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Inactivation of bacteria using ultraviolet irradiation in a recirculating salmonid culture system

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Abstract

The objective of this research was to determine the ultraviolet (UV) irradiation dosages required to inactivate bacteria in a commercial-scale recirculating salmonid culture system. Research was conducted in the commercial-scale recirculating system used for Arctic char growout at the Conservation Fund Freshwater Institute (Shepherdstown, West Virginia). This recirculating system uses a UV channel unit to treat 100% of the 4750 L/min recirculating water flow with an approximately 100–120 mW s/cm^2 UV irradiation dose. However, a second UV irradiation unit was operated at a constant intensity to treat a side-stream flow of water pumped from the commercial-scale recirculating system's low head oxygenator (LHO) sump. The side-stream water flow ranged from 0.15–3.8% (i.e., 7–180 L/min) of the entire recirculating flow so as to regulate the water retention time (i.e., from 3–70 s) within the UV irradiation unit and thus produce a range of UV irradiation doses (mW s/cm^2). UV irradiation doses of approximately 75, 150, 300, 500, 980, and 1800 mW s/cm^2 were applied to determine the dose required to inactivate total heterotrophic bacteria and total coliform bacteria. Total heterotrophic bacteria counts and total coliform bacteria counts were measured immediately before and immediately after the side-stream UV irradiation unit. Total heterotrophic bacteria in the recirculating system required a UV dosage in excess of 1800 mW s/cm^2 to achieve a not quite 2 LOG10 reduction (i.e., a 98.0 $\pm$ 0.4% reduction). In contrast, total coliform bacteria were more susceptible to UV inactivation and complete inactivation of coliform bacteria was consistently achieved at the lowest UV dose applied, i.e., at approximately 77 mW s/cm^2. These results suggest that: (1) the UV dose required to inactivate total heterotrophic bacteria—and thus disinfect a recirculating water flow—was nearly 60 times greater than the 30 mW s/cm^2 dose typically recommended in aquaculture and (2) inactivating 100% of bacteria in a given flow can be

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difficult, even at excessive UV doses, because UV irradiation cannot always penetrate particulate matter to reach embedded bacteria. We present a hypothesis that the recirculating system provided a selection process that favors bacteria that embed within particulate matter or that form bacterial aggregates that provides shading from some of the UV irradiation, because the bacteria in the recirculating water were exposed to approximately 100–120 mW s/cm^2 of UV irradiation every 30 min.

Keywords: Ultraviolet irradiation; Bacteria inactivation; Recirculating system; Water reuse; Aquaculture; Disinfection

1. Introduction

Water recirculating systems can support large populations of bacteria, protozoa, and micrometazoa. Some of these microorganisms metabolize waste organic matter found within the system and other microorganisms—especially bacteria—metabolize dissolved wastes that include dissolved organic compounds, ammonia, nitrite, and nitrate (Bullock et al., 1993, 1997; Blancheton and Canaguier, 1995; Sich and Van Rijn, 1997; Hagopian and Riley, 1998; Blancheton, 2000; Leonard et al., 2000, 2002; Nam et al., 2000). Many of these microorganisms live in biofilms that are located on surfaces within the biofilter and other pipes and vessels within the recirculating system, but they are also found within the water column. The majority of these microorganisms are an integral part of the dissolved waste treatment system used to manage water quality. However, pathogenic organisms may also occur in recirculating systems. Due to relatively little dilution with makeup water and to the large organic loading rates placed upon these system, these pathogens can accumulate to much higher concentrations within recirculating systems than in single-pass systems. Control of epidemics in recirculating systems can be challenging when chemotherapeutants recirculate—returning to the fish culture tank or passing through the biofilter when opportunities for flushing these compounds are reduced due to makeup water limitations—or if the entire system requires sterilization (Heinen et al., 1995; Noble and Summerfelt, 1996; Schwartz et al., 2000; Bebak-Williams et al., 2002).

Microorganisms are carried into the recirculating system through its makeup water supply (even from ground water sources), stocked eggs or fish, building air exchange, fish feed, animal and insect exposure, equipment used in and about the system, and staff/visitors that contact the system. Biosecurity procedures can be implemented to reduce the likelihood of introducing pathogenic organisms into recirculating systems (Summerfelt et al., 2001; Bebak-Williams et al., 2002). However, naturally occurring microorganisms can be opportunistic pathogens and may reside among the many other heterotrophic microorganisms within the system. Heterotrophic microorganisms obtain carbon and energy from organic compounds such as carbohydrates, amino acids, peptides and lipids. Whereas, autotrophic microorganisms derive carbon from CO_2 and energy from oxidation of an inorganic nitrogen, sulfur, or iron compound.

Populations of microorganisms may be reduced within the recirculating system by improving the effectiveness and speed of solids removal (Blancheton and Canaguier, 1995; Blancheton, 2000; Leonard et al., 2000, 2002). Efficient and rapid solids control can

minimize the amount of soluble organic compounds and ammonia that are released by decomposing waste feed and fecal matter. Fresh fecal matter and waste feed are often large and intact enough to be rapidly captured and removed from recirculating systems. However, the finer particles that are not removed can accumulate and constitute the majority of the organic solids within recirculating systems (Chen et al., 1993; Patterson et al., 1999; McMillan et al., 2003; Patterson and Watts, 2003a, b). Periodic flushing of all pipes and sumps can reduce the total reservoir for organic matter within the recirculating system, which may also reduce the reservoir of opportunistic pathogens within the system (Summerfelt et al., 2001). However, the largest reservoir of heterotrophic microorganisms in a recirculating system resides in the biofilter (Leonard et al., 2000).

