, the copper levels measured at maximum feed loading did not seem to have an effect on rainbow trout growth, as there were no significant differences in growth between high and low exchange treatments. However, statistical analysis of survival indicated a p-value (0.0495) bordering significance, with slightly greater mortality occurring within the low exchange WRAS. Therefore, an analysis of a potential relationship between copper concentration and mortality was conducted. Linear regression indicated a potential correlation between copper concentration (measured at maximum feeding and density) and survival percentage/mortality (p = 0.016, R² = 0.80) (Table 3). The linear regression was based on just one sampling event and assumes that differences in copper concentration between WRAS
were consistent throughout, and therefore is not extremely robust. However, it is at least interesting to note that WRAS 6, which had the greatest dissolved copper concentrations (0.056 mg/L), also had the greatest cumulative mortality (16) and thus the lowest survival rate (98.4%); and WRAS 1, for which no copper was detected, had the least cumulative mortalities (3) and thus the greatest survival (99.7%) (Table 3). Metals analysis was not conducted during previous low exchange trials in a commercial scale system at the Freshwater Institute, in which increasing but low-level mortality occurred with no apparent pathogenic agent. Therefore, it is reasonable to hypothesize that an accumulating metal such as copper could have contributed to increased mortality at low exchange rates within the commercial scale WRAS.

Copper piping was not used within the WRAS and make-up water contained only 0.004 ± 0.002 mg/L copper. However, copper proteinate was an ingredient in the feed; therefore, accumulation of copper in the WRAS was likely related to feed. The copper proteinate could end up in fish tissue, fecal matter, biofilm, or the water column. We cannot explain the phenomenon regarding the release of copper from feed into water. However, we can use a steady state mass balance to quantify the concentration of copper that accumulates within the system water, i.e., \( \frac{Cu_{out}}{Q} \) (mg/L), by subtracting the difference in the concentration of copper that washes out of the WRAS in its over-topping flow, i.e., \( Cu_{in} \) (mg/L), from the concentration of copper entering the system in the make-up water, i.e., \( Cu_{in} \) (mg/L):

\[
\frac{Cu_{in}}{Q} \left( \frac{10^6 \, \text{mg}}{1 \, \text{kg}} \right) \left( \frac{1 \, \text{day}}{1440 \, \text{min}} \right) = Cu_{out} - Cu_{in}
\]

where Q is the make-up water flow rate (L/min) through the WRAS. The mass balance assumes that some of the copper is incorporated into fish tissue or carried out of the system with backwashed biosolids and does not interact with the system water quality. Water samples collected from the culture tank indicate that approximately 56% of the zinc within the feed was released into solution, accumulating within culture water, and eventually discharging with the WRAS overtopping flow. Backwashed biosolids, collected in the present study and again in a later study, were tested and found to contain approximately 0.5–3.4 mg copper per kg dry solids. Unfortunately, fish tissue copper concentrations were not measured and the flow rates and TSS concentrations of the backwashed solids were not tracked; thus, a more comprehensive mass balance on copper could be completed.

Zinc was found in higher concentrations within the make-up water (0.013 mg/L) than was copper (0.004 mg/L). Water samples collected from the culture tank indicate that approximately 56% of the zinc in these samples was dissolved. The steady-state mass balance indicates that less than 1 mg/day of zinc was produced in the system’s over-topping flow in the high and low flushing systems, respectively. Fish feed contained 130 mg zinc per kg dry weight. Thus, much less than 1% of the zinc within the feed was released into solution and was not attributed to contributing to the zinc in the culture water. Backwashed biosolids, collected in the present study and again in a later study, were found to contain 1–13 mg zinc per kg dry solids. As with copper, a more comprehensive mass balance on zinc could not be completed.

Regardless of the origin of these metals, it is apparent that metals can accumulate to potentially harmful levels within low exchange WRAS and should be monitored. Interestingly, metal analyses of sludge waste at the Freshwater Institute indicates that a large portion of metals that are removed from the WRAS are particle bound. Future research will determine if ozonation of the recirculating water influences the accumulation of copper or other metals/elements and if the copper proteinate content of feed can be reduced without decreasing rainbow trout performance or health.

