

Ozonation of a recirculating rainbow trout culture system¹

I. Effects on bacterial gill disease and heterotrophic bacteria

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Abstract

Ozone was added to water in a recirculating rainbow trout (*Oncorhynchus mykiss*) culture system just before it entered the culture tanks in an attempt to reduce the numbers of heterotrophic bacteria in system water and on trout gills, and to prevent bacterial gill disease (BGD) in newly stocked fingerlings. During four 8-week trials, ozone was added to the system at a rate of 0.025 or 0.036–0.039 kg ozone/kg feed fed. In the control, where no ozone was added, and in previously published research, BGD outbreaks occurred within two weeks of stocking, and these outbreaks generally required three to four chemotherapeutant treatments to prevent high mortality. In three of four trials where ozone was added to the system, BGD outbreaks were prevented without chemical treatments, but the causative bacterium, *Flavobacterium branchiophilum*, still colonized gill tissue. The one ozone test where BGD outbreaks required two chemical treatments coincided with a malfunction of the ozone generator. Although ozonation did reduce BGD mortality, it failed in all trials to produce more than a one log₁₀ reduction in numbers of heterotrophic bacteria in the system water or on gill tissue. Failure of the ozone to lower numbers of heterotrophic bacteria or to prevent the causative BGD bacterium from occurring on gills was attributed to the short exposure time to ozone residual (35 s contact chamber) and rapid loss of oxidation caused by levels of total suspended solids. Rationale for ozone's success at preventing BGD mortalities are not fully understood but may in part be due to improved water quality. Use of the lower ozone

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dosing rate (0.025 kg ozone/kg feed) appeared to provide the same benefits as the higher dosing rate (0.036–0.039 kg ozone/kg feed fed); however, the lower ozone dosing rate was less likely to produce a toxic ozone residual in the culture tank and would also reduce ozone equipment capital and operating costs. © 1997 Published by Elsevier Science B.V.

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1. Introduction

Aquaculture is continuing to expand worldwide, but such growth is dependent on the availability of high quality water. The use of recirculating culture systems is one means of using available water more efficiently. For the past several years, The Conservation Fund's Freshwater Institute has researched recirculating system technology and production strategies to culture rainbow trout (*Oncorhynchus mykiss*). The current system being evaluated consists of cross-flow culture tanks, mechanical microscreen filters, carbon dioxide strippers, multi-stage low head oxygenators, and fluidized-sand biofilters. The goal of this research has been to develop cost effective and environmentally friendly technology. Recurring epizootics of bacterial gill disease (BGD) occur predictably each time fingerlings are stocked (Bullock et al., 1994). Although chemical treatments are available to control these epizootics (Bullock and Herman, 1991), they are not approved by the US Food and Drug Administration. Disinfection of culture water is another possible means of controlling disease outbreaks. Two commonly used procedures are ultraviolet irradiation (UV) and ozonation (Dupree, 1979; Anderson, 1982; Owsley, 1991). Although UV is widely used, water turbidity and algal or bacterial growth on the UV lamp jackets can severely limit its effectiveness.

Ozone has been used in single-pass aquaculture systems and to disinfect or sterilize water supplies and/or discharges in a few large federal salmonid hatcheries in the western US (Roselund, 1975; Colberg et al., 1977; Owsley, 1991; Cryer, 1992). These single-pass systems typically have a low ozone demand (i.e., low organic carbon content), and a three to four log₁₀ (i.e., 99.9–99.99%) reduction in pathogens can often be achieved.

Bacterial reduction and viral inactivation may be desirable within recirculating systems. However, to disinfect recirculating systems water with ozone could be very expensive due to: (1) the much higher ozone loading required to overcome the organic demand and to sustain a residual that would be sufficient to achieve significant bacterial and viral reductions; and (2) the need to strip any remaining residual ozone from the water before it is returned to the culture tank. The overall objectives of our research were to demonstrate what effect ozonation would have when added at levels that were obtained by creating 3–4% ozone within the existing oxygen feed gas before it is transferred into the system. It is significant that the ozone is generated and transferred within an oxygen feed gas that was already required to provide a dissolved oxygen supersaturation within each culture tank's influent.

