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Process requirements for achieving full-flow disinfection of recirculating water using ozonation and UV irradiation

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ABSTRACT

A continuous water disinfection process can be used to prevent the introduction and accumulation of obligate and opportunistic fish pathogens in recirculating aquaculture systems (RAS), especially during a disease outbreak when the causative agent would otherwise proliferate within the system. To proactively prevent the accumulation of fish pathogens, ozonation and ultraviolet (UV) irradiation processes have been used separately or in combination to treat water in RAS before it returns to the fish culture tanks. The objective of the present study was to determine the process requirements necessary to disinfect the full RAS flow, using ozonation followed by UV irradiation, just before the flow was returned to the fish culture tank(s). We found that a proportional-integral (PI) feed-back control loop was able to automatically adjust the concentration of ozone (O_3) generated in the oxygen feed gas (and thus added in the low head oxygenator) in order to maintain the dissolved O_3 residual or ORP at a pre-selected set-point. We determined that it was easier and effective to continuously monitor and automatically control O_3 dose using an oxidative reduction potential (ORP) probe (in comparison to a dissolved ozone probe) that was located at the outlet of the O_3 contact chamber and immediately before water entered the UV irradiation unit. PI control at an ORP set-point of 450 and 525 mv and a dissolved O_3 set-point of 20 ppb provided almost complete full-flow inactivation of heterotrophic bacteria plate counts (i.e., producing <1 cfu/mL) and improved water quality (especially color and %UVT) in a full-scale recirculating system. Achieving this level of treatment required adding a mean dose of approximately 29 ± 3 g O_3 per kg feed. However, because water is treated and reused repeatedly in a water reuse system, the mean daily O_3 demand required to maintain an ORP of 375–525 mV (or at 20 ppb dissolved O_3) was 0.34–0.39 mg/L, which is nearly 10 times lower than what is typically required to disinfect surface water in a single pass treatment. These findings can be used to improve biosecurity and product quality planning by providing a means for continuous water disinfection in controlled intensive RAS.

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1. Introduction

Without an internal disinfection process, obligate and opportunistic fish pathogens can accumulate in aquaculture systems that treat and reuse water, especially in the event of a disease outbreak when the pathogen is propagating and shedding from its host. Ultraviolet (UV) irradiation and/or ozonation can be used to treat and sometimes disinfect recirculated water before it returns to the fish culture tanks (Brazil, 1996; Bullock et al., 1997; Summerfelt et al., 1997; Christensen et al., 2000; Krumins et al., 2001a,b; Summerfelt, 2003; Sharrer et al., 2005; Summerfelt et al., 2004; Sharrer and Summerfelt, 2007). In recirculating aquaculture systems, UV irradiation has been shown to inactivate micro-organisms (Farkas et al., 1986; Zhu et al., 2002; Sharrer et al., 2005) and destroy dissolved O_3 (Summerfelt et al., 2004). The efficacy of UV irradiation depends on the particulate size and concentrations and UV transmittance of the water, as well as the dose response of a given micro-organism. Recent research indicates that a modest dose of O_3 (i.e., 0.1–0.2 min mg/L) followed by a more robust dose of ultraviolet irradiation (i.e., 42.5–112.7 mJ/cm^2) can produce nearly complete inactivation of total heterotrophic bacteria plate counts in a side-stream treatment process in a freshwater recirculating system (Sharrer and Summerfelt, 2007). In freshwater recirculating aquaculture systems, ozonation has also been shown to improve water quality by improving microscreen filter performance, by breaking refractory compounds and thereby eliminating the accumulation of water color, and by oxidizing nitrite to nitrate (Brazil, 1996; Summerfelt et al., 1997; Christensen

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et al., 2000; Krumins et al., 2001a,b). Achieving these benefits required adding approximately 15–25 g O_3 per kg of feed fed to the recirculating system (Brazil, 1996; Summerfelt et al., 1997). This level of ozonation was also reported to improve fish health, i.e., preventing recurring episodes of bacterial gill disease in rainbow trout without use of chemotherapeutic treatment, but without providing even a 1 $\log_{10}$ reduction in heterotrophic bacteria counts in the water column (Bullock et al., 1997). However, to achieve an O_3 residual concentration sufficient to produce significant bacteria reduction requires overcoming the O_3 demand of the nitrite and organic carbon found in the recirculating water. The O_3 demand required to maintain a 0.2 mg/L residual after 10 min was 2–3 mg/L of dosed O_3 in the Fishing Creek surface water supplied to the US Fish and Wildlife Service's Northeast Fishery Center in Lamar, Pennsylvania (Summerfelt et al., 2008). These results are similar to, or somewhat less than, the results that Cryer (1992) reported for ozonated surface water supplies at the Kitoi Bay Hatchery (Alaska) and the Cold Lake Fish Hatchery (Alberta, Canada). Organic carbon can accumulate to relatively high concentrations in an intensive water reuse system due to high levels of feed per unit of makeup water flow. Thus, we initially suspected that the O_3 demand of water from a recirculating system would be even higher than that exhibited in a relatively clean surface water. However, because water is treated and reused repeatedly in a water reuse system, it may also be possible that the frequent (e.g., every 30–60 min) ozonation of the recycled water would reduce the water's O_3 demand to something less than was required for a single pass of O_3 treatment. For example, an ozone demand of <1 mg/L was measured during a study (Sharrer and Summerfelt, 2007) that examined the disinfection of a small sidestream flow within a recirculating aquaculture system.

The O_3 dose required to overcome the O_3 demand and maintain a residual concentration at the end of the O_3 contact chamber may also change with feeding and waste production cycles in the recirculating aquaculture system. Potentially large spikes in fish metabolism and waste excretion can occur due to feeding events (Krumins et al., 2001a), or other stressful events such as fish crowding during harvests in intensive fish culture systems (Forsberg, 1994, 1995). To reduce the magnitude of diurnal spikes in waste excretion and to create a quasi-steady state and constant water quality in a water recirculating system, various management techniques are used including the use of 24 h lighting and increasing the number of feeding events in conjunction with feeding smaller portion size at equally spaced intervals. In addition, process control equipment can be used to automatically adjust the amount of O_3 generated in the oxygen feed gas (which is then transferred into the recirculating water at the head of the O_3 contact tank) in order to maintain the desired O_3 concentration or oxidative reduction potential (ORP) at the end of the O_3 contact chamber.

Although ozonation is an effective treatment option in aquaculture systems, O_3 gas is also very dangerous and 5 ppm can be immediately life-threatening to personnel. The United States Occupational Safety and Health Administration (OSHA) has set a time-weighted average for an 8-h exposure to O_3 gas at a maximum limit of 0.1 ppm and a 10-min short-term exposure limit of 0.3 ppm. Just as critical as human exposure is the risk of exposing fish to high O_3 concentrations, which can be lethal or produce gross tissue damage and even kill the fish. Thus, specific process requirements must be provided to effectively and safely apply O_3 in recirculating systems.

The objective of the present study was to determine the process requirements necessary to disinfect the full recirculating flow, using ozonation followed by UV irradiation, before the flow is returned to the fish culture tank(s). A primary goal was to determine O_3 process control requirements, i.e., (i) would it be more effective to continuously monitor and automatically control O_3 dose using a dissolved O_3 probe or an ORP probe located at the outlet of the O_3 contact chamber? (ii) Would a proportional-integral (PI) feed-back control loop be able to automatically adjust the concentration of O_3 generated in the oxygen feed gas (and thus added in the low head oxygenator) in order to maintain the dissolved O_3 residual or ORP at the pre-selected set-point? (iii) What dissolved O_3 and ORP set-point conditions would provide optimum full-flow bacteria inactivation and improve water quality in a full-scale recirculating system? and (iv) What O_3 dose, i.e., in mg/L and in mg per kg feed, must be applied to the recirculating flow in order to achieve various levels of disinfection.

