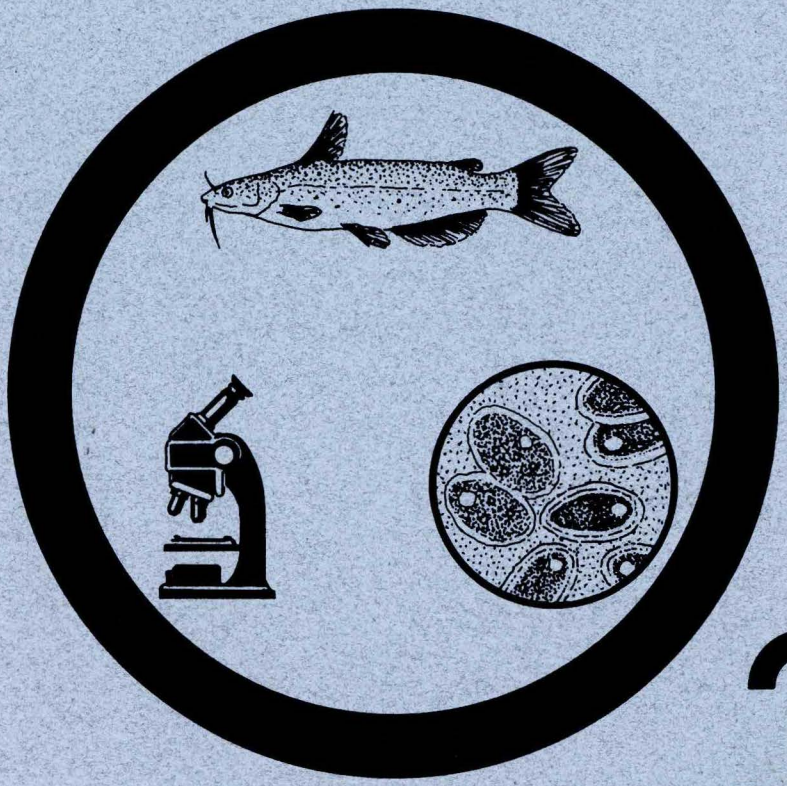
EFFECTS OF OZONE AND PHOTOZONE® ON WATERBORNE BACTERIA, FISH SURVIVAL, AND PLUMBING EQUIPMENT

Technical Bulletin No. 2



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Iowa Department of Natural Resources
Larry J. Wilson, Director
May 1990

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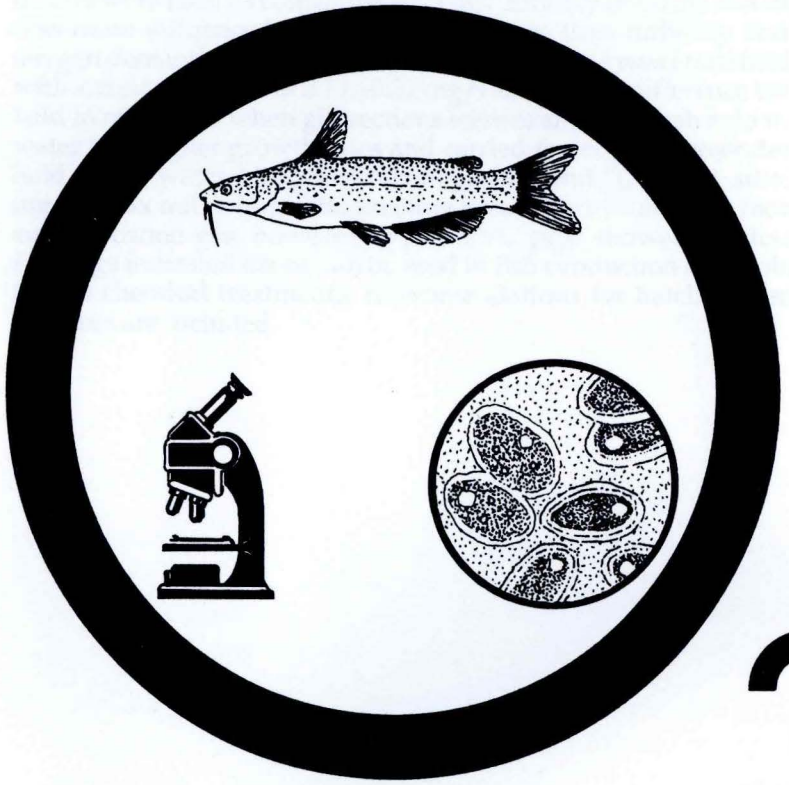

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ABSTRACT

A study was performed at Rathbun Hatchery to determine the effect of Photozone® and ozone gas on waterborne bacteria, fish survival, and plumbing materials. Systems tested were a baffle system, venturi system, counterflow, air diffusers, and an in-line static mixer which contained pall rings. Photozone® was not an effective disinfectant for a one pass hatchery system because low ozone concentrations required repeated water contact for effective disinfection. Ozone exhibited good disinfecting qualities, with bacteria colonies/ml reduced 51 to 91%, when air diffusers or in-line static mixers were used as contacting systems. Efficacy of ozone as a disinfectant was more influenced by water temperature than turbidity and chemical oxygen demand (COD). Channel catfish *(Ictalurus punctatus)* held in water with ozone residuals of 0.1 to 0.22 mg/l showed no difference between fish held in raw water when gill sections were examined. Fish held in ozonated water had higher growth rates and carried fewer gill trematodes than fish held in raw water. Neoprene rubber gaskets and "O" rings, silicone caulking, and six mil poly-plastic sheeting became brittle after six months exposure to ozone gas; however, type I PVC pipe showed no deterioration. Findings indicated ozone can be used in fish production as a viable alternative to chemical treatments; recommendations for hatchery use and cost analyses are included.