The objectives of the research reported here were to demonstrate the effects of ozone addition on outbreaks of BGD and on total heterotrophic bacteria concentrations. An

accompanying paper (Summerfelt et al., 1997) describes the effects of adding ozone on water quality and microscreen filter performance within this recirculating system.

2. Materials and methods

2.1. Recirculating system

The recirculating system consisted of one fluidized-sand biofilter, two multi-stage low-head oxygenators (LHO™²), two microscreen filters, one cascade aeration column, and two cross-flow fish culture tanks (C-1 and C-2). The system recirculated water in two parallel flow paths (a path for fish culture and a path for biofiltration and carbon dioxide stripping) connected within a common sump (Fig. 1). In the fish culture path, approximately 720 l/min were split into two parallel streams that were first pumped through a LHO™ unit, were carried by gravity through the cross-flow fish culture tank, and were finally passed through the Triangle™ filter unit (Model TF-12-RB with 80- μ m opening sieve panels; Hydrotech, Villinge, Sweden) before dropping back into the sump. Each cross-flow tank had a culture volume of 9.0 m³, which was replaced 2.3 times per hour or about 55 times per day. In the biofiltration and carbon dioxide stripping path, approximately 760 l/min were pumped through a fluidized-sand biofilter, and were then cascaded counter-current to air within the carbon dioxide stripping column before returning to the sump. Partitions were placed within the common sump to reduce mixing between the fish culture path and the biofiltration/stripping path. The sump design allowed for the independent operation of the fish culture and biofiltration flow paths, which was particularly important during chemical treatment of the fish culture tanks. A more complete description of a previous version of the same system was given by Heinen et al. (1996).

2.2. Ozone tests

Ozonation of the recirculating system was studied during four 8-week tests and an 8-week no ozone control (Table 1). During the first two ozone trials, ozone was added only to the flow passing through the LHO™ unit preceding tank C-1 (Fig. 1, Table 1). Adding ozone prior to only one of the two culture tanks let us study whether dosing location impacted system performance. Ozone was added to both LHO™ units during the third and fourth ozone tests (Fig. 1, Table 1), which allowed us to maximize the amount of ozone that could be added to this recirculating system without making additional structural modifications or without increasing oxygen usage beyond that required by the fish. Approximately 0.025 and 0.036–0.039 kg ozone were added per kg feed fed in the first two ozone tests and the last two ozone tests, respectively (Table 1). Ozone addition was relatively constant during each test, except in trial 2 when the ozone generator failed.

² Use of trade or manufacturer names does not imply endorsement.

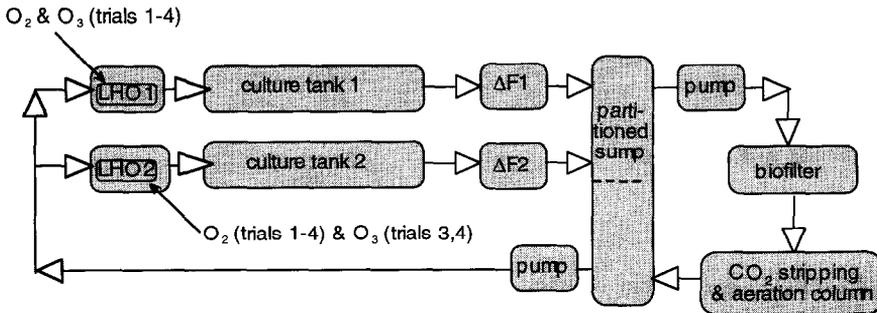


Fig. 1. Ozone was added within an LHO™ (LHO1 and LHO2) prior to each culture tank within the recirculating system: ΔF1 = Triangel™ filter 1; ΔF2 = Triangel™ filter 2.