### 3.1.2. Nitrogenous waste

TAN concentrations were significantly different between the high and low exchange treatments at maximum feed loading, 0.47 ± 0.02 and 0.84 ± 0.09 mg/L, respectively (Table 4). Total ammonia nitrogen (TAN) is a composite measurement of the two ammonia species, the ionized (NH₄⁺) form and the un-ionized (NH₃) form, which is more toxic to fish. Concentrations of NH₃-N were significantly greater within the low exchange WRAS during maximum feed loading and density as well as throughout the study (Tables 4 and 5 and Fig. 4). However, mean NH₃-N concentrations measured within the low exchange WRAS at high feed loading and throughout the study did not exceed 0.004 mg/L (Tables 4 and 5) which is well below recommended limits for salmonids. Piper et al. (1982) recommended an upper limit of 0.0125 mg/L and Wedemeyer (1996) recommended an upper limit of 0.02–0.03 mg/L for salmonids. Recent literature suggests that un-ionized ammonia levels within commercial production systems could be >0.04 mg/L without negative consequences to gills (Colt, 2006). In any case, the mean un-ionized ammonia levels measured within the low exchange WRAS at high feed loading and throughout the study were well below these limits.

Due to efficient nitrification within the fluidized-sand biofilters, nitrite nitrogen levels, i.e., 0.030 ± 0.005 and 0.013 ± 0.005 mg/L in the high and low exchange WRAS, respectively, were well below recommended maximum safe limits at maximum feed loading and density (Table 4). Nitrite nitrogen was significantly greater within the low exchange WRAS during the week at maximum feed loading and density, as well as throughout the study (Tables 4 and 5 and Fig. 4). Piper et al. (1982) suggest an upper limit for nitrite nitrogen of 0.2 mg/L in hard water. However, the presence of calcium and chloride ions as well as the pH of the culture water can buffer the toxic effect of nitrite nitrogen (Wedemeyer and Yasutake, 1978; Colt and Tomasso, 2001; Wedemeyer, 1996). Observations of nitrite nitrogen toxicity during biofilter acclimation at the Freshwater Institute have

**Table 3**

<table>
<thead>
<tr>
<th>Recirculating system</th>
<th>High or low exchange?</th>
<th>Copper (mg/L)</th>
<th>Cumulative mortalities</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRAS 1</td>
<td>High</td>
<td>0.000</td>
<td>3</td>
<td>99.7</td>
</tr>
<tr>
<td>WRAS 2</td>
<td>Low</td>
<td>0.037</td>
<td>8</td>
<td>99.2</td>
</tr>
<tr>
<td>WRAS 3</td>
<td>Low</td>
<td>0.041</td>
<td>8</td>
<td>99.2</td>
</tr>
<tr>
<td>WRAS 4</td>
<td>High</td>
<td>0.011</td>
<td>7</td>
<td>99.3</td>
</tr>
<tr>
<td>WRAS 5</td>
<td>High</td>
<td>0.005</td>
<td>4</td>
<td>99.6</td>
</tr>
<tr>
<td>WRAS 6</td>
<td>Low</td>
<td>0.056</td>
<td>16</td>
<td>98.4</td>
</tr>
</tbody>
</table>
shown that rainbow trout do not exhibit brown blood disease until nitrate nitrogen concentrations equal or exceed 0.8 mg/L, at a total hardness of approximately 300 mg/L as CaCO₃ (Noble and Summerfelt, 1996).

Nitrate nitrogen concentrations were 99 ± 3 mg/L during the week at maximum feed loading within the low exchange WRAS compared to 14 ± 0 mg/L within the high exchange systems (Table 4). The nitrate nitrogen concentration measured in a WRAS is an indicator of the intensity of water reuse when no active denitrification process is provided, as is the case here, because nitrate nitrogen accumulation is directly proportional to the feeding rate and the mean hydraulic retention time of the system. Thus, nitrate nitrogen can accumulate to relatively high concentrations within WRAS with low make-up water rates. Fig. 4 illustrates the accumulating nitrate nitrogen and differences in nitrate nitrogen concentration between the two treatments over the six-month study period. During the week at maximum feed loading, nitrate nitrogen concentrations were almost ten times greater within the low exchange systems compared to 14 ± 0 mg/L within the high exchange systems (Table 4). Nitrate nitrogen concentrations were not expected to be toxic to rainbow trout as the 96-h LC₅₀ for rainbow trout is 1360 mg/L (Westin, 1974). Losordo (1991) reported a recommended upper limit for nitrate nitrogen of 1000 mg/L within recycle systems, but the maximum level would certainly depend upon fish species. Results from the present study indicated that nitrate levels of approximately 100 mg/L within the low exchange WRAS did not cause any noticeable adverse effects on rainbow trout. However, more research regarding the chronic effects of accumulating nitrate on fish cultured within recirculating systems is needed.

