Impact of Ozonation on System Performance and Growth Characteristics

of Hybrid Striped Bass (Morone chrysops (f) x Morone saxatilis (m))

Reared in Recirculating Aquaculture Systems

by
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(ABSTRACT)

This study was conducted to evaluate the impact of sustained ozone dosages rates (0, 3, 13, 25, and 45 g O_3 per kg feed delivered per day) on water quality profile, fish physiology, and growth during a production cycle of hybrid striped bass reared in pilot scale recirculating systems. Nitrogenous wastes and dissolved organic compounds increased linearly ($p < 0.01$) throughout the trial under both ozonated and unozonated conditions. Ozone treatments 13 and 25 g O_3 / kg feed received 170 kg more feed than the control treatment (0 g O_3 / kg feed) by the end of trial. The ozonated system received an average 1 kg more feed per day than did the unozonated system, although the unozonated system received 25% more freshwater during the trial. Ozonation increased nitrification efficiency which resulted in a 25% reduction in residual ammonia measured per kilogram of feed delivered. Biologically degradable organics ($CBOD_5$) were lowered 45% under ozonation when adjusted for daily feed input. Microbial activity as determined by the rate of degradation of DOC was 43% higher in the ozonated environments.

Increased environmental quality provided through ozonation did not result in measurable physiological improvements (p 0.05). All parameters monitored except plasma protein and hematocrit values varied significantly ($p > 0.05$) over time. Hemoglobin, plasma protein, and hematocrit values in fish under all rearing conditions were 10.2 ± 0.4 g/dL, 7.1± 0.6 g/dL, and

52.9 ± 1.9%, respectively. Final mean serum chloride level of 115.8 ± 3.4 mE/L and mean glucose level of 186.3 ± 2.1 mg/dL were measured in fish reared at treatments 0, 13, and 25 g O_3.

Fish reared under ozonated conditions possessed an overall mean growth rate of 2.3 g/day and FCR of 1.58:1. Simultaneously, fish in the unozonated environment gained 1.8 g/day at an FCR of 1.90:1. At the conclusion of the trial, the final mean weight (443 ± 11.6 g) of fish reared in the unozonated environment was significantly lower ($p < 0.01$) than fish reared in the ozone treatments 13, and 25 g O_3 (combined mean of 576.6 ±16.4 g).

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CHAPTER 1

Application Of Ozone To Recirculating Aquaculture Systems[1]

Introduction

Water in recirculating aquaculture systems is continuously filtered and reused to maintain a suitable rearing environment for aquatic organisms. Water reuse increases the mass of fish that can be produced using a limited water resource and allows more control over the culture environment. Also, because of intensive water reuse, systems that recirculate water have effluents that are relatively low in volume, but contain proportionally higher nutrient and organic levels, which can be treated for discharge more readily (on a total mass basis) than other aquaculture effluents.

Use of recirculating systems to achieve environmental control is not without drawbacks. Reusing the majority of water each day requires processes that remove both dissolved and particulate matter to maintain water quality. Focus is placed on ammonia removal within a biofilter and on processes that remove settleable and filterable solids. However, as production intensifies and water reuse increases, the removal of nitrite, certain dissolved organics termed refractory organics, and colloidal solids left by conventional filtering becomes increasingly important (Rosenthal and Kruner 1985). Intensifying production in recirculating systems generally places a greater organic and nitrogenous load on the biofilter, which can reduce its

[1]This was first referenced as: Brazil, B.L., S.T. Summerfelt, and George S. Libey. 1996. Application of ozone to recirculating aquaculture systems. Pages 235- 245 in G.S. Libey and M.B. Timmons, eds. Proceedings from Successes and Failures in Commercial Recirculating Aquaculture Facilities. Roanoke, Virginia. Modifications were made to the original content and format to incluede this body of work in the dissertation.

capacity to complete the two-step serial conversion of ammonia to nitrite and nitrite to nitrate (nitrification). Because the increased loading increases competition for space and oxygen to the detriment of the bacteria that convert nitrite into nitrate, a net increase in nitrite concentration across the biofilter and within the recirculating system as a whole can occur (Okabe et al. 1995). These increased nitrite levels can be toxic to fish (Mazik et al. 1991).

Refractory organics accumulate because they are not readily biodegradable due to their size or chemical nature and because daily replacement of water is usually less than 15 percent. Colloidal solids levels increase in systems with low water exchange because they are generally not removed by common settling and filtration units (Chen et al. 1993). Elevated concentrations of refractory organics and colloidal solids may elicit physiological responses that lead to restricted growth and eventually to increased mortality rates (Smith 1982).

Nitrite, refractory organic compounds, and colloidal organic materials that accumulate in recirculating systems can be removed by increasing the daily water exchange rates or by introducing oxidizing agents. To avoid high water exchange rates, some closed-system culturists have chosen to apply ozone because of ozone's attractive physio-chemical characteristics. Traditionally, ozone has been used to remove pathogens from discharges and/or water supplies of aquaculture systems (Owsley 1991). Ozone can be added to recirculating systems to support water treatment by: (1) directly oxidizing nitrite to nitrate, (2) breaking relatively non-biodegradable refractory organic compounds into smaller and more biodegradable compounds, and (3) precipitating dissolved organic molecules and micro-flocculating colloidal organic matter, which improves their removal via settling, filtration or foam fractionation.

Physical Properties

Ozone is a metastable molecule of oxygen possessing three atoms rather than two. This unstable and highly reactive species is produced by the reaction:

$$O + O_2 \Rightarrow O_3$$

which is initiated by a high energy field (Bablon et al. 1991). The standard free energy of formation G^o (1 atm) = 161.3 kJ / mol, reveals that thermal activation is impossible, although, heat is used to decompose ozone (Bablon et al. 1991).

Rice et al. (1988) reported that in the atmosphere the half-life of ozone was approximately 12 hours, making storage of ozone difficult. Ozone must be generated on site because the heat generated during compression destroys any ozone present and because ozone gas concentrations of 70% or greater can spontaneously explode (Bablon et al. 1991). Additionally, when liquid oxygen containing dissolved ozone evaporates, the ozone separates, creating a mixture which has exploded at concentrations as low as 30% (Kinman 1972).

Generation

High energy sources such as cheminuclear sources, corona discharge, electrolytic processes, and ultraviolet light (wavelengths less than 200 nm) can excite the electrons of the oxygen molecule (Bablon et al. 1991). Generation of ozone in corona fields, a high energy field established between two dielectric metals, is the most common method used to produce large quantities of ozone (Bablon et al. 1991). When dried air or gaseous oxygen is passed through the energy field, a portion of the diatomic oxygen molecules are excited, creating triatomic ozone (Rosenthal 1981; Bablon et al. 1991). Ultraviolet light generators are less expensive to purchase,

but energy requirements per unit of ozone generated basis can be 30 times greater than corona generation. However, improvements in lamp technologies may reduce production costs (Bablon et al. 1991).

Either air or pure oxygen can be used as a feed gas. However, 2 to 3 times more energy is required to produce ozone at similar concentrations using air rather than purified oxygen (Bablon et al. 1991). Additionally, the output concentration from a generator can be roughly doubled by using pure oxygen rather than air, because pure oxygen contains up to 77% more oxygen per unit volume than air (Masschelein 1982). Corona discharge generators have been reported to produce 6-8% ozone, but more commonly produce 2-3% (Hudlicky 1990). The efficiency of generation depends upon the concentration of oxygen in the feed gas and the percentage of ozone produced; it requires about 10 kWh of electricity to produce 1 kg ozone at a concentration of 4-6% in an oxygen feed gas (Carlins and Clark 1982).

Reliable and efficient generation of ozone requires that the feed gas has a dew point temperature less than 65°C, and is free of particles and coalescible oil mists (Dimitriou 1990). These impurities foul the dielectrics within the corona discharge cells and react with the ozone, which can reduce generator output. Experience at the Freshwater Institute (Shepherdstown, West Virginia) revealed that hydrocarbon contamination of the liquid oxygen feed gas reduced ozone generation efficiency and output concentrations (Bullock et al. 1996). Hydrocarbons can be retained during the distillation of liquid oxygen and, on rare occasion, can result in concentrations greater than 20 ppm in industrial grade liquid oxygen. Use of hospital grade oxygen (99.9% pure), although more expensive, will alleviate this problem, extending efficient

operational periods. Dimitriou (1990) suggested a limit of 5 ppm hydrocarbon in the oxygen feed gas.

Treatment Applications

The decision to use ozone should be made realizing it is not a panacea to all production problems. The culturist must precisely identify the goals of ozone injection for economically effective use.

Organic Oxidation The presence of dissolved organic compounds is readily identified by the yellow/brownish coloration of culture water. The main coloring agents, humic acid, amino acids, carboxylic acids, carbohydrates, etc., are collectively referred to as humic substances (Hirayama et al. 1988). Accumulation of dissolved organic compounds, solids and oxygen-demanding matter in recirculating systems is dependent on feed input, water exchange rate, solids removal in the clarifier, and to a lesser degree biofiltration. Organic concentrations increase in proportion to daily and cumulative feed inputs (Hirayama et al. 1988). Easter (1992) observed maximum CBOD, COD, and DOC levels approaching 25, 100, 40 mg/l, respectively, in recirculating systems rearing hybrid striped bass. Exact sublethal and lethal concentrations of dissolved organics have not been identified; however, the accumulation of dissolved organics has been implicated as a possible cause for reduced fish growth rates and reduced nitrification efficiencies (Hirayama et al. 1988; Morrison and Piper 1988; Easter 1992; Nunely 1992; Bosworth 1994).

Ozone and its reaction by-products are capable of oxidizing many organic substances (Rice et al 1981; Bablon et al. 1991). Ozone has been reported to effectively decrease the

accumulation of non-biodegradable organic compounds in recirculating systems (Rosenthal and Otte, 1980). First order reaction rates describe the oxidation of organic substrates (M) with ozone:

Equation 1.1. $d[M] / dt = k [O_3] [M]$

where the rate limiting substance can be either ozone or the substrate (Bablon 1991). The oxidation potential of M must be high for degradation to occur. The reactivity with ozone is usually more pronounced than with other oxidizing agents (Bablon 1991). Sites of initial reaction are multiple bonds (C≡C, C=C, R) C=C) X) or negatively charge atoms (N, P, O, S, and nucleophilic carbons), therefore, strong initial reactivities are anticipated for molecules possessing OH, CH_3, or OCH_3 groups and weaker reactivities with NO_2, CO_2H or CHO constituents (Richard and Brener 1984; Bablon 1991).

Ozone addition also reduces the accumulation of TSS and COD within recirculating systems, probably due to improved filtration resulting from ozone-induced micro-flocculation and precipitation of dissolved organic compounds (Summerfelt et al. 1996).

Ammonia Removal Reduction of ammonia in the biofilters within recirculating systems is a major concern in recirculating systems because unionized ammonia is extremely toxic to fish at low levels (Lucchetti and Gray 1988). Ozone can directly oxidize ammonia to nitrate:

Equation 1.2. $4O_3 + NH_3^- \Rightarrow NO_3^- + 4O_2 + H_3O^+$

However, the reaction rate constants for this process are extremely slow at pH levels below 9.3 (Richard and Brener 1984; Bablon et al. 1991). Conditions conducive to direct ammonia oxidation are not typical in recirculating aquaculture systems, freshwater or marine, which operate

at a pH level between 6.8 and 8.4.

Even so, ozone addition may change the water chemistry within a recirculating system sufficiently to improve ammonia removal within the biofilter (Malley et al. 1993). Autotrophs of primary concern, *Nitrosomonas sp.* and *Nitrobacter sp.*, are responsible for the nitrification process (Wheaton et al. 1994). In this aerobic process, *Nitrosomona sp*. oxidize ammonia to nitrite which is further oxidized to nitrate by *Nitrobacter sp.* Ozone oxidation of non-biodegradable organic compounds makes them more biodegradable, i.e., the oxidized organic molecules can be more readily assimilated by heterotrophic microorganisms. Additionally, ozonation improves the removal of solids within recirculating systems through precipitation of dissolved organic matter and micro-floclation of colloidal solids (Summerfelt et al. 1996), which reduces the total organic loading on the biofilter compared with an unozonated system. Research at Virginia Tech on freshwater recirculating systems employing rotating biological contactors (RBCs) showed ammonia removal efficiencies nearly 26% higher in systems receiving ozonation than in non-ozonated systems (unpublished data). Sutterlin et al. (1984) and Paller and Lewis (1988) also reported improved nitrification when ozone was added to recirculating systems. The authors suggest that reducing the total load of organics on the biofilter may reduce the growth of heterotrophic bacteria and allow for more growth of autotrophic nitrifying bacteria. Collins et al. (1975) demonstrated that autotrophic bacteria had significantly lower growth rates than heterotrophic bacteria, which is a disadvantage to autotrophic bacteria when competing for space and oxygen with heterotrophic bacteria. Limiting the nutrient base available to heterotrophs may restrict their growth and allow autotrophic colonies to expand (Wheaton et al. 1994).

Nitrite Removal Unlike the reaction rates of ammonia oxidation, direct ozone oxidation of nitrite to nitrate proceeds readily:

Equation 1.3. $NO_2^- + O_3 \Rightarrow NO_3^- + O_2$

The reaction rate constant, $3.3 - 3.7 \times 10^5$ M^{-1} s^{-1}, is largely pH independent (Bablon et al. 1991). Ozone stoichiometrically oxidizes nitrite to nitrate (Bablon et. al. 1991); therefore, addition of ozone to recirculating systems is beneficial because it reduces nitrite levels compared with systems that do not receive ozone (Rosenthal 1981; Sutterlin et al. 1984, Rosenthal and Kruner 1985; Paller and Lewis 1988; Summerfelt et al. 1996). If on occasion the nitrification in the biofilter is lost, ozonation of the system will help prevent the accumulation of nitrite, which is a substantial benefit. Because routine ozonation reduces the nitrite concentration going to the biofilter, over a long period ozonation also reduces the quantity of nitratifying bacteria in the biofilter; thus, reducing the total nitrite removal capacity of the biofilter. Interruption of regular ozone addition would result in nitrite rapidly accumulating within the recirculating system, which can produce serious fish health problems (Summerfelt et al. 1996).

Disinfection The inactivation of pathogens is a function of the residual ozone concentration, residence time, pathogen load, temperature, pH, and alkalinity (Sproul 1975; Legeron 1984; Masschelein 1992). Ozone and by-product radicals react with substances preferentially based on oxidation potential and stoichiometric amounts of the substrates present (Bablon et al. 1991). However, because there is so much more organic carbon present than typical amounts of ozone added to recirculating systems, ozone's effect on microbial reductions in recirculating systems is limited (Kinman 1972; Rosenthal 1981; Legeron 1984; Bullock et al.

1996).

Residual concentrations ranging from 0.5 to 4.0 mg/l are typically used to eradicated water borne pathogens (Kinmam 1972). In pure water systems, residual concentrations between 0.01 and 0.4mg/l have proven effective in reducing *E. coli* and *S. faecalis* (Kinman 1972; Block 1982). Kinman (1972) demonstrated that in the pure water systems a contact time as short as 15 seconds was sufficient to destroy 100% of the microbes present. However, most systems exert an ozone demand which increases the amount of ozone that must be added to sustain an ozone residual for a given contact time. For example, secondary effluent from a municiple wastewater plant contacted for 10 minutes with greater than 50 mg of ozone per liter reduced bacteria counts by 99%; the increased ozone demand from dissolved organics accounted for the large amount of ozone that must be added just to achieve a 2 $\log_{10}$ reduction (Kinman 1972). Increasing either residual ozone concentration or contact time are required to produce significant disinfection in situations with high levels of organic matter (Kinmam 1972; Block 1982; Bablon et al. 1991; Bullock et al. 1996). Complete bacterial reduction is necessary when treating water for human consumption, but may not be desirable in the treatment of fish-culture water.

Seasonal variation in the pathogen load is often a characteristic of surface waters, rendering the source unusable for certain aquaculture applications without disinfection (Conrad et al. 1975; Piper et al. 1982). Hatcheries using surface waters are particularly interested in reducing pathogen loads to reduce mortalities and improve the growth and quality of fish. Roselund (1975), rearing rainbow trout, reported that maintaining residual ozone concentrations between 0.1 to 0.6 mg/l in the effluent of their contactor insured complete removal of bacteria.

Conrad (1975), artificially increasing the pathogen load of *Flexibacter columnaris*, observed a 99% reduction in bacteria counts after ozonation. While complete sterilization was not accomplished significant increases in survival rates of fish from 60 to 96% were observed. Conrad et al. (1975) remarked that water low in sediment and organic matter enhanced the bactericidal efficacy of the low level ozonation used. Seasonal disease outbreaks of *Ceratomyxa shasta* at the Cowlitz hatchery (Washington) resulting in a 62.5% mortality rate were significantly reduced to 1.4% through ozonation; the authors reported that steelhead and cutthroat trout reared in water ozonated with a contact time > 0.84 were free of infection (Tipping 1988).

Collins (1992) reported that attempts to achieve disinfection failed when an ozone dose of 0.1 to 0.15 mg/l was applied to marine mammal exhibition pools. The author indicated that total ozone demand of the system required higher dosages to effect disinfection. In closed systems, organic loads can reach levels that might make disinfection economically prohibitive; therefore, achieving a reduction in pathogen loading or other improvements in water quality should be the goal of ozonation.