2.3. Addition of ozone

Because the LHO™ and oxygen distribution and control mechanisms were already in place, adding ozone only required the addition of an ozone generator and accompanying ozone distribution, monitoring, and control mechanisms. Ozone was generated by passing the oxygen feed gas through a corona type generator (model G-1, PCI Ozone and Control Systems, West Caldwell, NJ) rated at 1.2 kg/d when producing 3% ozone output within 20.5 l/min feed gas at standard temperature and pressures. Approximately 7.9 l/min oxygen that contained 4.5% ozone were added to the LHO™ preceding C-1 during trials 1 and 2; approximately 7.1 l/min oxygen that contained 3.5% ozone were added to each LHO™ unit during trials 3 and 4. The 7.1–7.9 l/min oxygen feed was all of the oxygen added to each LHO™ during normal operating conditions. However, an automatic oxygen control system monitoring each fish culture tank would, on occasion, cause more oxygen (free of ozone) to be added to the appropriate LHO™ units to

Table 1

Ozone dosing during each treatment: ozone concentration added to the flow passing through each LHO™, total daily ozone addition, and the ratio of daily ozone addition to daily fish feed fed within the recycle system. Feed weights are reported as total feed fed (with moisture), not on a dry weight basis

Trial	8-Week	Ozone added before each culture tank		Ozone dose (kg/d)	Ozone:feed ratio (kg ozone/kg feed)
		C-1 (mg/l)	C-2 (mg/l)		
1	1/5/94–3/1/94	1.3	0.0	0.68	0.025:1
control	3/2/94–4/26/94	0.0	0.0	0.0	0.000:1
2 ^a	4/27/94–6/22/94	1.3	0.0	0.68	0.025:1
3	8/17/94–10/11/94	1.0	1.0	1.0	0.039:1
4	10/12/94–12/13/94	1.0	1.0	1.0	0.036:1

^aApproximately 40% of ozone generation capacity was lost towards the end of this treatment due to hydrocarbons in the oxygen feed gas that fouled the dielectrics within the corona discharge cell of the ozone generator.

counter short-term increases in oxygen demand. Ozone concentrations within the feed gas were measured continually with an UV-based instrument (model HC-12, PCI Ozone and Control Systems).

Generated ozone was moved through stainless steel pipes to either one or both LHO™ units and was transferred to the recirculating flow just prior to entry into the culture tank. Adding ozone to the LHO™ within this configuration resulted in an ozone contact time within the water of only 35 s before it entered the cross-flow culture tank. Because cross-flow culture tanks are characterized as completely mixed vessels (Watten and Johnson, 1990), the ozone that entered the culture tank was immediately diluted to the concentration leaving the culture tank. Therefore, the culture tank provided additional time for ozone reaction and destruction.

Dissolved ozone was measured three times a week at the water inflow immediately after ozonation and within culture tanks using Hach Chemical Ozone Reagent Ampoules and a Hach DR/2000 spectrophotometer (Hach Chemical, Loveland, CO). As an added safety measure, oxidation/reduction potential (ORP) based control systems were used to prevent ozone residual from accumulating to toxic levels within the culture tanks. The Stranco model 4-2F Automatic ORP Controller (Bradley, IL) was used to track ORP and control ozone addition.

2.4. *Determination of water quality*

Total suspended solids (TSS), total ammonia nitrogen, and nitrite nitrogen in the water leaving the culture tanks were measured three times weekly, for at least six weeks of each eight week trial. Dissolved oxygen, pH, and temperature were measured continuously with calibrated probes. TSS concentrations were measured using APHA (1989) method 209°C. Total ammonia nitrogen and nitrite nitrogen were measured by the Nessler and diazotization methods, respectively, using Hach Chemical reagents and either a DR2000 or DR3000 spectrophotometer.

2.5. *Fish and feeding*

Rainbow trout were raised using a continual culture strategy as described by Summerfelt et al. (1997). A mean biomass of about 2000 kg was maintained within each 9.0-m³ culture tank during these studies. At the beginning of each trial, approximately 2250 fingerlings (mean total length = 100 mm) were stocked into each of the two culture tanks. Fish were fed a Hi-Fat Trout Grower diet (Zeigler Brothers, Gardener, PA) with demand feeders. Fingerlings were fed 2.4-mm (3/32-in.) pellets for 8 weeks and, thereafter, received 3.2-mm (1/8-in.) pellets. Daily system feed consumption averaged 31.2, 29.5, 29.4, 26.2, and 28.3 kg, respectively, for the no-ozone control test and ozone tests one through four. Daily mortality records were kept, and if mortality from BGD exceeded 20 fish per day, trout were treated with 1-h bath treatments of either 12 mg/l chloramine-T (*n*-chloro-4-methylbenzenesulfonamide sodium salt) or 2 mg/l Roccal™ (dimethyl benzyl ammonium chloride).