2. Materials and methods

2.1. Recirculating system

The process requirements necessary for disinfecting the full recirculating flow using ozonation followed by UV irradiation were determined in the commercial-scale recirculating system at the Conservation Fund Freshwater Institute (Shepherdstown, WV). The recirculating system (Fig. 1) has been described elsewhere (Davidson and Summerfelt, 2005; Sharrer et al., 2005). In brief, the recirculating system used two 5-HP centrifugal pumps to move 4640 L/min of water from the system's lowest hydraulic grade line elevation, i.e., the pump sump, to the system's highest elevation, i.e., at the top of the Cyclo Bio™ fluidized-sand biofilter. Water exiting the top of the biofilter flowed by gravity through a forced-ventilated cascade aeration column, a low head oxygenation (LHO) unit, a LHO sump, and a channel UV irradiation unit (Fig. 2) before the water entered a 150 m^3 fish culture tank. Water exited the fish culture tank by gravity and flowed through a microscreen drum filter (installed with 90-μm sieve panels) and into a pump sump, where the water was pumped again. The water flow rate was selected to exchange the water volume in the fish culture tank approximately once every 30 min. Makeup water flow into the system was approximately 4% of the total recirculating flow, i.e., 185 L/min, and exchanged the total system volume approximately once every day. Ozone was generated in the 99.5% pure oxygen feed gas. The ozonated-oxygen feed gas was subsequently injected into the recirculating system at the LHO, where the pure oxygen feed gas was used to supplement dissolved oxygen levels to increase the carrying capacity of the system.

2.2. Photoperiod, feed, and fish

The recirculating system (Fig. 1) was used to grow out rainbow trout (Oncorhynchus mykiss) from a mean initial size of 710 g to a maximum mean final size of 1620 g during this study. A constant 24-h photoperiod was provided. In addition, to produce a nearly constant biological respiration (Fig. 3) and waste production rate, timer-controlled mechanical feeders were used to feed fish equivalent portions during eight feeding events daily, i.e., approximately one feeding every 3 h. Mean daily feed rates ranged from 72 ± 6 to 93 ± 8 kg/day during the study. Culture densities were maintained at approximately 50–80 kg/m^3 by selectively top-grading the largest fish from the system approximately once every 3–6 weeks, as required.

2.3. Continuous in situ monitoring

Three sc100 Universal Controllers (Hach Company, Loveland, CO) were connected to receive data from six digital sensors, with two sensors assigned to each controller. Dissolved O_3 was
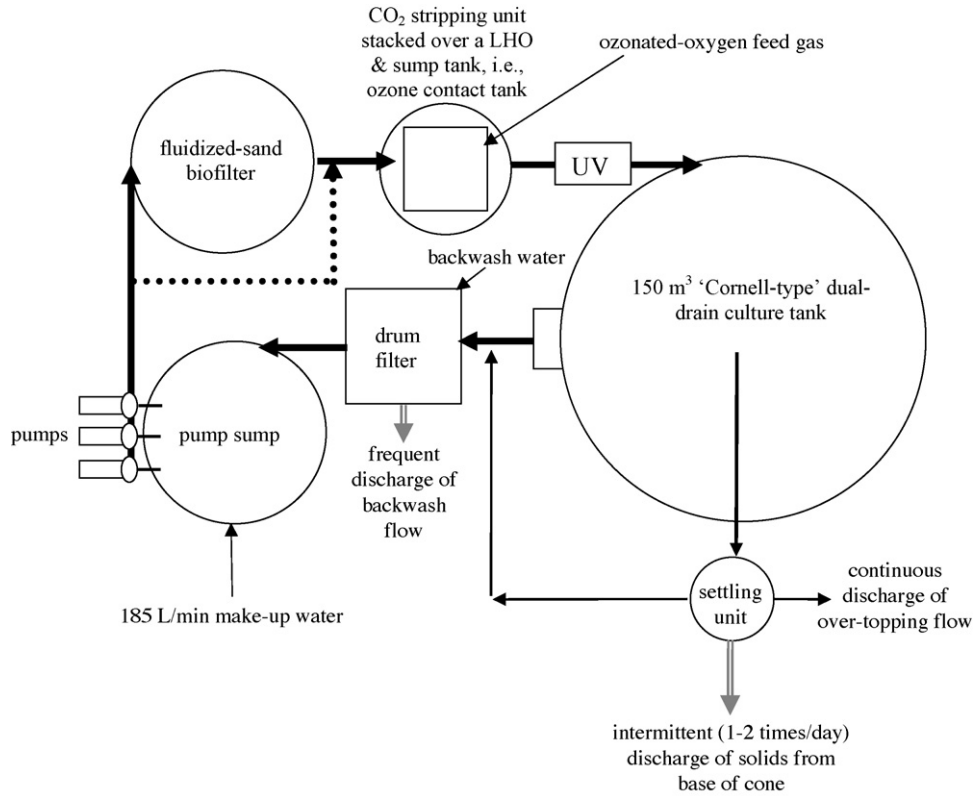
Fig. 1. Process flow drawing of the recirculating salmonid growout system (after Davidson and Summerfelt, 2005).
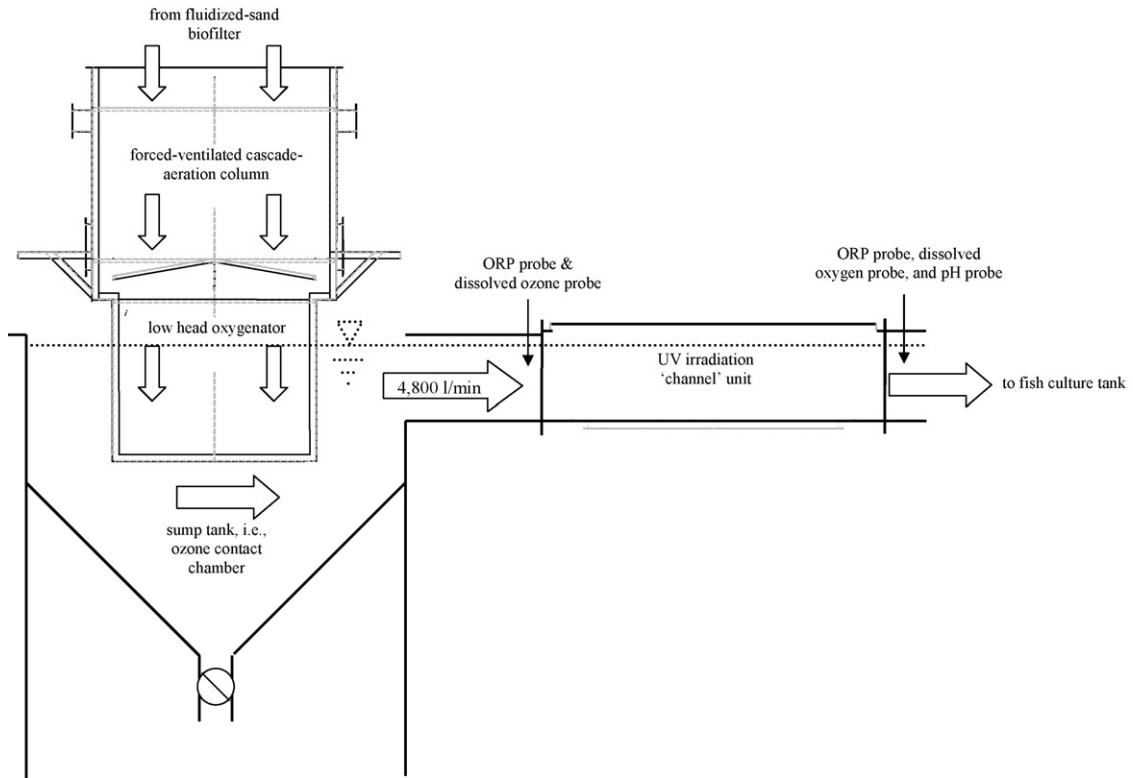

Fig. 2. Water flowing out of the top of the fluidized-sand biofilter cascaded down through the forced-ventilated aeration column and then through the low head oxygenator (LHO) and into the sump tank. An ozonated oxygen feed gas was transferred into the water in the LHO. The sump tank and channel immediately before the UV irradiation unit served as the ozone contact chamber. An ORP probe and a dissolved ozone probe were located in the channel immediately before water entered the UV irradiation unit. Probes to measure ORP, dissolved oxygen, and pH were located in the channel immediately after the UV irradiation unit and again in culture tank sidewall box outlet (not shown).
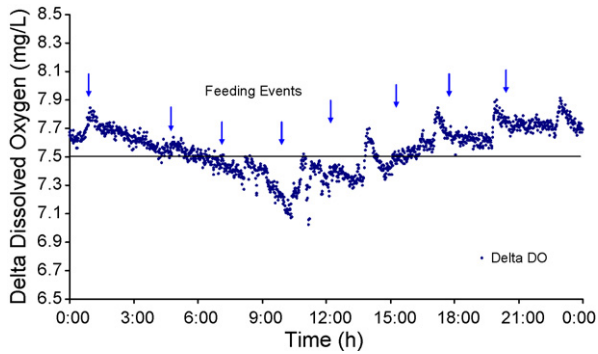
Fig. 3. Use of a continuous 24-h photoperiod and mechanical feeders that fed fish approximately once every 3 h produced a nearly constant (e.g., 7.5 mg/L in this mean data from a 5-day interval) dissolved oxygen respiration across the culture tank.