Injection Regime

Pivotal to effective ozone use are the method and location of injection and amount of ozone injected. Table 1.1 presents terminology used in the following sections. Ozone treatments can be applied as a batch injection, as a series of daily injections, or continuously supplied throughout the day. The method and location of injection, along with the amount of ozone to be injected, should be chosen to meet the primary treatment goals characteristic of a given recirculating system .

Method Batch injection refers to the practice of using one continuous injection period of duration less than one day to apply the ozone treatment. Serial injection periods can be used to apply the ozone treatment over a greater portion of the day by injecting ozone periodically throughout the day. With continuous injection, ozone can be added to the system 24 hours per day. The decision to use one method over another depends on the culturist's management strategy, which may be linked to the feeding schedule.

Introduction of feed initiates the degradation of water quality. Easter (1992) and Herbst (1994) conducted diurnal studies of recirculating systems to evaluate the impact of feed delivery on rearing conditions. Approximately 3 to 4 hours after the delivery of feed, ammonia (Easter 1992) and dissolved organics (Herbst 1994) concentrations peaked (Figures 1.1 and 1.2, respectively).

Dissolved oxygen concentrations dropped to their lowest levels within minutes of feeding and required up to 2 hours to return to preferred levels. From these results, we believe that batch treatment can be employed to improve water quality if no more than three moderate feed allotments are presented per day. Based upon experiences at Virginia Tech, injection should begin just prior to final feeding and extend for at least 3 hours (Chapter 3).

Batch ozone injection (Figure 1.3) produces larger fluctuations in water quality than serial ozone injection would produce (Figure 1.4). Continuous ozone addition produces the least fluctuation in water quality, especially if fish are fed 24 times per day (Figure 1.5). Also, providing a given ozone dose based on feed loading (i.e., daily mass of ozone added is proportional to the daily mass of feed added) with continuous ozone addition requires less ozone

addition per unit time than a batch or serial injection strategy in order to achieve a particular water quality management objective.

However, ozone added by batch typically is introduced when water quality has reached its worst cyclical condition within the system; therefore, batch ozone addition may produce more water quality benefits per unit of ozone added than are produced by either serial or continuous ozone addition.

Implimenting batch injection, if done manually, is less complicated and cheaper than employing a serial injection regime. Implicit in serial injection is the situation that the ozone generator will be cycled off and on. An automated controlled system employing a central computer to calculate the proper amount of ozone to for each culture system would be best suited to control ozone distribution .

Location Because ozone can be used for many different functions, the site of ozone injection can have a large effect on how the recirculating system responds to the treatment. Arguments can be made for injecting ozone in several locations within a recirculating system, particularly adding ozone just before the biofilter versus generating and adding ozone within the oxygen feed used to supersaturate the water just before the fish culture tank Regardless of where ozone is injected, however, there could be problems with residuals concentrations affecting either the fish within the culture tank or the bacteria within the biofilter.

Direct ozonation of the rearing tank to achieve an ozone residual is not recommended. Ozone is extremely toxic at low concentrations. Researchers have reported 96-hour LC50s of 9.3 and 80 µg/l for rainbow trout (Wedemeyer et al. 1979) and striped bass larvae (Hall et al. 1981),

respectively. Wedemeyer et al. (1979) reported gill pathology and reduced feeding at a residual concentration of 5 µg/l. Most recently, Bullock et al. (1996) reported that ozone destroys gill lamellar epithelium, promoting ionic imbalances that lead to death. Adding ozone before the biofilter may allow ozone residual to enter the biofilter; this residual would expend itself on the biosolids present. In this manner, the biofilter can be used to shield the culture tank from potentially harmful ozone residuals. Research at Virginia Tech University shows that measurable ozone residual levels entering the biofilters did not adversely affect biofilter performance (Chapter 2). However, in certain instances, it is possible that an ozone residual could damage the microorganisms within the biofilter, which would be indicated by an increase in ammonia or nitrite concentrations.

Possibly the most economical way of adding large quantities of ozone to a recirculating system is to add ozone within the feed gas used to oxygenate the water just before entering the fish culture tanks (Bullock et al. 1996; Summerfelt et al. 1996). Adding ozone within the oxygen feed gas takes advantage of an oxygen supply and gas-transfer unit that the fish already require and allows the ozone to act upon and reduce the nitrite and pathogens just before the water contacts the fish. Adding ozone just before the fish culture tank, however, has the potential of exposing fish to ozone residuals (Bullock et al. 1996). The risk of exposing fish to ozone was reduced when lower ozone injection rates were used and when an oxidation/redox (ORP) controller was used to prevent ozone accumulation within each culture tank (Bullock et al. 1996). Additionally, the risk of exposing fish to ozone can be reduced by using an ozone contact chamber to retain the water for several minutes before passing the water to the fish culture tank.

Ozonation can increase biodegradability and nitrification rates; yet, it is unknown whether or not it is necessary to add ozone directly before the biofilter to positively affect nitrification within the unit. However, it is likely that the point of ozone injection within the recirculating system does not have a large affect on oxidation of non-biodegradable organic compounds and their subsequent assimilation within the biofilter. Additionally, ozone will oxidize dissolved organic compounds and colloidal solids within the water regardless of where ozone is injected; However, microflocculation and precipitation of the destabilized compounds is affected by the length of contact time before the solids removal device. The culture tank can double for the chamber to provide contact time for microflocculation and precipitation.

Amount Conrad et al. (1975), Tipping (1988), Blogoslawski (1992), and Rueter and Johnson (1995) have suggested treatment regimes to remove fish and crustacean pathenogentic organisms from influent culture waters based on the C•T concept (Legeron 1982). There has been little published work to suggest appropriate dosage rates for the reduction of organics in recirculating aquaculture systems. Ozonation rates in published works (Williams et al. 1982; Sutterlin et al. 1984; Morrison and Piper 1988; Poston and Williams 1988; Poston and Williams 1990) appear to be based on generator production capacities to evaluate ozone treatment or treatment regimes used in the water treatment industry.

The authors believe that unless disinfection is the objective of ozone injection, ozone should be injected to reduce organic loads and increase biodegradability, as is the case in wastewater treatment. Research at the Freshwater Institute (Sheppardstown, WV) also showed that the ozone dosing rate was not sufficient to produce greater than a 1 log10 reduction in the

numbers of heterotrophic bacteria in the system water or on gill tissue. Rapid loss of oxidation capacity (ozone half-life < 1-15 sec) caused by levels of nitrite and organic carbon was blamed for the failure of ozone to reduce numbers of heterotrophic bacteria.

Research in a recirculating system used to culture rainbow trout at the Freshwater Institute indicated that an ozone dosing rate of 25 g of ozone per kilogram of feed improved water quality and microscreen filtration (Summerfelt et al. 1996) and reduced bacterial gill disease (BGD), associated mortalities, and number of chemical treatments required to control bacterial gill disease (BGD) epizootics (Bullock et al. 1996). Adding ozone at higher rates, 36-39 g of ozone per kilogram of feed, had roughly the same effect on water quality, microscreen filtration, and BGD epizootics, but was much more likely to produce fish mortality when on occasion ozone accumulated to toxic levels (Bullock et al. 1996).

Water quality was best in recirculating systems receiving treatment ratios of 25 and 45g of ozone per kg of feed delivered per day. However, diminishing returns were discovered for growth rates (not significantly different) at dosage rates greater than 13g of ozone per kilogram of feed (chapter 2). Dissolved organic carbon (DOC) concentrations did not differ significantly when compared with levels measured in systems not receiving ozone injection; however, the ratio of DOC: accumulated feed input differed significantly. Increased growth rates and decreased turbidities were positively correlated to ozone dosage rate, indicating that ozonation enhance environmental quality.

Summary

Specific water treatment goals must be set before implementation of an ozonation regime. The culturist must understand the limitations of ozone and the impact that it will have on system performance. Our discussion was intended to be a starting point to suggest injection strategies and dispel a few myths about ozone. Ozone can be an aid to increasing production, but it can also lead to mortality of production stock if not used properly.

Concepts paramount to the efficient water treatment in closed systems are unit process (filtration technique) optimization and diminishing returns. Optimization is accomplished by accurately sizing the process according to the total waste demand generated by the introduction of feed. Ozone dosage rates should be no exception.

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Table 1.1. Definition of terminology used to describe ozone treatment.

Parameter	Definition
C·T	a measure of microbial reduction potential based upon the product of the ozone residual concentration times the contact time
Dosage rate	amount of ozone injected per unit of substrate (g of ozone / M) where M is the substrate targeted for oxidation
Injection period	the length of time ozone is injected (time)
Injection rate	amount of ozone in the gas stream supplied to the contactor per hour (mg/l /h, g/ m^3 / h)
Ozone concentration	ozone present within the gas stream (% ozone by weight, mg /l)
Ozone production	the amount of ozone generated daily (g/day)
Ozone residual	concentration of measurable ozone leaving the contactor (mg/l)
Ozone treatment	total amount of ozone to be injected daily (g/day) = specific dosage rate * M, M = kg of feed/day

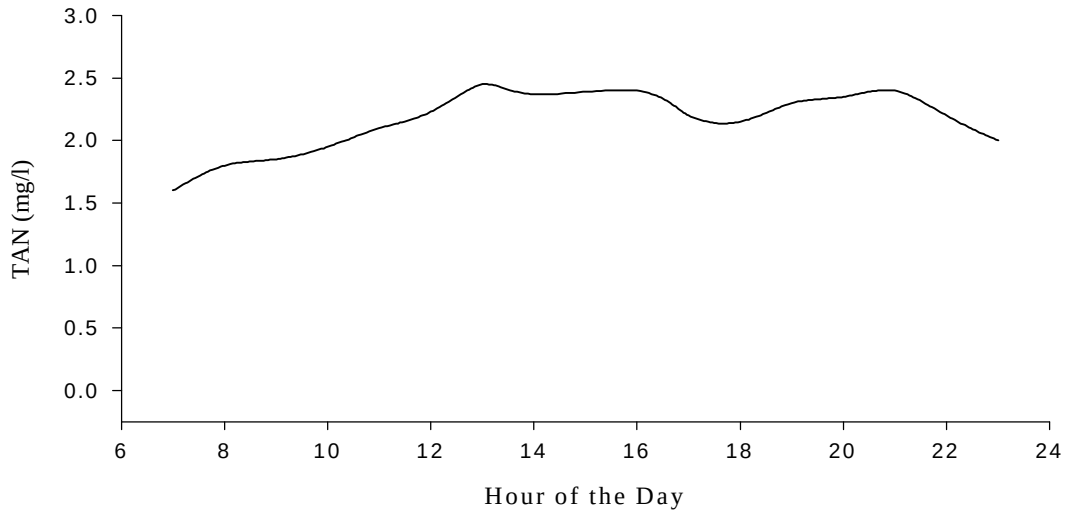

Figure 1.1. Diurnal study of total-ammonia-nitrogen concentrations in recirculating systems rearing hybrid striped bass. Fish were fed at 9:00am and 5:00pm daily. TAN concentrations peaked approximately 3 to 4 hours after feed delivery, leading to the poorest water quality conditions (Easter 1992).

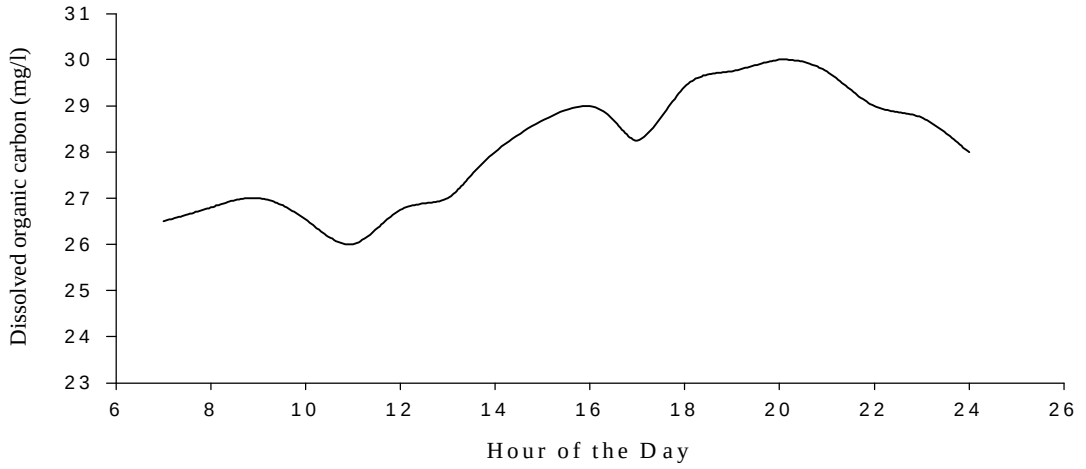

Figure 1.2. Diurnal study of dissolved organic carbon concentrations in recirculating systems rearing hybrid striped bass. Fish were fed at 8:30am, 12:00pm, and 5:00pm. Concentrations began to increase approximately 3 to 4 hours after the first feeding and continued to rise throughout the day, peaking 11 hours after the first feeding (Herbst 1994).

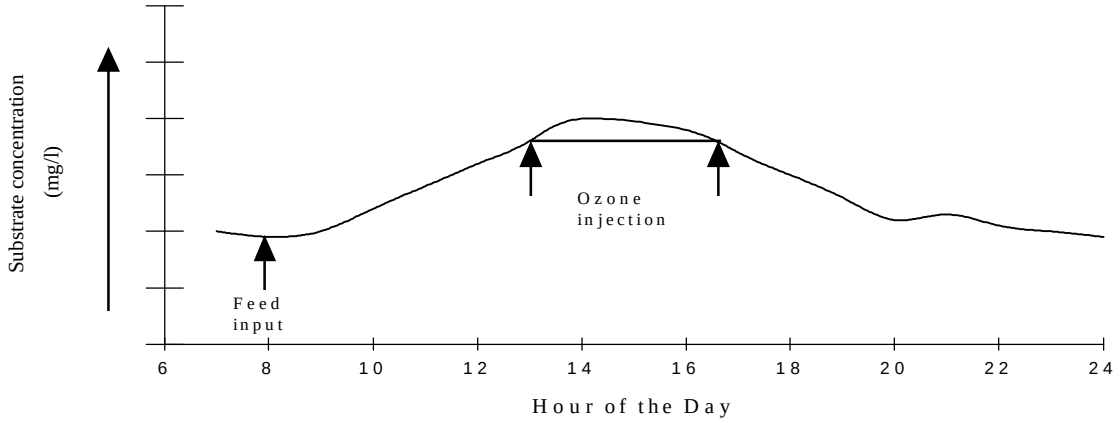

Figure 1.3. Idealized batch injection regime implemented with a single feeding for the day. Substrate concentration refers to ambient concentrations of waste products. Contrived data used to simulate diurnal waste profile.

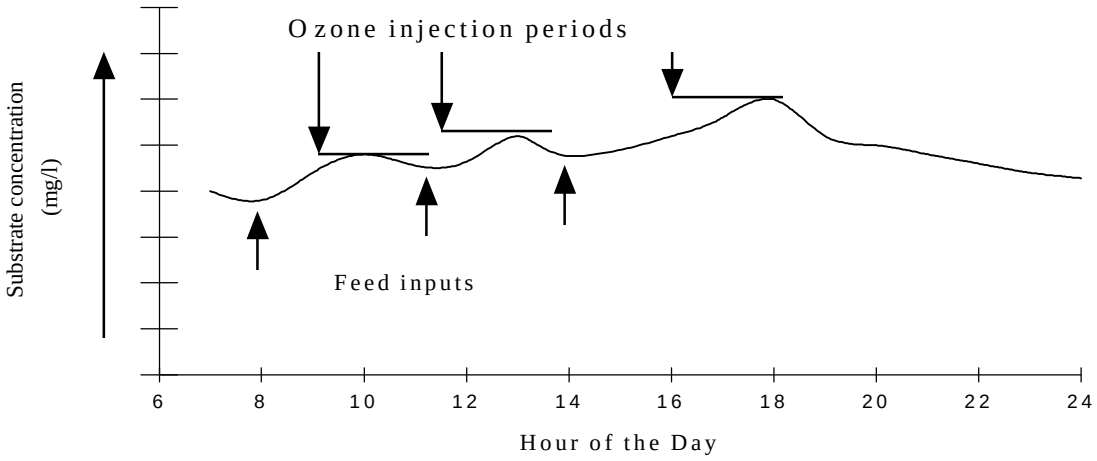

Figure 1.4. Idealized serial injection regime implemented with three feedings per day. Substrate concentration refers to ambient concentrations of waste products. Contrived data used to simulate diurnal waste profile.