2.6. Enumeration of heterotrophic bacteria and *flavobacterium branchiophilum*

The effect of ozone on the numbers of heterotrophic bacteria in the recirculating water and on rainbow trout gills and the presence of *F. branchiophilum* on gill tissue was determined as follows: The day before the fish were stocked, five were randomly selected, euthanized in tricaine methanosulfonate, and gill tissue was aseptically removed. A gill smear was prepared to detect *F. branchiophilum* by the indirect fluorescent antibody test (IFAT) as described by Bullock et al. (1994). Each stained smear was examined under oil immersion, using a fluorescence microscope with epi-illumination, and the number of clumps (three or more cells) of *F. branchiophilum* was counted in 50 microscope fields. For enumeration of heterotrophic bacteria, 0.48–0.52 g of gill tissue was aseptically weighed into a sterile 15-mm × 75-mm tube. Cold, sterile, pH 7.2 phosphate buffered saline (PBS) was added to prepare a 1:10 dilution. Each sample was then sonicated as described by Bullock et al. (1993) to remove bacteria, and serial log₁₀ dilutions were prepared. Using the drop plate technique of Miles et al. (1938), six 50- μ l drops each of selected dilutions were placed onto a 15-mm × 100-mm culture plate of plate count agar (PCA; Difco Laboratories, Detroit, MI). Plate cultures were incubated at 25°C for 72 h, colonies were counted in each dilution, multiplied by the appropriate dilution factor, and reported as colony forming units (CFU) per gram of gill tissue. In the recirculating culture system water, samples were taken just prior to and immediately after the points of ozone addition (i.e., one or both LHO™ units) and from water within the culture tanks. Ten-fold dilutions were prepared using PBS; plate counts were performed as previously described, and bacteria reported as CFU/ml of water.

For each of the four ozone tests and the no ozone control, gill and water samples were taken on day 7, 10, 14, 17, 24, 28, 35, 42, and 49 post stocking. In ozone tests one and two and the no ozone control, five fish from C-1 and from C-2 were examined each sample day for heterotrophic counts and IFAT examination. In ozone tests three and four, five fish were sampled for heterotrophic counts but, because of limited supply of antiserum, only three fish per tank were examined by IFAT.

3. Results

The addition of ozone in the four tests did not prevent colonization of *F. branchiophilum* on the gills or completely prevent mortality from BGD (Table 2). Additionally, ozone did not appear to reduce the numbers of heterotrophic bacteria on gill tissue or in the water by more than 1 log₁₀ (Table 2). As described in the accompanying paper (Summerfelt et al., 1997), ozone reduced water color and the concentration of nitrite, and oxidized the total suspended solids improving their removal across the Triangle™ microscreen filters. In the culture tanks, water pH ranged from 7.1–7.3, oxygen from 9.1–12 mg/l, total ammonia nitrogen from 1.1–1.3 mg/l, TSS from 2.9 to 6.3 mg/l, nitrite from 0.024–0.265 mg/l, and temperature from 14.3–16.3°C.

Table 2

Effect of ozone addition on occurrence of bacterial gill disease, fish mortality, and water quality parameters