### 3.1.3. Alkalinity

Alkalinity was maintained well within safe limits, but was significantly different between the high and low exchange treatments (192 ± 3 and 163 ± 4), respectively, at maximum feed loading (Table 4). Alkalinity was also significantly different between treatments as measured throughout the study (Table 5). However, alkalinity alone is not expected to threaten fish health. Alkalinity interacts with other water quality parameters, preventing wide swings in pH and carbon dioxide, as well as buffering the effects of toxicants such as nitrite nitrogen and metals (Wedemeyer, 1996). At very low alkalinities, water loses its capacity to buffer the toxic effects of heavy metals, pH, and CO₂ (Piper et al., 1982). In recirculating systems that use biofiltration to control the accumulation of TAN, alkalinity is consumed during the nitrification process and must be supplemented to maintain homeostatic conditions (Loyless and Malone, 1997). During the current study, 0.15 kg of sodium bicarbonate was added per kg of feed delivered in the low make-up systems. Piper et al. (1982) and Heinen (1996) recommend optimal alkalinity levels of 100–400 mg/L for fish culture, whereas alkalinities of at least 80 mg/L are required to prevent a reduction in nitrification (Paz, 1984). The alkalinity levels measured during the present study were not expected to impact fish health or nitrification (Tables 4 and 5).

### 3.1.4. Total suspended solids and particles

Total suspended solids were significantly different between the high and low exchange treatments, 3 and 14 mg/L, respectively, during the week of sampling at maximum feed loading (Table 4). Total suspended solids were also significantly different between treatments as measured throughout the study (Table 5). Suggested TSS criteria for salmonids range from <15 to 2000 mg/L (Heinen, 1996).

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**Table 4**

Mean water quality values at the tank side drain outlets for high and low water exchange treatments when the system was operated at maximum feed loading and fish density during study #1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>High exchange</th>
<th>Low exchange</th>
<th>Recommended limits (mg/L)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAN (mg/L)b</td>
<td>0.47 ± 0.02</td>
<td>0.84 ± 0.09</td>
<td>–</td>
</tr>
<tr>
<td>Un-ionized ammonia (mg/L)b</td>
<td>0.002 ± 0.000</td>
<td>0.004 ± 0.000</td>
<td>–0.012–0.030</td>
</tr>
<tr>
<td>Nitrate nitrogen (mg/L)b</td>
<td>0.030 ± 0.005</td>
<td>0.013 ± 0.005</td>
<td>–0.2–1.0 in hard water</td>
</tr>
<tr>
<td>Nitrate nitrogen (mg/L)b</td>
<td>14 ± 0</td>
<td>99 ± 3</td>
<td>&lt;1000</td>
</tr>
<tr>
<td>Alkalinity (mg/L)b</td>
<td>192 ± 3</td>
<td>163 ± 4</td>
<td>10–18 (optimum)</td>
</tr>
<tr>
<td>eCODs (mg/L)b</td>
<td>3 ± 0</td>
<td>13 ± 1</td>
<td>NA</td>
</tr>
<tr>
<td>TSS (mg/L)b</td>
<td>3 ± 0</td>
<td>14 ± 0</td>
<td>&lt;80 mg/L</td>
</tr>
<tr>
<td>CO₂ (mg/L)</td>
<td>11 ± 0</td>
<td>13 ± 1</td>
<td>&lt;25</td>
</tr>
<tr>
<td>O₂ (mg/L)</td>
<td>9.8 ± 0.1</td>
<td>9.2 ± 0.2</td>
<td>6.5 mg/L (saturating)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>12.4 ± 0.0</td>
<td>12.3 ± 0.1</td>
<td>NA</td>
</tr>
<tr>
<td>Total organic carbon (mg/L)b</td>
<td>4.64 ± 0.14</td>
<td>20.52 ± 1.65</td>
<td>NA</td>
</tr>
<tr>
<td>Dissolved organic carbon (mg/L)b</td>
<td>2.61 ± 0.14</td>
<td>17.29 ± 0.97</td>
<td>NA</td>
</tr>
<tr>
<td>True color (Pt–Co units)b</td>
<td>16 ± 1</td>
<td>103 ± 5</td>
<td>NA</td>
</tr>
<tr>
<td>UV transmittance (%)b</td>
<td>86 ± 0</td>
<td>45 ± 1</td>
<td>NA</td>
</tr>
<tr>
<td>Heterotrophic bacteria (cfu/mL)b</td>
<td>1016 ± 286</td>
<td>5487 ± 1203</td>
<td>NA</td>
</tr>
</tbody>
</table>