monitored using one Hach 9185sc Ozone Analyzer. ORP was monitored using two Hach Differential ORP Sensors. Dissolved oxygen was monitored using an Advanced Hach LDO® Process Probe. One of the ORP sensors and the LDO probe were located immediately after the UV irradiation unit and the other two sensors – one for ORP and one for dissolved O_3 – were located at the end of the O_3 contact chamber, i.e., just before the water entered the UV irradiation unit (Fig. 2). In situ water quality measurements of pH were also taken. pH was measured using a Hach digital pH sensor in the after UV irradiation and RAS sidebox locations.

Lookout version 4.5 data process control software installed on a PC (National Instruments, Austin, TX) provided a central location to continuously monitor and record the following real-time data: O_3 gas concentration exiting the generator; O_3 generator output level; dissolved O_3 concentration, ORP, and temperature of the water just before it exited the O_3 contact chamber; and ORP, dissolved oxygen concentration, and temperature of water exiting the UV irradiation unit, just before it was returned to the fish culture tank.

A pipe mounted ultrasonic flow meter (Transport Model PT868 Portable Flowmeter, Panametrics, Inc., Waltham, MA) was used to measure the total recirculating flow rate.

2.4. Ozone generation and control systems

Ozone application required the following processes: a purified oxygen feed gas supply, an O_3 generator, a gas dissolution system (i.e., a LHO), a vessel to provide hydraulic contact time for O_3 reaction, an O_3 destruction unit (i.e., a UV irradiation unit), an in situ sensor (i.e., an ORP or dissolved O_3 sensor) to monitor the water exiting the O_3 contact chamber for use in a feed-back O_3 control loop, and an in situ sensor (i.e., an ORP sensor) to monitor and then stop O_3 addition if O_3 residual was detected in the water about to enter the fish culture tank. To protect staff from O_3, off-gas from the gas dissolution unit (i.e., LHO) was vented (through 2.5 cm diameter PVC piping) outside the building. In addition, automatic fail-safes were installed that would (1) alarm in case elevated levels of O_3 were detected using room air monitors or (2) turn off the O_3 supply upon loss of water flow to the LHO. Additionally, spot-check O_3 measurements were taken in various areas of the room and around the valves, tubing, and fittings of the O_3 gas generation and delivery system using a Porta Sens II Gas Leak Detector (Analytical Technology, Inc., Collegeville, PA).

A PCI-Wedeco Environmental Technologies (West Caldwell, New Jersey) model GSO40 water-cooled O_3 generator was used. The generator had a total production capacity of approximately 4.0 kg/day of O_3 at a concentration of 6% by weight. Ozone was generated in a 99.5% pure oxygen feed gas that was supplied from a

liquid oxygen tank. After exiting the generator, the ozonated-oxygen feed gas was transported to the LHO using 6.4 mm (1/4-in. nominal) diameter 316 stainless steel tubing and fittings. Just before the LHO, the ozonated-oxygen feed gas was transported through a 316 stainless steel check valve, solenoid valve, and flow control assembly.

The flow control assembly consisted of a borosilicate glass variable-area rotameter (Model K-03217-78, Cole-Parmer Instrument Company, Vernon Hills, Illinois) with sapphire float and integral teflon inlet valve and fittings, a 316 stainless steel pressure gauge, and a 316 stainless steel needle valve. The needle valve followed the pressure gauge on the O_3 gas supply line and was used to regulate the back-pressure on the rotameter so that the flow control assembly could be calibrated to standard conditions. Thus, the flow control assembly was used to both measure and control the ozonated-oxygen feed gas before it entered the LHO unit.

A normally closed, 6.4 mm (1/4-in. nominal) diameter, 316 stainless steel solenoid valve (Model 8262G220NV, ASCO Red Hat, Florham Park, New Jersey) was installed immediately before the flow control assembly on the ozonated feed gas piping. The solenoid valve was wired to close whenever the water level above the LHO distribution plate dropped to a low level, as indicated by a float switch installed above the LHO distribution plate, or, when the ORP monitored at the outlet of the UV irradiation unit exceeded a set-point of 375 mV. The solenoid valve would only open when the water level above the LHO distribution plate rose above the low water level, as indicated by the float switch, and when the ORP value at the outlet of the UV irradiation unit was less than 375 mV.

A 4–20 mAmp analog input signal from the O_3 controller (i.e., the Hach sc100) was wired to the O_3 generator to adjust ozone output from 1 to 100% of full capacity. The ozone controller used a PI control loop to adjust the percentage of O_3 generated in order to maintain a selected set-point of either dissolved O_3 or ORP in the water exiting the O_3 contact tank. Set-points were adjusted directly at the O_3 controller. Using observational experience, we adjusted the values for the Proportional (P) and Integral (I) on the controller unit we achieved an acceptable dose–response behavior around our set-point for ORP values or dissolved ozone concentration. PI control was tuned as necessary for each ORP value or O_3 concentration. One tuning was generally adequate over a wide range of biomass.

A modular O_3 gas detector (STX-PA Gas Monitor, Pure-Aire Monitoring Systems, Inc., Lake Zurich, IL) was installed in the room between the fish culture tank and the LHO for detecting O_3 gas in the surrounding air space. The modular O_3 gas detector was programmed to alarm – powering a strobe light and loud buzzer – in the event that O_3 gas levels in the room reached 0.07 ppm.

The handheld PortaSens II Gas Leak Detector described above was used to further protect workers against potential leaks and confined zones of O_3 accumulation in the room. These spot-checks were performed periodically, and this instrument would also sound an audible alarm if the ambient O_3 gas levels were measured at or above 0.07 ppm.

2.5. Experimental treatments

A PI control loop was used to automatically adjust O_3 generator output to produce the following O_3 and UV treatment conditions: an ORP value of 375, 450, or 525 mV, or a dissolved O_3 concentration of 20 ppb immediately before the water entered the operational UV irradiation unit. These treatment conditions, along with a 'no O_3/no UV' control, were randomly distributed in time and were each replicated at least three times (Table 1). However, some conditions (i.e., 450 mV and 20 ppb) were replicated four times and one condition was replicated five times

Table 1
Mean (±S.E.) water temperature, feed rates, and culture tank dissolved oxygen inlet, outlet, and consumption for each treatment condition.

Treatment	Temp. (°C)	Feed rate (kg/day)	Tank O_2 in (mg/L)	Tank O_2 out (mg/L)	Tank ΔDO (mg/L)
No ozone/no UV	13.5 ± 0.2	73 ± 7	19.1 ± 0.1	10.9 ± 0.4	8.2
Ozone at 375 mV and no UV	15.4 ± 0.1	72 ± 6	19.1 ± 0.2	11.8 ± 0.4	7.3
Ozone at 375 mV and UV	14.0 ± 0.4	93 ± 8	19.6 ± 0.2	11.0 ± 0.4	8.6
Ozone at 450 mV and UV	14.1 ± 0.3	90 ± 11	19.5 ± 0.2	11.1 ± 0.2	8.4
Ozone at 525 mV and UV	14.3 ± 0.3	92 ± 6	19.3 ± 0.2	11.2 ± 0.3	8.1
Ozone at 20 ppb and UV	14.5 ± 0.3	82 ± 2	18.9 ± 0.0	11.1 ± 0.2	7.8

(i.e., 525 mV). In addition, at the end of these combined O_3 and UV trials, three replicates of an 'O_3 only (at 375 mV) control' was replicated three times.

Treatment conditions were randomized on a weekly basis. For each experimental treatment, the recycle system was operated for 5–12 days before bacteriological and water quality samples were collected for three consecutive sampling days. Samples were collected on days 5, 6, and 7 or days 12, 13, and 14, during instances in which randomized treatments fell on consecutive weeks.