Parameter	Control (no ozone)	Ozone trial			
		1	2	3	4
<i>BGD-induced mortalities, %</i>					
Tanks C-1 + C-2	4.3	4.1	10.1	3.3	1.7
<i>Treatments to control BGD^a, #</i>					
Tank C-1	4	0	2	0	0
Tank C-2	4	0	4	0	0
<i>Presence of F. branchiophilum on gills, percent positive (#/#)^b</i>					
Tank C-1	54 (27/50)	8 (4/50)	54 (27/50)	39 (9/23)	40 (12/30)
Tank C-2	44 (22/50)	24 (13/50)	46 (23/50)	57 (12/21)	30 (9/30)
<i>Average number of clumps of F. Branchiophilum per 50 fields on infected trout gills</i>					
Tank C-1	4.0	0.45	8.0	5.0	5.0
Tank C-2	3.0	1.8	8.0	4.0	4.0
<i>Heterotrophic bacteria on gills, CFU/g tissue × 10⁴ ± s.e.</i>					
Tank C-1	58.3 ± 8.4	37.9 ± 12.4	425 ± 166	425 ± 229	167 ± 111
Tank C-2	39.5 ± 7.4	29.2 ± 8.7	223 ± 49	205 ± 88	129 ± 75
<i>Heterotrophic bacteria in water, CFU/ml × 10³ ± s.e.</i>					
Tank C-1	30.8 ± 10.3	6.8 ± 3.2	4.5 ± 1.1	4.8 ± 1.5	3.6 ± 2.2
Tank C-2	30.6 ± 8.2	18.0 ± 2.2	13.8 ± 2.0	3.1 ± 1.3	3.8 ± 2.5
<i>Ozone concentration, µg/l ± s.e.</i>					
Tank C-1 influent	0	50.0 ± 12.9	180.0 ± 29.6	33.6 ± 15.6	87.3 ± 29.0
Tank C-2 influent	0	0	0	18.2 ± 7.5	65.5 ± 22.0
Tank C-1	0	10.0 ± 6.4	24k.5 ± 1.6	5.5 ± 3.9	11.8 ± 8.0
Tank C-2	0	0	0	3.6 ± 3.6	10.9 ± 6.7
<i>Ozone-induced mortalities, %</i>					
Tanks C-1 + C-2	0	0	0	3.9	5.0
Temperature,	15.2 ± 0.2	14.3 ± 0.1	15.6 ± 0.1	16.3 ± 0.1	15.2 ± 0.1
°C ± s.e.					

^a12 mg/l chloramine-T for 1 h or 2 mg/l Roccal for 1 h.^bnumber of trout positive/number trout examined.

3.1. Bacterial gill disease

F. branchiophilum was not detected on gill tissue before fish were stocked. Once fish were stocked, *F. branchiophilum* was detected on gill tissue within 10 days in all trials (Table 2). The control and test two had a slightly higher percentage of *F. branchiophilum* positive fish. Some mortality from BGD occurred in all trials; but it was slightly higher in ozone trial two (when the ozone generator malfunctioned) and during the no ozone control. The higher percentage of fish carrying the bacterium and the necessity of chemical treatment in test two coincided with a 40% reduction in ozone production due

to fouled dielectrics in the corona discharge cell of the ozone generator. In the control, four chemical treatments were required in each culture tank to prevent increased mortalities. However, multiple chemical treatments were not required to control mortality from BGD in three of the four tests where ozone was added to the system (Table 2). In tests one, three, and four, mortality from BGD was self limiting, and no treatments were required.

3.2. Heterotrophic bacteria

Heterotrophic bacterial counts in C-1 and C-2 tank water during the control trial contained 3.1×10^4 bacteria/ml water, while gill samples from fish in the two culture tanks contained $3.9\text{--}5.8 \times 10^5$ bacteria/g tissue. Counts during the 8-week period for tests one and two showed a slight reduction of bacteria in culture tank water. The range in C-1 water was 4.5×10^3 to 6.8×10^3 CFU/ml; the sample site was directly before the point of ozone addition. The range in C-2 water was 1.4×10^4 to 1.8×10^5 CFU/ml; water in this tank should not have received any direct exposure to residual ozone in tests one and two. In tests three and four, when both tanks received ozone, counts ranged from 3.1×10^3 to 4.8×10^3 CFU/ml (Table 2).

There was no apparent effect of ozone on numbers of heterotrophic bacteria on gill tissue during the trials; counts ranged from 2.9×10^5 to 4.2×10^6 CFU/g tissue (Table 2).

3.3. Residual ozone

Residual ozone entering tanks C-1 and C-2 for the four tests ranged from 0 to 0.25 mg/l, and the means ranged from 0.03 to 0.18 mg/l for the four ozone tests. During the four ozone tests, ozone levels within the fish culture tanks receiving ozonated water averaged $\leq 12 \mu\text{g/l}$ (Table 2). Dissolved ozone concentrations taken from the culture tanks receiving ozonated flow were variable (Fig. 2), and ozone levels could rise from 0 to 0.03 mg/l in less than 1 h.