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**Table 5**

Mean water quality values at the side drain outlets for high and low water exchange tanks over the six-month duration of the study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>High exchange</th>
<th>Low exchange</th>
<th>Recommended limits (mg/L)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAN (mg/L)a</td>
<td>0.29 ± 0.00</td>
<td>0.48 ± 0.05</td>
<td>–</td>
</tr>
<tr>
<td>Un-ionized ammonia (mg/L)a</td>
<td>0.002 ± 0.000</td>
<td>0.004 ± 0.000</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate nitrogen (mg/L)a</td>
<td>0.04 ± 0.01</td>
<td>0.10 ± 0.00</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate nitrogen (mg/L)a</td>
<td>12 ± 0</td>
<td>70 ± 4</td>
<td>–</td>
</tr>
<tr>
<td>Alkalinity (mg/L)a</td>
<td>226 ± 1</td>
<td>214 ± 1</td>
<td>–</td>
</tr>
<tr>
<td>CO₂ (mg/L)</td>
<td>7 ± 0</td>
<td>9 ± 0</td>
<td>–</td>
</tr>
<tr>
<td>eCODs (mg/L)a</td>
<td>2 ± 0</td>
<td>6 ± 1</td>
<td>–</td>
</tr>
<tr>
<td>True color (Pt–Co units)a</td>
<td>11 ± 1</td>
<td>74 ± 9</td>
<td>–</td>
</tr>
<tr>
<td>UV transmittance (%)a</td>
<td>89 ± 0</td>
<td>53 ± 4</td>
<td>–</td>
</tr>
<tr>
<td>Phosphorous (mg/L)a</td>
<td>0.52 ± 0.01</td>
<td>3.11 ± 0.22</td>
<td>–</td>
</tr>
<tr>
<td>TSS (mg/L)a</td>
<td>16 ± 0.1</td>
<td>16 ± 1.2</td>
<td>–</td>
</tr>
<tr>
<td>Total particles (0–60 μm)a</td>
<td>6618</td>
<td>13,849</td>
<td>–</td>
</tr>
</tbody>
</table>

³ Significant difference between high and low exchange WRAS.
Suspended solids within recirculating systems can originate from a number of sources, including feed and fecal particles, detached biofilm, clay, silt, and precipitates, such as calcium carbonate. In the present study, suspended solids likely originated from feed, fecal matter, and detached biofilm. Based on these studies, the concentration of suspended solids measured within low exchange WRAS (14 mg/L during the week at max feed loading) could have caused minor damage to gill tissue; however, Good et al. (in press) found no significant differences in gill damage between the high and low exchange treatments.

Mean total particle counts (2–60 μm) collected throughout the study were two times greater within the low exchange WRAS vs. the high exchange WRAS, i.e., 13,849 vs. 6,618 counts/mL, respectively (Table 5). The majority of particles numbers were sized ≤20 μm, indicating that the 60 μm microscreen filters captured almost all of the particles ≥20 μm. Particle size distributions of culture water from the WRAS in this study were similar to the findings of Patterson et al. (1999), who reported that a greater number of fine particles are present within recirculating systems.

3.1.5. Carbonaceous biochemical oxygen demand

Carbonaceous BOD is an indirect measurement of organic concentration and the resulting oxygen demand by microorganisms that consume organic matter. Carbonaceous BOD within the high and low exchange treatments was significantly different during the week of sampling at maximum feed loading, 3 and 13 mg/L, respectively (Table 4). Carbonaceous biochemical oxygen demand was also significantly different between treatments over the course of the study (Table 5). Carbonaceous BOD concentrations tracked TSS concentrations (Tables 4 and 5), which would be as expected if the majority of carbonaceous BOD was associated with particles. These results suggest that little dissolved carbonaceous BOD accumulation occurred, even in the low exchange WRAS, likely because the FSBs were effective at removing the dissolved carbonaceous BOD. Carbonaceous BOD alone does not impact fish health, but it does provide a substrate to support growth of heterotrophic bacteria and protozoa. Carbonaceous BOD can also inhibit WRAS performance, particularly nitrification efficiency of biofilters (Zhu and Chen, 2001) which could lead to suboptimal ammonia or nitrite nitrogen concentrations. Fortunately, the resulting TAN and nitrite nitrogen concentrations were never a concern.