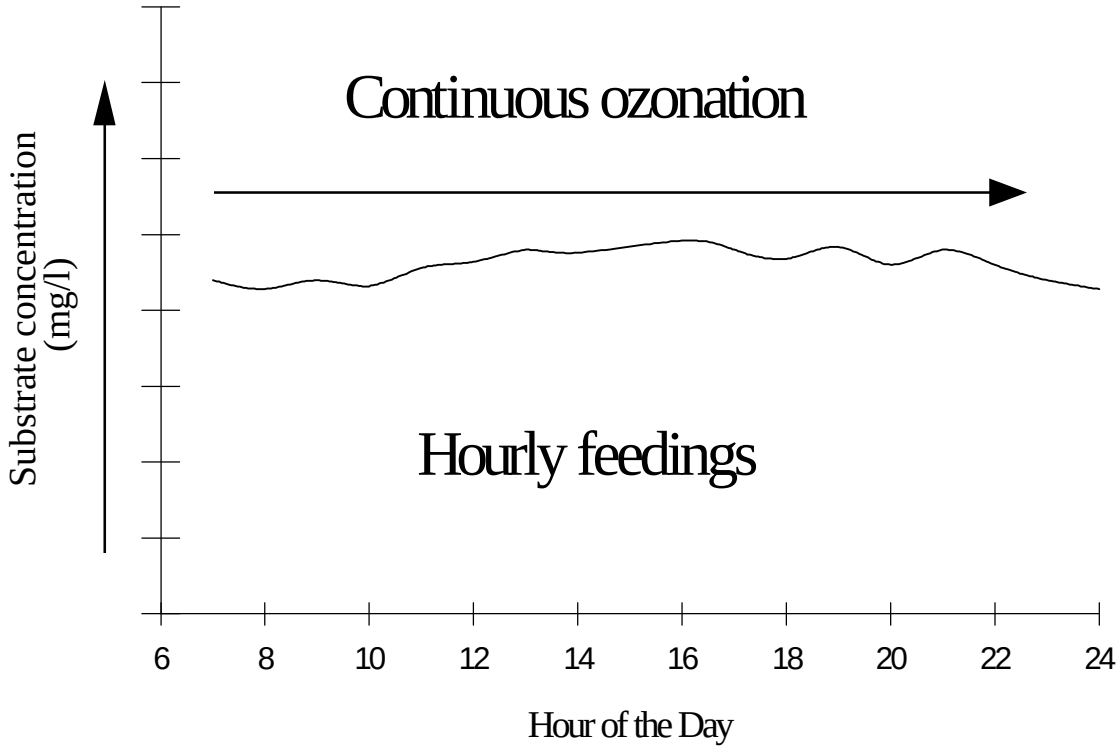

Figure 1.5. Idealized continuous ozonation regime implemented with twenty-four feedings per day. Substrate concentration reflects ambient waste loads. Contrived data used to simulate diurnal waste profile.

CHAPTER 2

The Impact of Ozonation on Feed Input Rates and Dissolved and Suspended Organic Compounds In Pilot Scale Recirculating Aquaculture Systems Rearing Hybrid Striped Bass (Morone chrysops (f) x Morone saxatilis (m)).

Abstract

The application of ozone to recirculating systems has gained considerable attention in recent years due to increases in production intensity. However, research is lacking that provides quantitative information establishing waste production (feed input)-based ozonation treatment regimes. In this study, feed input and environmental quality characteristics of recirculating aquaculture systems receiving different ozone treatments were investigated to assess the impact of ozonation during a production trial. Nitrogenous wastes and dissolved carbon compounds increased significantly ($p < 0.01$) through time in a relatively linear fashion under all ozonation treatment regimes. Mean daily feed inputs (e.g., fish consumption rates) of 4.0 , 4.7, and 5.0 kg / day were obtained in systems receiving ozone treatments 0, 3, and 25 g O_3 / kg feed, respectively, and resulted in the delivery of 170 kg more feed to the ozonated treatments. Differences in cumulative and daily feed inputs between the ozonated and unozonated systems were not reflected in ambient total ammonia nitrogen (TAN) concentrations. Overall TAN means were not significantly significantly different (p-value > 0.05). During a six-day period midway through the trial, a continuous flow of fresh water was used in the unozonated system to improved enivornmental quality. This did not result in a drop in $CBOD_5$, however, DOC and COD levels drop 65 and 80%. Adjusting TAN for weekly feed input suggested that the systems receiving

ozone treatment experienced a 25% increase in the nitrification removal rate not observed in the unozonated treatment. A similar transformation on $CBOD_5$ and DOC revealed that 45% fewer refractory compounds accumulated in the ozonated environments per unit mass of feed delivered than was observed accumulating in the unozonated environment. The rate of biological degradation of organic compounds remained significantly higher ($p = 0.07$) in the ozonated systems than observed in the unozonated system . Particle analysis showed that particles smaller than 30 microns comprised more than 90 % of all particles remaining in solution in all systems. The cumulative size distribution of particles showed that in the ozone treatment of 25 g O_3 / kg feed increased the percentage of particles within the size range of 30 and 50 microns. Ozonation of the rearing environment facilitated greater TAN and dissolve carbon compound removal, allowing for sustained higher feed input (fish feed consumption) than oxygen injection alone.

Introduction

Water quality in recirculating aquaculture systems is governed by the feed input rate and the efficiency at which filtering devices remove or detoxify waste products. By increasing rearing intensity, the rate of water quality deterioration escalates in proportion to the unit mass of feed input rate. As a result, traditional treatment processes (nitrification and clarification) have had difficulty in sustaining adequate environmental quality to promote fish growth under commercial production scale pressures.

Hirayama et al. (1988) described the accumulation of dissolved organic substances in recirculating systems as a function of the water exchange rate and feeding rate. Their conclusions suggested that increased levels of dissolved organic compounds could restrict production in closed system aquaculture. Nunley (1992) and Bosworth (1994) culturing hybrid striped bass, reported that measured water quality parameters were within acceptable limits, yet daily growth rates slowed as the production cycle progressed. Both noted that changes in water color may have suggested shifts in microbial populations and/or the accumulation of refractory organic substances coinciding with lowered growth.

Enhancing water quality with supplemental ozonation has been shown to improve biological filtration and solids removal (Rosenthal and Otte 1979), thereby helping to increase production capabilities of recirculating aquaculture systems (Williams et al. 1982; Sutterlin et al. 1984; Poston and Williams 1988; Poston and Williams 1990). The oxidation of refractory substances generates biologically assimilable byproducts that are more easily removed through biofiltration (Rosenthal and Otte 1979; Hirayama et al. 1988). Rosenthal and Kruner (1985)

concluded that the gradual increase of dissolved substances over time was counteracted through side stream ozonation of recirculated water. In addition, they reported that ozonation supported the second stage of nitrification by the direct oxidation of nitrite to nitrate.

Dissolved organics provide a nutrient base for heterotrophic bacteria that out compete autrophic bacteria for limited substrate area on biofilter media (Oga et al. 1991; Honn and Chavin 1976). Okabe et al. (1995) observed that biofilm thickness increased as refractory organic compounds accumulated, resulting in changes in the spatial distribution of colonizing bacteria. Autotrophic bacteria tended to migrate toward the bottom of the biofilm where ammonia and oxygen transfer were restricted, hindering the nitrification process. Limiting potential population expansion of heterotrophs by lowering dissolved organic compound levels through ozonation, promoting increased numbers of nitrifying colonies leading to elevated nitrogen removal efficiencies (Hirayama et al 1988; Morrison and Piper 1988; Wheaton et al. 1994; Furumai and Rittmann 1994). Thus, ozonation may facilitate the first stage of nitrification as well.

Suspended particulates, also, accumulate in recirculating systems operating with reduced daily fresh water exchanges (Weeks 1992). Coche (1981) suggested that the accumulation of suspended solids can induce physiochemical problems and increased mortality of fish as total suspended solids (TSS) levels approached and exceeded 15 mg/l. Micro screen filtration (Chen et al. 1993a) and foam fractionation (Chen et al. 1993b) have increased the removal of suspended solids (< 5 microns); however, Herbst (1994) cited Ghan et al. (1976) as having demonstrated that the ozonation of a liquid waste stream resulted in a 70 % higher reduction of the total suspend solids and a 40 % higher reduction in the turbidity than achieved with air floatation (e.g.,

foam fractionation).

It has been demonstated that ozone possesses potential to improve water quality in recirculating systems. However, the high capital expense of commercial scale ozone generators combined with the toxic nature of ozone (to fish and humans) demands that ozonation be conducted at safe and economically efficient treatment rates. In this study, ozone was applied at different treatment rates to characterize water quality associated with routine ozonation during a production trial hybrid striped bass.

Methods

Five independent pilot scale recirculating aquaculture systems operated at the Virginia Tech Aquaculture Center (Blacksburg, VA) were used to examine the impact of ozonation on water quality. Four ozone treatment rates (3, 13, 25, and 45 g of O_3 injected per kg of feed per day) and a control (0 g O_3 per injected kg feed per day, pure oxygen injection) were evaluated during the production of hybrid striped bass, Morone chrysops x M. saxatilis (Chapter 3). Each system, stocked at 150 fish/m^3 (average weight 19 g), consisted of a rearing tank, multi-tube clarifier (sump), a rotating biological contactor (RBC), an ozone contactor, and two U-tube aeration devices (Figure 2.1). Detailed operational procedures are presented in Chapter 3.

Ozonation protocol

Ozone injection was done in a side stream fashion. Water directed to the ozone contactor was pumped from the top of the multi-tube clarifier, received ozone treatment, and then entered the RBC. Once in the biofilter vessel, the recently ozonated water was mixed with clarified water pumped directly to the RBC (refer to Figure 2.1). Approximately 35% of the system flow was contacted per pass with a hydraulic retention time in the contactor of nearly four minutes.

Contactor design and injection. The ozone contactor was constructed of schedule 40 PVC pipe (4.5 m X 46 cm dia.). The middle 4.2 m of the column was filled with plastic media (5 cm x 5 cm dia., Norpac, NSW Corp., Roanoke, VA) held in place by two 1.3 cm thick perforated plastic plates. Packing was used to prevent direct vertical ascent of the ozone gas bubbles (Figure 2.2).

Water entered the contactor 28 cm from the top and exited at the bottom, 4.3 m from the

top. Inlet and outlet ports were 3.8 cm diameter. Approximately 20 % of the water passing through the contactor was recirculated with a 0.4 kW pump and reentered the chamber opposite the exit port. A baffle prevented “short circuiting”. Recirculated water passed through a Model 978 injector (Mazzie Corp., Bakersfield, CA) aspirating the O_3/O_2 gas mixture for contact. The use of counter-current contact with the packed column enhanced transfer and contact efficiency of the chamber (Le Sauze et al., 1992) However, ozone transfer efficiencies were not measured. To prevent pressurization of the contact chamber undissolved gasses were vented to a thermal destruct unit.

Ozone production. Ozone was produced from pure oxygen using four corona discharge generators (one OZP-1 and two OZP-2, Clearwater Tech., San Luis Obispo, CA and one OZ6, Ozo-Tech, Yreka, CA). The output from each machine was directed into a manifold to a computer controlled distribution board. Ozone production was monitored with an HC-400 Ozone Monitor (PCI Ozone and Control Systems, Caldwell, NJ). The production concentration ranged from 1.8 and 2.5 % ozone by weight at flow rates between 27 and 32 lpm, which yielded an average ozone concentration of 24.5 mg/l injected into each system.

The different ozone treatment levels were achieved by extending the length of time ozone was injected into each system. Daily ozone treatment requirements and generation times were calculated as followed:

Equation 1. $O_{3sysi} = feed_{sysi} \times O_{3trti}$

Equation 2. $Gt_{abs} = O_{3dmd} \times O_{3gas}$

a. $O_{3gas} = O_{3prod} \div n$

Equation 3. $Ti_{sysi} = O_{3sysi} \div O_{3gas}$

where:
- i = individual system and/or treatment,
- $feed_{sysi}$ = feed input to the system (kg/day),
- Gt_{abs} = time required to generate ozone for daily injections (min),
- O_{3gas} = quantity of ozone in gas stream injected (g/hr),
- O_{3prod} = ozone production rate (g/hr),
- O_{3sys} = quantity of ozone injected into the system (g/day),
- O_{3trt} = ozone treatment ratio (g O_3 / kg feed / day),
- O_{3dmd} = largest ozone injection requirement (g/day),
- n = number of systems receiving ozone injection, and
- Ti_{sysi} = length of time ozone is injected into a system (min/day).

Gt_{abs} was administered during four injection periods lasting three hours each starting at 1200, 1500, 1800, and 2100 hours. Ti_{sysi} also was divided into four equal portions applied during each of the injection periods. Once the ozone treatment for a system during an injection period was achieved, the O_3/O_2 gas stream was shunted to the thermal destruct unit. Pure oxygen then was injected at the same flow rate as O_3/O_2 until Ti_{sysi} was accomplished for the other systems (Appendix 2.A.1 for an example of these calculations).

The four injection periods were established to reflect scheduled feed inputs (Chapter 3). Initiating ozone injection when the waste loads peak would optimize the oxidation capabilities of ozone, as suggested by diurnal studies conducted Easter (1992).

Data collection.

Chemical analysis. Daily and weekly measurements of nitrogenous wastes (total ammonia nitrogen [TAN], nitrite, and nitrate) were made using a DR/2000 spectrophotometer (HACH Co., Loveland, CO). Daily pH measurements were made using an HACH pH pen, twice a weekly alkalinity and hardness levels were monitored by titration following HACH kit methology. Dissolved oxygen and temperature were measured with a portable oxygen meter (Model 58 oxygen meter, YSI Co., Yellow Springs, OH).

Secchi disk depth and light absorbance were measured periodically to quantify changes in water turbidity and clarity. Light absorbance, reported in Formazin Turbidity Units (FTU), was measured with an DR/2000 spectrophotometer at a wave length of 450 nm (Hach method 750).

Organic matter. Measurements of dissolved organic matter were reported as five-day carbonaceous biological oxygen demand ($CBOD_5$), dissolved organic carbon (DOC), and chemical oxygen demand (COD) (data also reported in Herbst 1994). Particulate organic matter was characterized as total suspended solids concentration and particle size distribution. Analyses were conducted approximately every 28 days and more frequently during the latter stages of the production cycle.

Unfiltered water samples were used in measuring $CBOD_5$ following the Standard Methods (1992) procedure 5210 B for the 20°C test. Nitrification was inhibited through the addition of 2-chloro-6 (trichloro methy) pyridine (TCMP).

DOC and COD concentrations were determined from water samples filtered through 0.45 μm glass fiber filters. DOC was measured with a Model DC 80 Total Organic Carbon analyzer

(Dohrmann Inc., Santa Clara, CA) immediately following sample collection. Otherwise, samples were acidified with phosphoric acid (pH < 2) and frozen until analysis could be conducted. On days 113 and 138, biological degradation of DOC was tracked in 500 mL water samples drawn from the culture tank. Following determination of initial DOC values, water samples were aerated with subsequent analyses made daily for the next seven days and then on alternate days over the next two weeks. Distilled water was added to the batch reactor making up for daily evaporative losses. Linear regression (Proc REG, SAS Inc., Cary, NC) was used to calculate best fit lines whose slopes approximated the rate of degradation (Herbst 1994). COD levels were determined following the closed flux titrimetric method, procedure 5220 C (Standard Methods 1992).

Molecular weight distributions of dissolved organic substances were determined through low pressure liquid chromatography (gel filtration) from 1 mL aliquots dispensed on the top of the gel column. Sephadex G-25 gel (Sigma Chemical Co., St. Louis, MO) was used to fractionate compounds between 1000 - 5000 daltons. Column effluent samples were collected at 2 min intervals and absorption measured at 200 nm with a spectrophotometer (Beckman DU640, Beckman Instruments, Inc., Fullerton, CA). The absorption value obtained for each sample was fitted to a standard curve to determine the molecular weight of materials in each effluent sample (Herbst 1994).

Total suspended solids (TSS) and particle size distributions were examined in water samples collected from the culture tanks. TSS concentrations were determined following Standard Methods (1992) procedure 2540 D for solids dried at 103 - 105 °C. Size distributions were determined with a Hiac Model PC-320 Particle Size Analyzer (Hiac/Royco Instruments,

Menlo Park, CA). Diluted samples were analyzed to prevent clogging of the analyzer (Herbst 1994).

Data Analysis

Differences in water replacement (cumulative system volumes exchanged) and feed input rates confounded direct comparisons of environmental characteristics between ozone treatments. This divergence was in response to differential growth rates in hybrid striped bass described in Chapter 3. To compensate for these differences, organic and ammonia parameter values were adjusted for the cumulative feed input the seven days prior to analysis as follows:

$$\text{parameter value}_{adj} = \text{parameter mass quantity (mg/l x system volume)} \div \text{total weight of feed delivered the previous 7 days (kg)}$$

$$= \text{g parameter / kg feed}$$

Statistical analysis was conducted using general linear model procedures (Proc GLM, SAS Institute, Cary, NC) to describe trends in water quality. Differences in the slopes of lines were compared using full and reduced model prodecures (Ott 1988) with an alpha level of 0.1. Treatment means were compared using single degree of freedom orthogonal contrast and analysis of variance for two-way classification without replication (Stoodley et al. 1980). Pump failure not related to the injection of ozone resulted in the premature termination of treatments 3 and 45 g O_3 by day 117, therefore, separate analyses where conducted. Results from all treatments were analyzed from day 0 through day 112 and from treatment 0, 13, and 25 g O_3 from day 0 through 236.