Three different brands of oxidation-reduction potential (ORP) probes and controllers were evaluated in an attempt to measure and control the oxidizing potential of the water within the fish culture tanks in order to prevent the accumulation of toxic levels of dissolved ozone. Only one of the ORP controllers evaluated (Stranco model 4-2F Automatic ORP Controller), the most expensive controller of the group, satisfactorily tracked ORP under conditions both preceding the accumulation of ozone and in the presence of residual ozone.

ORP-based control of ozone residual within the culture tank water was complicated in our research system because the system contained two cross-flow culture tanks, with each culture tank divided into two sections by a mesh screen. The purpose of the divider was to keep the newly stocked fingerlings separated from the older and larger cohorts that had been stocked previously. This barrier produced two regions within each tank where rates of ozone accumulation were different. Only one ORP probe and controller

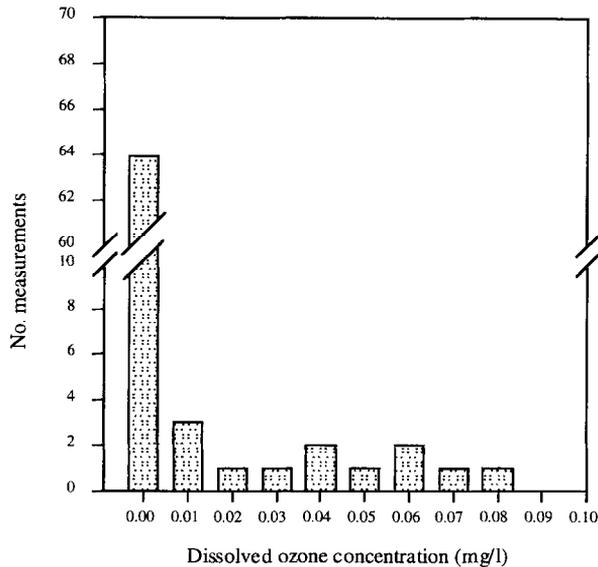


Fig. 2. Histogram of all dissolved ozone concentrations measured in the culture tanks receiving ozonated flow during the study.

was used to regulate ozone addition to the system. And, unfortunately, accumulations of dissolved ozone could not be controlled in any tank region that did not contain an ORP probe and controller. Lack of ORP controllers in both portions of both culture tanks resulted in ozone-induced mortalities on five occasions caused by direct ozone toxicity in tests three (3.9%) and four (5.0%) (Table 2). No ozone induced mortalities occurred during tests one and two.

The first signs of exposure to toxic concentrations of dissolved ozone were noticeable changes in fish behavior. Fish stopped feeding and congregated near the surface of the water and sometimes 'gasp' for air. Eventually, erratic swimming behavior occurred and became progressively worse. Attempts to jump out of the tank increased, and some fish showed darting behavior followed by listless swimming. Fish eventually lost equilibrium and also became pale, with vertical patches of dark pigment on the sides of the body. Fish which reach this latter condition rarely survived. Gills of fish exposed to high levels of ozone showed excess mucus, hyperplasia, and aneurysms.

4. Discussion

Prior to ozonation, BGD was a constant problem among newly stocked fish. During an 11-month period previous to ozonation, five groups of rainbow trout were stocked, and up to 30% of each group died because of BGD or a secondary amoebic infection (Bullock et al., 1994) despite regular chemotherapeutic treatments. In the ozonation

study, BGD associated mortalities also occurred on a regular basis when ozone was not added or insufficient ozone was added. Adding ozone appeared to lower total mortality and the number of clumps of BGD bacteria on gill tissue in tests one, three and four, compared to that in the control and test two, when the ozone generator failed. A total of 14 treatments were required to reduce BGD mortality in the two tanks in the control and test two, while no treatments were needed in the other trials. After ozone addition, only 1.7–4.1% of stocked fish died because of BGD, and chemical treatments were rarely required (Table 2).