Some have questioned whether or not disinfecting the water within recirculating systems is actually achievable or beneficial. Continuous disinfection of the recirculating flow would be beneficial if it controlled or eliminated the accumulation of pathogenic organisms. Reducing the numbers of less harmful populations of heterotrophic bacteria might reduce the in situ demand for dissolved oxygen, which can be equal to the dissolved oxygen demand expressed by the fish (Blancheton, 2000; Timmons et al., 2002). However, continuous disinfection may not be necessary if biosecurity practices have excluded specific pathogens from the system, if fish are never stressed, and if the water flow rates and treatment efficiencies of the unit processes always maintain excellent water quality. The decision to disinfect in such a scenario would be based upon an analysis of the consequences and risk of a breach in biosecurity, on the fixed and capital cost required to achieve disinfection, and on whether continuously disinfecting the recirculating water would then prevent an epidemic.

Depending upon which microorganisms must be eliminated, continuous disinfection of an entire recirculating flow can be expensive and difficult (Bullock et al., 1997; Summerfelt, 2003; Summerfelt et al., in press). Ozonation and ultraviolet (UV) irradiation have been used to treat relatively large aquaculture flows, including flows within recirculating systems (Blancheton, 2000; Liltved, 2002; Summerfelt, 2003; Summerfelt et al., 2004a, b, in press). UV irradiation treatment of recirculating flows is more common in salmon egg incubation, fry, and smolt recirculating systems and, according to Blancheton (2000), in Mediterranean hatcheries and growout facilities used to produce turbot and sea bass. Except for UV applications for ozone destruction (Summerfelt et al., 2004b), little research has been published to quantify the performance or benefits of UV irradiation within these commercial-scale recirculating systems (Farkas et al., 1986; Zhu et al., 2002; Summerfelt, 2003). Farkas et al. (1986) presented data on UV irradiation treatment of facultative fish pathogens (*Aeromonas* [*hydrophila* and *punctata*] and *Flexibacter columnaris*), total heterotrophic aerobic bacteria, and facultative anaerobic bacteria, obligate anaerobic bacteria within a recirculating aquaculture system operated at 20–25 °C. In the other case, Zhu et al. (2002) presented a comprehensive mathematical model that describes microorganism inactivation within recirculating systems, which is dependent upon UV irradiation input, recirculating flow rate, water UV transmittance, and the first-order inactivation rate constant for a given organism.

UV irradiation can denature the DNA of microorganisms, causing death or inactivation (Liltved, 2002). Inactivation can be achieved at UV wavelengths from 100 to 400 nm, although a wavelength of 254 nm is most effective. Most UV lamp systems (e.g., low-

pressure lamps) supply monochromatic irradiation specific to the 254 nm wavelength. The intensity of UV irradiation applied is described in terms of milliwatts per square centimeter (mW/cm^2). The dose of UV irradiation required to inactivate a specific microorganism is usually described by a UV irradiation intensity multiplied by the exposure time (i.e., mW s/cm^2 or mJ/cm^2), because UV inactivation of microorganisms normally follows approximately first-order kinetics with respect to UV intensity (White, 1992). Low-pressure UV lamp systems typically provide exposure times of 6–30 s (White, 1992), although longer exposure times may be provided when higher UV irradiation doses are required. However, medium-pressure UV lamp systems provide such high intensities that exposure times are typically even lower than those provided by low-pressure lamp systems. Depending upon the target organism and the required kill rate, UV irradiation doses used in aquaculture can vary from only 2 mW s/cm^2 to more than 230 mW s/cm^2 (Wedemeyer, 1996). Wedemeyer (1996) and Liltved (2002) report that many fish pathogens are inactivated by UV doses of 30 mW s/cm^2. However, they also report that microorganisms such as *Saprolegnia,* white spot syndrome baculovirus, and IPN virus can require UV doses that are 4–10-fold higher in order to achieve inactivation.

During this study, no obligate fish pathogens were present within the commercial-scale recirculating system. Also, it was not practical to introduce an obligate fish pathogen into the system. Indicator organisms have been used to determine the relative effectiveness of a given disinfection process; justification for the use of indicator organisms has been provided by Zhu et al. (2002). Therefore, this research was conducted to determine the UV irradiation dosages required to inactivate total heterotrophic bacteria and total coliform bacteria, which were already present within the commercial-scale recirculating salmonid culture system at the Conservation Fund Freshwater Institute.

2. Materials and methods

2.1. System details

The UV irradiation dosages required to inactivate total heterotrophic bacteria and total coliform bacteria were determined during studies that were carried out within the fully recirculating system (Fig. 1) located at the Conservation Fund Freshwater Institute (Shepherdstown, West Virginia). At the time of these studies, the system was used for Arctic char growout (Summerfelt et al., 2004a). The recirculating system was maintained in a room receiving a continuous 24 h photoperiod. In order to ensure a nearly continuous waste production rate, fish were fed on average approximately 120 kg feed per day in equal portions distributed eight times daily, i.e., one feeding every 3 h, using micro-processor controlled mechanical feeders. The Arctic char were maintained at a culture density of approximately 100–130 kg/m^3 using biannual stocking and selective harvest events that occurred approximately once every 2–3 weeks (Summerfelt et al., 2004a). The recirculating system had been operating for more than 12 months at the time this study was conducted. Prior to its stocking with Arctic char, the recirculating system was thoroughly cleaned (including replacing all of the sand in the fluidized-sand biofilters) and disinfected with >100 mg/L of chlorine for approximately 4 h. The chlorine was completely neutralized with sodium