Effects of Ozone and Photozone® on Waterborne Bacteria, Fish Survival, and Plumbing Equipment

by
Alan A. Moore

Technical Bulletin No. 2
Fish and Wildlife Division
Iowa Department of Natural Resources
Wallace State Office Building
Des Moines, IA 50319-0034
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INTRODUCTION

The purpose of this study was to investigate Photozone® and ozone as a water disinfectant at the Rathbun Fish Hatchery. This warm water facility produces channel catfish, 5-inch largemouth bass, tiger muskellunge, and walleye fry for Iowa public lake and stream stockings. Water flow through the one pass intensive culture hatchery ranges from 3,200 to 7,000 gpm and originates in the 11,000 acre Rathbun Reservoir. Intensive culture of warm water fish has advantages over extensive culture, but the culture techniques are not without problems. Outbreaks of disease, causing fish loss and reduced growth, increased the need for an effective method of disinfection of the hatchery water supply. Experimental evaluations of ozone as a water supply disinfectant for salmonids and salmonid hatcheries have been conducted during the past 15 years. These projects were designed to evaluate the sensitivity of fish, and general waterborne environmental bacteria to ozone. According to Rosenlund (1974, 1975) rainbow trout *Oncorhynchus mykiss* held in water with ozone concentrations of 0.01 and 0.06 ppm succumbed within hours. Ozone has been shown to destroy more bacteria and algae than ultraviolet light; however, the concentration of ammonia may increase (Colberg et al., 1977, 1978). During the same experiment, a residual of 0.3 mg/l ozone caused gill edema and damage to the liver, kidney, and heart of steelhead. In addition, *Aeromonas salmonicida* and *Yersinia rucheri* bacteria showed a 99% mortality at ozone concentrations of 0.15 ppm and a 60 second contact time. The acute 96 hour Lc_{50} of ozone to rainbow trout was found to be 9.3 µg/l while chronic exposure at 2 µg/l ozone for three months caused no mortalities and only minor gill changes (Wedemeyer et al., 1979). According to Smith and Dwyer (1980), Photozone® type ozone used in aquariums caused severe gill damage and 15 to 30% mortality to rainbow trout after a three day exposure. Photozone® gave no significant ammonia nitrogen removal or oxygen addition. Photozone® significantly reduced *Escherichia coli* and *Staphylococcus aureus* in aquariums at 2.1 mg O_3 equiv l^{-1} min^{-1} (Lohr and Gratzek, 1981). In contrast, dosages of 0.2 to 0.4 mg O_3 equiv l^{-1} min^{-1} failed to prevent the spread or development of clinical *Ichthyopthirius multifiliis*. Hall et al. (1981) revealed a 70% mortality in striped bass *(Morone saxatilis)* exposed 12 hours to 0.1 mg/l ozone, while a 7 day ozonization of secondary sewage effluent at 0.2-0.3 ppm was required to kill fathead minnows *(Pimephales promelas)* (Arthur and Mount, 1973). Little information was available concerning the effects of Photozone® and ozone on warm water fish health, waterborne bacteria, and plumbing systems. This study was designed to determine the effect of Photozone® and classic ozone on a broad spectrum of microorganisms, warm water fish, and fish hatchery plumbing materials. The investigational approach consisted of determining the ozone levels necessary to reduce bacteria levels, followed by the effect of these levels on fish health and plumbing materials. Bacteria reduction was determined by standard plate count; fish health by total mortalities, gill tissue damage, and growth; and plumbing deterioration by microscopic observation and physical failure.

METHODS

System Design

The design of the experimental testing system included a water treatment and/or detoxification reservoir (3 m x 1.2 m x 0.6 m) and four fish holding troughs (0.3 m x 0.3 m x 2.4 m) located directly below the reservoir. The water to the experimental system was taken directly from the hatchery raw water supply, with three troughs receiving ozonated raw water and the control trough receiving untreated raw water. Conditioning of the raw water, prior to use in the experimental system, consisted of macroscreening for removal of fish and large debris, aeration, microscreening for removal of small debris, and periodic flocculation with non-ionic Hercofloc or anionic Separan. Bacteria content of water used for fish production inside the hatchery building (start tank water) was identified as the minimum acceptable level of water purification needed at Rathbun Hatchery. In addition to the screening described above, hatchery start tank water was flocculated, filtered, and treated with ultraviolet radiation. Initital testing of Photozone® and ozone was designed to reveal the most effective contacting system. Subsequent investigations concentrated on monitoring contacting system effectiveness, water temperature and turbidity, and how fish and plumbing were affected. Statistical analyses of data were performed by T-test.

Disinfection Monitoring

The effectiveness of the disinfection regime was determined by bacteria standard plate count according to the APHA "Standard Methods for the Examination of Water and Waste Water" (15 ed.). Microbiological techniques permitted growth of a broad spectrum of microorganisms, many of which were not specific fish pathogens. Water samples were taken from the raw water trough inlet (control), the Photozone® or ozone treated water trough inlets and, when available, the hatchery start tank water inlet. Dilutions were made when colony counts were above 300/ml. Water samples were taken in triplicate from each sample point and pipetted into petri plates which contained 10 ml melted Standard Methods Agar. Plates were rotated five times in one direction and then rotated an equal number of times in the opposite direction. Control plates inoculated with sterile water blanks were used to check sterility of media and plate inoculation techniques. Plates were incubated at 35°C ± 0.5°C for 48 ± 3 hours. Colonies were counted using a method similar to a Quebec Colony Counter. Colonies per milliliter were expressed as the product of sample colonies per plate and the dilution rate. Water quality parameters monitored periodically at the time of sampling were temperature, turbidity, COD, and suspended solids.

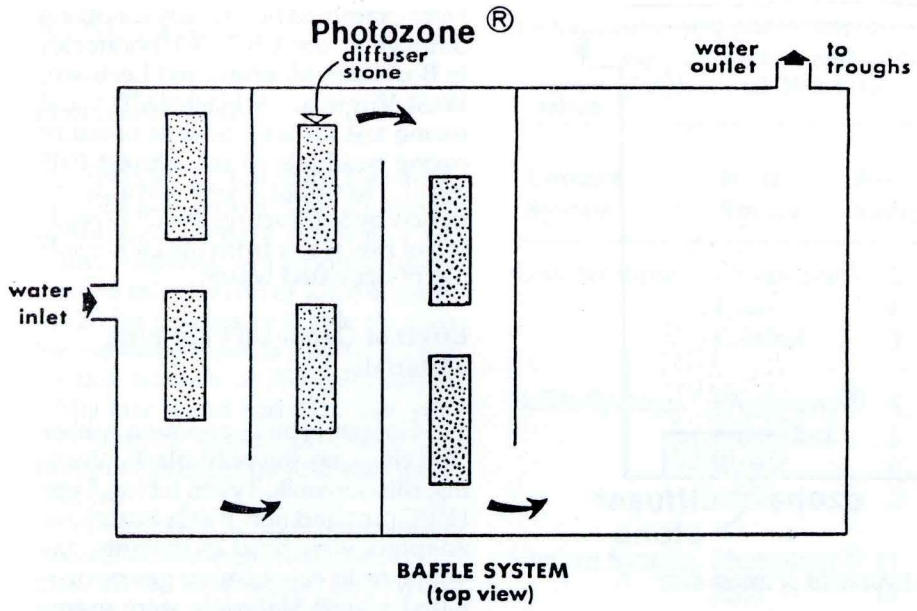

Figure 1. Configuration of baffle system used in Photozone® treatment of hatchery water.