Results

During the experimental period, monitored water quality parameters remained within known acceptable limits (Nicholson et al. 1990). However, all treatment environments experienced decline in water quality as the cumulative feed input increased to the completetion of the study. Table 2.1 presents mean water quality conditions at the start of the trial and at the final sampling period.

Between days 70 and 113 water clarity declined significantly ($p > 0.05$) from 65 cm in the unozonated system. On day 113, fish in the control system rejected feed delivered to the tank; simultaneous measurement of water clarity with secchi disk revealed a transparency of 5 cm. The reduced feeding led to a continuous addition of fresh water (9 lpm for 18 hours) to the system (Fish husbandry, Chapter 3). The following day (114), fish fed slugglishly, consuming less than one-quarter of the day's first feed allotment. Therefore, a continuous water exchange (9 lpm for 8 hrs / day) was begun to improve water quality conditions. Water exchange each day was not begun until it was apparent that fish would not consume the day's first feed. This remedy was employed for 6 consecutive days resulted in a 187 % water replacement by day 119 when feeding behavior and feed consumption returned to normal. In addition to the resumption of feeding, water clarity improved to nearly 45 cm, with water coloration returning to a brown or amber (tea colored). Experience suggested that the reestablishment of this water color signaled a return to a relatively healthy environment.

Concurrently, systems receiving ozonation remained tea colored with average visibility of 40 cm for treatments 3, 13, and 25 g O_3. The highest sustained visibility of 80 cm was observed

under the ozone treatment of 45 g O_3 / kg feed. Feed consumption in ozonated environments slowed, however, daily feed input remained above 1.75 % of the biomass present (average fish weight 280g) (Figure 2.3). Water transparencies of 15, 46, 55 cm for treatments 0, 13, 25 g O_3 / kg feed, respectively, were measured at the conclusion of the study.

Nitrification. Ambient levels of nitrogenous based metabolites, pH, and alkalinity levels changed significantly over time ($p > 0.01$). Regression analysis estimated a positive linear relationship for TAN with time and daily feed input time, and a negative relationship between pH and cumulative feed (Table 2.2). A combined ammonia accumulation rate of 0.03 mg/l/day was observed at treatment rates of 0, 13, and 25 g O_3 / kg feed even though slightly higher ambient concentrations 1.12 ± 0.5 , 1.19 ± 0.4 mg/l were observed in the ozonated environments at the end of the trial (Figure 2.3a). This was attributed to differences in the cumulative feed input between treatments reflecting differential growth rates of hybrid striped bass (Chapter 3).

Mean monthly TAN measurements increased linearly with daily feed at approximately the same rates in indicated in Figure 2.4a. TAN concentrations were generally lower in ozonated environments than in the unozonated system receiving similar cumulative feed inputs and similar cumulative water exchange rates through day 113. (Figure 2.4b). Adjusted TAN measurements plotted against cumulative feed input revealed that ozone stabilized ammonia removal through biofiltration (Figure 2.5).

Dissolved Organics. Both readily biodegradable and refractory organics increased significantly ($p < 0.01$) in all treatments by the end of the study (Figure 2.6). Regresssion anylsis revealed that organic concentrations possessed significant curvilinear responses overtime which

was influenced by cumulative amount of feed delivered 7 days prior to sampling and amount of fresh water replaced ($p < 0.01$). No significant treatment effects ($p > 0.05$) were observed in $CBOD_5$ and DOC values. The influence of feed of daily feed input and water exchanges were most pronounced in DOC measurements, particularly in the unozonated system. Treatments 0 and 3 g O_3 / kg feed showed an average linear increase of 0.179 mg/L/day in DOC values that was 18.9 % faster than the higher ozone treatments and 31% faster than the increase observed at the highest ozone treatment (45 g O_3 / kg feed / day). DOC and COD levels in the unozonated system decreased immediately following continuous fresh water placement; however, the lowest measurements were not observed until day 162 (Figures 2.6b and c).

Adjusted $CBOD_5$ values from the ozonated systems were largely consistent, while values observed in the control system increased through day 162 before dropping sharply through completion of the trial. In contrast, adjusted DOC levels in the control system reached a maximum value of 16.5 g DOC / kg feed by day 120 before returning to values similar to those obtained from the ozonated environments. Unlike the relatively consistant series of normalized $CBOD_5$ levels, at the end of the study adjusted DOC values had increased 3-fold over initial values (Figure 2.7).

Biodegradation rates of dissolved carbons were positively correlated to the level of ozone treatment. On day 113, microbial reduction rates were -0.52 and -0.48 mg/l/day for treatments 13 and 25 g O_3 / kg feed and -0.37 and -0.38 mg/l/day for treatments 0 and 3 g O_3 / kg feed. Batch tests shown in Figure 2.8, revealed that microbial degradation activity remained higher in the ozonated systems ($0.05 < p < 0.1$).

Suspended solids characterization . TSS increased significantly ($p < 0.01$) throughout the course of the trial, in both the unozonated and ozonated systems. The initial mean concentration was 3 mg/l for all treatments. At the end of the study suspended solids levels were 15.8, 15.3, and 20.8 mg/l at treatments levels 0, 13, and 25 O_3 / kg feed (Figure 2.9a). TSS in all treatments followed a similar pattern, although levels in the control system were more than 48 mg/l higher by day 175, before dropping sharply to levels near those observed in the ozonated environments. Adjusted TSS from ozonated systems were relatively constant throughout the study. Values obtained from the control system approximated those from systems receiving ozone treatment except between days 140 and 200. Adjusted TSS values spiked higher than 173 g / kg feed and then returned to range consistant with the ozonated systems (Figure 2.9b).

No differences in molecular weight and particle size distributions similar in all environments Molecular weights of particles ranged between 4,000 and 10,000 g/mol. Approximately 90% of the particles in solution were smaller than 20 microns (Figure 2.10). The cumulative distribution curve for the treatment 25 g O_3 / kg feed was shifted to the right indicating a lower percentage of particles smaller than 40 μm.

Discussion.

Improvements in water quality in the ozonated as compared to control systems were easily observed through day 106 when feed input and water exchange rates were similar between treatments. However, direct comparison of ambient conditions across treatments beyond day 106 suggested that ozonation increased the rate of water quality degradation (referring to Figure 2.4). This apparent negative impact reflected higher daily feed inputs resulting in higher cumulative feed inputs and less water replacement with ozone treated.

Adjusted TAN levels and the rate of decline in pH suggested that the nitrification process in the ozonated systems had been augmented, which yielded an improved ammonia removal efficiency. This probably resulted from the reduction of organic carbon compounds through ozonation and this restricted the expansion of heterotrophic bacteria colonies (Rice et al. 1986). Heterotrophs (nurished by carbon compounds) grow faster than nitrifiers allowing their numbers to increase relatively unchallenged, thereby relegating the more desirable nitrifier colonies to the inner layer of the biofilm (Okabe et al. 1995). The reduced heterotrophic nutrient base created through ozonation may have limited biofilm thickness which enabled autotrophic populations to expand or more easily migrate to the outer layers of the biofilm. This would explaining the increased rates the improved nitrification process observed in this study and by others (Rosenthal and Otte 1979), rather than the direct oxidation of ammonia to nitrate as documented in Honn and Chavin (1976).

Honn and Chavin (1976), working in an marine environment ($8.5 < pH < 10$), reported that ammonia concentrations in biofiltered effluent increased 200 % within 24 hours of the

termination of ozonation. It was further observed that the resumption of ozonation significantly reduced total and un-ionized ammonia levels to pre-termination ranges within 6 hours. Colberg and Lingg (1978) found the oxidation rate of ammonia by ozone was pH dependent, increasing as waters became more alkaline (pH > 8.5), commonly measured in marine systems. This could possibly explain the almost immediate reduction in ammonia levels observed by Honn and Chavin (1976). However, during this study, an increasingly acidic environment indicated that the rate of direct ammonia oxidation by ozone occurs too slowly to significantly reduce TAN levels, thereby lending support to the conclusion that an enhanced nitrifier community resulted from ozonation.

Rosenthal and Otte (1979) measured a net increase in the biological oxygen demand (BOD) loading in water exiting their ozone contactor. They concluded that less readily assimbliable organic compounds were oxidized by ozone into more biologically available organics, thereby elevating the BOD in the system. Batch tests conducted during this study partially confirmed Rosenthal and Otte's (1979) conclusions. Biological oxidation rates of DOC in the ozonated systems were nearly double the rates measured from the unozonated system. However, there was no measurable increase in $CBOD_5$ from the ozone contact chamber effluent. Rather, adjusted dissolved organic values, suggested that the accumulation of $CBOD_5$ and DOC were reduced through ozone injection as a result of increased biodegradability. Dissimilar ozonation regimes (i.e. length of contact, contactor design, and injection concentration) may account for differences observed in the effect on $CBOD_5$ measurements reported presently and those documented by Rosenthal and Otte (1979).

Soluble organic concentrations that limit fish growth have not been established, yet in the

unozonated system feed consumption (i.e. feed input) slowed and eventually stop as adjusted $CBOD_5$ and DOC levels surpassed 10 and 15 mg/l, respectively. Other researchers have implicated increased refractory organic levels as growth inhibiting substances to aquatic animals (Honn and Chavin 1976; Hirayama et al. 1988; Morrison and Piper 1988; Poston and Williams 1990; Arbiv and van Rajin 1995). Their research concluded that ozone treatment reduces the accumulation of recalcitrant organics, facilitates increased removal efficiencies of both suspended solids and ammonia, in addition to reducing pathogen loads in recirculated waters. These factors permitted the elevation of stocking densities and promoted faster growth and increased survival of culture organisms compared to animals reared in unozonated recirculated waters.

Easter (1992) and Chen et al. (1993a) suggested that suspended particulates concentrate in recirculating systems not employing micro screen filtration. Coche (1981) recommended that suspended particle concentrations not exceed 15 mg/l. This maximum safe level was surpassed in the unozonated system by nearly 3-fold, while the maximum TSS level observed in the ozonated treatments reached 21 mg/l. Easter (1992) reported that the suspended solids profile was a function the type of removal device employed and that multi-tube clarifiers tended to promote the accumulation of particles smaller than 30 microns. Particle size analysis conducted during this study revealed that greater than 90 % of the particles remaining in solution were smaller than 30 microns in all treatments, in agreement with Chen et al. (1993a). However, in the 25 g O_3, treatment the percentage of particles below 40 μm was reduced by nearly 10 %. Rice et al. (1986) reported similar findings and conclued that ozonation promoted micro-flocculation, thereby improving solids removal. Ozonation causes a partial charge reversal allowing particles to

attract, creating larger, more easily settleable solids (Rueter and Johnson 1995).

The results of this study clearly demonstrated the utility of waste production-based ozonation treatment regimes. The ozonated water quality remained within acceptable limits and allowed for sustained feeding rates not observed in the unozonated environment. Even more importantly, an approximate doubling of the ozone treatment dosage from 13 to 25 g O_3 / kg feed produced only minor differences in environmental qualilty. Capital cost of ozonation equipment has thus far restricted widespread use of ozonation. Results of this study demonstrated that it was not necessary to produce a sterile environment to maintain water quality that would allow sustained feeding. Finally, daily feed inputs did not differ significantly between treatment 13 and 25 g O_3 ($p > 0.05$), therefore, not justifying cost associated with doubling ozone production capacity for increased treatment.

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Table 2.1. Mean ± standard error values presented for chemical analysis of ozonated and unozonated recirculating aquaculture systems rearing hybrid striped bass. Overall mean values for treatments 3 and 45 g O_3 were calculated through day 112. Overall means for a given water quality parameter with the same letter are not significantly different.

Day of Study	Treatment g O_3	Temperature °C	D O mg / L	pH	TAN mg / L	NH^{-}_{3}- N mg / L	NO^{-}_{2}- N mg / L	NO^{-}_{3}- N mg / L	Alkalinity mg / L	Hardness mg / L
0	0 g	22.8	12.8	8.2	0.2	0.02	0.3	5.0	174.0	423.0
	3 g	23.2	11.9	8.1	0.2	0.01	0.1	4.7	128.0	414.7
	13 g	23.7	12.6	8.3	0.2	0.02	0.1	4.8	192.0	378.0
	25 g	23.4	12.4	8.3	0.3	0.02	0.2	5.3	182.3	399.0
	45 g	23.4	12.8	8.0	0.5	0.03	0.5	7.5	147.0	487.0
236	0 g	24.0 ± 0.1	14.5 ± 0.5	7.3 ± 0.03	1.3 ± 0.04	0.02 ± 0.01	1.1 ± 0.1	107.0 ± 6.5	117.5 ± 16.4	385 ± 12.2
	13 g	24.0 ± 0.1	13.8 ± 0.6	7.1 ± 0.1	1.4 ± 0.1	0.01 ± 0.001	0.6 ± 0.1	166.3 ± 10.8	95.4 ± 8.2	349 ± 6.6
	25 g	24.2 ± 0.1	13.5 ± 0.4	7.0 ± 0.03	1.6 ± 0.01	0.01 ± 0.001	1.0 ± 0.1	154.1 ± 7.7	90.8 ± 10.9	349 ± 12.2
Over all mean	0 g	25.6 ± 0.1a	13.0 ± 0.2a	7.6 ± 0.02a	0.9 ± 0.03a	0.04 ± 0.002a	0.4 ± 0.05a	86.1 ± 8.5a	156 ± 5.2a	435 ± 14.6a
	3 g	26.7 ± 0.2a	12.9 ± 0.2a	7.6 ± 0.04a	0.8 ± 0.04a	0.03 ± 0.003a	0.3 ± 0.05a	61.2 ± 10.2a	113.6.6a	447 ± 23.4a
	13 g	25.6 ± 0.1a	12.4 ± 0.2a	7.5 ± 0.04a	1.1 ± 0.04a	0.02 ± 0.002a	0.9 ± 0.1a	95.1 ± 7.9a	133 ± 4.7b	435 ± 14.1a
	25 g	25.3 ± 0.2a	12.9 ± 0.2a	7.5 ± 0.04a	1.2 ± 0.05a	0.02 ± 0.002a	0.6 ± 0.08a	101.4 ± 8.5a	128 ± 4.8b	411.± 13.9a
	45 g	26.5 ± 0.2a	12.3 ± 0.3a	7.6 ± 0.04	0.8 ± 0.04a	0.02 ± 0.002a	0.6 ± 0.3a	81.0 ± 15.5a	135 ± 6.8	446 ± 32.6a
Linear change p-value		> 0.05	> 0.05	< 0.01	<0.01	>0.05	<0.01	<0.01	<0.01	>0.05

Table 2.2. Linear regression parameter estimates (A) determined from ozonated and unozonated recirculating environments rearing hybrid striped bass. Time (day of study) and daily feed inputs were used to predict ambient TAN concentrations and pH levels. Slopes for all treatments were significantly different from zero ($p < 0.01$)

	TAN[B]						pH		
	Time			kg feed / day			Time		
Treatment	b_o	b_1	r^2	b_o	b_1	r^2	b_o	$b_1 \times 10^{-3}$	r^2
0 g	0.55	0.02	0.58	0.36	0.17	0.38	7.7	- 1.1	0.23
13 g	0.38	0.03	0.69	-0.04	0.23	0.50	7.8	- 2.8	0.57
25 g	0.54	0.03	0.65	0.42	0.17	0.27	7.8	- 4.1	0.81
Combined[C]	0.46	0.03	0.64	0.46	0.03	0.63	7.8	- 2.7	0.47

A. Regression parameter values: b_o = intercept, b_1 = slope, r^2 = coefficient of determination.

B. TAN = total ammonia nitrogen.

C. All treatment measurements used to determine regression values.

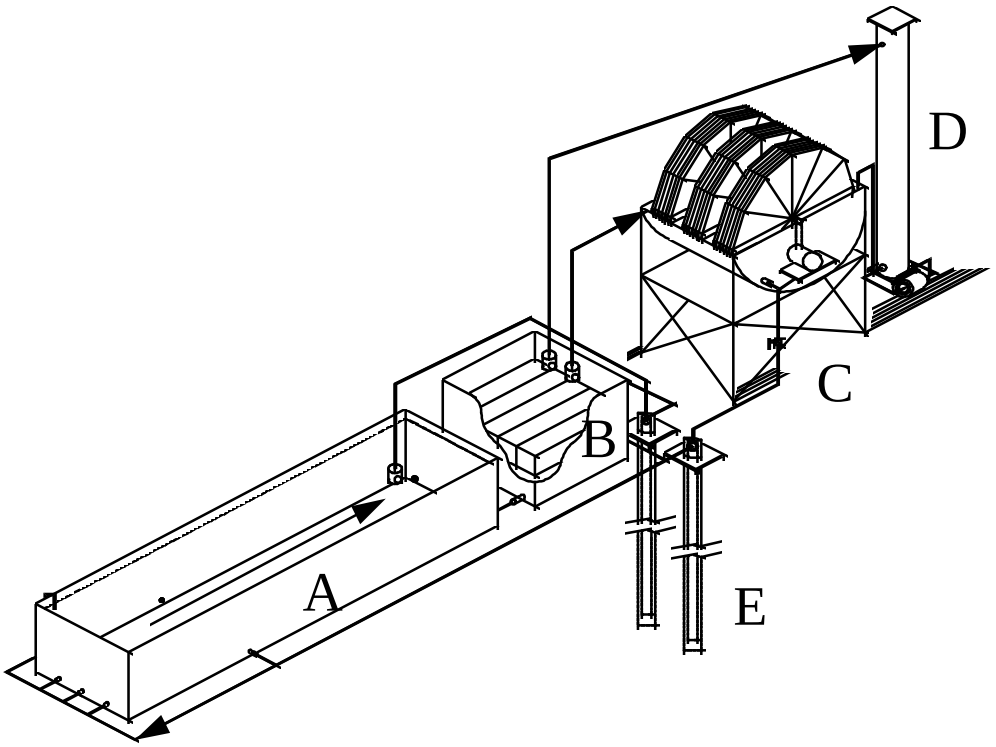

Figure 2.1. Pilot-scale recirculating aquaculture system configuration used to rear hybrid striped bass under ozonated and unozonated conditions. Water flowed from the rearing tank (A) to an upflow multi-tube clarifier (B) for solids removal. Clarified water then was pumped through either through the ozone contactor (D) to the first stage of the RBC or directly to the first stage of the RBC (C). Water exited the RBC vessel down the primary U-tube device (E) where pure gaseous oxygen was injected. Oxygenated water then traveled the length of the rearing tank to re-enter through five ports positioned at the bottom of the from wall of the tank. Undissolved oxygen from primary aeration was re-injected down the secondary U-tube via water pumped directly from the rearing tank.