2.6. UV irradiation unit

A custom UV channel unit was used to irradiate 100% of the 4640 L/min recirculating water just before it was returned to the fish culture tank (Sharrer et al., 2005). The UV unit contained twenty-four 200 W low-pressure, high-output lamps (Emperor Aquatics Inc., Pottstown, Pennsylvania). UV dose at the beginning of the study (i.e., when operated with new lamps) was estimated at approximately 100 mJ/cm^2, based on expected bulb output, water flow rate, and %UV transmittance of the water.

2.7. Bench-top water quality analysis

Dissolved O_3 concentrations were monitored before the LHO and at the outlets of the O_3 contact chamber, the UV irradiation unit, and the culture tank using a Hach Company DR/4000U spectrophotometer and Hach Company's low range Ozone AccuVac® Reagent Ampuls—Indigo Method.

The relative effectiveness of each of the disinfection treatments was determined using plate counts of indicator micro-organisms, i.e., total coliform bacteria and total heterotrophic bacteria, as justified by Zhu et al. (2002). Bacteria counts were assessed in samples collected from four locations: (i) makeup water, (ii) immediately before O_3 transfer (before the water entered the LHO), (iii) at the end of the O_3 contact channel (before the water entered the channel UV irradiation unit), and (iv) after UV irradiation (before the water entered the fish culture tank). Samples were collected from the before O_3 transfer site by placing a sterile borosilicate glass sample bottle upside down in the water just prior to it passing over the biofilter weir and approximately 0.5 m below the water surface and then inverting the bottle to collect the sample. The post-O_3 transfer samples and post-UV samples were taken by placing the sterile sample bottle (upside down) into the water channel and inverting the bottle approximately 0.5 m below the water surface. The makeup water samples were taken from a 1.3 cm (0.5 in.) valve located on the makeup water supply pipe within 5 m upstream of the recirculating system. The makeup water valve was opened and flushed at 2–4 L/min for approximately 1 min before the sterile sample bottle was placed under the water flow. Plate counts for both heterotrophic and total coliform bacteria were evaluated and processed according to the respective Membrane Filtration Techniques found in Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Heterotrophic bacteria were incubated for 48 h at 35 °C with TGE

(tryptone glucose extract) broth w/TTC (tetrazolium chloride) indicator (Millipore Corporation, Billerica, MA), counted using a low-power microscope, and reported in colony forming units (cfu) per 1-mL sample. Total coliform bacteria were incubated for 24-h at 35 °C with m-ColiBlue24® broth (Hach Company), counted with a low-power microscope, and reported in colonies per 100-mL sample. Counts were recorded as zero when no bacteria colonies were present and when counts were calculated to be <1 cfu per mL (for total heterotrophic bacteria) and <1 per 100 mL (for total coliform bacteria). A mean (±S.E.) of the bacteria count data at each location was then calculated for each condition. These mean counts were used to calculate the bacteria removal efficiency according to the following equation:

$$\text{bacteria removal}\,(\%) = \frac{\text{count}_{\text{inlet}} - \text{count}_{\text{outlet}}}{\text{count}_{\text{inlet}}} \times 100$$

Log$_{10}$ reduction in bacteria across the treatment system was then calculated using the equation:

$$\log_{10}\text{reduction} = -\log_{10}\left(1 - \frac{\%\text{removal}}{100}\right)$$

Statistical analyses assessed homogeneity of means at the 'after UV' sampling site for total heterotrophic and total coliform bacteria counts under all experimental treatments. A Shapiro-Wilk test was performed to determine data normality, which indicated the datasets for total heterotrophic and total coliform bacteria were not normally distributed. Data transformation did not facilitate a normal distribution. As a result, a non-parametric Kruskal–Wallis test was performed to assess homogeneity of means. Post hoc analysis was performed to determine specific differences in bacterial population ranked non-transformed means at each experimental treatment applying Tukey's multiple comparison procedure. Statistics were performed using SYSTAT 11 (2004).

Water samples were also analyzed to determine water quality within the recirculating system. Analyses were performed once weekly from four locations: from the system's make up water, in the flow before ozonation, at the outlet of the O_3 contact chamber, and at the outlet of the UV irradiation unit. Samples collected pre-ozonation, post-ozonation, and post-UV irradiation were analyzed for total ammonia nitrogen (TAN), nitrite–nitrogen, and nitrate–nitrogen. TSS, total alkalinity, true color, percentage UV transmittance (%UVT), and particle size distribution (PSD) were also measured once weekly, but only on the samples collected post-UV irradiation. Make up water was analyzed for total alkalinity and nitrate–nitrogen once weekly.

TAN was assessed utilizing Hach Company's Nessler Method and a DR4000/U spectrophotometer. Nitrite–nitrogen and nitrate–nitrogen were assessed using Hach Company's diazotization and cadmium reduction methods, respectively. Total suspended solids (TSS) concentrations were determined according to Standard Methods procedure 2540 D (APHA, 2005). True color was determined according to Hach Company's Platnium-Cobalt Standard Method and %UVT by the direct reading method

(λ = 253.7 nm). PSD was measured utilizing a Hach Company 2200 PCX Particle Counter and modified set up consisting of a peristaltic pump, flow dampener, and stir plate. Total alkalinity was determined by digital titration and endpoint pH according to Hach Company's phenolphthalein and total alkalinity method using sulfuric acid.

2.8. Salt-tracer tests to determine HRT of O₃ contact chamber

In order to determine the mean hydraulic retention time (HRT) of the recirculating flow as it passed through the LHO and O₃ contact chamber (i.e., LHO sump and O₃ contact channel up to the entry into the UV irradiation unit), 4 L of sodium chloride solution was added in a single pulse as the flow entered the LHO distribution plates and specific conductance was then recorded every 5 s at the outlet of the O₃ contact channel. The salt tracer study was replicated three times, one trial per day. The mean HRT of the water flowing through the O₃ contact chamber was estimated by calculating the area under the curve (Fig. 4) and determining the point (i.e., HRT) when equal areas occurred on either side of this point.

2.9. Mass balance to determine O₃ application rate and dose

The concentration of O₃ generated in the oxygen feed gas (%O₃) was continuously logged every 5 min throughout the study. These data were then used to calculate the daily mean %O₃ generated in the oxygen feed gas during each treatment. Also, the flow of the ozonated-oxygen feed gas (Q_{gas}) was kept constant and was logged once daily, along with the back-pressure on the rotameter. The Q_{gas} was pressure compensated to standard temperature (21.1 °C) and 1 atm pressure. The daily mass of O₃ applied was calculated from Q_{gas} and %O₃ using the following equation:

$$\text{mass applied} = \left\{ Q_{gas}, \frac{L}{min} \right\} \cdot \left\{ \frac{m^3}{1000\,L} \right\} \cdot \left\{ \frac{1.331\,kg\,O_2}{m^3} \right\}$$
$$\cdot \left\{ \frac{1440\,min}{day} \right\} \cdot \left\{ \frac{\%O_3}{100}, \frac{kg\,O_3}{kg\,feed\,gas\,(i.e.,\,O_2)} \right\}$$
$$= \frac{kg\,O_3}{day}$$

The O₃ dose per unit feed input, i.e., mg O₃ per kg feed, that was applied to the recirculating flow was calculated by dividing the

mean daily mass of O₃ applied by the mean daily fish feed rate (as is, not a dry weight) during each trial period, i.e.,

$$\frac{mgO_3}{kg\,feed} = \left\{ \text{mass ozone applied} \frac{kg\,O_3}{day} \right\} \cdot \left\{ \frac{10^6\,mg}{kg} \right\} \cdot \left\{ \frac{1}{4640}\,\frac{min}{L} \right\}$$
$$\cdot \left\{ \frac{day}{1440\,min} \right\}$$
$$= \frac{mg}{L\,of\,ozone\,applied}$$

The mean concentration of O₃ applied, i.e., the dose applied, was calculated from daily mass of O₃ applied divided by the treated water flow rate, 4640 L/min, according to the following:

$$\text{dose applied} = \left\{ \text{mass ozone applied} \frac{kg\,O_3}{day} \right\} \cdot \left\{ \frac{10^6\,mg}{kg} \right\}$$
$$\cdot \left\{ \frac{1}{4640}\,\frac{min}{L} \right\} \cdot \left\{ \frac{day}{1440\,min} \right\}$$
$$= \frac{mg}{L\,of\,ozone\,applied}$$

The ozone Ct was calculated from the product of the mean concentration of dissolved O₃ (measured at the outlet of the O₃ contact chamber) times the mean HRT, i.e.,

$$C \cdot t = \left\{ \text{ozone residual concentration}, \frac{mgO_3}{L} \right\}$$
$$\cdot \{\text{contact time}, min\}$$
$$= \frac{mgO_3}{L} \cdot min$$

3. Results and discussion

During this study, mean water temperature, mean feed rates, and mean tank dissolved oxygen consumption ranged from 13.5 to 15.4 °C, 72–93 kg/day, and 6.9–8.6 mg/L O₂ consumed with each pass through the culture tank, respectively (Table 1) during all treatments.