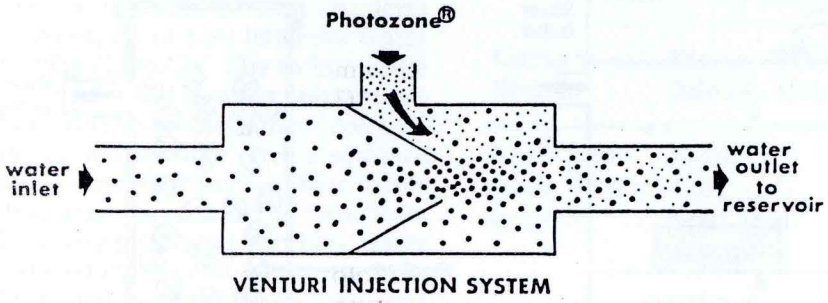

Figure 2. Design of venturi system used for Photozone® injection in hatchery water supply.

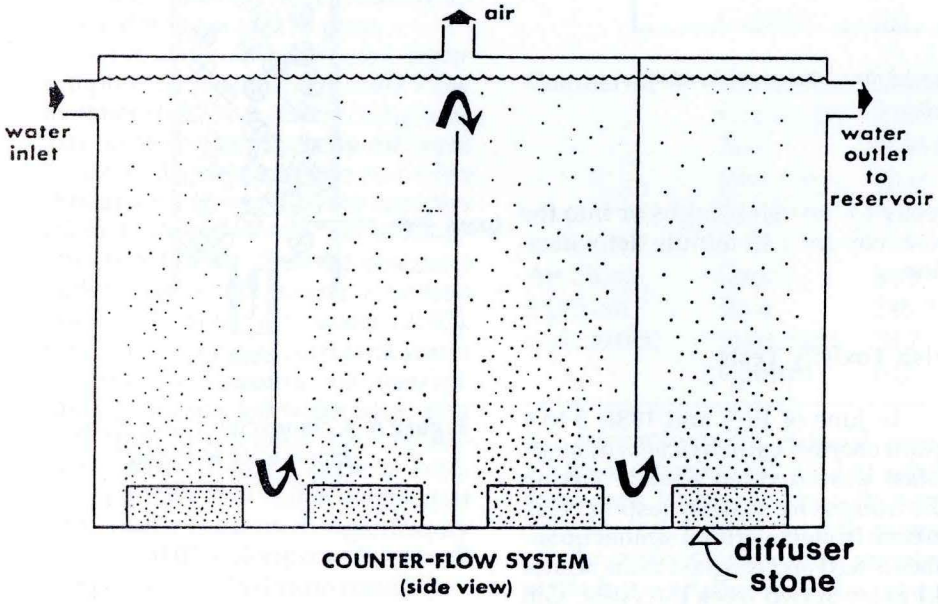

Figure 3. Configuration of counter-flow system used in Photozone® treatment of hatchery water.

Photozone®System Tests

Photozone®, an inexpensive and commercially available form of activated oxygen, is produced by passing compressed ambient air through a PVC chamber irradiated by an ultraviolet lamp. The oxygen is disassociated to form new activated oxygen molecules, one of which is "classic" ozone (Ionization International Inc., personal communication).

A P-140 Photozone®water purifier, capable of treating 40 gpm, was installed to allow treatment of water in the reservoir or in the pipe delivering water to the reservoir. Water flow to the fish holding troughs was regulated to increase or decrease the Photozone®contact time. Maximum flow was 30 gallons per minute. Air passage over the ultraviolet lamp was a constant 50 SCFH.

Three Photozone ® contact system designs were tested; 1) a baffle system with minimum contact time of 40 minutes (Figure 1); 2) a venturi in-line injection system with a contact time of 30 seconds (Figure 2); and 3) a counter- flow system which provided a minimum contact time of 30 minutes (Figure 3). Water from the counter-flow unit was held in the reservoir for an approximate 12 minute detoxification period before flowing to the fish troughs. This treatment regime was recommended by the manufacturer. Diffuser stones used in systems 1 and 3 were made of Porex®plastic.

Ozone System Tests

An electric, tubular type, water cooled, ozone generator complete with in- line static-mixer (reactor) was used in the second phase of the project. The generator was capable of producing 19 grams/hour ozone, 2% by weight, with an air flow of 12.8 L/min at 15 psig. Maximum treatable water flow, at 100% of generator capacity, was 20-35 gpm with an ozone dosage of 4.1 to 2.4 ppm, respectively.

The ozone contact systems used for treatment were; 1) counter-flow system as previously described for Photozone®; 2) an airstone system that bubbled ozone through four Porex ® airstone diffusers located

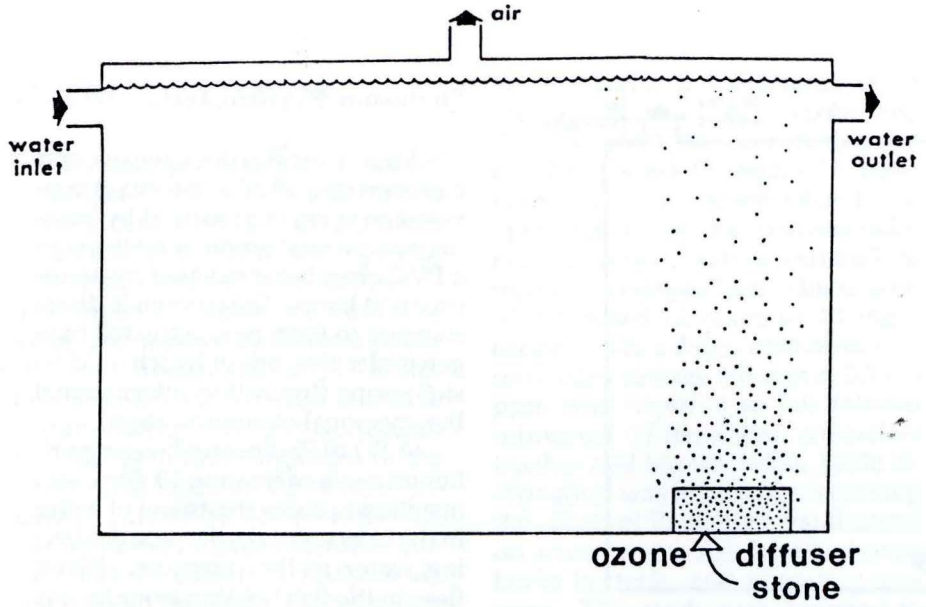

Figure 4. Design of airstone system for ozone treatment of water near reservoir outlet.