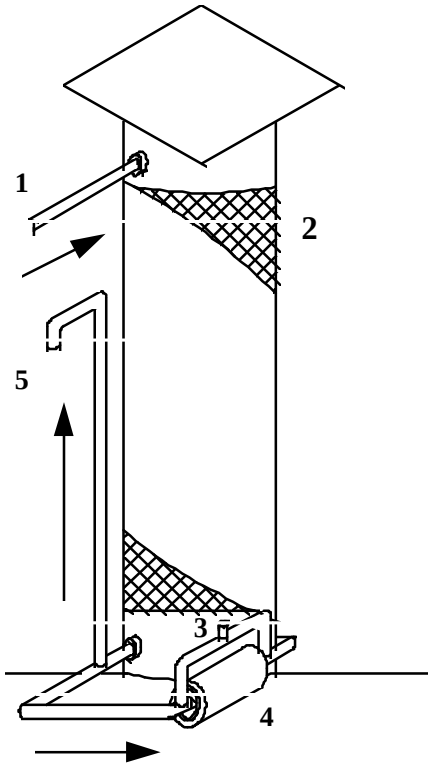

Figure 2.2. Ozone contactor used on a pilot scale recirculating aquaculture systems. Water entered through a port (1) positioned at the top of the contactor and flowed downward across packing material (2). Ozone / oxygen gas was aspirated into the contactor through a venturi injector (3) operated with a recirculation pump (4). Treatment was affected in countercurrent fashion, with water exiting (5) to biofilter.

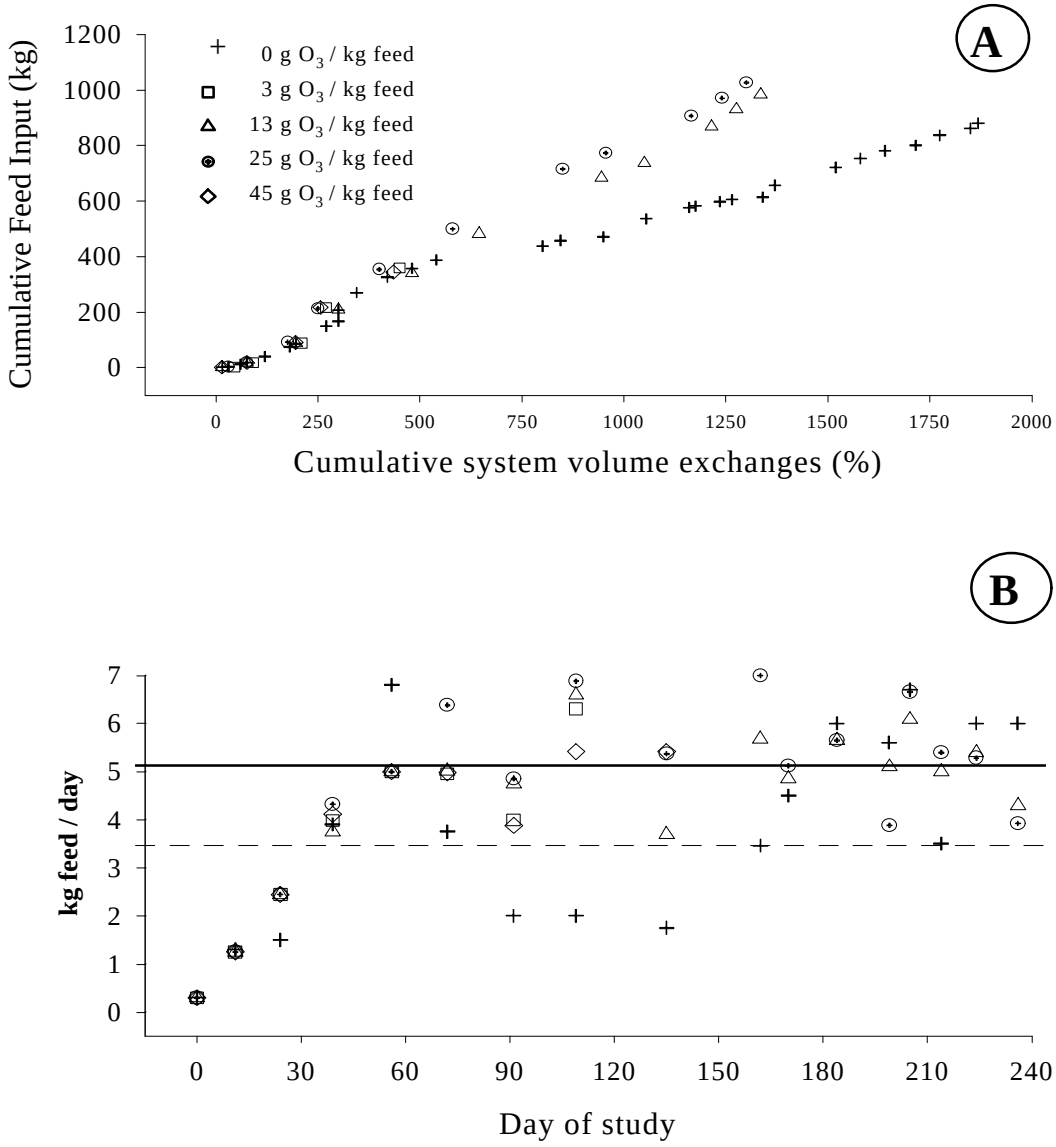

Figure 2.3. (A) Time profiles of cumulative feed inputs and fresh water replacements and (B) daily feed inputs (calculated at 7 day intervals) observed during the rearing of hybrid striped bass in pilot scale recirculating aquaculture systems receiving ozone injection. Solid and dotted horizontal lines mark the average daily feed delivered to the ozonated and unozonated systems, respectively.

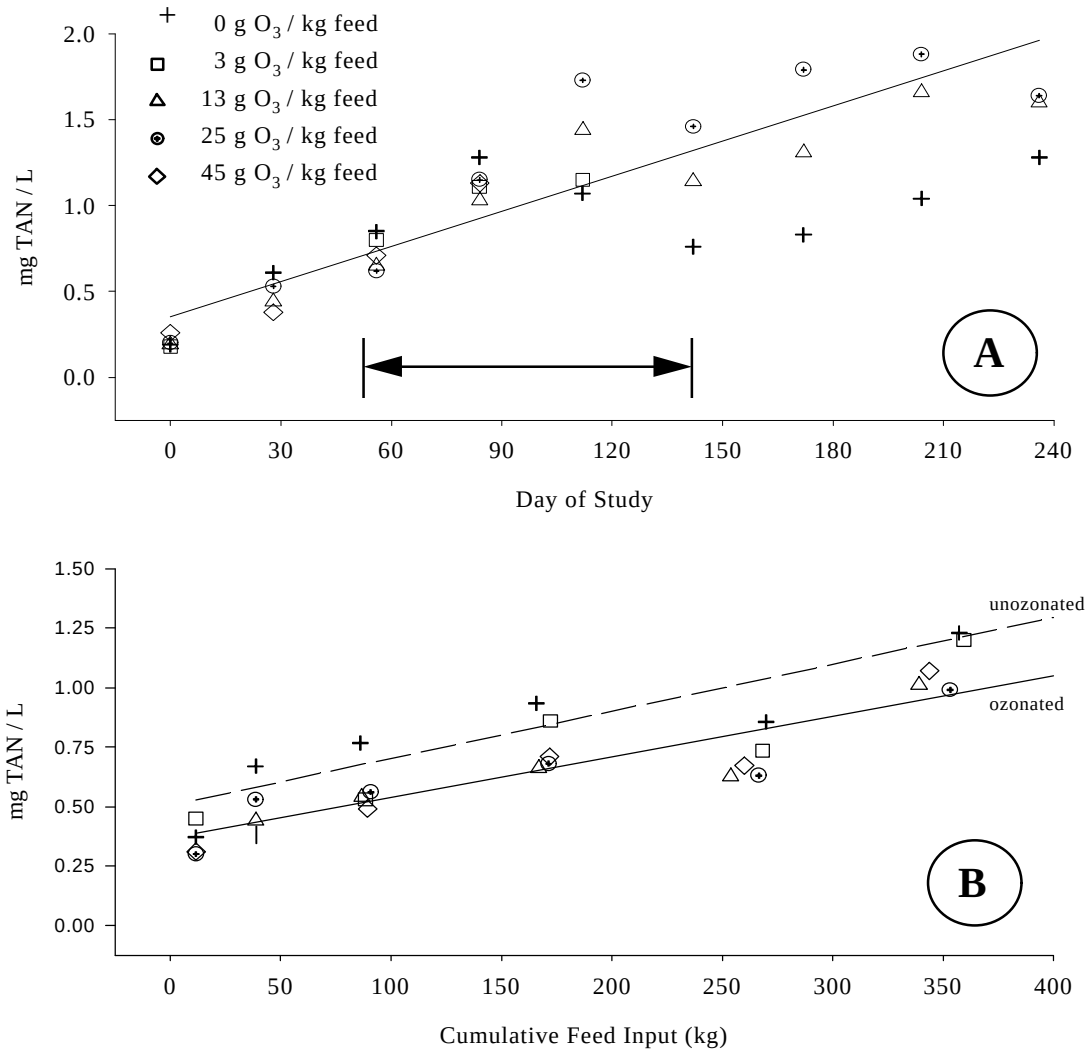

Figure 2.4. (A) Time profile of ambient environmental conditions in pilot scale recirculating aquaculture systems rearing market size hybrid striped bass receiving ozone treatment. Area denoted with arrows represents the period when feed consumption slowed and semi-continuous water exchanges occurred in the unozonated system. Plot (B) presents ambient TAN levels as a function of cumulative feed input between days 0 and 113 when water cumulative exchange rates were approximately equal.

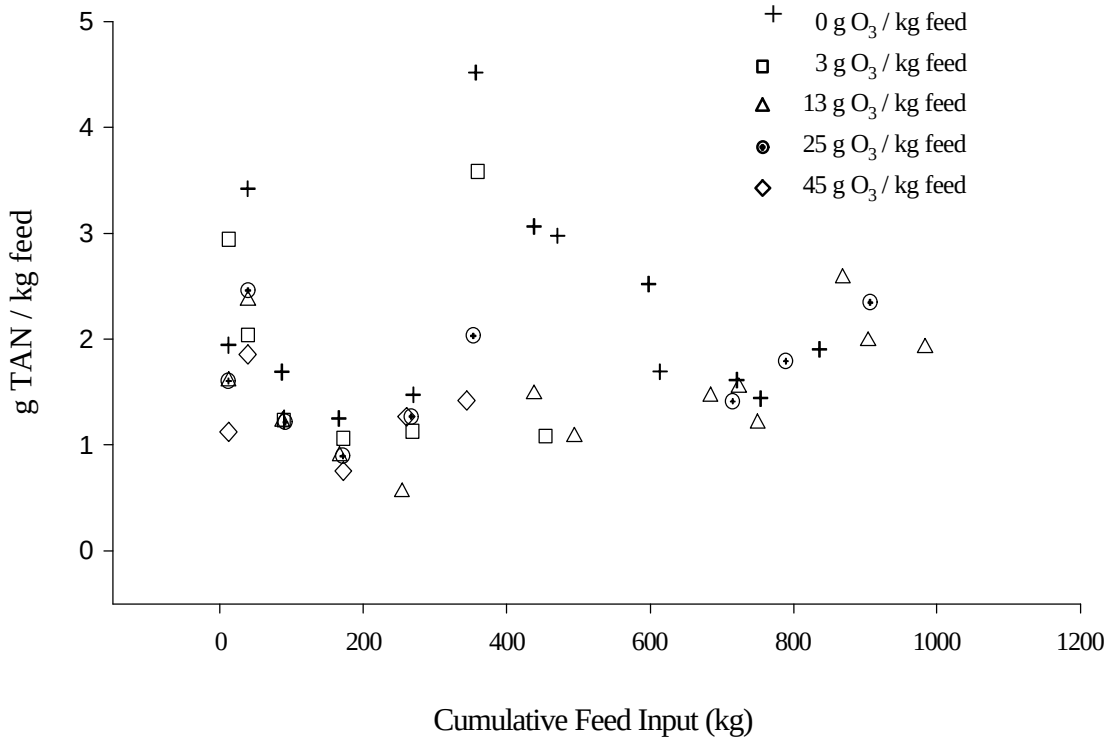

Figure 2.5. Ambient TAN levels adjusted against weekly cumulative feed input. Hybrid striped bass under ozonated conditions in pilot scale recirculating aquaculture systems grew at faster rates than fish reared under unozonated conditions which resulted in a higher daily feed input. Adjusted TAN levels were plotted against cumulative feed inputs to account for differences in daily feeding rates over time.

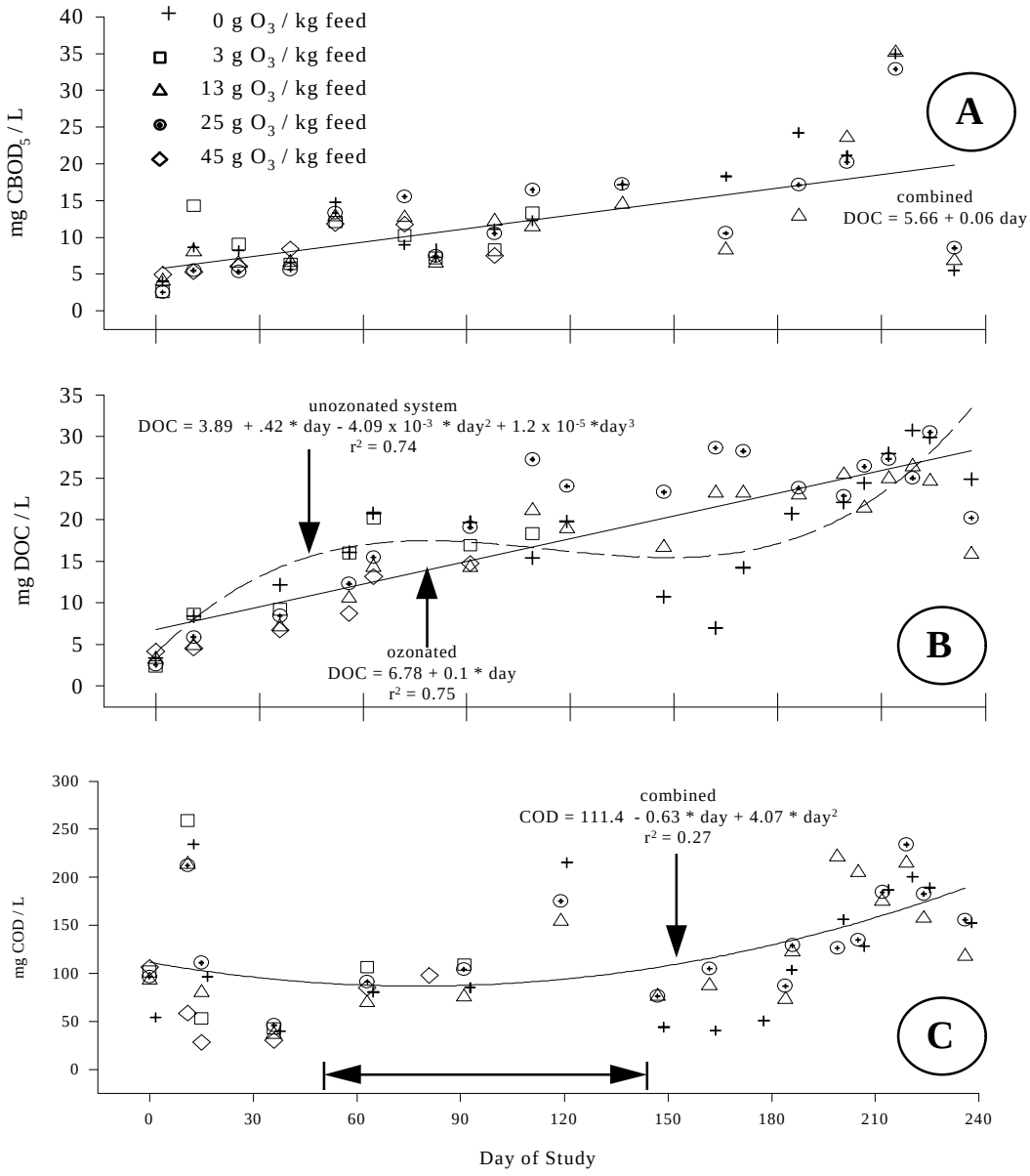

Figure 2.6. Time profiles of organic compound concentrations measured as (A) readily biologically available and recalcitrant (plots B and C) compounds observed in ozonated and unozonated pilot-recirculating aquaculture systems rearing hybrid striped bass. Area denoted by arrows indicates the period of reduced feed input and increased water exchange in the unozonated treatment (0 g O_3 / kg feed).