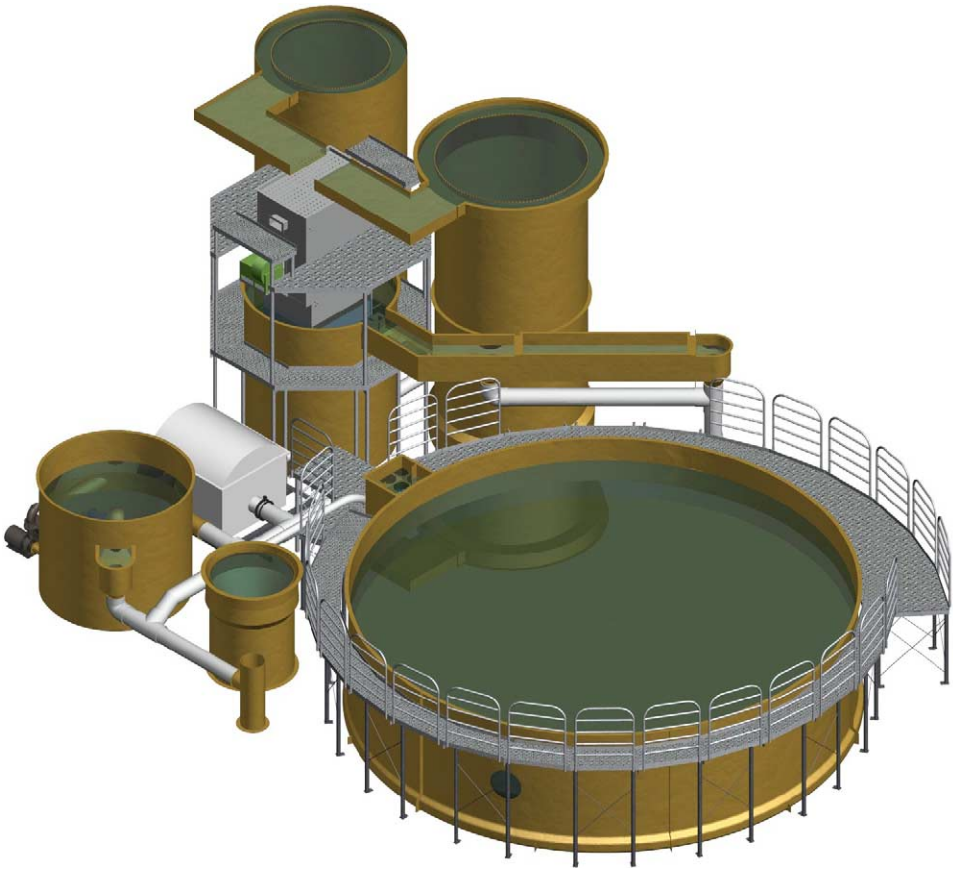

Fig. 1. The 4800 L/min recirculating system at the Freshwater Institute (from Summerfelt et al., 2004a, b). Drawing courtesy of Marine Biotech Inc. (Beverly, MA).

thiosulfate and the recirculating system was then flushed. The biofilters were not inoculated with a commercial bacteria solution, but rather the biofilter inoculation occurred naturally from bacteria carried into the system from the spring water supply or from bacteria present in feed that was added to the system, along with ammonia chloride, approximately four weeks in advance of fish stocking.

The recirculating system pumped 4750 L/min of water through a fluidized-sand biofilter. Water exiting the top of the fluidized-sand biofilter then flowed by gravity through a series of unit treatment processes (i.e., forced-ventilated cascade aeration column, low head oxygenation unit, and UV channel unit) before the water entered the 150 m^3 fish culture tank. Water flowed out of the culture tank's bottom-center drain (approximately 7% of the total flow) and side wall drain (approximately 93% of the total flow) and passed through a swirl separator on the bottom-drain flow and a microscreen drum filter on the recombined culture tank discharge. Water exiting the microscreen drum filter was returned to the pump sump where the water recirculation process began again.