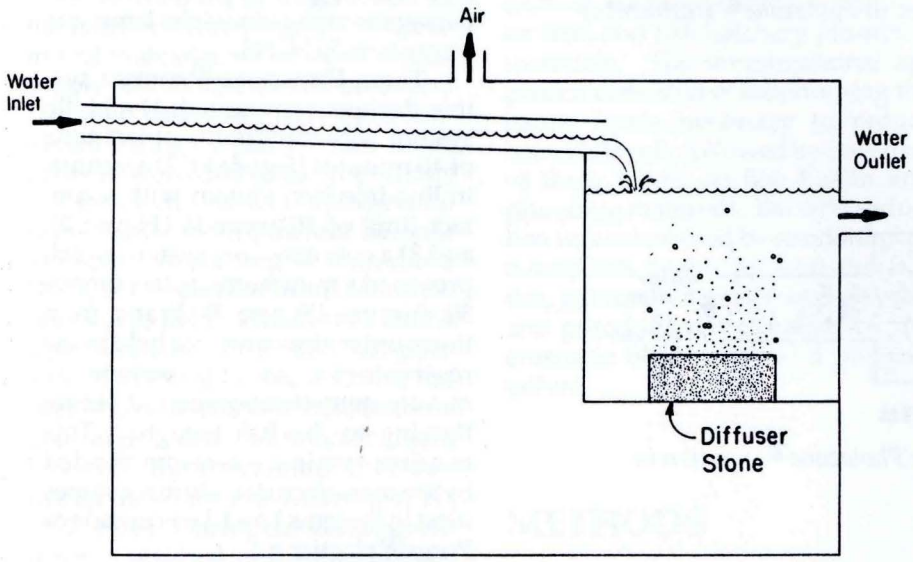

Figure 5. Configuration of airstone system used for ozone treatment of water in a 30-gallon contact chamber.

near the reservoir outlet to the troughs (no contact time calculated) (Figure 4); 3) a second airstone system with two 12 inch Porex® airstone diffusers placed in a 30 gallon treatment box so that water entered the large reservoir moved into the treatment box and was contacted by ozone for 12 to 15 minutes (Figure 5); and 4) a commercial in-line static mixer which contained pall rings to create turbulent mixing of flowing water and ozone (Figure 6). Water flowed through the mixer was contacted approximately four seconds and then flowed directly to the fish troughs or into the reservoir for a 45 minute detoxification.

Fish Toxicity Tests

In June of 1985 and 1986, 3 to 4 gram channel catfish *(Ictalurus punctatus)* were divided equally among the troughs for toxicity testing. Fish in each trough were fed similar quantities of feed and weighed to the nearest 0.1 gram at two week intervals. Gill samples of five fish from each trough were examined biannually for ozone damage at the USFWS laboratories in Bozeman, Montana and Leetown, West Virginia. A Hach DPD OZ-2 ozone test kit was used to measure ozone residuals to the nearest 0.05 mg/l. Residual levels were determined by subtracting the DPD reading of raw water from the DPD reading of ozonated water.

Effect of Ozone on Plumbing Materials

Copper tubing, common rubber "O" rings, six mil poly-plastic sheeting, silicone caulk, Tygon tubing, Type I PVC pipe and non-PVC plastic hose adapters were used as sealants, gas vents, or to carry ozone gas or ozonated water. Materials were examined under a dissecting microscope at monthly intervals to determine deterioration.

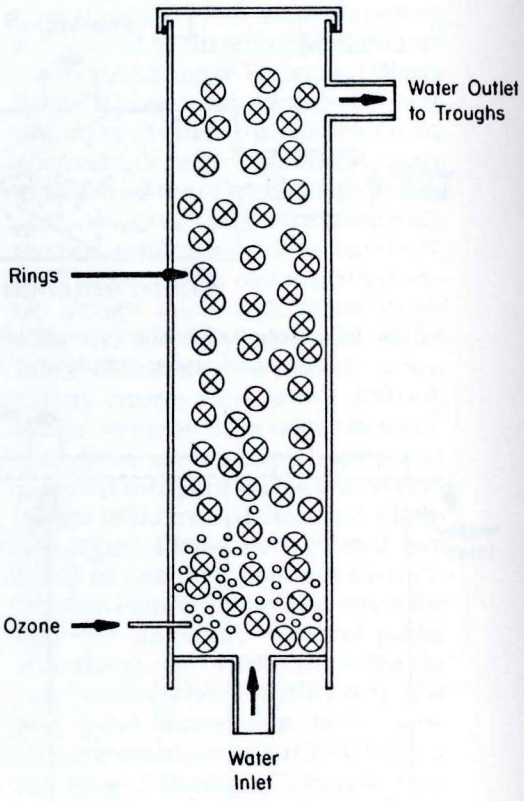

Figure 6. Commercial in-line static mixer used to contact water with ozone.

RESULTS

Photozone® Tests

The use of Photozone® to disinfect hatchery water proved unsuccessful in one-pass systems. The counter-flow system produced mean bacteria colonies/ml of 122 compared to 128 for raw water (Table 1). Photozone® treatment in a baffle system yielded a mean of 785 colonies/ml while raw water had 715. The venturi contact system produced 172 bacteria colonies/ml and raw water was 161.

There were no significant differences ($P>0.05$) in mean bacteria colonies/ml of raw water and water treated with Photozone®.

Table 1. Standard plate counts of bacteria colonies found during experimentation with various Photozone® contact systems at the Rathbun Fish Hatchery.

Contact System	Water Source	No. Samples	Mean Colonies/ml	Mean Temp (°C)	Mean Turbidity	pH
Counter Flow	Photozone®	5	122	9.3	18.0	8.5
	Raw	6	128	9.3	15.0	8.5
	Control	3	0	--	----	---
Baffle System	Photozone®	4	785	24.5	27.0	9.0
	Start Tank	1	79	24.5	27.5	9.0
	Raw	4	715	24.5	30.0	9.0
	Control	2	0	----	----	---
Venturi System	Photozone®	11	172	18.0	14.0	8.5
	Raw	12	161	18.0	14.5	8.5
	Control	3	0	----	----	---

Ozone Tests - Preliminary

Ozone treatment significantly reduced ($P<0.05$) the mean bacteria colonies/ml of raw hatchery water in 1984 (Table 2). The in-line static mixer followed by a 45 minute retention in the reservoir reduced colony/ml counts by 40%, while samples taken directly from the static mixer discharge gave a 95% reduction. Hatchery processed start tank water reduced colony/ml counts by 94%. Ozonated water and hatchery start tank water had significantly fewer ($P<0.05$) bacteria colonies/ml when compared to raw water. Maximum ozone dosage was 1.76 ppm.