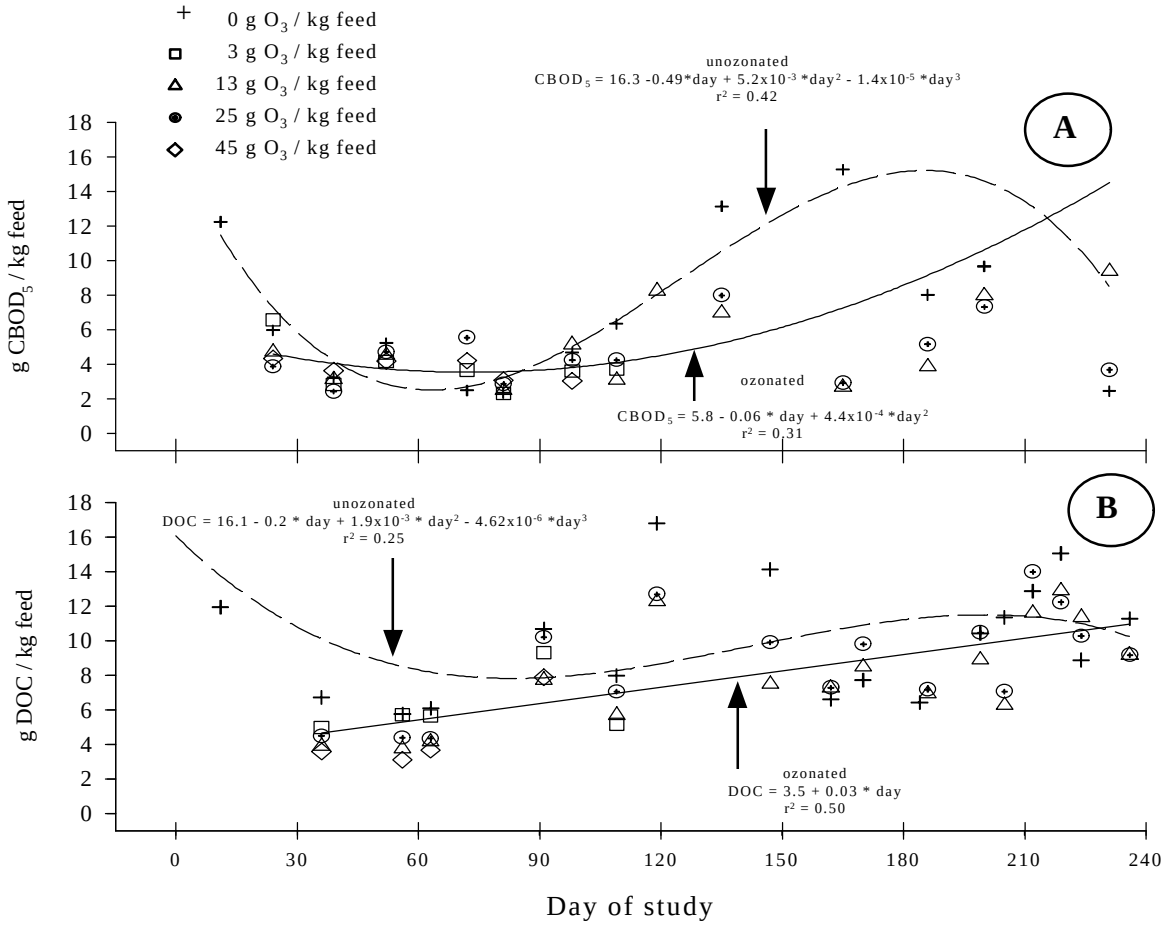

Figure 2.7. Adjusted (A) $CBOD_5$ and (B) DOC levels observed in ozonated and unozonated pilot scale recirculating aquaculture systems rearing hybrid striped bass. Regression estimates provided the best fit based on r^2 value. Ozonation provided relatively more consistant environmental conditions, as suggested by the lower degree polynomial regression equations.

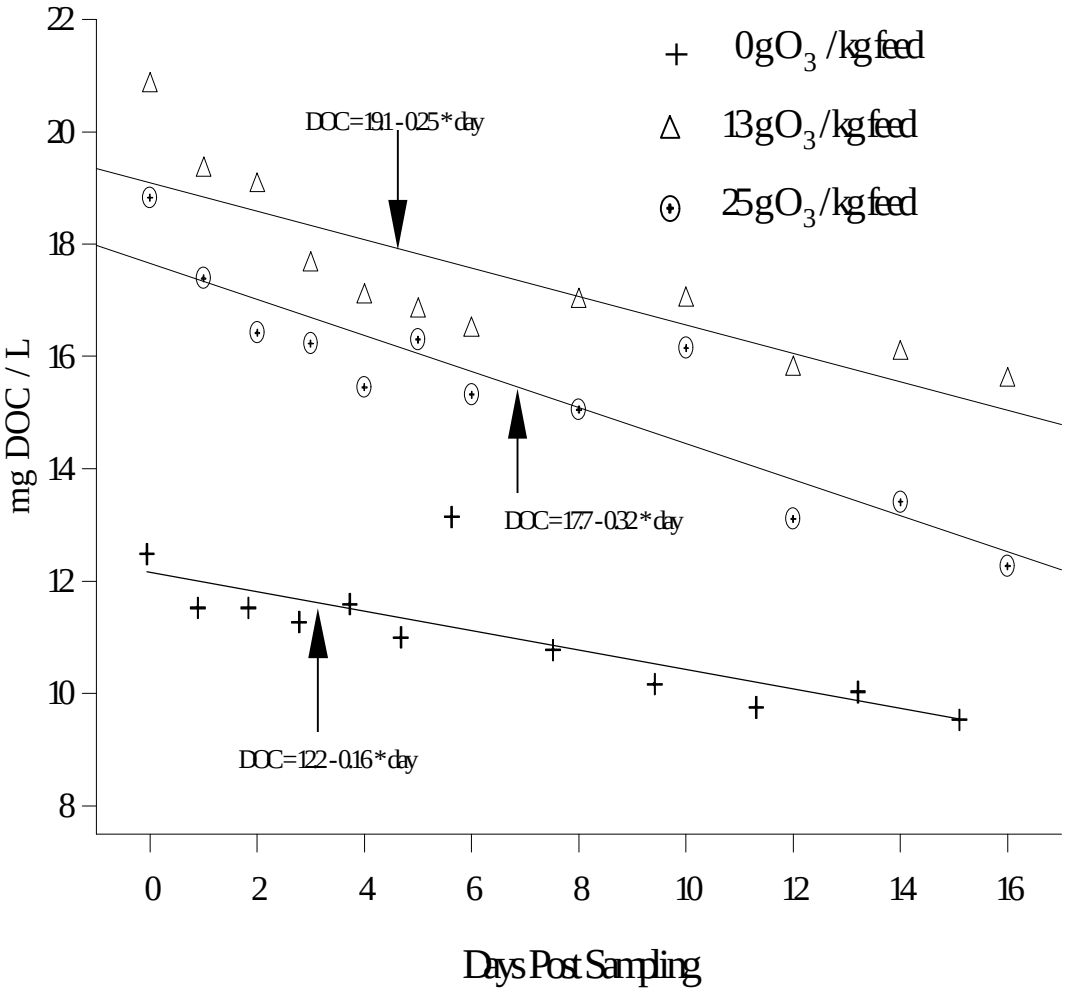

Figure 2.8. Batch tests indicated higher biological degradation rates of dissolved organic carbon (DOC) compounds in ozonated pilot scale recirculating aquaculture systems than in the unozonated system.

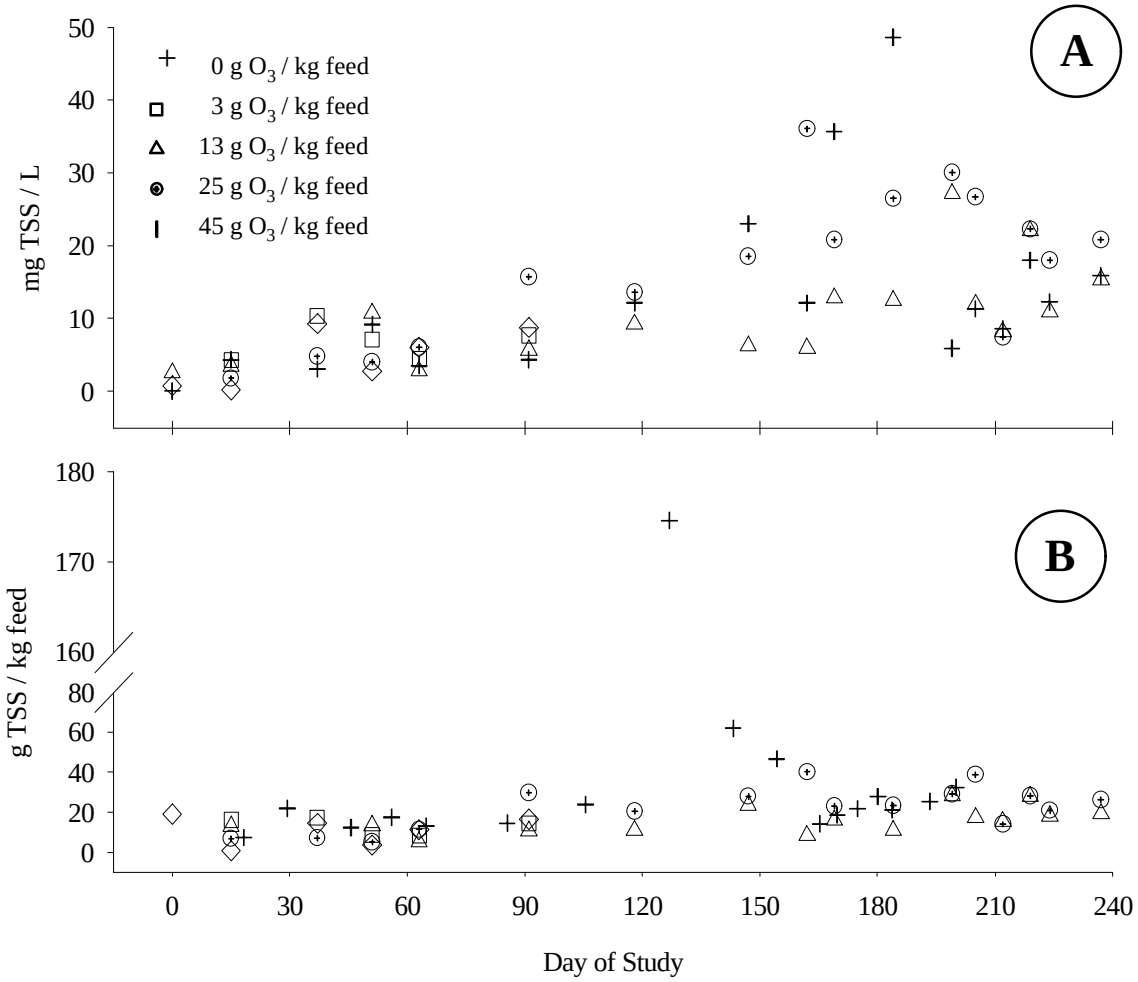

Figure 2.9. Suspended organic solids quantified as (A) total suspended solids were (B) normalized for weekly cumulative feed input and profiled during the production of hybrid striped bass in pilot scale recirculating aquaculture systems.

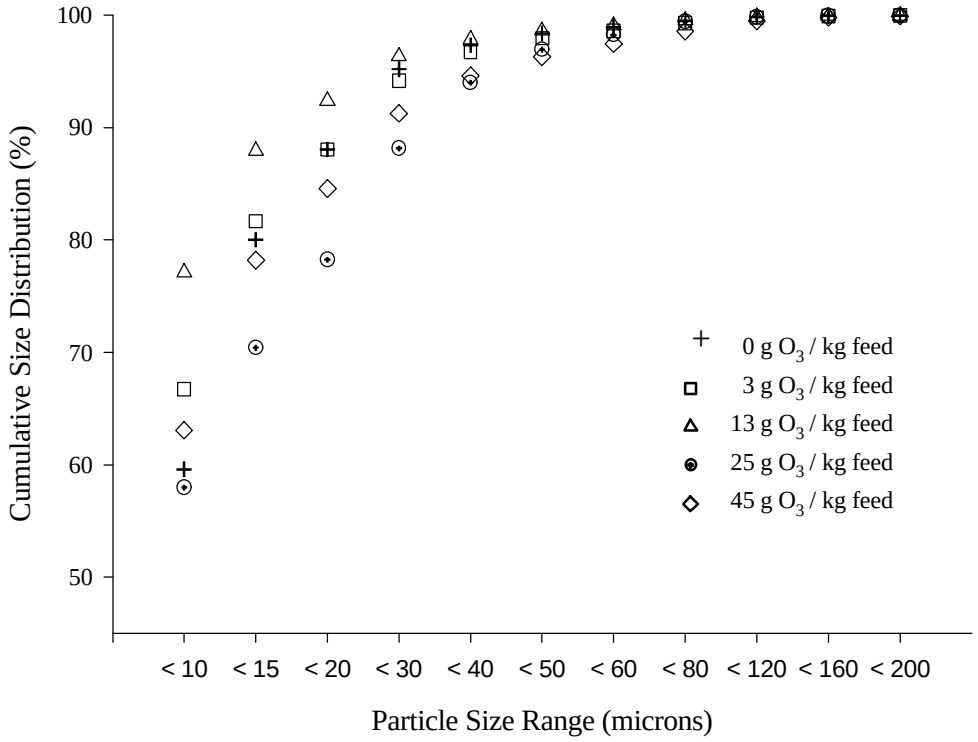

Figure 2.10. The range of the suspended particles shown as a cumulative distribution function under ozonated and unozonated conditions in pilot scale recirculating aquaculture systems rearing hybrid striped bass.

Appendix 2.A.1

Presented below is sample exercise of how ozone treatments would be calculated and then applied to systems as described in the methods (ozone injection regime). Values presented in the exercise below are simplied to ease understanding of calculations. Design parameters for one day's treatment:

a). ozone treatment ratios: 0, 5, 10, 15

b). 1 kg of feed delivered twice daily to each system = total feed input 4 kg

c). maximum ozone generation capacity = 720 g / day (30 g/ hr) @ 864 lph O_2

Step 1.

Equation 1. $O_{3sysi} = feed_{sysi} \times O_{3trti}$

$O_{3sys0} = 4$ kg x 0 g of O_3 / kg feed / day = 0 g / day

$O_{3sys5} = 4$ kg x 5 g of O_3 / kg feed / day = 20 g / day

$O_{3sys10} = 4$ kg x 10 g of O_3 / kg feed /day = 40 g / day

$O_{3sys15} = 4$ kg x 15 g of O_3 / kg feed /day = 60 g / day

Step 2.

Equation 2. $Gt_{abs} = O_{3dmd} \div O_{3gas}$

$= O_{3dmd} \div (O_{3prod} \div n)$,

Dividing $O_{3\ prod}$ by the number of systems receiving ozone (n) accounts for the proportional decrease in O_3/O_2 gas flow injected into each system (providing each recieves an equal gas flow rates). A reduction in the gas flow reduces the amount of ozone injected per unit of time.

$= 60$ g $\div$ (30 g / hr $\div$ 3)

$= 60$ g $\div$ (10 g / hr)

$= 6$ hr @ 4.8 L / min

Appendix 2.A.1 continued.

Step 3.

Equation 3. $Ti_{sysi} = O_{3sys15} \div O_{3gas}$

$Ti_5 = 20 \text{ g / day} \div 10 \text{ g / hr} = 120 \text{ min}$

$Ti_{10} = 40 \text{ g / day} \div 10 \text{ g / hr} = 240 \text{ min}$

$Ti_{15} = 60 \text{ g / day} \div 10 \text{ g / hr} = 360 \text{ min}$

Step 4.

Ti_{sysi} is then divided by 4 and administered during each of the injection periods as shown in the following figure. Once the Ti_{ssys} for a particular system was met, the ozone is shunted away and replaced with oxygen until Ti_{sys} has been met for the remaining systems.

Appendix 2.A.1. continued.