Salt-tracer studies determined that the O₃ contact chamber, i.e., water passing through the LHO, LHO sump, and contact channel immediately before the UV irradiation unit, provided a mean hydraulic HRT of approximately 2.0 min (Fig. 4). In addition, O₃
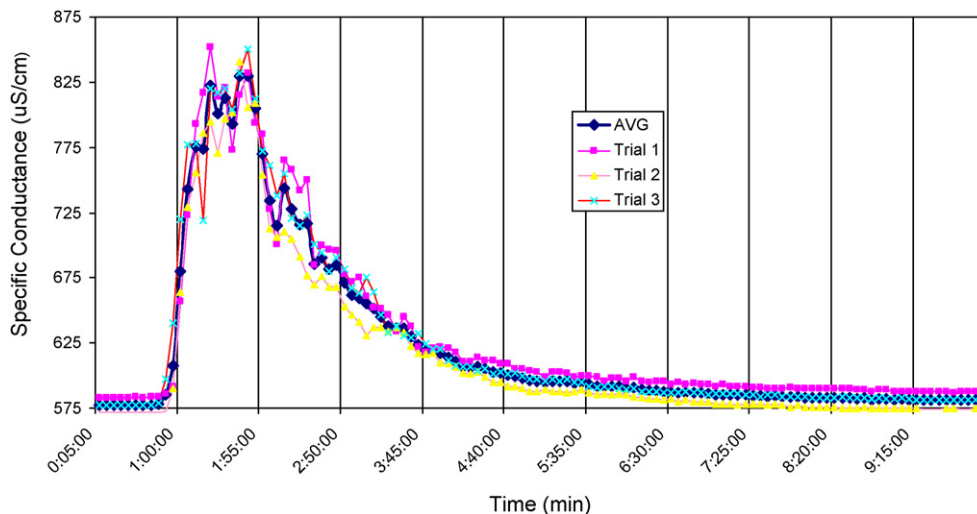

Fig. 4. Plot of the specific conductance measured in the recirculating flow for each water sample taken at the outlet of the ozone contact channel; time zero represents when the salt solution was added above the LHO distribution plates; mean hydraulic retention time of the ozone contact chamber was approximately 2 min.

transfer efficiency within the LHO approached 100%, as indicated by the lack of O_3 in the LHO off-gas.

3.1. Treatment efficacy—bacteria inactivation

PI control of O_3 dose to create ORP set-points of 450 and 525 mV and a dissolved O_3 set-point of 20 ppb (equivalent to a mean ORP of 607 mV), when followed by UV irradiation, provided complete full-flow inactivation of heterotrophic bacteria plate counts, i.e., mean total heterotrophic bacteria counts of <1 cfu/mL and $3+ \log_{10}$ reduction in heterotrophic plate counts (Table 2). These same O_3 dosages, when followed by UV irradiation, reduced total coliform bacteria counts to 3–5 cfu/100 mL and achieved 2.7–$3.1 \log_{10}$ reductions in counts (Table 2). In addition, PI control of O_3 dose to create an ORP set-point of 375 mV, when followed by UV irradiation, reduced the mean total heterotrophic bacteria counts to 3 ± 1 cfu/mL (a $1.6 \log_{10}$ reduction) and reduced the total coliform bacteria counts to 26 ± 15 cfu/100 ml (a $2 \log_{10}$ reduction). However, when only PI control of O_3 dose to create an ORP set-point of 375 mV was used (i.e., without UV irradiation), the mean total heterotrophic bacteria counts were only reduced to 21 ± 3 cfu/mL (a $0.4 \log_{10}$ reduction) and the total coliform bacteria counts to 636 ± 304 cfu/100 mL (a $0.4 \log_{10}$ reduction). Even adding O_3 at dosages that achieved the higher ORP set-points of 450 and 525 mV, which were treatments used immediately before the water entered the UV irradiation unit, did not by themselves account for even $1 \log_{10}$ of the reduction in the total heterotrophic or total coliform bacteria counts (Table 2).

A Kruskal–Wallis test assessing homogeneity of means indicated a statistical difference in total heterotrophic bacteria count means ($p = 0.000$, $\alpha = 0.05$) at the 'post-UV' site under all experimental treatments. Closer examination of specific differences in mean heterotrophic bacteria counts applying Tukey's post hoc analysis is indicated in Table 2. In summary, statistical differences in total heterotrophic plate counts appear when comparing the 'no ozone and no UV' experimental treatment to all other treatments, indicating some level of heterotrophic bacterial inactivation applying ozone with or without UV irradiation. Further, statistical differences are also evident when comparing the 'ozone at 375 mV and no UV' treatment to all other treatments indicating that ozone at a low level provides moderate total heterotrophic bacterial disinfection. However, when comparing application of ozone (at any of the doses applied) in combination with UV irradiation, statistically different mean heterotrophic plate counts were observed when compared to the 'no ozone and no UV' and 'ozone at 375 mV and no UV' treatments.

Applying the Kruskal–Wallis test to assess homogeneity of means for total coliform bacteria indicated a statistical difference ($p = 0.000$, $\alpha = 0.05$) in mean plate counts at the 'post-UV' site under all experimental treatments. Examination of specific differences in mean total coliform bacteria counts is summarized in Table 2, and display trends similar to heterotrophic bacteria disinfection. Specifically, the 'no ozone and no UV' treatment indicated a statistically significant difference in total coliform

Table 2
Mean ($\pm$S.E.) total heterotrophic plate counts, total coliform plate counts, and bacteria removal efficiencies for each treatment condition.

Site	Total heterotrophs (cfu/mL)	%Removal	$\log_{10}$ removal	Total coliform (cfu/100 mL)	%Removal	$\log_{10}$ removal
No ozone and no UV						
Pre-O_3 contactor	466 ± 147			27203 ± 7458		
Post-O_3 contactor	509 ± 139			30065 ± 8209		
Post-UV (unit off)	530 ± 145[a]	N/A	N/A	31123 ± 8327[b]	N/A	N/A
Ozone at 375 mV and no UV						
Pre-O_3 contactor	48 ± 9			1293 ± 326		
Post-O_3 contactor	22 ± 5			571 ± 229		
Post-UV (unit off)	21 ± 3[a]	56.3	0.35	636 ± 304[c]	55.8	0.35
Ozone at 375 mV and UV						
Pre-O_3 contactor	124 ± 27			2800 ± 665		
Post-O_3 contactor	81 ± 18			2293 ± 763		
Post-UV (unit on)	3 ± 1[d]	97.6	1.6	26 ± 15[e]	99.1	2.04
Ozone at 450 mV and UV						
Pre-O_3 contactor	50 ± 12			2702 ± 1054		
Post-O_3 contactor	22 ± 4			864 ± 236		
Post-UV (unit on)	0 ± 0[f]	100	N/A	5 ± 2[g]	99.8	2.7
Ozone at 525 mV and UV						
Pre-O_3 contactor	386 ± 348			1418 ± 505		
Post-O_3 contactor	225 ± 209			439 ± 107		
Post-UV (unit on)	0.4 ± 0.3[h]	99.90	3.0	3 ± 2[i]	99.8	2.7
Ozone at 20 ppb and UV						
Pre-O_3 contactor	47 ± 11			3195 ± 939		
Post-O_3 contactor	8 ± 2			498 ± 272		
Post-UV (unit on)	0 ± 0[j]	100	N/A	3 ± 1[k]	99.91	3.1