Raw water and ozonated water samples taken from the counter-flow treatment box gave colony/ml counts that were not significantly different ($P>0.05$). Bacteria colonies/ml were similar for ozonated water samples taken 1) directly from the counter-flow box (2158), 2) at the reservoir outlet after a 12 minute retention (3360), and 3) for raw water (2550). Hatchery processed start tank water produced 80 bacteria colonies/ml. Samples of ozonated water taken after a 12 minute retention had significantly more ($P<0.05$) bacteria colonies/ml than raw water, while start tank water contained significantly fewer ($P<0.05$) colony numbers in all comparisons. Maximum ozone dosage was 5.5 ppm.

Airstone injection of ozone near the reservoir outlet reduced mean bacteria colonies/ml by 160 when compared to raw water. Bacteria colonies/ml (ozone vs raw water) were significantly lower in ozonated water but were significantly greater ($P<0.05$) than those produced by start tank water.

Table 2. Mean standard plate counts of bacteria colonies found during experimentation with various ozone contact systems at the Rathbun Fish Hatchery.

Contact System	Water Source	Mean Colonies/ml	Mean Turbidity	Water Temp.(°C)	% Generator Capacity (X)
In-line Static Mixer - 45 min. retention	Ozone	86.5	6.0	5.2	88
	Raw	142.8	6.0	5.2	--
	Start Tank	2.0	3.0	5.2	--
	Control	0.0	--	--	--
In-line Static Mixer - direct	Ozone	61.3	18.6	19.4	95
	Raw	1316.8	18.6	19.4	--
	Start Tank	81.6	----	19.4	--
	Control	0.0	----	----	--
Counter-flow	Ozone Box	2158.0	----	22.1	93
	Ozone Res.	3360.0	----	22.1	93
	Raw	2550.0	----	22.1	--
	Start Tank	80.0	----	22.1	--
	Control	0.0	----	----	--
Air Stone Injection (res. outlet)	Ozone	84.5	16.3	16.4	95
	Raw	245.2	16.3	16.4	--
	Start Tank	38.7	----	16.4	--
	Control	0.0	----	16.4	--

Ozone Tests - Extended Airstone Injection

Airstone injection of ozone during January and March, 1985 reduced bacteria colonies/ml by 68.4% when compared to raw water (Table 3).

Table 3. Mean standard plate counts of bacteria colonies/ml produced by water sampled during experimentation with ozone utilizing air stone injection at Rathbun Fish Hatchery. (Generator operating capacity was 100%.)

Treatment Period	Water Source	Mean Colonies/ml	Mean Turbidity	Water Temp. (°C)
January-March	Ozone	60.8	9.9	9.9
	Raw	192.3	10.1	9.9
	Control	0.0	----	---
April-June	Ozone	47.1	16.9	16.9
	Raw	230.6	17.6	17.6
	Start Tank	4.3	12.6	12.6
July-September	Ozone	354.0	11.7	23.1
	Raw	1331.8	11.2	23.1
	Start Tank	115.9	5.5	23.1
October-December	Ozone	31.0	7.9	7.9
	Raw	93.3	9.6	7.9

Table 4. Mean standard plate counts of bacteria colonies/ml produced by water sampled during experimentation with ozone utilizing an in-line static mixer at Rathbun Fish Hatchery. (Generator operating capacity was 100%.)

Treatment Period	Water Source	Mean Colonies/ml	Mean Turbidity	Water Temp. (°C)
January-February	Ozone	43.3	14.1	3.2
	Raw	145.0	14.7	3.2
	Control	0.0	----	---
May-June	Ozone	103.6	7.1	19.5
	Raw	256.8	7.7	19.5
	Start Tank	12.2	6.6	19.3
	Control	0.0	----	----
July-August	Ozone	19.9	5.8	24.4
	Raw	245.8	6.7	24.4
	Start Tank	78.9	4.0	24.4
	Control	0.0	----	----
October-December	Ozone	29.0	11.5	9.7
	Raw	59.7	14.3	9.7
	Start Tank	5.6	6.7	9.7
	Control	0.0	----	----

Raw water contained a significantly greater ($P<0.05$) number of bacteria colonies/ml compared to ozonated water.

During second quarter testing (April, May, and June), ozonated water produced 47.1 bacteria colonies/ml while raw water had 230.6 colonies/ml (Table 3). Ozonated water bacteria counts were significantly lower ($P<0.05$) than those produced by raw water; however, hatchery processed start tank water had significantly fewer bacteria colonies/ml when compared to both water sources ($P<0.05$).

Testing during July, August, and September, revealed water processed for hatchery start tanks again significantly reduced bacteria contamination when compared to the other water sources tested ($P<0.05$) (Table 3), while bacteria counts in ozonated water were significantly lower ($P<0.05$) than that produced by raw water. When compared to raw water, ozonated water had a reduction of 73.5%, while hatchery start tank water exhibited a 91.3% reduction.

Airstone injection system testing during the fourth quarter (October, November, December) revealed bacteria colonies/ml of 31.1 while mean bacteria colonies/ml of raw water was 93.3. Ozonated water had significantly fewer ($P<0.05$) colonies compared to raw water.

A quarterly breakdown of disinfection effectiveness revealed ozonated water reduced total bacteria colonies 66.8 to 79.6% when compared to raw water.

Start tank water contained fewer bacteria and counts showed a 91.4 to 98.1% reduction when compared to raw water. Mean ozone treatment rates and corresponding ozone residuals, in mg/l were: 33.3 and 0.17, respectively.

Ozone Tests - Extended In-line Contactor

The first quarter (January, February) in-line static mixer evaluation revealed mean bacteria colonies/ml of 43.3 for ozonated water and 145 for raw water (Table 4). The effectiveness of the 70.2% reduction was significant when colony/ml counts were compared ($P<0.05$).

Second quarter (May and June) mean bacteria colonies/ml were 103.6 for ozonated water, 12.2 for start tank water, and 256.8 for raw water (Table 4). Hatchery start tank water contained significantly fewer ($P<0.05$) bacteria colonies than all other samples, while ozonated water was significantly lower ($P<0.05$) than raw water.

Bacteria reduction in hatchery start tank water was less during third quarter (July and August) comparisons (78.9 colonies/ml). Ozonated water colony/ml counts were 19.9 while raw water had 245.8. Ozo-

nated water counts were significantly lower ($P<0.05$) than all other samples.