3.1.6. Total heterotrophic bacteria plate count

Total heterotrophic bacteria counts from samples taken from the side drain of the tanks showed that there were significantly greater total heterotrophic bacteria plate counts (approximately five times more) in the low exchange treatment, i.e., 5,487 ± 1203 cfu/mL, than in the high exchange treatment, i.e., 1,016 ± 286 cfu/mL (Table 4). Histopathology of the spleen, heart, and skin indicated inflammation and other pathology that is typically associated with increased immune challenge (Good et al., in press). The greater bacteria challenge presented within the low exchange WRAS could have contributed to the observed pathology. Thus, the accumulating heterotrophic bacteria loads that occur when WRAS are operated at low make-up water exchange rates could be problematic, and control should be considered through ozonation and/or UV filtration.

Additionally, elevated populations of heterotrophic bacteria can outcompete autotrophic bacteria that are responsible for nitrification and thus lead to suboptimal ammonia and nitrite nitrogen concentrations within WRAS (Zhu and Chen, 2001; Ling and Chen, 2005; Michaud et al., 2006). In the present study, nitrification was inhibited within the low exchange WRAS; however, TAN and nitrite nitrogen concentrations remained within safe recommended limits.
3.1.7. Other water quality parameters

Carbon dioxide was controlled and was $9 \pm 0$ mg/L for both treatments during the six-month study (Table 5). During the week of intensive water sampling CO$_2$ was $11 \pm 0$ and $13 \pm 1$ mg/L for the high and low exchange treatments, respectively (Table 4). These CO$_2$ concentrations are not expected to negatively impact rainbow trout growth or survival (Wedemeyer, 1996; Westers, 2001; Danley et al., 2005). Carbon dioxide was $30 \pm 2$ mg/L during previous studies operating the commercial scale recirculating system at the Freshwater Institute at low water exchange (Table 1), when increased mortality occurred. Chronic CO$_2$ concentrations $\geq 34$ mg/L could negatively impact rainbow trout growth (Danley et al., 2005), but alone have not been shown to cause mortality. An additional study using the same WRAS operated at approximately 10 and 25 mg/L CO$_2$, with both treatments at low water exchange, has been conducted and will be submitted for publication in the near future.

Other water quality concentrations were significantly greater within the low exchange WRAS, including total organic carbon, dissolved organic carbon, true color, and phosphorous. However, there is no literature that suggests that these water constituents would negatively impact rainbow trout growth or survival. Additionally, UV transmittance was significantly lower within the low exchange WRAS, but like true color this is simply a reflection of water clarity and would not directly impact rainbow trout.

3.2. Growth and survival

Growth rates and mean final size were exceptional for both the high and low exchange WRAS and were similar between treatments. Final rainbow trout weights (372 days post-hatch) for the high and low exchange WRAS at the conclusion of the study were $1401 \pm 23$ and $1366 \pm 33$ g, respectively ($p_{treat} = 0.402; p_{time\times treat} = 0.768$). Mean thermal growth coefficients for rainbow trout in the high and low exchange WRAS over the six-month study were $2.64 \pm 0.13$ and $2.62 \pm 0.17$, respectively. The growth curves overlapped for much of the study and began to diverge slightly over the final month (Fig. 5), with the high exchange trout growing slightly faster than the low exchange trout. This slight divergence in growth could have been a potential indication of a chronic impact of some of the higher water quality concentrations such as dissolved copper, TSS, fine particles, heterotrophic bacteria, or a combination of these parameters.