[a] Statistical difference ($p < 0.003$, $\alpha = 0.05$) compared to all other treatments.
[b] Statistical difference ($p = 0.000$, $\alpha = 0.05$) compared to all other treatments except ozone at 375 mV and no UV.
[c] Statistical difference ($p = 0.000$, $\alpha = 0.05$) compared to all other treatments except no ozone and no UV.
[d] Statistical difference ($p < 0.015$, $\alpha = 0.05$) compared to all other treatments except ozone at 525 mV and UV.
[e] Statistical difference ($p = 0.000$, $\alpha = 0.05$) compared to all other treatments except ozone at 450 and UV, ozone at 525 and UV, and ozone at 20 ppb and UV.
[f] Statistical difference ($p < 0.015$, $\alpha = 0.05$) compared to all other treatments except ozone at 20 ppb and UV and ozone at 525 mV and UV.
[g] Statistical difference ($p = 0.000$, $\alpha = 0.05$) compared to all other treatments except ozone at 375 and UV, ozone at 525 and UV, and ozone at 20 ppb and UV.
[h] Statistical difference ($p = 0.000$, $\alpha = 0.05$) compared to all other treatments except ozone at 20 ppb, ozone at 525 mV and UV, and 375 mV and UV.
[i] Statistical difference ($p = 0.000$, $\alpha = 0.05$) compared to all other treatments except ozone at 375 and UV, ozone at 450 and UV, and ozone at 20 ppb and UV.
[j] Statistical difference ($p < 0.007$, $\alpha = 0.05$) compared to all other treatments except ozone at 450 mV and UV and ozone at 525 mV and UV.
[k] Statistical difference ($p = 0.000$, $\alpha = 0.05$) compared to all other treatments except ozone at 375 and UV, ozone at 450 and UV, and ozone at 525 mV and UV.

counts compared to all other treatments suggesting some degree of disinfection when applying ozone alone or in combination with UV irradiation. Also, statistical differences are evident when applying the 'ozone at 375 mV and no UV' treatment with all other experimental treatments indicating moderate total coliform disinfection when applying ozone alone. Finally, when comparing application of ozone (at any of the doses applied) in combination with UV irradiation, statistically different mean total coliform bacteria counts were evident when compared to the 'no ozone and no UV' and 'ozone at 375 mV and no UV' treatments.

The two highest O_3 doses applied (i.e., at an ORP set-point of 525 mV or a dissolved O_3 set-point of 20 ppb) only produced an O_3 $c \times t$ at the end of the contact tank of approximately 0.01 and 0.03 mg/L·min, respectively, which is an extremely low $c \times t$ to expect much bacteria disinfection in fish culture water (Sharrer and Summerfelt, 2007). However, results indicate that ozonation helped to improve the efficacy of the downstream UV treatment, possibly by increasing the %UVT of the water or by reducing total particle counts.

A follow-up study is being conducted to determine the bacteria inactivation and water quality achieved by UV irradiation of the entire recirculating flow, when the system is not ozonated.

In comparison, previous research on a small side-stream flow in the same system using UV dosages of 78, 150, 303, 493, and 980 mJ/cm^2 (and no O_3) could only achieve 0.4–0.9 log$_{10}$ reduction in total heterotrophic bacteria counts, while a UV dose of 1800 mJ/cm^2 achieve a 1.7 log$_{10}$ reduction, leaving 181 ± 71 cfu/mL in the water exiting the UV irradiation unit (Sharrer et al., 2005). Sharrer et al. (2005) hypothesized that UV inactivation of heterotrophic bacteria was reduced because the bacteria that survived in the system had embedded within particulate matter or had formed bacterial aggregates that effectively shielded them from UV irradiation. In contrast, treating the full recirculating flow using ozonation followed by UV irradiation can produce practically complete disinfection of total heterotrophic bacteria plate counts.

3.2. Treatment efficacy—water quality improvements

Ozonation at all dosages tested, when followed by UV irradiation, improved water quality (especially color and %UVT) without resorting to high daily water exchange rates (Table 3). In comparison to the control (no O_3 and no UV), the water returning to the culture tank in all of the treatments that combined ozonation and UV irradiation exhibited a mean drop in NO_2–N from 0.06 mg/L (control) to 0.01–0.02 mg/L (test conditions), in true color from 9.5 Pt-Co to 0.7–1.7 Pt-Co, and in TSS from 4.0 mg/L to 2.1–2.5 mg/L. Meanwhile, the UV transmittance rose from 90.2% to 94.9–96.8%, respectively, between the control (no O_3 and no UV) and the ozonation treatments.

Even without ozonation, NO_2–N concentrations in the recycle system were maintained at extremely low levels (i.e., <0.1 mg/L) by the highly efficient fluidized-sand biofilter, which is similar to what others have reported when these fine sand biofilters are used in salmonid systems (Summerfelt, 2006). Yet, ozonation of the system reduced NO_2–N concentrations even further to levels near the limits of the method of detection. Likewise, reduction in recycle system water color upon ozonation has been widely reported (Summerfelt et al., 1997; Christensen et al., 2000). In addition, ozonation has also been reported to improve solids removal via foam fractionation and settling (as reviewed by Summerfelt and Hochheimer, 1997). Summerfelt et al. (1997) reported that adding just 25 g per kg feed improved microscreen filter performance: TSS removal was increased by 33%, wash cycles were reduced by 35%, sludge water production was reduced by 53%; and sludge water settled sludge volume was reduced by 77%.

3.3. Ozone concentration applied (mg/L) and dose per unit feed

The O_3 concentration applied and the O_3 dose per unit feed input (i.e., O_3 process requirements) were determined for each treatment (Table 4). Results indicate that a daily mean dose of 27–

Table 3
Mean ($\pm$S.E.) TAN, nitrite nitrogen, nitrate nitrogen, TSS, true color, and UV transmittance for each treatment condition.

Site	TAN (mg/L-N)	Nitrite (mg/L-N)	Nitrate (mg/L-N)	TSS (mg/L)	Color (Pt-Co)	UVT (%)
No ozone and no UV						
Pre-O_3 contactor	0.05 ± 0.01	0.027 ± 0.009	11.9 ± 1.4			
Post-O_3 contactor	0.10 ± 0.02	0.036 ± 0.008	11.7 ± 1.4			
Post-UV (unit off)	0.11 ± 0.01	0.060 ± 0.025	11.7 ± 1.5	4.0 ± 0.9	9.5 ± 2.2	90.2 ± 1.5
Ozone at 375 mV and no UV						
Pre-O_3 contactor	0.03 ± 0.01	0.006 ± 0.002	10.0 ± 0.4			
Post-O_3 contactor	0.09 ± 0.01	0.008 ± 0.006	9.9 ± 1.8			
Post-UV (unit off)	0.10 ± 0.01	0.020 ± 0.010	11.3 ± 1.4	3.0 ± 1.2	0.3 ± 0.3	95.7 ± 0.3
Ozone at 375 mV and UV						
Pre-O_3 contactor	0.06 ± 0.01	0.026 ± 0.003	13.8 ± 1.4			
Post-O_3 contactor	0.12 ± 0.02	0.009 ± 0.002	16 ± 1.7			
Post-UV (unit on)	0.13 ± 0.02	0.020 ± 0.006	15 ± 2.2	2.1 ± 0.4	1.7 ± 0.3	94.9 ± 0.2
Ozone at 450 mV and UV						
Pre-O_3 contactor	0.03 ± 0.01	0.010 ± 0.004	15.0 ± 1.0			
Post-O_3 contactor	0.11 ± 0.01	0.012 ± 0.009	14.7 ± 1.9			
Post-UV (unit on)	0.11 ± 0.01	0.008 ± 0.006	15.5 ± 2.0	2.5 ± 0.5	0.7 ± 0.3	95.3 ± 0.2
Ozone at 525 mV and UV						
Pre-O_3 contactor	0.05 ± 0.00	0.013 ± 0.004	15.5 ± 0.6			
Post-O_3 contactor	0.13 ± 0.03	0.004 ± 0.001	14.7 ± 1.2			
Post-UV (unit on)	0.14 ± 0.02	0.012 ± 0.007	14.8 ± 0.6	2.4 ± 0.6	1.0 ± 0.6	95.9 ± 0.3
Ozone at 20 ppb and UV						
Pre-O_3 contactor	0.04 ± 0.02	0.018 ± 0.014	12.6 ± 1.0			
Post-O_3 contactor	0.11 ± 0.02	0.006 ± 0.002	11.9 ± 1.2			
Post-UV (unit on)	0.10 ± 0.02	0.015 ± 0.010	12.1 ± 1.0	2.2 ± 0.2	1.7 ± 0.3	96.8 ± 1.0

Table 4
Tabulated values of the mean (±S.E.) concentration of ozone applied (in mg/L) and the dose of ozone applied per unit feed fed (in g/kg) for each treatment. Tabulated values are also included for the ORP and dissolved ozone concentrations measured using both probe and wet chemistry techniques at the end of the ozone contact chamber.