Efficiency of ozonization in reducing bacteria contamination decreased during fourth quarter testing (October, November, December) (Table 4); however, raw water again contained a significantly greater ($P<0.05$) number of colonies/ml than ozonated water. Bacteria colonies/ml in the hatchery start tank water were significantly less ($P<0.05$) than either of the above samples.

Bacteria reduction exhibited highly variable results in 1986. Bacteria disinfection obtained in ozonated water ranged from 51.4 to 91.8% while hatchery start tank water effectiveness was 67.9 to 91.6%. The ozone disinfection rate was 5.5 mg/l and residuals measured 0.168 mg/l.

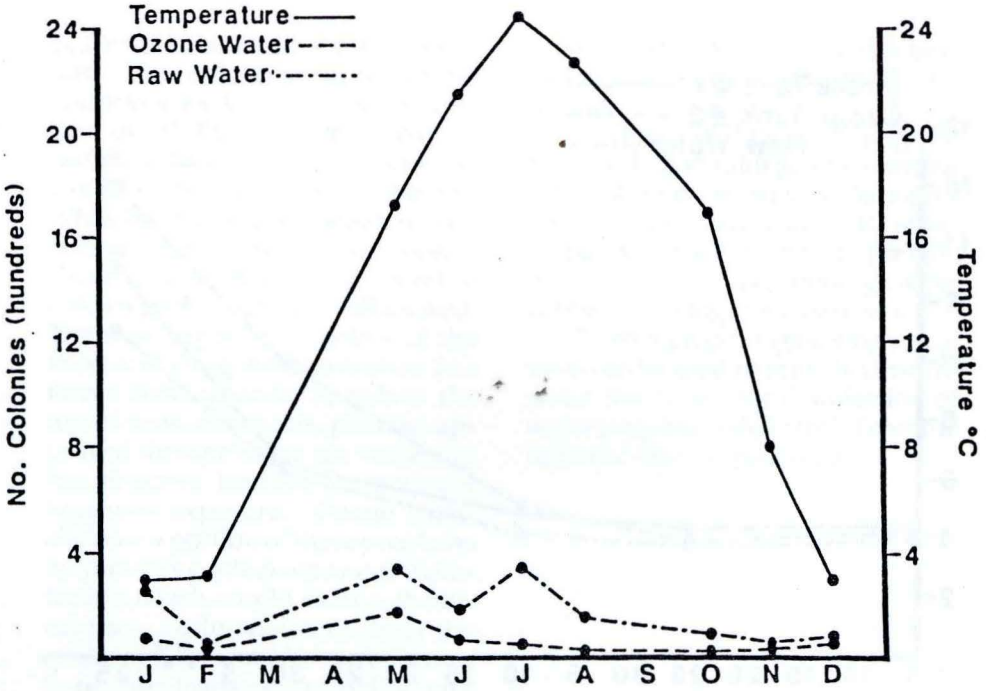

Figure 7. Effect of water temperature on bacteria growth and ozone disinfection at Rathbun Hatchery in 1985.

Water Quality

Turbidity did not seem to have a direct impact on ozone efficiency. Reduction in bacteria colonies/ml of water was 95.4% at the 3.5 turbidity (mg/l SiO_2) and 94.3% at a turdibity level of 30.0. Temperature produced the largest effect on ozone efficiency. At temperatures above 20°C, reduction of bacteria colonies was greatest when bacteria numbers were at a peak (Figures 7 and 8). As temperatures declined, ozone effectiveness declined, even though treatment rates remained constant.

Fish Toxicity Tests

Ozone residuals found in the water flowing to the fish troughs ranged from 0.1 to 0.25 mg/l in 1985 and 0.17 to 0.13 mg/l in treatment tanks 1 and 2, respectively, in 1986. Residuals at mid-trough in 1986 were 0.075 mg/l. Fish mortality was zero in the ozonated troughs and the control trough in 1985, while in 1986, 5 fish died in ozonated trough 1; and, 4 fish died in trough 2. Trough 3, the control, produced 3 fish mortalities. Histological examination of gill tissue samples in 1985, revealed no difference between control fish and fish maintained in ozonated water. In 1986, fish in the control and treatment tanks exhibited the same degree of gill hypertrophy (cellular

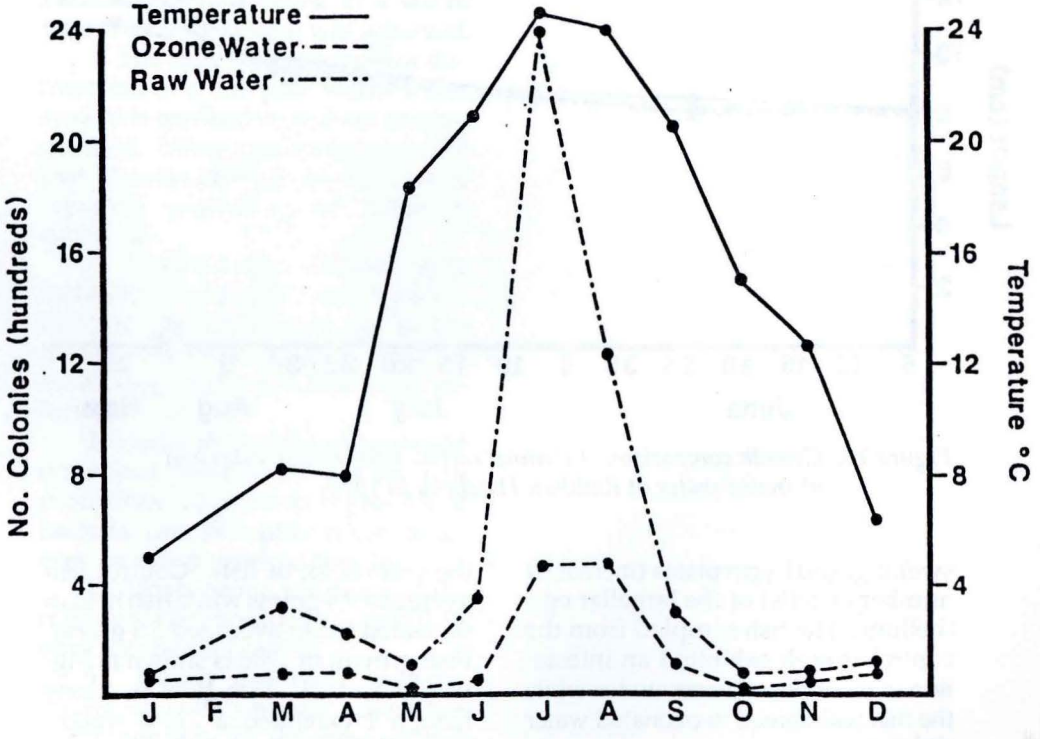

Figure 8. Effect of water temperature on bacteria growth and ozone disinfection at Rathbun Hatchery in 1986.