The benefits of adding ozone to our system were an overall improvement in water quality entering the culture tanks (Summerfelt et al., 1997) and, more importantly, a reduction of mortality due to BGD and a reduction in the need for chemotherapeutic treatments. The improvement in water quality from ozonation may, at least indirectly, affect mortality from BGD. MacPhee et al. (1995) found that feeding played an important role in BGD mortality; fish fed after being challenged with *F. branchiophilum* developed clinical signs of BGD and had high levels of mortality, while those that were not fed after the challenge developed only moderate clinical signs and were generally normal 72 h post challenge. They proposed that feeding promotes active excretion of urea and ammonia which accumulates in the mucus and static water layer surrounding the gills, and this provides a nutrient-rich environment that allows colonization and growth of BGD bacteria on gill tissue. They also proposed that acidification of the mucous boundary layer of the gill, which can be produced from increased carbon dioxide excretion as a result of feeding, may play an important role in *F. branchiophilum* attachment and colonization of the gills. Because MacPhee et al. (1995) used a single-pass system, it is unlikely that deterioration of water quality or environmental stresses favored the development of BGD. Within our recirculating system, however, it is more likely that the nitrogenous and organic substrates in the water affected the growth of *F. branchiophilum*. Better water quality (Summerfelt et al., 1997) and reduced BGD mortalities both appeared to result from system ozonation; but the connection between the two was not shown. Although limiting nutrients to *F. branchiophilum* may be a reason for reduced BGD mortality, other factors are probably involved.

Several factors contributed to the failure of ozone to eliminate *F. branchiophilum* and the general failure to reduce numbers of heterotrophic bacteria in our recirculating system by even one \log_{10} . Bacterial reduction can be predicted from the product of the dissolved oxidant concentration and the exposure times, as described by the Chick–Watson model (Watson, 1908). Within our system, ozone was co-transferred with oxygen in LHO™ units and short (35 s) contact times were provided for ozone reaction after transfer to the flow before entering the culture tank. Even the roughly 55 daily exposures of recirculated water to ozone within the LHO™ units did not off-set the short contact time each pass.

The other factor that limited bacterial reductions was the low ozone residuals (means ranged from 0.02 to 0.180 mg/l) at the end of the ozone contact tank (Table 2). Within our recirculating system, ozone demand produced by suspended solids, nitrite, and color (dissolved organic molecules) reduced ozone's half-life to levels that were generally too short to measure. The longest half-lives measured were only 15 s. In contrast, the half-life of ozone in a solution of pure water is about 165 min at 20°C (Rice et al.,

1981). The ozone demand of the water in the recirculating system consumed the ozone's oxidative power and thus shielded the bacteria from direct oxidation. The shortened half-life reduced the effective concentration and the time of ozone contact within solution and thus reduced the predictor of ozone disinfection power, the product of residual concentration and contact time.

The product of the contact time and range of ozone concentrations in these trials were less than those reported by others. In the studies by Owsley (1991), the water supply was treated with 0.2 mg/l ozone for 10 min to kill infectious hematopoietic necrosis virus (IHN); after treatment, water was degassed in packed columns to reduce ozone to a safe level for the fish. Liltved et al. (1995) reported 99.99% inactivation (4 log reductions in viable count) of four bacteria (*Aeromonas salmonicida* subsp. *salmonicida*, *Vibrio anguillarum*, *Vibrio salmonicida*, and *Yersinia ruckeri*) and the infectious pancreatic necrosis virus (IPNV) within 180 s at residual ozone concentrations of 0.15 to 0.20 mg/l within distilled water in bench-top studies. Tipping (1988) reported that a contact time and ozone concentration product of 1 mg/l · min was necessary to kill the protozoan *Ceratomyxa shasta* from the water entering a trout hatchery. And, Colberg and Lingg (1978) reported 99% kill of four bacterial fish pathogens (*A. salmonicida* subsp. *salmonicida*, *A. liquefaciens*, *Pseudomonas fluorescens*, and *Y. ruckeri*) when exposed to 0.1 and 1.0 mg/l ozone for 60 s in simulated recirculating system water.