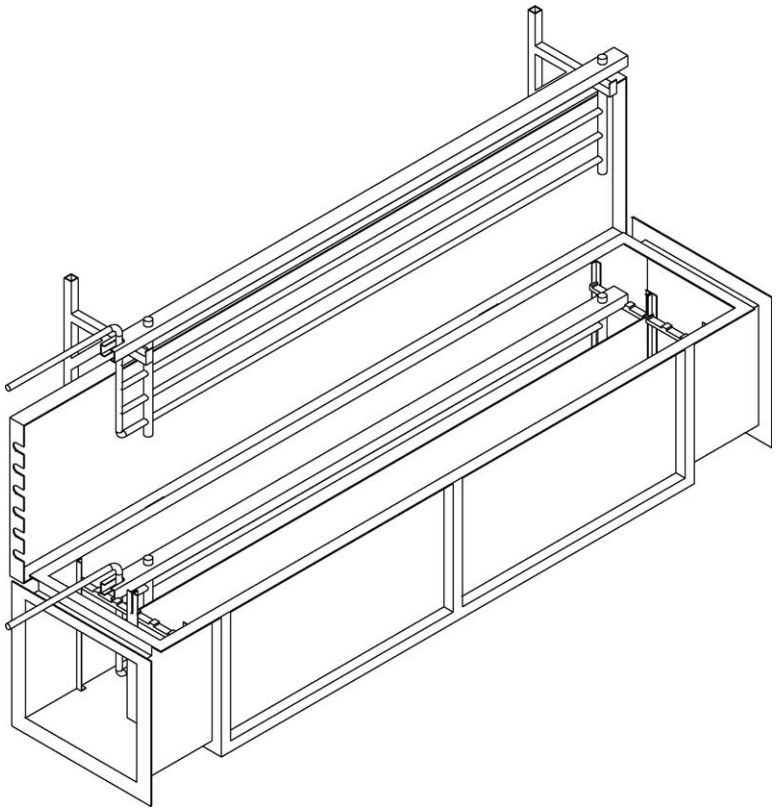

Fig. 2. The horizontal UV channel filter shown here (with one of its lamp-racks removed for service) was installed to irradiate the full 4800 L/min recirculating flow before it returned to the fish culture tank within the recirculating system (from Summerfelt et al., 2001). Drawing courtesy of PRAqua Technologies Ltd., Nanaimo, British Columbia.

PRAqua Technologies LLC (Nanaimo, British Columbia, Canada) and Emperor Aquatics Inc. (Pottstown, Pennsylvania) jointly supplied the custom UV channel unit that was installed to irradiate 100% of the 4750 L/min recirculating water flow (Fig. 2). The UV channel unit contained twenty-four 200 W low-pressure, high-output lamps that supplied a total UV dose of approximately 100–120 mW s/cm^2. However, this study also employed a second UV irradiation unit (UVLogic, Model No. 02AM15, Trojan Technologies Inc., London, Ontario, Canada) that was operated at a constant intensity while treating a side-stream flow of water pumped from the recirculating system's low head oxygenator (LHO) sump (Fig. 3). The UV logic unit was a tube-and-shell design that contained two 254 nm Amalgam lamps, a calibrated UV intensity monitor, and a manual wiper system. The side-stream water flow that was pumped through the UV irradiation unit ranged from 0.15–3.8% (i.e., 7–180 L/min) of the entire recirculating flow. The various water flow rates that were pumped through the side-stream UV irradiation unit produced different water retention times (i.e., from 3–70 s) within the UV irradiation unit and thus produced a range of UV irradiation doses (Tables 1 and 2).

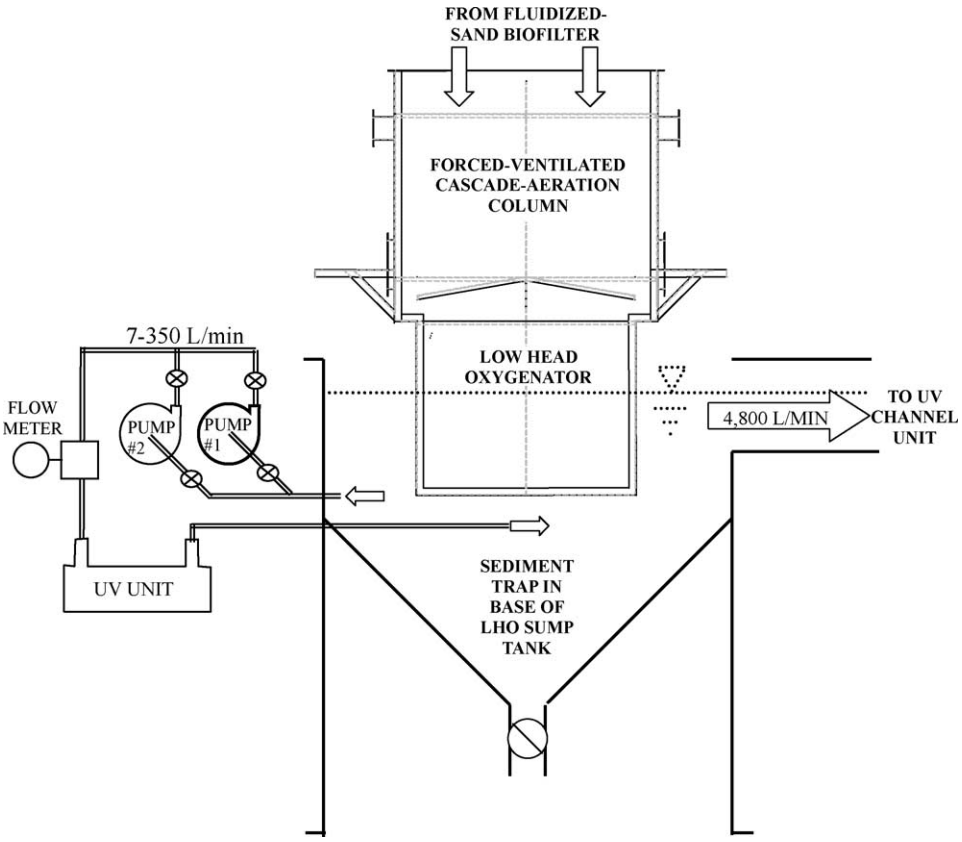

Fig. 3. One or two pumps were used to impel water from the low head oxygenator (LHO) sump tank past a flow meter and then through the UV irradiation unit before this water was returned to the opposite end of the LHO sump. The UV irradiation unit output a constant intensity, so the water flow was adjusted from 7 to 180 L/min in order to adjust the dose of UV applied to the flow.