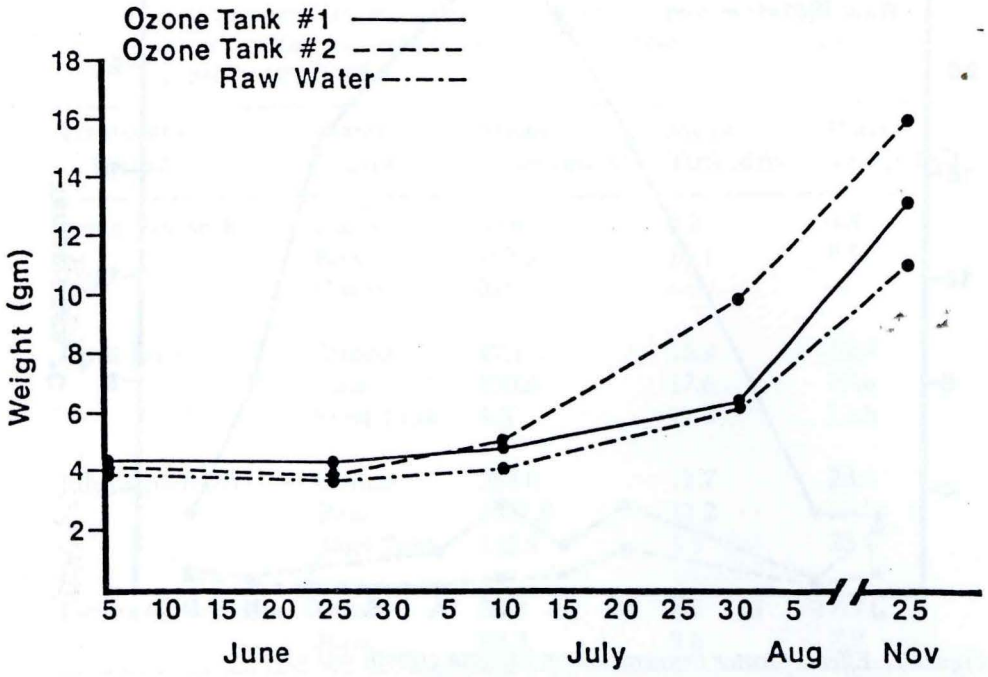

Figure 9. Growth comparison of channel catfish held in raw water and ozonated water at Rathbun Hatchery in 1986.

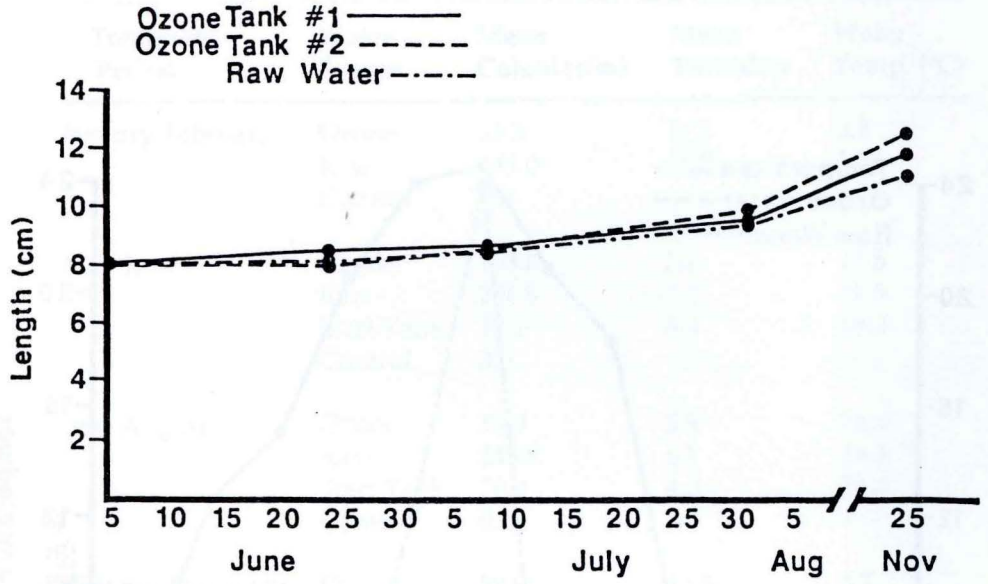

Figure 10. Growth comparison of channel catfish held in raw water and ozonated water at Rathbun Hatchery in 1986.

swelling) and hyperplasia (increased number of cells) of the lamellar epithelium. The fish sampled from the control trough exhibited an infestation of monogenetic trematodes while the fish maintained in ozonated water did not.

In 1985, fish growth in water treated with ozone was slower than the control lot of fish. Control fish averaged 7.4 grams while fish held in ozonated water averaged 5.4 grams. Fish growth in 1986 is shown in Figures 9 and 10. Fish held in ozone trough 1 exhibited a 210% weight increase while the fish in ozonated trough 2 and the control increased 292 and 198%, respectively.

Total length increase was 47.6 and 58.0% for fish in ozonated troughs 1 and 2 and 41.0% for the control fish.

Effect of Ozone on Plumbing Materials

Only Tygon tubing and Type I PVC pipe failed to decompose after direct exposure to ozone gas. The rubber "O" rings, silicone caulk, and plastic sheeting became brittle and crumbled or broke on contact. Ozone gas oxidized holes in copper tubing, and crystallized non-PVC plastic hose adapters. The Tygon tubing, while showing some discoloration, was serviceable after 10 months and Type I PVC pipe revealed no deterioration after carrying ozone gas for almost one year. Submerged rubber "O" rings and neoprene gaskets exposed at the trough inlet showed no ill effects; however, similar material tested in the water-ozone contact chamber cracked but was still serviceable. Ozone residuals in the treated water ranged from 0.1 mg/l to 0.25 mg/l.

DISCUSSION

The Photozone® generator failed to produce the required amounts of pure ozone necessary to disinfect water in the one-pass systems; even though, flows did not exceed those recommended by the manufacturer. Disinfection may be achieved if water was repeatedly contacted by Photozone® in a closed water system or recirculation system. This fact is in agreement with the findings of Lohr and Gratzek (1981).