Interestingly, rainbow trout condition factor was generally greater within the low exchange treatment, despite relatively equal fish weights and growth (Fig. 6). Mean condition factors for the high and low exchange treatments to conclude the study were $2.12 \pm 0.02$ and $2.22 \pm 0.06$, respectively (Fig. 6). Significant differences in mean condition factor were detected when comparing treatments as well as a time $\times$ treatment interaction ($p_{treat} = 0.047; p_{treat\times time} = 0.038$). The authors have observed increased swimming speed and exercise within low exchange WRAS (unpublished data), which could have cause increased muscle mass and thus condition factor. More research is needed to investigate whether low exchange WRAS increases swimming speed in comparison to high exchange WRAS.

Additionally, there were no significant differences in feed conversion ratios (Fig. 7) between treatments throughout the study ($p_{treat} = 0.236; p_{time\times treat} = 0.901$). However, FCRs were generally better for the high exchange treatment (Fig. 7). Mean feed conversion ratios over the duration of the study were $1.38 \pm 0.02$ and $1.41 \pm 0.05$ for the high and low exchange treatments, respectively. Feed conversion ratios could have increased over time for both treatments for a variety of reasons including: a change in feed formulation (i.e., after being forced to switch from 45:20 to 42:16 protein-fat when the higher energy feed was no longer available) at day 257 post-hatch (Fig. 7), purposeful intensive feeding, increasing fish size, and decreasing fish numbers relative to density control.

Cumulative survival for the six-month study was $99.5 \pm 0.1$ and $98.9 \pm 0.4\%$, respectively, for the high and low exchange treatment ($p = 0.0495$). Since the $p$-value only borders significance the authors are hesitant to declare a real difference in mortality between treatments. As previously mentioned linear regression analysis of
copper concentration and mortality indicated a significant relationship, with the highest cumulative mortality occurring within a low exchange WRAS that had the highest copper concentration (Table 3). Although some low-level mortality occurred during the present study, anecdotal observations of increasing mortality and declining fish health within our commercial scale WRAS operated at low exchange were not replicated during the present study at the same severity. Dissolved metals were not evaluated within the commercial scale WRAS when these observations occurred. Based on results from the present study, we hypothesize that accumulating copper levels could have contributed to mortality and declining fish health observed within the commercial scale WRAS.

More detailed analysis of the fish health aspects of these studies (histopathology and blood chemistry) is available in Good et al. (in press).

4. Conclusions

Important information regarding water quality between high and low exchange WRAS was gleaned during the study. All water quality parameters existed at significantly greater concentrations within the low exchange systems during the week of maximum feeding load, as well as throughout the study, aside from temperature, dissolved oxygen, and dissolved carbon dioxide concentrations. The majority of constituents found at significantly greater levels within low exchange WRAS were within safe recommended limits as described by the literature. However, more research is necessary to determine chronic toxicities of water quality parameters that accumulate within WRAS including CO₂, total ammonia nitrogen, nitrate nitrogen, nitrite nitrogen, TSS, and heavy metals. Although, no acute effects of water quality were observed within the present studies, the accumulating fine particles (<20 μm and suspended solids) could potentially cause detrimental effects to fish gills and fish performance within systems operated at low exchange. Additionally, accumulating solids, fine particles, and other constituents create an environment that enhances heterotrophic bacteria loads as evidenced in this paper. Thus, opportunistic bacteria could become more prevalent within low exchange WRAS and could pose potential fish health risks. UV or ozone could be incorporated within low exchange systems to safeguard against this problem.

Most notably this study indicated that dissolved copper accumulated to levels within the low exchange WRAS that could likely cause sublethal effects on rainbow trout. Fish farmers operating low exchange recirculating systems, particularly over long periods of time without shutdown or significant water replacement should be aware that residual metals originating from feed, make-up water, or dissolving system components can accumulate to potentially toxic levels. If aquaculture facility budgets do not allow for the periodic evaluation of heavy metals within WRAS, then water exchange should be increased or periodic flushing should be done to avoid toxic accumulation. Additionally, fish farmers operating WRAS at low exchange should avoid using copper piping and components within their systems. New fish farms should also test their potential water source (make-up water) for a suite of dissolved metals during the design phase to identify potential problems ahead of time. Treatment options for reduction/removal of heavy metals from water are available.

This work has helped to identify and possibly rule out compounds that can accumulate and affect fish health within water recirculating systems operated at low flushing rates and has provided crucial background data for future studies. Future studies are required to evaluate ozonation within low exchange WRAS as a method to reduce concentrations of potentially harmful water quality constituents such as fine particulates, metals, and high counts of opportunistic bacteria, such as flavobacteria.

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