Greater reductions in bacteria within our recirculating system, with its high oxidation demand, would have required ozone loading rates greater than those used here (i.e., > 0.039 kg ozone/kg feed), which would be difficult to achieve without: (1) wasting excess oxygen to carry more ozone to the LHO™ unit, and/or (2) replacing the ozone generator with a larger unit that could produce a higher ozone concentration in the oxygen feed gas (6–10% instead of 4–5%), and/or (3) installing an ozone removal unit (air stripper, UV light, or large hydraulic retention chamber) to prevent the increased ozone residual from reaching toxic levels in the culture tank.

One of the main reasons that ozone is not widely used in aquaculture is its toxicity and a manager's unwillingness to risk losing fish to an accidental overdose. Residual ozone is highly toxic to fish at low levels. Ozone destroys epithelium covering the gill lamella which results in a rapid drop in serum osmolality (Paller and Heidinger, 1979; Wedemeyer et al., 1979) and, if mortality does not occur immediately, can leave the fish highly susceptible to microbial infections (Paller and Heidinger, 1979). Wedemeyer et al. (1979) reported that an ozone residual of 0.002 mg/l would be a safe level of ozone when culturing rainbow trout. Based on the literature, the exact level of ozone that damages gills or kills rainbow trout is between 0.008–0.06 mg/l (Roselund, 1975; Wedemeyer et al., 1979). In our research, ozone concentration rose to lethal levels on five occasions when we attempted to maximize ozone dosages in trials three and four (Table 2). The high ozone concentrations were caused by variable ozone demand in the water and the short hydraulic retention time provided before each fish culture tank. Ozone levels as high as 0.08 mg/l were measured during fish mortalities; however, higher ozone levels probably occurred but were not measured because staff would first attempt to restore ozone-free water flow to protect the fish; measuring ozone residual was less important. Ozone mortalities were not observed in tests one and two, probably because the ozone dosing rate per unit feed fed was lower than those in tests three and

four (Table 1). Additionally, we observed that when fish stopped feeding from the demand feeders after being stressed (for example, just after selective harvest of the fish greater than about 0.34 kg) ozone accumulated more readily within the region that was harvested. This indicated that the production of organic compounds during and after feeding affected the rate that ozone reacted, which decreased ozone concentrations.

Occurrence of ozone produced mortalities illustrates a serious liability of ozone technology—the lack of instrumentation to continually detect ozone at levels < 0.1 mg/l and the lack of chemical tests to readily measure ozone in water grab samples at concentrations < 0.01 mg/l. At present, there is no fail-safe system to directly measure and control ozone in solution. An indirect measure of residual ozone is the water's oxidation reduction potential (ORP), which is a measure of a water's potential to oxidize and is thus a measure of the water's potential to disinfect or to kill fish. ORP can be monitored and used to control ozone addition to ensure that the desired treatment objective has been achieved and to ensure that ozone residual is not in the fish culture tank. A safe ORP for freshwater appears to be between 300–350 mV, depending upon pH. Our attempts to indirectly measure ozone residuals by ORP control strategies were only partially successful. An ORP control system was identified that could prevent ozone residual from accumulating in the culture tank within the region of the ORP probe. However, because our recirculating system contained two culture tanks, each partitioned into two areas to isolate fingerlings from larger fish, a single ORP controller, no matter how accurate, could not prevent mortalities from occurring within a given region of a culture tank unless a probe was in that region. In a single completely mixed freshwater environment, a good automatic ORP controller could probably help to obtain maximum oxidative treatment with minimum toxicity to fish.

These results may indicate that adding ozone at a lower rate (0.025 kg ozone/kg feed) could provide about the same benefits as a higher dosing rate (0.036–0.039 kg ozone/kg feed): e.g., reduced BGD associated mortalities and no required use of non-approved chemical treatments to control BGD epizootics. Yet, the lower ozone dosage rate apparently did not kill fish from ozone toxicity because ozone had such a short half-life and its residual quickly reacted away. Accordingly, the lower ozone addition rate could allow use of a shorter ozone contact time before the completely mixed culture tanks and also avoid the use of ozone residual removal units and the dependence upon expensive and sometimes unreliable ORP control technologies. Hence, use of the lower dose could provide all of the benefits but also reduce capital and operating costs associated with the higher ozone dosing rate.

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