2.2. Determinations of UV dosages and bacterial reductions

UV irradiation doses of approximately 75, 150, 300, 500, 980, and 1800 mW s/cm^2 were applied to determine the dose necessary to inactivate total heterotrophic bacteria and total coliform bacteria. The UV irradiation dosages applied were each calculated from the product of the average UV irradiation intensity (i.e., UV intensity, mW/cm^2) detected in the irradiation chamber, multiplied by the exposure time—which is the volume of the UV irradiation chamber (i.e., $V_{\text{vessel}} = 9.4$ L) divided by water flow rate (i.e., Q, L/min)—multiplied by a transmittance factor, as shown in the following equation:

$$\begin{aligned}\text{UV dose} &= (\text{UV intensity})(\text{exposure time})(\text{transmittance factor}) \\ &= (\text{UV intensity})\left(\frac{V_{\text{vessel}}}{\text{Q}}\right)(\text{transmittance factor}) \\ &= \text{mW s/cm}^2\end{aligned}$$

Table 1
Number of sampling events and mean (±standard error) UV dose, hydraulic residence time within the UV chamber, total heterotrophic bacteria counts entering and exiting the UV chamber, percentage reduction of total heterotrophic bacteria passing through the UV chamber, and LOG10 reduction in total heterotrophic bacteria

Mean UV dose (mW s/cm^2)	Hydraulic residence time within UV unit (s)	Number of sampling events	Total heterotrophic bacteria counts before UV (cfu/1 mL)	Total heterotrophic bacteria counts after UV (cfu/1 mL)	Reduction in total heterotrophic bacteria counts across UV[a] (%)	LOG10 reduction in total heterotrophic bacteria across UV
1821 ± 86	70.1 ± 2.8	4	9038 ± 3225	181 ± 71	98 ± 1	1.7
980 ± 17	36.2 ± 1.1	4	1708 ± 441	192 ± 68	87 ± 7	0.9
493 ± 20	22.3 ± 0.3	8	8580 ± 2463	5612 ± 1952	57 ± 14	0.4
303 ± 12	12.8 ± 0.0	7	2259 ± 1269	416 ± 209	81 ± 5	0.7
150 ± 9	6.4 ± 0.1	3	7953 ± 3672	328 ± 311	81 ± 19	0.7
78 ± 1	3.1 ± 0.0	3	3688 ± 2342	2678 ± 2586	65 ± 29	0.5

[a] Mean removal efficiencies were calculated from all of the data from each treatment, which provides higher removal efficiencies than if they were calculated from the mean inlet and outlet concentrations shown above.

Table 2
Number of sampling events and mean (±standard error) UV dose, hydraulic residence time within the UV chamber, total coliform bacteria counts entering and exiting the UV chamber, percentage reduction of total coliform bacteria passing through the UV chamber, and LOG10 reduction in total coliform bacteria

Mean UV dose (mW s/cm^2)	Hydraulic residence time within UV unit (s)	Number of sampling events	Total coliform bacteria counts before UV (cfu/100 mL)	Total coliform bacteria counts after UV (cfu/100 mL)	Reduction in total coliform bacteria counts across UV (%)	LOG10 reduction in total coliform bacteria across UV
1821 ± 86	70.1 ± 2.8	4	228 ± 144	<1	100	na
990 ± 21	35.7 ± 1.3	3	60 ± 25	<1	100	na
524 ± 23	22.3 ± 0.4	5	46 ± 21	<1	100	na
303 ± 12	12.8 ± 0.0	7	56 ± 19	<1	100	na
150 ± 9	6.4 ± 0.1	3	100 ± 55	<1	100	na
77 ± 1	3.2 ± 0.0	2	215 ± 205	<1	100	na

To account for resistance to transmittance through the quartz sleeve and the surrounding water, the side-stream UV irradiation unit was supplied with an integral UV irradiation monitor. This monitor continuously measured the UV irradiation intensity at a single location within the irradiation chamber. The transmittance factor was calculated using a proprietary spreadsheet provided by Trojan Technologies, but this calculation was based on the percentage of 254 nm UV irradiation transmitted across a 1-cm path length (%UVT) and a correlation for lamp spacing. The UV irradiation intensity data was combined with the transmittance factor in the proprietary software program to calculate the average UV irradiation intensity supplied within the irradiation chamber.

Water flow rates were measured during each test using a magnetic flow meter (model IFS/020F, Krohne Inc., Peabody, Massachussets). Percentage of 254 nm UV irradiation transmitted across a 1-cm path length (%UVT) was measured by placing water samples into a clean cuvette with a 1-cm path length and then placing the cuvette into a spectrophotometer (model DR/4000U, Hach Chemical Company, Loveland, Colorado) set to display transmittance at a wavelength of 254 nm.

Total heterotrophic bacteria counts and total coliform bacteria counts were measured in water samples collected immediately before and immediately after the side-stream UV irradiation unit. The inlet and outlet samples were collected from 1.3 cm diameter sample valves that were located within 1 m of the inlet and outlet of the UV irradiation unit. Water samples were first collected from the outlet of the UV irradiation unit by opening the sample valve and allowing approximately 2–4 L/min of water flow to dump to the floor. Water flowing out of the sample port was collected in a sterile glass bottle without touching the sample port and after the sample port had been flowing for at least three minutes. The sample valve at the outlet of the UV irradiation unit was then closed and the same water sampling procedure was again initiated by opening the sampling valve at the inlet of the UV irradiation unit. Water samples were immediately used to produce 2–4 different dilutions that were assayed separately for total heterotrophic bacteria and total coliform bacteria. Heterotrophic bacteria were evaluated using Hach Membrane Filtration method 8242 m-TGE Broth with TTC indicator. After incubation, colonies were counted with a low-power microscope and were reported in number of colony forming units (cfu) per 1 mL sample. Similarly, coliform bacteria were analyzed using Hach Membrane Filtration method 8074 (m-Endo Broth). Water samples were not pre-filtered before they were assayed for bacteria using Membrane Filtration methods 8242 and 8074. Coliform colonies were counted with a low-power microscope and were measured in number of cfu per 100 mL sample. Calculation of coliform concentration followed the American Public Health Association (APHA) (1998) *Membrane Filter Technique for Members of the Coliform Group* using membrane filters with an ideal count range of 20–80 coliform colonies and not more than 200 colonies of all types per membrane by the following equation:

$$\frac{\text{coliforms}}{100\,\text{mL}} = \frac{(\text{coliform colonies counted})}{\text{sample filtered (mL)}} \times 100$$