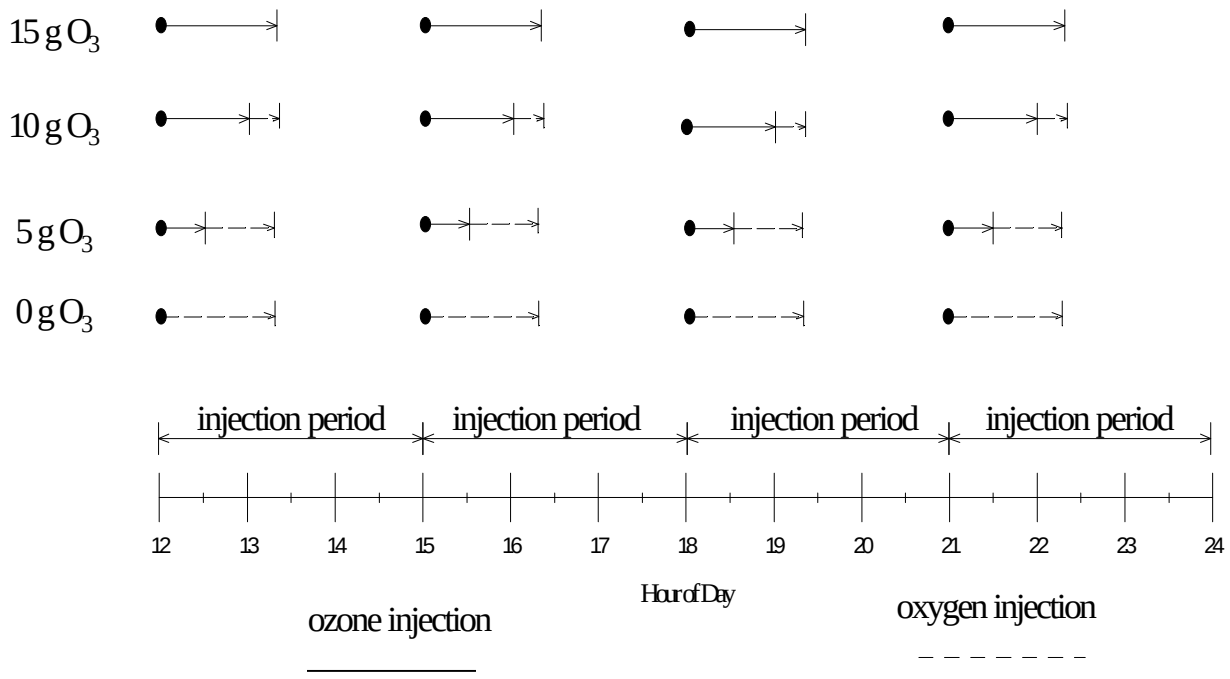

Figure 2.A.1. Presented is the ozone injection regime for the example and described in the in methods (ozone injection). The four injection periods denote the maximum time allowed, however in this problem the entire time was not required. Ozone would be injected at a rate of 10 g/hr at 4.8 lpm.

CHAPTER 3

Growth and Physiological Profiles of Hybrid Striped Bass (Morone chrysops (f) x M. saxatilis (m)) Reared in Pilot Scale Ozonated Recirculating Aquaculture Environments.

Abstract

Five ozone treatments (0, 3, 13, 25, and 45 g of O_3 per kg of feed per day) were evaluated to asses their impact on water quality and fish physiology and growth during the production of hybrid striped bass. Through day 112, mean fish growth in all treatments were not significantly different (averaging 2.1 g/day, $p > 0.05$). However, from day 84 to 142 feed consumption of fish reared at the treatment 0 g O_3 / kg feed / day slowed, resulting in a 59% reduction in growth (0.96 ± 0.1 g/day), while during that same period, fish reared in the ozonated environments sustained an average daily growth rate 2.3 ± 0.4 g. Overall growth of fish in the high ozonated environments (13 and 25 g O_3 / kg / feed) was statistically greater (averaging 2.36 g / day, $p < 0.01$) than fish growth in the unozonated environment. Final feed conversion ratios of 1.90, > 1.58 = 1.59: 1 were calculated for fish reared at treatments 0 , 13, and 25 g O_3 / kg feed / day, respectively. At harvest, fish raised at 0 g O_3 treatment weighed an average 443.5 g, whereas those in high ozone environments weighed significantly more, 576.6 g ($p < 0.01$).

The highest overall mean white blood cell counts, 124 ± 9.4 x 10^3 cells/μL, were observed in fish reared at the 0 g O_3 treatment ($p < 0.01$). No treatment differences were detected in hemoglobin content, plasma protein, and hematocrit values, exhibiting grand means of 10.2 ± 0.4 g dL^{-1}, 7.1 ± 0.6 g dL^{-1}, and 52.9 ± 1.9 %, respectively. Serum chloride and glucose values

(overall means of 115.8 ± mE/L and 186.3 mg/dL, respectively) exhibited no significant differences between fish reared in an ozonated environment and unozonated conditions. Microscopic examination of gill tissue revealed a statistically higher incidence of pathology ranging from telangiectasis to complete mucus erosion in fish reared in the unozonated environment. Generally, the evaluation of the physiological profile trends revealed a significant decrease in the health condition in fish from all treatments. However, sustained higher growth in fish at treatments 13 and 25 g O_3 indicated that relatively "healthier" (environmental conditions allowing for sustainted growth) rearing conditions were maintained through ozonation.

Introduction

Diminishing supplies of ground water and increasingly stringent effluent discharge standards have shifted the focus of aquaculture towards more efficient water usage methods. Brune and Gunther (1981) suggested that the techniques of water reclamation and conservation (e.g., biofiltration, solids removal, and disinfection) used in water and wastewater treatment be used in food fish production. Recirculating aquaculture systems employ biofiltration and suspended solids removal devices to maintain adequate environmental quality to sustain fish growth rates. However, recent research suggests that additional filtration may be required as production intensity increases and growth rates slow in response to declining water quality (Sutterlin et al.1984).

The continuous recirculation degrades water quality, thereby exposing the cultured fish to chronic stress that can disrupt physiological homeostasis, reduce growth, and increase the incidence of disease outbreaks (Wedemeyer and McLeay 1981; Robertson et al. 1987). Nunley (1992) observed slower growth of hybrid striped bass raised at higher densities, and Bosworth (1994) found that fish growth "plateaued" during the latter stages of the production cycle. Both researchers suggested that extended exposure to the harsh rearing conditions and ambient concentration of dissolved compounds may have inhibited growth.

The use of ozone treatment in recirculating aquaculture system water has increased the productivity in closed systems by helping to reduce levels of nitrite, biological oxygen demand (BOD), suspended solids, humic compounds (Rosenthal et al. 1979; Williams et al. 1982; Rosenthal 1981; Sutterlin et al. 1984; Rueter and Johnson 1995), and pathogen loads (Tipping

1988; Colt 1994). Their findings described the efficacy of ozone treatment, yet none have recommended treatment regimes which account for temporal changes in waste production (daily feeding schedule) and system loading (associated increasing fish weight and feeding).

Waste production and system loading are influenced by the frequency and amount of feed input and the water replacement rate (Tanaka and Kadowaki 1995; Hirayama et al. 1988). Changes in either factor can affect the rate of accumulation of oxidizable compounds, thereby minimizing the qualitative effect of ozonation on the environment. This becomes of particular importance when attempting to disinfect recirculating water, because refractory compounds exert an oxidation demand that consumes ozone before sufficient microbial reduction occurs (Vestergard 1994).

Effective pathogen reduction is accomplished by sustaining an ozone residual between 0.1 and 0.4 mg/l with a contact time of between 10 seconds to 5 minutes (Hass 1990). Humic substances and other dissolved organic compounds create an ozone demand significantly increasing dosage requirements (Bablon et al. 1992). Kinman (1972) demonstrated with secondary wastewater effluent that a 10 minute contact time at 50 mg/l injected ozone concentration was required to achieve a 2-$\log_{10}$ reduction in bacterial count. Comparisons between data collected by Easter et al. (1992) and water chemistry characteristics of secondary municipal discharge (Metcalf and Eddy 1991) indicate that water quality in recirculated waters in some instances resembled dilute waste water. This suggested that attempting to sustain ozone residuals in production systems could be economically prohibitive. Therefore, ozone injection may be more practically applied to attenuate the rate of environmental decline during a production

cycle by eliminating potentially harmful waste compounds before they accumulate in the system.

This study was conducted to profile the impact of water quality as affected by ozonation on fish physiological and growth profiles of hybrid striped bass. Constant ozone treatment ratios were maintained throughout the production cycle to assess temporal changes in water quality, fish physiology and growth characteristics.

Methods

Five pilot-scale recirculating aquaculture systems (RAS) housed at the Virginia Tech Aquaculture Center were used to rear hybrid striped bass (Morone chrysops (f) x M. saxatilis (m)) to market size (weight ≥ 567.5 g, Coale et al. 1993). Daily ozone treatment ratios of 0, 3, 13, 25, and 45 grams of ozone per kg of feed per day were examined for their impact on water quality (Chapter 2) and fish physiological and growth profiles. Fish were stocked and allowed to acclimate for two weeks prior to the start of the study.

System Design and Management. Each recirculating system (Figure 3.1) contained approximately 13,000 L. Water flowed from a 7,560 L rearing tank to a multi-tube clarifier (1893 L) containing corrugated polyvinyl chloride blocks (BIOdeck 12060, Munters Corp., Fort Meyers, FL) for solids removal. A 0.186 kW pump elevated clarified water 2.13 m to a three staged rotating biological contactor (RBC). A second 0.186 kW pump elevated clarified water 3.7 m to the ozone contactor chamber for treatment. Water exiting the 240 L ozone contact chamber entered the first stage of the 1,930 L RBC vessel, where it was mixed with water that had been pumped directly to the RBC from the multi-tube clarifier. RBC media was constructed of Norpac (NSW, Roanoke, VA) disks (30 cm X 1.83 m dia) rotated at three revolutions min^{-1} by a 0.186 kW Dayton electric gear motor. Water gravity flowed through the RBC vessel at approximately 227 lpm and down a 12.2 m deep U-tube aeration device receiving pure oxygen injection. Re-oxygenated water passed through a gas collector where undissolved gas was vented before reentering the rearing tank.

Isolation and wash down of the clarifier to remove settled solids was conducted after the

delivery of 3 kg of feed to the unozonated system and 6 kg to the ozonated systems. After wash down, the clarifier was refilled with municipal water containing less than 30 mg/l of both alkalinity and hardness. To sustain system wide alkalinity levels greater than 100 mg/l and hardness above 150 mg/l, 1.5 kg of sodium bicarbonate and 1 kg of calcium chloride were added, respectively, during each water exchange.

If water quality parameters exceeded known toxic limits (Table 3.1), a continuous input of fresh water (9 lpm) and feed delivery stopped until safe limits were reached. Additionally, daily observations of fish behavior for abnormal cessation of feed consumption (consumption of less than one-half desired feeding rate, lasting more than three consecutive days) combined with lethargy would initiate continuous fresh water replacement.

Fish husbandry. Hybrid striped bass, approximately 18 g, were arbitrarily stocked at 150 fish per m^3 and fed a floating pellet (Souther States Cooperative, Inc., Richmond, VA.) containing minimum of 36% crude protein and 7% fat levels. Daily feeding rates declined from 4.5 % to less than 1 % of the total body weight. The daily ration was divided into three or four equal allotments ($\leq$ 1.5 Kg) delivered approximately 3.5 hours apart. Feed remaining on the surface after five minutes was removed and the weight subtracted from the total administered.

Data Collection. Ambient water temperature, dissolved oxygen (DO), total-ammonia-nitrogen (TAN), and pH were measured daily before the first feeding. A portable DO meter (Yellow Springs Instrument, Yellow Springs, OH) was used to measure oxygen and temperature. Alkalinity, hardness, nitrite (NO^-_2 - N), and nitrate (NO^-_3-N) levels were measured twice weekly. TAN, nitrite, and nitrate concentrations were measured with a spectrophotometer (DR/2000,

Hach Company, Loveland, CO), pH with a pH pen (Hach Company). Alkalinity and hardness were measured following Standard Methods procedures 403 and 314, respectively (Standard Methods 1992).

Length (± 0.1 mm) and weight (± 0.1 g) measurements were taken at 28 to 30 day intervals to the nearest. A minimum of 5% of the total population was arbitrarily netted for sampling and anesthetized with 70 mg/l of tricaine methanesulfonate (MS-222, Sigma Chemical Co., Saint Louis, MO) dissolved in a 4000 mg/l saline bath.

Growth was characterized as follows:

mean weight = $(w_t - w_{t-1}) \div (t_1 - t_{t-1})$,

mean length = $(l_t - l_{t-1}) \div (t_1 - t_{t-1})$,

specific growth = $(w_t - w_{t-1}) \div (t_t - t_{t-1})$,

relative growth = $((w_t - w_{t-1}) \div w_t) \times 100$,

condition factor = $K = (w_t \times 100) \div l_t^3$, and

feed conversion ratio (FCR) = (weight of feed delivered from t_{t-1} to t_t) $\div$ $w_t - w_{t-1}$,

where l_t = total length at time t,

l_{t-1} = total length at time t-1,

w_t = weight at time t, and

w_{t-1} = weight at time t-1.

Physiological parameters were measured on a sub-sample of six fish netted for routine length and weight measurements. Blood and tissue samples from fish were collected immediately following the loss of equilibrium by the fish. Measurements included total length, weight, liver

somatic index, gill evaluation, serum chloride and glucose, hemoglobin, hematocrit, plasma protein, and blood cell counts. Blood samples were collected from the caudal blood vessels and transferred to serum separators and EDTA tubes for storage in an ice-water bath until sampling was completed. Clotted blood was centrifuged in serum separators for five minutes at 14,000 x g, after which the serum was removed and frozen at - 20 °C until analysis. Blood placed in EDTA tubes was used for blood chemistry profiles.

Gill lamellae were wet mounted on glass slides and examined for evidence of trauma which included epithelial destruction, telangiectasis, and clubbing. Gill filaments were collected from the second gill arch located on the left side of the head. A ranking system of 1 to 5 (1= mostly excess mucus production, 2 = approximately 10 - 20 % of gill lamella examined possessed telangiectasia, 3 = telangiectasia observed in 30 - 50 % of the lamella, 4 = three quarters of the gill tissue possess telangiectasia and discoloration, and 5 = the erosion of mucus from the lamella and clubbing) was assigned to gill tissue samples to quantify any damage sustained.

Hematocrit values were measured with non-heparinized micro-hematocrit tubes filled 2/3 full of blood and sealed (Hemato-Seal, Fisher Scientific, Pittsburgh, PA). Micro-hematocrit tubes were then centrifuged for 5 minutes at 14,000 x g and the packed cell volume read with a micro hematocrit reader. Separated plasma was then placed into a refractometer (Reichert-Jung, Leica, Buffalo, NY) measuring the protein level. Colorimetric diagnostic procedures 461, 510, and 525 (Sigma Diagnostics, Sigma Chemical, St. Louis, MO) were used to analyze serum chloride, glucose levels and total hemoglobin concentrations, respectively.

Serum and whole blood samples were thawed at room temperature in a water bath before

analysis. Day 0 serum analysis was conducted on pooled serum samples collected from three batches of ten fish. This was necessary to acquire at least 1 ml of serum required for analysis. Blood cells were counted as erythrocytes, leukocytes, and thrombocytes with leukocytes further differentiated as small and large lymphocytes, neutrophils, and monocytes. Cell counts were made on days 0, 84, and 236. Blood stored in EDTA-microtainer tubes was used to obtain total red and white blood cell counts. Direct smears for differential white blood cell counts were made immediately following blood collection. Cell counts were obtained manually with a hemocytometer using Natt-Herrick's solution for dilution (Natt and Herrick 1952).

Data analysis. Treatment and ozone effects were evaluated in a unreplicated randomized block design. Comparisons of temporal changes between treatments were accomplished by examining the slopes of linear regression lines (Ott 1988) using proc GLM (SAS Institute, Cary, North Carolina). Pooled treatment means were used to compare ozonated environments against the control in single degree of freedom orthogonal contrasts. Analyses examining all treatments were conducted over the first 112 days, and comparisons from day 0 to 236 were made between treatments 0, 13, and 25 g O_3.

Results

Experiments in recirculating systems receiving ozone treatments of 3 and 45 g O_3 per kg feed per day ended on days 117 and 106, respectively, due to mechanical failure unrelated to the injection of ozone. Both populations experienced mortality rates greater than 94%. After 236 days, fish reared in the remaining ozone treatments were market size (mean weight $\leq$ 567.5) and the study ended.

Water quality. Water quality parameters displayed significant changes over time ($p < 0.01$) Table 3.3 (also refer to Chapter 2). Monthly TAN and pH plots illustrate the general linear decline in water quality (Figures 3.2a and b). The cumulative feed inputs and system volume exchanges differed significantly among treatments ($p = 0.02$).