As temperature and bacteria numbers increased during the summer months, the effectiveness of ozone to reduce bacteria numbers became evident. This fact was substantiated by a PCI Corporation report (no date) which indicated that even though ozone solubility increased with decreasing water temperature, the degree of disinfection improved remarkably at higher temperatures. The report further stated in-line static mixers were the most rapid means of transferring ozone into water; and disinfection occurred within a contact time of less than 2 seconds. The capacity of the ozone generator used

in fish hatchery situations must be adequate to control bacteria numbers at both high and low temperatures. Conrad et al. (1975) also showed ozone effectively destroyed high concentrations of *F. columnaris* at temperatures as high as 21°C.

Several authors reported considerable fish mortality at ozone residual concentrations of 9.3 µg/l to 0.1 mg/l (Rosenlund 1975, Wedemeyer et al., 1979, and Hall et al., 1981). The organic content of the warm water may explain why catfish in this study demonstrated no mortality at residuals as high as 0.25 mg/l ozone. Organic matter seemed to reduce ozone, as exhibited by a mid-tank residual of 0.075 mg/l compared to an incoming residual of 0.17 mg/l. Also, catfish were observed inhabiting this mid-tank area on a routine basis. A decrease in growth rate of channel catfish was noted in 1985 when residuals peaked at 0.25 mg/l; however, in 1986, growth was enhanced in ozonated water when residuals did not average above 0.17 mg/l. Growth was further increased when ozone levels were 0.13 mg/l. The improved growth was probably the result of less fish stress due to lower organic loads and fewer trematode parasites on the gills.

The effects of ozonization on plumbing materials, found in this study, were consistent with those found by Rosenlund (1975). PVC Type I, Tygon tubing, and stainless steel appeared to be the best materials for ozone transportation. Airstone diffusers must be made of materials resistant to ozone because certain porous materials become very brittle after prolonged contact.

Installation cost of an ozone generator, air dryer, compressor, diffuser and alarms required to treat the Rathbun Hatchery water supply range from $155,000 to $251,000. These units would be large enough to produce the 168 to 200 pounds of ozone per day needed to treat 7,000 gpm at 3 mg/l; however, a unit of this size may not be large enough to allow for extra dosage when midsummer bacteria numbers increase. Electrical energy needs would be 10 to 12 kwh/lb O_3/day and cost approximately $144 per day or $26,352 for a 6 month period. Current fish therapeutics cost for a similar period is $15,000, plus $2,000 labor necessary for application. The use of ozone to control epizootics would allow the production of 65,000 additional channel catfish, valued at $10,000; these fish would normally be lost to disease. While this monetary evaluation may not prove cost effective, ever present chemical availability and chemical efficacy problems must be discussed. The slow registration policy of the Food and Drug Administration has forced fish culturists to reduce the use of some chemicals, plus the approved therapeutants are becoming less effective because of continual organism exposure. Ozone could alleviate a portion of these problems by providing adequate water disinfection which would reduce chemical use. Additional benefits of the ozone system would be a reduction in organic loads and turbidity, and improved aeration of the water due to the use of packed column contactors.

MANAGEMENT CONSIDERATIONS

The primary goal of investigating the efficacy and behavior of Photozone® and ozone in a warm water hatchery system was achieved.

1. The use of Photozone® for disinfection of a one pass warm water system is ineffective and not recommended. Other studies have shown that Photozone® can be effective if repeated contacting of water is achieved.

2. Counter-flow systems seem ineffective for warm water disinfection and should not be used. In-line mixing contactors or airstone diffuser contacting systems proved more effective.

3. Ozonization of the water should occur just prior to its use for fish production to prevent regrowth of bacteria; care should be taken, however, to avoid residual toxicity.

4. Mean ozone residuals of incoming water should not exceed 0.15 ppm for channel catfish.

5. Ozone generator capacity should be sufficient and adjustable to compensate for the increase and decrease in total bacteria numbers as corresponding water temperatures increase and decrease. Overall contact time is not as important as total dosage.

6. Only PVC I plastic piping and fittings, Tygon tubing, and stainless steel should be exposed to ozone gas. Caution should be taken when using ozonated water to prevent deterioration of neoprene gaskets, rubber "O" rings and airstones.

7. With proper engineering, ozone units can be used effectively in warm water hatcheries for disinfection of water supplies and control of chronic bacterial disease problems.

LITERATURE CITED

Arthur, J.W., and D.I. Mount. 1973. Toxicity of a disinfected effluent to aquatic life. In: Ozone for Water and Waste Water Treatment. R.G. Rice and M.E. Browning editors. The International Ozone Institute, Inc., Waterbury, Connecticut pp. 778-782.

Colberg, P.J., and A.J. Lingg. 1978. Effect of ozonation on microbial fish pathogens, ammonia, nitrate, nitrite, and BOD in simulated reuse hatchery water. J. Fish. Res. Board. Can. 35:1290-1296.

Colberg, P. J., and A. J. Lingg. 1978. Effect of ozonation on microbial fish pathogens, ammonia, nitrate, nitrite, and BOD in simulated reuse hatchery water. J. Fish. Res. Board. Can. 35:1290-1296.

Conrad, J. F., R. A. Holt and T. D. Kreps. 1975. Ozone disinfection of flowing water. Progressive Fish Culturist 37(3):134-136.

Hall, L. W. Jr., D. T. Burton, L. B. Richardson. 1981. Comparison of ozone and chlorine toxicity to the developmental stages of Striped Bass, *Morone saxatilis*. Can. J. Fish Aquat. Sci. 38:752-757.

Lohr, A. L. and J. B. Gratzek. 1981. Bactericidal and parasiticidal effects of an activated air oxidant in a closed aquatic system. Aquaculture.

Ozone treatment of potable water. P.C.I. Corporation, West Caldwell, New Jersey. P II C-1 - II C-23.

Rosenlund, B. D. 1974. Disinfection of hatchery water supply by ozonation and the effects of ozonated water on rainbow trout. Preliminary Studies for Abottsford Hatchery, B.C.

Rosenlund, B. D. 1975. Disinfection of hatchery influent by ozonation and the effects of ozonated water on rainbow trout. In: Aquatic Applications of Ozone, Blogoslawski, W. J. and R. G. Rice, Eds. International Ozone Institute, Inc., Syracuse, NY pp. 59-65.

Smith, C. E., and W. P. Dwyer. 1980. Photozone ® toxicity in rainbow trout, *Salmo gairdneri*. Bozeman Information Leaflet 13.

Wedemeyer, G. A., N. C. Nelson, and W. T. Yasutake. 1979. Physiological and biological aspects of ozone toxicity to rainbow trout *(Salmo gairdneri)*. Journal Fisheries Research Board of Canada. 36(6):605-614.

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