Counts falling below the ideal range were recorded and were used if the other dilutions tested did not produce a bacteria count of <200 colonies per membrane. However, if no coliform colonies were observed, the coliform colonies counted were reported as

<1 coliform/100 mL. Calculation of total heterotrophic density followed the American Public Health Association (APHA) (1998) *Heterotrophic Counting and Recording* procedure using membrane filters with an ideal count range of 30–300 colonies. Counts falling below the ideal range were recorded and were used if the other dilutions tested did not produce a bacteria count of <300 colonies per membrane. However, if no heterotrophic colonies were observed, the total heterotrophic colonies counted were reported as <1 cfu/mL.

In an earlier study, total heterotrophic bacteria counts were also quantified immediately before and after the full-flow UV channel unit. Counts of total heterotrophic bacteria were also measured in the makeup water supplied to the recirculating system. Total heterotrophic counts were performed on the above water samples by making serial 10-fold dilutions in phosphate buffered saline (PBS) and plating samples by drop or spread plate technique on R2A agar (Difco Laboratories Inc., Detroit MI). In the drop plate procedure 25 μl of each dilution was placed on a single R2A plate, and after the liquid was absorbed into the medium, the plate was inverted and incubated for 5 days at 20 °C. With the spread plate technique 25 μl of each dilution was placed on each of three R2A plates and a sterile bent glass rod was used to spread the drop over the surface of the medium. Plates were then inverted and incubated the same as drop plates. After incubation those dilutions showing 5–20 colonies on drop plates, and 30–300 on spread plates were counted, multiplied by the dilution factor and reported as cfu/ml of water. This procedure was more time consuming than the Hach Membrane Filtration method 8242, which was why the Hach method was later used to measure total heterotrophic bacteria counts.

The bacteria removal efficiency across the UV irradiation unit was calculated using the following equation:

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

Then, the LOG10 reduction of bacteria was calculated using the following equation:

$$\text{LOG10 reduction} = -\log_{10}\left(1 - \frac{\text{percent removal}}{100}\right)$$

As an example, a 1.0 LOG10 bacteria reduction would correspond to a 90% removal efficiency and a 2.0 LOG10 bacteria reduction would correspond to a 99% removal efficiency.

Water samples were collected during the bacteria sample collection. Total suspended solids (TSS), total dissolved solids (TDS), total ammonia nitrogen (TAN), and alkalinity, along with the water's pH were measured. TSS and TDS concentrations were measured according to standard methods procedures 2540 D and 2540 C, respectively (American Public Health Association (APHA), 1998). TAN concentrations were measured using the Nessler method using Hach Chemical Company reagents and a DR4000 spectrophotometer (Hach Chemical Company). Alkalinity of water samples was measured by titration (American Public Health Association (APHA), 1998). The pH of water was measured using a pH probe and a Fisher Scientific Accumet pH meter 915 (Pittsburgh, PA) that was calibrated against standard buffer solutions of known pH.

3. Results and discussion

3.1. UV irradiation of the full-recirculating flow

In the first study, reductions in total heterotrophic bacteria were monitored across the full-flow UV channel unit, i.e., the UV unit that was used to treat the entire recirculating flow. During this period, the UV channel unit achieved an $85.8 \pm 3.3\%$ (<1 LOG10) reduction in total heterotrophic bacteria at a UV irradiation dose that was estimated at approximately 100–120 mW s/cm^2. The concentration of total heterotrophic bacteria entering the UV channel unit was relatively high, averaging $21{,}360 \pm 4500$ cfu per 1 mL. During this same period, the makeup water contained, on average, 1940 ± 220 cfu of total heterotrophic bacteria per 1 mL. Therefore, even with full-flow UV irradiation, the organic load within the recirculating system increased the total heterotrophic counts by approximately 10-fold (1 LOG10).