Treatment	ORP[a] (mV)	Dissolved ozone probe[a] (ppb)	Dissolved ozone ampoule[a] (ppb)	Ozone applied per feed (g/kg)	Ozone dose applied (mg/L)
No ozone/no UV	333 ± 15	0 ± 0	0 ± 0	N/A	N/A
375 mV/no UV	356 ± 19	1 ± 1	1 ± 1	21 ± 2	0.23 ± 0.03
375 mV	375 ± 0	3 ± 0	0 ± 0	28 ± 4	0.38 ± 0.04
450 mV	450 ± 0	7 ± 2	2 ± 1	29 ± 3	0.39 ± 0.06
525 mV	525 ± 0	12 ± 3	7 ± 2	29 ± 2	0.34 ± 0.04
20 ppb	607 ± 32	20 ± 0	22 ± 3	27 ± 3	0.34 ± 0.05

[a] Measured at the end of the ozone contact chamber.

29 g O_3 per kg feed had to be applied to achieve an ORP of 375 mV (28 ± 4 mg/kg), 450 mV (29 ± 3 g/kg), or 525 mV (29 ± 2 g/kg) or a dissolved O_3 concentration of 20 ppb (27 ± 3 g/kg) in the flow immediately before UV irradiation treatment (Table 4). Without UV irradiation, a daily mean dose of 21 ± 2 g O_3 per kg feed was required to achieve an ORP of 375 mV at the end of the O_3 contact chamber (Table 4).

Previous research (Brazil, 1996; Summerfelt et al., 1997) indicates that only 15–25 g O_3 per kg feed was required to achieve water quality control benefits in a recirculating fish culture system. The 15–25 g O_3 per kg feed level of ozonation was also reported to improve fish health (Brazil, 1996; Bullock et al., 1997), i.e., preventing recurring episodes of bacterial gill disease in rainbow trout without use of chemotherapeutic treatment, without providing even a 1 $\log_{10}$ reduction in heterotrophic bacteria counts in the water column (Bullock et al., 1997). The present research indicates that when ozonation is followed by UV irradiation, a daily mean dose of at least 27–29 g O_3 per kg feed (Table 4) was required to produce a minimal O_3 residual concentration (as indicated by an ORP of 375–525 mV or a dissolved O_3 of 20 ppb), overcoming the O_3 demand of the nitrite and organic carbon found in the RAS waters. These results suggest that using UV irradiation immediately following ozonation can increase the amount of O_3 that must be added, i.e., 27–29 g O_3 per kg feed was required when ozonation was followed by UV irradiation, but only 21 ± 2 g O_3 per kg feed was required without UV irradiation (Table 4). This increase may be due to destruction of hydroxyl radicals or other ozone residuals when the flow passes through the UV irradiation.

To maintain an ORP of 375, 450, or 525 mV or a dissolved O_3 concentration of 20 ppb in the water about to flow into the UV irradiation unit (i.e., after an approximately 2 min HRT in the O_3 contact chamber) required adding a daily mean O_3 dose of 0.34–0.39 mg/L (respectively, 0.38 ± 0.04, 0.39 ± 0.06, 0.34 ± 0.04, or 0.34 ± 0.05 mg/L). Without UV irradiation, a daily mean dose of 0.23 ± 03 mg/L had to be added to achieve an ORP of 375 mV at the end of the O_3 contact chamber (Table 4). These mean O_3 dosages were nearly 10 times lower than the approximately 3 mg/L O_3 dose that has been required to overcome O_3 demand during disinfection of surface water in a single pass treatment (Summerfelt et al., 2008). We think that the mean daily O_3 dose required to overcome the O_3 demand of the recirculating water was unexpectedly low (i.e., 0.34–0.39 mg/L vs. approximately 3 mg/L), because water is treated and reused nearly 50 times daily. In previous side-stream studies in the same system, approximately 0.75–1.2 mg/L of O_3 had to be transferred into flow to maintain 0.05, 0.1, and 0.2 mg/L of O_3 at mean HRT's through the contact column of 8 and 16 min (Sharrer and Summerfelt, 2007). The daily mean O_3 dose was higher in the side-stream study, most likely because the majority of flow was not ozonated sufficiently to overcome O_3 demand when it was by-passed around the O_3 disinfection process.

3.4. Feed-back control using ORP vs. dissolved O_3 probe

The proportional-integral (PI) feed-back control loop was successful at automatically adjusting the concentration of O_3 generated in the oxygen feed gas (and thus added in the low head oxygenator) in order to maintain the mean dissolved O_3 residual or ORP at the desired pre-selected set-point, as shown in Table 4. Dissolved O_3 residual concentration correlated well with ORP measurements at the same location and time, especially when mean values were compared (Fig. 5). We determined that it was easier and just as effective to continuously monitor and automatically control O_3 dose using an ORP probe in comparison to a dissolved O_3 probe. The ORP probe was considerably less expensive than the O_3 probe. The ORP probe was also easier to calibrate and maintain than the O_3 probe. Specifically, due to initial membrane fouling, the dissolved ozone probe was removed from the flow cell provided by the manufacturer and placed directly in the process water with a modified probe body designed to protect the probe sensor without interfering with water flow across the membrane. Dissolved ozone probe calibration required probe immersion in oxidant-free process water for 24 h before introduction into ozonated process water for approximately 3–4 h. ORP probe calibration procedure required probe immersion in a 200 mV ORP standard solution for 1–2 h. Calibration confirmation procedure for both the dissolved ozone and ORP probes using the Hach sc100 unit required less than 5 min. The ORP and dissolved O_3 probes were similar to tune for PI control. However, the ORP probe was just as effective as the dissolved O_3 probe at monitoring and automatically controlling O_3 dose. In addition, the dissolved O_3 probe was operated at just above its minimum detection limit (approximately 1 ppb), which made accurate calibration more difficult. However, the dissolved O_3 probe was quick to respond to
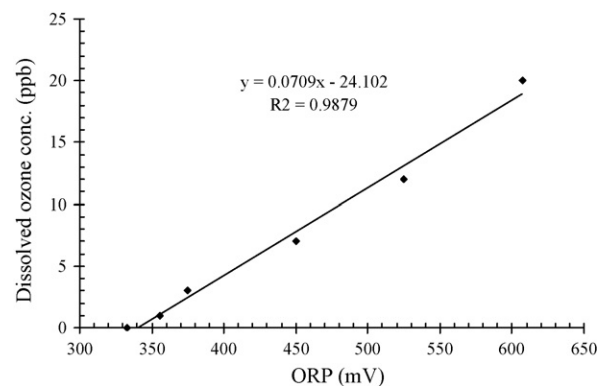
Fig. 5. Plot of the mean dissolved ozone concentrations vs. mean oxidative reduction potential (ORP) measurements that were logged at the same time and location, i.e., at the end of the ozone contact chamber, using in situ probes. A linear regression shows the relationship between dissolved ozone and ORP, i.e., dissolved ozone concentration, ppb = 0.0709 (ORP, mV) – 24.1.
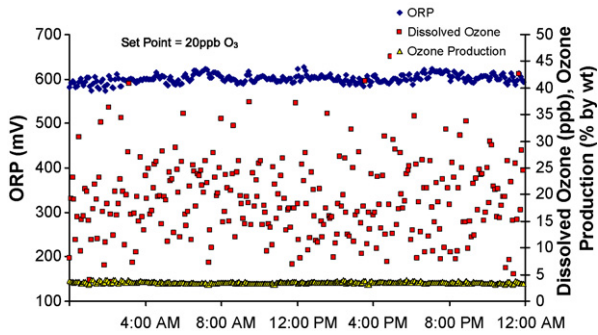
Fig. 6. Sample of data measurements recorded for the dissolved ozone probe, ORP probe, and the percent ozone produced in the oxygen feed gas (by weight) required to maintain a dissolved ozone set-point of 20 ppb.