3.2. UV dosages necessary for bacteria inactivation

In the second study, a relatively small side-stream water flow was used to investigate the impact of UV irradiation dose on inactivation of total heterotrophic bacteria and total coliform bacteria. Results indicate that the total coliform bacteria in the recirculating system were susceptible to UV inactivation and that complete inactivation of coliform bacteria was consistently achieved at all UV doses applied, even at the lowest dose of 77 mW s/cm^2 (Table 2). Achieving total inactivation of total coliform bacteria at a dose of $\geq$77 mW s/cm^2 was not surprising, because others (Oppenheimer et al., 1997; Emerick et al., 1999) have reported 3–4 LOG10 inactivation of total coliform bacteria at similar UV dosages. In contrast, a UV dosage in excess of 1800 mW s/cm^2 was required to achieve a not quite 2 LOG10 reduction, i.e., a $98 \pm 1\%$ reduction in total heterotrophic bacteria (Table 1). This level of bacteria inactivation required a mean hydraulic residence time within the UV irradiation chamber of approximately 70 s (Table 1) at a constant UV irradiation level of approximately 26 mW/cm^2. In comparison, Farkas et al., (1986) reports no inactivation or inconsistent inactivation of heterotrophic bacteria, *Aeromonas* [*hydrophila* and *punctata*], and *Flexibacter columnaris* across a UV irradiation within a recirculating system. In the present study, the relatively low inactivation of heterotrophic bacteria measured was surprising, because the UV dose required to achieve nearly a 2 LOG10 reduction in total heterotrophic bacteria was nearly 60 times greater than the 30 mW s/cm^2 dose typically recommended in aquaculture. It was also surprising that UV irradiation was not as effective at reducing heterotrophic bacteria because Oppenheimer et al. (1997) and Emerick et al. (1999) report a 3–4 LOG10 reduction in heterotrophic bacteria at a UV dose of near 78 mW s/cm^2. Granted, the UV inactivation of heterotrophic bacteria data they reported were collected from effluents of publicly owned wastewater treatment facilities. Emerick et al. (1999), Loge et al. (1996), and Liltved and Cripps (1999) have noted that inactivating 100% of bacteria in a given flow can be difficult, even at excessive UV doses, because UV irradiation cannot always penetrate particulate matter to reach embedded bacteria.

Table 3
Mean (±standard error) water quality during these UV inactivation tests

pH	7.53 ± 0.02
Alkalinity (mg/L as $CaCO_3$)	219 ± 3
Total suspended solids (mg/L)	3.5 ± 0.4
Total dissolved solids (mg/L)	410 ± 10
UV transmittance (%)	90 ± 1
Total ammonia nitrogen (mg/L as nitrogen)	0.44 ± 0.06

During our study, the concentration of total suspended solids entering the UV irradiation unit averaged 3.5 ± 0.4 mg/L (Table 3). These solids were likely smaller than 90 μm in size, because larger solids would have been removed by the 90 μm opening in the sieve panels of the microscreen filter. However, this relatively low TSS concentration must still have contained sufficient imbedded bacteria to reduce the effectiveness of UV irradiation at dosages approaching 1800 mW s/cm^2. Was it possible that our recirculating aquaculture system had embedded heterotrophic bacteria that could be resistant to a UV irradiation dose that was 20–50 times greater than what Oppenheimer et al. (1997) reported necessary to inactivate the heterotrophic bacteria? Consider that in our study the recirculating water flow was frequently passed through a UV channel unit that supplied in excess of 100 mW s/cm^2 of UV irradiation. Based on mean hydraulic residence times within the system, bacteria suspended in the recirculating water would pass through the UV channel unit approximately once every 0.5 h. We present the hypothesis that this frequent exposure to approximately 100–120 mW s/cm^2 of UV irradiation provided a process that selects for bacteria that are embedded within particulate matter or that form bacterial aggregates, because some of the embedded bacteria would be shaded from the full UV dose. Other hypotheses could be formulated to explain the mechanism that allowed the heterotrophic bacteria to resist UV irradiation dosages of up to 1000 mW s/cm^2 in the recirculating aquaculture system. This phenomena merits further study.

It is important to note that the total coliform bacteria were always inactivated at the UV irradiation dosages applied, which indicates that at least certain microorganisms are always inactivated under the conditions tested. It remains yet to be seen whether the majority of fish pathogens, which are reported to be inactivated by UV irradiation doses of less than 30 mW s/cm^2 in single-pass applications (Wedemeyer, 1996; Liltved, 2002), will respond more like the total coliform bacteria reacted in the recirculating systems—and be susceptible to UV inactivation—or like the total heterotrophic bacteria encountered during this study.

4. Acknowledgments

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experimental protocol and methods used in this study were in compliance with Animal Welfare Act (9CFR) requirements and are approved by the Freshwater Institute Institutional Animal Care and Use Committee.

References

American Public Health Association (APHA), 1998. Standard methods for the examination of water and wastewater, 20th ed. APHA, Washington, DC.

Bebak-Williams, J., Noble, A., Bowser, P.A., Wooster, G.A., 2002. Fish health management. In: Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., Vinci, B.J. (Eds.), Recirculating Aquaculture Systems. second ed. Cayuga Aqua Ventures, Ithaca, NY, Chapter 13, pp. 427–466.

Blancheton, J.P., 2000. Developments in recirculating systems for Mediterranean fish species. Aquacult. Eng. 22, 17–31.

Blancheton, J.P., Canaguier, B., 1995. Bacteria and particulate materials in recirculating seabass (*Dicentrarchus labrax*) production system. Aquaculture 133, 215–224.

Bullock, G., Hankins, J.A., Heinen, J., Starliper, C., Teska, J., 1993. Qualitative and quantitative bacteriological studies on a fluidized sand biofilter used in a semi-closed trout culture system. Biological Report 17, U.S. Fish and Wildlife Service, Washington, DC, p. 24.

Bullock, G.L., Summerfelt, S.T., Noble, A., Weber, A., Durant, M.D., Hankins, J.A., 1997. Ozonation of a recirculating rainbow trout culture system: I. Effects on bacterial gill disease and heterotrophic bacteria. Aquaculture 158, 43–55.

Chen, S., Timmons, M.B., Aneshansley, D.J., Bisogni Jr., J.J., 1993. Suspended solids characteristics from recirculating aquaculture systems and design implications. Aquaculture 112, 143–155.

Emerick, R.W., Loge, F.J., Thompson, D., Darby, J.L., 1999. Factors influencing ultraviolet disinfection performance. Part II. Association of coliform bacteria with wastewater particles. Water Environ. Res. 71, 1178–1187.