Fig. 7. The mean ORP measurements collected at the end of the ozone contact chamber, at the outlet of the UV irradiation channel, and within the well-mixed circular culture tank for each treatment condition.

small changes in dissolved O_3 concentration (Fig. 6). Conversely, the ORP probe registered stable values with minimal oscillation around set points (Fig. 6). The ORP probe rapidly responded to increasing dissolved O_3 concentrations but was slow to respond to sudden drop in dissolved O_3.

3.5. UV and ORP control to protect the aquatic-animals

The risk of exposing fish to high O_3 concentrations must be avoided as this exposure can produce gross tissue damage and even kill the fish. The dissolved O_3 concentration that damages gills has been reported to range from 1 to 6 ppb (Roselund, 1974) and that the lethal threshold level in rainbow trout was approximately 8 ppb (Wedemeyer et al., 1979a,b). At these levels, dissolved O_3 destroys the epithelium covering the gill lamella, resulting in a rapid drop in serum osmolality, and if mortality does not occur immediately, the tissue damage can leave fish highly susceptible to microbial infection (Roselund, 1974; Paller and Heidinger, 1979; Wedemeyer et al., 1979a,b). The first signs of exposure to toxic concentrations are noticeable changes in fish behavior (Bullock et al., 1997). Fish stop feeding and congregate near the surface and sometimes gasp for air. Their swimming behavior becomes progressively erratic, attempts to jump out of the tank increase, and some fish show darting behavior followed by listless swimming. Fish then lose vertical equilibrium and become pale, with vertical patches of dark pigment on the sides of the body (Bullock et al., 1997). During the present study, none of these adverse behavioral changes were observed. In fact, in the present study the applied UV irradiation dose was sufficient to destroy any O_3 residual concentration exiting the O_3 contact chamber before the water entered the culture tank, as indicated by the ORP measurements logged at the outlet of the UV chamber (Fig. 7). This supports previous research that indicates a UV irradiation dose of 50 mJ/cm^2 will consistently remove all of the dissolved O_3 entering the UV unit at a concentration of $\leq$0.1 mg/L under similar conditions (Summerfelt et al., 2004). To protect against a failure of the UV irradiation unit (i.e., after a power outage the UV irradiation unit in the present study had to be manually switched back on), ORP was measured at the outlet of the UV irradiation channel and a controller was used to close a solenoid valve on the ozonated oxygen feed gas supply if the ORP exceeded 375 mV. This fail-safe system protected the fish in the culture tank from exposure to even low levels of dissolved O_3. The ORP measured at the outlet of the UV irradiation unit and within the completely mixed fish culture tank average approximately 300 mV (Fig. 7), which corresponds to a dissolved O_3 concentration of less than 1 ppb (Table 4) and is considered safe for rainbow trout in
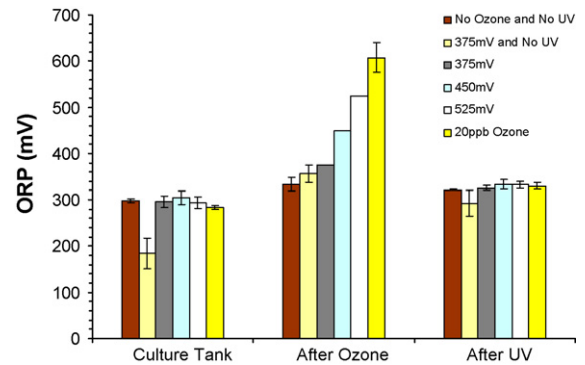
freshwater (Bullock et al., 1997). Note that when UV irradiation does not follow ozonation, extra fail-safes (such as a back-up ORP probe and a lower ORP set-point) must be considered to reduce the risk of an O_3 over-dose that would harm fish.

3.6. Processes to protect human safety

Transferring the ozonated-oxygen supply gas from the O_3 generator to the LHO required proper design, selection, and installation of the piping and gas–liquid contacting systems to both protect human safety and provide reliable service. Once all gas leaks had been eliminated after installation, the 316 stainless steel piping (with compression fittings) and valves (with Teflon gaskets and seals) that were used were found to reliably and safely transfer the ozonated-oxygen feed gas from the generator to the LHO.

In the event that water flow to the LHO decreased dramatically or ceased, then the float switch that had been installed above the LHO distribution plate dropped, which triggered the closing of the solenoid valve carrying ozonated oxygen feed gas to the LHO, and, thus, prevented a continuous flow of O_3 gas from escaping into the atmosphere in the room. In addition, to further ensure worker safety, LHO off-gas was constantly vented from the building to limit opportunities for O_3 to accumulate in the room.

A modular gas detector installed between the fish culture tank and the LHO was also used to protect staff from potentially dangerous levels of O_3 gas in the room air space. The room air O_3 gas detector was programmed to alarm-activating a siren and a strobe light when a room air O_3 concentration of greater than or equal to 0.07 ppm is detected. In addition, a switch to remotely turn OFF the O_3 generator was located beside the entry door to the room containing the O_3 generator and recirculating aquaculture system used in this study. As a further precaution, the room ventilation fan was run continuously whenever O_3 was being applied.

4. Conclusions

Complete inactivation of total heterotrophic bacteria plate counts can be achieved by first ozonating and then UV irradiating the full recirculating flow just before the flow returns to the fish culture tank. The ozonation process can be controlled using a PI control loop that adjusts the amount of O_3 generated and transferred into the system. Maintaining an ORP set-point of 450 or 525 mV or a dissolved O_3 set-point of 20 ppb at the end of a 2 min contact chamber produced the best disinfection following UV irradiation. The majority of the bacteria removal was due to the UV irradiation process, but ozonation did contribute to the

bacteria inactivation and also appears to improve the efficacy of the downstream UV treatment, possibly by increasing the %UVT of the water or by reducing total particle counts. Ozonation also improves water quality (especially TSS, NO_2-N, color, and %UVT) in the recirculating systems without resorting to high daily water exchange rates. A daily mean O_3 dose of 0.34–0.39 mg/L (equivalent to 27–29 g O_3 per kg feed) was required to achieve an ORP of 375, 450, or 525 mV or a dissolved O_3 concentration of 20 ppb in the flow immediately before UV irradiation treatment. However, without UV irradiation, a daily mean O_3 dose of 23 ± 3 mg/L (equivalent to 21 ± 2 g O_3 per kg feed) was required to achieve an ORP of 375 mV at the end of the O_3 contact chamber. These results suggest that using UV irradiation immediately following ozonation can increase the dose of O_3 required to achieve the ORP or dissolved O_3 set-points at the end of the O_3 contact chamber.

This paper should reinforce the importance of providing a control system that can automatically adjust O_3 output to match the O_3 demand of the system, which changes with fish feeding rate. Fine tuning the control system could assist in optimizing the control response during both system start-up and normal operation. In addition, when a proportional control system is used to automatically adjust the percentage of O_3 generated in the oxygen feed gas, providing excess O_3 generation capacity is required to maintain the desired O_3 concentration at the outlet of the contact tank when water quality deteriorates. We also found that calibration of the dissolved O_3 probe was time-consuming and its measured output more variable than ORP. In addition, ORP probes demonstrated a more stable output (except that they were slow to respond to a decrease in ORP) and were less likely to drift with time compared to the dissolved O_3 probes.

Precautions and fail-safes must be taken to use O_3 safely. However, the process requirements that were provided in this paper will offer insight into design and operation of an ozonation system.

These findings can be used to improve biosecurity and product quality by providing a means for continuous water disinfection in controlled intensive recirculating aquaculture systems. However, the cost effectiveness of O_3 and UV irradiation systems was not examined in this paper. We think that O_3 and UV irradiation technologies would most likely find application in recycle systems that produce relatively high value fingerling fish (such as salmon smolt or other valuable seed-stock) or even in fry or fingerling quarantine systems. Nevertheless, few large growout facilities, unless raising high value niche marketed species, would likely justify the cost of ozonating and UV irradiating the entire recycle flow.

Acknowledgements

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