Farkas, J., Oláh, J., Magyar, K., 1986. Effect of ultraviolet lamp on the microflora of a closed warm-water recycling system. Aquacult. Hung. (Szarvas) 5, 191–199.

Hagopian, D.S., Riley, J.G., 1998. A closer look at the bacteriology of nitrification. Aquacult. Eng. 18, 223–224.

Heinen, J.M., Weber, A.L., Noble, A.C., Morton, J.D., 1995. Tolerance to formalin by a fluidized-bed biofilter and rainbow trout *Oncorhynchus mykiss* in a recirculating culture system. J. World Aquacult. Soc. 26, 65–71.

Leonard, N., Guiraud, J.P., Blancheton, J.P., 2000. Populations of heterotrophic bacteria in an experimental recirculating aquaculture system. Aquacult. Eng. 22, 109–120.

Leonard, N., Guiraud, J.P., Gasset, E., Cailleres, J.P., Blancheton, J.P., 2002. Bacteria and nutrients—nitrogen and carbon—in a recirculating system for sea bass production. Aquacult. Eng. 26, 111–127.

Liltved, H., 2002. Ozonation and UV-irradiation. In: Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., Vinci, B.J. (Eds.), Recirculating Aquaculture Systems. second ed. Cayuga Aqua Ventures, Ithaca, NY, Chapter 12, pp. 393–426.

Liltved, H., Cripps, S.J., 1999. Removal of particle-associated bacteria by prefiltration and ultraviolet irradiation. Aquacult. Res. 30, 445–450.

Loge, F.J., Emerick, R.W., Heath, M., Jacangelo, J., Tchobanoglous, G., Darby, J.L., 1996. Ultraviolet disinfection of secondary wastewater effluents: prediction of performance and design. Water Environ. Res. 68, 900–916.

McMillan, J.D., Wheaton, F.W., Hochheimer, J.N., Soares, J., 2003. Pumping effect on particle sizes in a recirculating aquaculture system. Aquacult. Eng. 27, 53–59.

Nam, T.K., Timmons, M.B., Montemagno, C.D., Tsukuda, S.M., 2000. Biofilm characteristics as affected by sand size and location in fluidized bed vessels. Aquacult. Eng. 22, 213–224.

Noble, A.C., Summerfelt, S.T., 1996. Diseases encountered in rainbow trout cultured in recirculating systems. Annu. Rev. Fish Dis. 6, 65–92.

Oppenheimer, J.A., Jacangelo, J.G., Laine, J.-M., Hoagland, J.E., 1997. Testing the equivalency of ultraviolet light and chlorine for disinfection of wastewater to reclamation standards. Water Environ. Res. 69, 14–24.

Patterson, R.N., Watts, K.C., Timmons, M.B., 1999. The power law in particle size analysis for aquacultural facilities. Aquacult. Eng. 19, 259–273.

Patterson, R.N., Watts, K.C., 2003a. Micro-particles in recirculating aquaculture systems: particle size analysis of culture water from a commercial Atlantic salmon site. Aquacult. Eng. 28, 99–113.

Patterson, R.N., Watts, K.C., 2003b. Micro-particles in recirculating aquaculture systems: microscopic examination of particles. Aquacult. Eng. 28, 115–130.

Schwartz, M.F., Bullock, G.L., Hankins, J.A., Summerfelt, S.T., Mathias, J.A., 2000. Effects of selected chemotherapeutants on nitrification in fluidized-sand biofilters for cold water fish production. Int. J. Recirc. Aquacult. 1, 61–81.

Sich, H., Van Rijn, J., 1997. Scanning electron microscopy of biofilm formation in denitrifying, fluidized bed reactors. Water Res. 31, 733–742.

Summerfelt, S.T., 2003. Ozonation and UV irradiation—an introduction and examples of current applications. Aquacult. Eng. 28, 21–36.

Summerfelt, S.T., Bebak-Williams, J., Tsukuda, S., 2001. Controlled systems: water reuse and recirculation. In: Wedemeyer, G. (Ed.), Second Edition of Fish Hatchery Management. American Fisheries Society, Bethesda, Maryland, pp. 285–395.

Summerfelt, S.T., Wilton, G., Roberts, D., Savage, T., Fonkalsrud, K., 2004a. Developments in recirculating systems for arctic char culture in North America. Aquacult. Eng. 30, 31–71.

Summerfelt, S.T., Sharrer, M.J., Hollis, J., Gleason, L.E., Summerfelt, S.R., 2004b. Dissolved ozone destruction using ultraviolet irradiation in a recirculating salmonid culture system. Aquacult. Eng. 32, 209–224.

Summerfelt, S.T., Bebak-Williams, J., Fletcher, J., Carta, A., Creaser, D. Description of the surface water filtration and ozone treatment system at the Northeast Fishery Center. In: Fisheries Bioengineering Symposium IV. American Fisheries Society, Bethesda, Maryland, in press.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., Vinci, B.J., 2002. Recirculating Aquaculture Systems, second ed. Cayuga Aquaculture Ventures LLC, Ithaca, NY, p. 769.

Wedemeyer, G.A., 1996. Physiology of Fish in Intensive Culture. International Thompson Publishing, New York, NY.

White, G.C., 1992. Handbook of chlorination and alternative disinfectants. Van Nostrand Reinhold, New York.

Zhu, S., Saucier, B.B., Chen, S., Durfey, J.E., 2002. Evaluation of UV disinfection performance in recirculating systems. Int. J. Recirc. Aquacult. 3, 61–79.