Feed consumption. Through day 106, feed inputs at all treatments were approximately equal, averaging 4.2 kg/day. This resulted in a cumulative feed delivery of 432.5 kg (Figures 3.3 a and b). During the next 70 days, the average daily feed delivered to the treatment of 0 g of O_3 / kg feed declined to 2.9 kg as fish feeding slowed. Concurrently, fish reared at treatments of 13 and 25 g O_3 / kg feed sustained an average daily feed consumption of 5.5 kg, significantly higher than that consumed by fish in the control ($p > 0.03$). On day 113, feed consumption of fish reared in the unozonated (0 g of O_3) system stopped and appeared lethargic, even though measured water quality parameters where within acceptable limits presented in Table 3.1. (water clarity fell below 5 cm). To correct this apparent water quality difficulty, a continuous flow of well water through the rearing tank at 9 lpm was initiated during the next 18 hours replacing nearly 75 % of the system volume. Fish fed sluggishly during the first feeding of the day, and

would not eat for the remainder of the day. Therefore, a continuous flow-through (9 lpm, 8 hrs/day) was implemented until normal feeding returned. This episode lasted six days, during which 187 % of the system volume of water was exchanged. Feed consumption in treatments 13 and 25 g of ozone slowed, but remained above 4 kg day.

Survival and Growth. For the three groups of fish cultured through day 236, survival at harvest was greater than 98%. Before mechanical failure in treatments 3 and 45 g O_3 / kg feed, no significant losses occurred. Total biomass production was positively correlated to treatment (Pearson correlation coefficient = 0.83).

Final harvest yielded 1886 kg from the three remaining rearing systems (Table 3.2). Mean length and weights were approximately equal in all treatments through day 112. Orthogonal contrasts comparing pooled weights of fish reared at 0 and 3 g of O_3 / kg / feed against pooled weights of fish reared at 13, 25, and 45 g O_3 / kg feed revealed a greater mean weight of fish reared at the higher ozone treatments ($p < 0.01$). Fish weights at harvest averaged 443.5 < 568.8 = 584.4 g for treatments of 0, 13, and 25 g O_3 / kg feed, respectively (Figure 3.4a).

Minor treatment differences in daily weight gain existed through day 112. Fish reared at the treatment of 3 g O_3 / kg feed displayed the lowest growth, 1.92 g/day, while daily growth rates of 2.34, 2.19, 2.26, and 2.49 g/day were calculated for treatment ratios 0, 13, 25, and 45 g O_3 kg feed / day, respectively. Between days 84 and 142, the growth rate of fish reared in the unozonated environment averaged 0.96 g/day. Fish reared in the ozonated systems were significantly higher ($p = 0.03$), averaging 2.3 g/day, 57 % faster than fish in the control system.

During the final 94 days, daily growth of fish reared under unozonated conditions more than doubled to 2.1 g/day (Figure 3.4b).

The condition factor (K) of fish was similar for all treatments ($p > 0.05$) until the growth rate of fish began to decline in the unozonated system. As the trial proceeded, K improved under ozonated conditions while fish from the unozonated system sustained lower K values for similarly sized fish from day 112 through the end of the study (Table 3.3).

Hematological profiles. Erythrocytes were the most numerous cell type followed by thrombocytes and small lymphocytes. All white blood cell types increased at similar increments in unozonated and ozonated environments, however, red blood cells increased dramatically from the second to final sampling (Table 3.4). At harvest, fish reared in the unozonated environment had the lowest red blood cell count, 12.7×10^6 cells/μL, yet erythrocyte counts exceeded the upper reference limit proposed by Hrubec et al. (1995a). Total leukocyte counts increased 75% during the study in fish reared in the unozonated environment, while fish reared in ozonated conditions experienced only a 40% increase in white blood cell counts. Thrombocyte counts showed greater treatment and sample variation than other cell types ($p < 0.05$). No treatment differences were detected in final blood cell counts.

Plasma protein levels were similar among fish from all rearing environments; however, fish raised in the unozonated environment (mean plasma protein 6.7 ± 0.2 g/dL) routinely exhibited lower values than those measured from other treatments. Protein levels in all treatment ratios increased through day 56, when the highest concentration of 9.9 g/dL was measured from fish reared at the 13 g O_3 / kg feed treatment before declining.(Figure 3.5a).

Hemoglobin and hematocrit values showed greater variation between treatments as exposure to the rearing environments continued (Figures 3.5b and c), reflecting significant increases in erythrocyte numbers in fish. Hemoglobin concentration increased significantly ($p < 0.01$), with values doubling by the end of the study. The lowest mean value, 6.5 g/dL, was recorded from the unozonated environment on day 142 while hemoglobin concentrations from the ozonated environments never fell below 8 g/dL. Unlike other physio-chemical indices, hematocrit values varied erratically in all treatments during the trial (range, 19 to 78%, $\bar{x} = 48.3 \pm 1.0$ %), with initial and final values having no significant difference ($p > 0.05$). The highest value was measured at a treatment of 13 g / O_3 / kg feed; however, hematocrit levels in fish reared in the unozonated system increased to 62.8 % in 56 fewer days.

Serum chemistry profiles. Serum chloride and glucose profiles from all rearing environments were typical of long term exposure to chronic stressors. Chloride levels of fish reared in the unozonated environment fell below 102 mEq/L (day 142) before recovering to levels similar to those in fish from the ozonated environments (Figure 3.6a). No treatment effects were detected, although chloride levels decreased significantly over time ($p < 0.01$). A similar pattern was observed in serum glucose, where the unozonated environment elicited a greater rate of change in glucose than was observed in ozonated environments. Glucose levels peaked at 205 mg/dL (day 84) before declining. The largest difference in glucose levels was 29.5 mg/dL which occurred between fish reared in the treatment of 0 g O_3 / kg feed and those reared at 13 g O_3 / kg feed on day 84 (Figure 3.6b).

Tissue examination. Liver somatic indices (LSI) were similar for similar-sized fish between treatments ($p > 0.05$). Fish reared at the treatment 25 g O_3 / kg feed were found to have a somatic index of 22%, which was nearly twice that measured at treatment ratios 0 and 13 g O_3 / kg feed on day 142 (Table 3.5). Values calculated for treatments 0 and 13 g O_3 / kg feed were lowest, coinciding with observed reductions in growth rates. Gross observations detailed no major differences in the appearance of livers between treatments, however, liver coloration (pale and mottled with white splotches) was characteristic of that seen in other hybrid striped bass reared intensively (S. Smith, Virginia/Maryland Regional College of Veterinary Medicine). Smith indicated that the discoloration of the liver was caused by the deposition of dietary fat, however, histological examination was not conducted.

The gross examination of gill filaments indicated harsher environmental conditions without ozone treatment (Table 3.6), however there was only a slight statistical difference ($p = 0.06$) Nearly 84% of the individuals examined from the unozonated environment were given a damage rating of three (or higher). The majority of the gross pathology was in the form of increased mucus and telangiectasis. Rankings of three or higher indicated presence of telangiectasis, and mucus erosion (Figure 3.7). Clubbing was observed at very low incidence only in fish reared in the unozonated system.

Discussion

This study examined temporal changes in fish physiochemical indices and growth characteristics of hybrid striped bass reared under ozonated conditions. Overall, ozone treatment of recirculating waters promoted relatively healthy gill condition and growth rates obtained by hybrid striped bass from the ozonated systems.

Environmental conditions, in addition to nutrition, size, age, and sex, can affect significant changes in biochemical analytes in fish (Larsena and Snieszko 1961; Tomasso et al. 1980; Ellsaesser and Clem 1987; Bucher and Hofer 1990). In addition, Hrubec et al. (1995a) suggested that these factors may promote differential response levels accounting for the sometimes wide variations in reported hematological reference values for striped bass (Courtois 1975, 1976; Hunn and Greer 1990) and other teleost fishes (Strange and Schreck 1978; Lane 1979; Smit and Schoonbee 1988).

Changes in physiochemical indices of hybrid striped bass reared in ozonated and unozonated environments followed similar response patterns similar to those described for other fishes exposed to acute and chronic stressors (Strange et al. 1977; Tisa et al. 1983; Robertson 1987). Abrupt changes in physiology preceded significant ($p < 0.01$) decreases in feed consumption and daily weight gained by fish at the treatment level 0 g O_3 / kg feed between days 84 and 142. A similar significant decrease in feed consumption was not observed in fish from the ozonated environments. Occasionally, unozonated conditions associated with greater variation in physiology (with respect to rate and magnitude of change) between sample periods; however, final and mean values were similar across all treatments. The latter was possibly reflective of

similarities in ambient environmental conditions between treatments and the small sample size per treatment. Hrubec et al. (1995b) suggested variation in blood chemistry could be influenced by a low number of individuals used to establish "normal" ranges.

Sheehan and Lewis (1986) and Kjartansson (1988) both reported that intensification of production resulted in extended exposure to poor water quality disrupting physiological homeostasis within fish. Hrubec et al. (1995b) examining the impact of culture technique, reported that hybrid striped bass obtained from recirculating aquaculture systems were in poorer physiological condition than hybrids cultured in ponds and cages. Mean erythrocyte counts, leukocyte counts, and hematocrit observed during this study from fish under all environmental conditions exceeded the upper limit of reference intervals put forward by Hrubec et al. (1995b). This tended to suggest hemoconcentration in the hybrids striped bass, which also has been documented in channel catfish after exposure to poor environmental conditions associated with intensive culture techniques (Sheehan and Lewis 1986). Increased stocking density elicited the highest hematocrit levels in Atlantic salmon (Kjartansson 1988). In contrast, goldfish (Murray and Burton 1979) and chinook salmon (Mazur and Iwama 1993) maintained at the lowest rearing densities exhibited higher hematocrit values than fish held at higher densities. These discrepancies may reflect differences in species-specific adaptive responses (Warner and Williams 1977), and the length of exposure to deteriorated water quality and water quality, oxygen, ammonia, carbon dioxide, (Swift and Lloyd 1974). In recirculating systems, the critical factors probably include interactions between stocking density, length of exposure poor water quality, and nutritional deficiencies.

Determining the exact cause of hemoconcentration was difficult. Final erythrocyte levels where nearly three times higher than established reference intervals in the ozonated and unozonated environments. Increased hemoglobin, hematocrit, and red blood cell counts are typical responses to an oxygen deficit after exercise or hypoxia (Hall 1928; Ostroumova 1954; Stevens 1968) neither of which were experienced during this trial. It was observed that the mean erythrocyte hemoglobin values declined over time, suggesting haemolytic anaemia (Scarano et al. 1984). The release of red blood cells into the blood stream could have been a compensatory mechanism used to increase the oxygen carrying capacity of blood. Stevens (1968) suggested that in acute situations, releasing red blood cells from storage organs (i.e., spleen and liver) significantly increased the blood stream oxygen content; however, the sampling schedule followed during this trial was unable to confirm or exclude this possibility. The steady increase in erythrocyte levels in conjunction with chronic exposure to low concentrations of ammonia and nitrite lend greater support to an increase in erythropoietic activity (Swift and Lloyd 1974).

It was possible that oxygen transfer limitations across gill lamellae, reducing the oxygen content of the blood, may have resulted from the gill pathology observed, excess mucus production. In addition, mean erythrocyte hemoglobin content decreased during the trial. This may have simulated exposure to hypoxic conditions. The suspected causative agents, such as chronic sublethal concentrations of ammonia and nitrite, and acidification (Colberg and Lingg 1978; Hirayama et al. 1988; Rueter and Johnson 1995) including total suspended solids levels which surpassed 15 mg / L (chapter 2), were persistent characteristics of both the ozonated and unozonated environments. However, it was observed that gross gill pathology was more severe

in the unozonated system, supporting the assertions that ozone treatment enhanced water quality.

Not only does epithelial destruction of lamellae restrict oxygen transfer but it results in changes in serum electrolytes (chloride) and plasma metabolites (glucose and protein) concentrations. Warner and Williams (1977) measured lower chloride levels in channel catfish from intensive raceway culture systems than reared in ponds. Striped bass exposed to chronic stress displayed a slow decline in serum chloride levels rather than the sharp decrease observed after exposure to acute stressors (Tommasso et al. 1980). In this study, the chloride levels declined under all treatments, although, the rate of decline was slower under the ozone treatments, suggesting the amelioration of harsher environmental elements.

Temporal changes in plasma metabolite concentrations of hybrid striped bass reared in unozonated and ozonated environments also were observed during this investigation. Glucose and plasma protein levels showed no treatment differences, although variations within-treatment variation was higher for glucose measurements than observed in plasma protein measurements. Ellsaessare and Clem (1987) cited nutrition as a factor influencing plasma glucose and protein levels. Segregating environmental impacts from nutritional influences on plasma chemistry proves to be problematic for intensively cultured fish. Roberts and Bullock (1989) suggested that nutritional diseases are more commonly encountered as culture techniques intensified. Warner and Williams (1977) found higher levels of glucose in raceway-cultured catfish, but those levels were within the “normal range” for pond-reared catfish. Additionally, significantly lower total protein levels were measured from raceway fish. It was theorized that increased widths of normal ranges were associated with the variations in nutritional states of the pond reared fish. The results

of this study showed glucose and protein levels were within the established normal ranges reported for hybrid striped bass reared in recirculating systems and cages (Hrubec et al. 1995a), but the values were higher than those reported for wild striped bass (Tisa et al. 1983).

Final evaluation of any water treatment technique must be based the quality and consistency of the product produced. Therefore, the ultimate determination of environmental quality resides in survival and growth performance. Fish reared under the ozone treatments of 13 and 25 g O_3 averaged 2.3 and 2.4 g/day, respectively, compared with the gain of 1.8 g/day by fish reared in the unozonated environment. Overall, growth rates obtained by hybrid striped bass from all rearing environments examined were comparable to rates measured for hybrid striped bass from other culture systems. Williams et al. (1981), Kerby et al. (1983), Woods et al. (1983), Nunley (1992), and Bosworth (1994) reported growth rates ranging between 0.9 and 2.6 g/day under pond, raceway, net-pen, and closed system culture techniques.

Results obtained by Nunley (1992), investigating the effect of density on hybrid striped bass growth, are ideal for direct comparison, as his work was conducted in closed systems of similar configuration and management strategy. The rearing density (150 fish per m^3) examined during this current study fell between the medium and high densities (119 and 238 fish per m^3) range investigated by Nunley (1992). Interestingly, the mean growth rate of fish cultured in an ozonated environment (current study) was equal to that reported by Nunley (1992) from the medium density (Figure 3.8), supporting the assertion that ozone treatment of recirculated water helps to increase fish growth by enhancing the quality of the culture environment (Williams et al. 1982; Sutterlin et al. 1984; Poston and Williams 1990).

Sutterlin et al. (1984) defined enhanced water quality as the increase in removal efficiency of inorganic nitrogen through biofiltration. Ozone oxidizes refractory organics present in the system, thereby limiting heterotrophic bacteria growth within the biofilter providing additional room for nitrifying colonies. Coche (1981) reported that besides increasing bio-degradability of dissolved substances and oxidizing nitrites to nitrates, ozone decreases diurnal fluctuations of dissolved organics promoting greater water quality consistency. Rosenthal and Otte (1979) counteracted the accumulation of low degradable substances through ozonation, thereby providing ideal conditions for fish growth in brackish water recycle systems. They concluded that long-term operation of high density, recycle systems was possible with ozonation supplementing biological filtration, reducing the need for continuous water exchanges.

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Table 3.1. Toxic concentrations of routinely monitored water quality parameters for striped bass (Morone saxatilis) and/or it's hybrids. When environmental conditions existed which exceeded any of the minimum limits, freshwater exchanges were conducted to help lower ambient concentrations.

Parameter	Minimum Toxic Concentration (mg/l)	Source
Total-Ammonia-Nitrogen	1.5 at pH 7.5	Nicholson et al. 1990
Nitrite	163	Maziket et al. 1990
Nitrate	200	Nicholson et al. 1990
Dissolved Oxygen	5.0	Lewis et al. 1981
Alkanlinity	No know limit	----------
Hardness	No know limit	----------
Total Suspended Solids[1]	15	Chen et al.
Dissolved Organic Compounds	No know limit	----------

1. Concentration was established as a minimum limit which induced gill pathology.

Table 3.2. Monthly mean ± standard error water quality measurements observed in pilot-scale recirculating aquaculture systems under differing ozone treatment ratios for each sampling period. Measurements reflect ambient conditions prior to the day's first feeding.

Time Treatment	Temperature (°C)	Dissolved O_2 (mg/L)	pH	TAN (mg/L)	NH_3-N (mg/L)
Day 0					
0g	23.6 ± 2.3	12.8 ±2.1	8.2 ± 0.5	0.2 ± 0.03	0.02 ± 0.02
3g	23.6 ± 2.3	9.6 ± 2.1	8.6 ± 0.5	0.2 ± 0.03	0.01 ± 0.02
13g	24.9 ± 2.3	9.6 ± 2.1	8.2 ± 0.5	0.2 ± 0.03	0.01 ± 0.02
25g	23.9 ± 2.3	10.4 ± 2.1	8.2 ± 0.5	0.3 ± 0.03	0.01 ± 0.02
45g	23.5 ± 2.3	12.2 ± 2.1	7.9 ± 0.5	0.5 ± 0.03	0.01 ± 0.02
Day 0 - 28					
0g	26.3 ± 2.5	11.5 ± 0.6	7.7 ± 0.1	0.4 ± 0.03	0.02 ± 0.01
3g	26.2 ± 2.5	12.4 ± 0.3	7.8 ± 0.1	0.4 ± 0.04	0.02 ± 0.01
13g	26.0 ± 2.5	12.3 ± 0.3	7.9 ± 0.1	0.4 ± 0.04	0.02 ± 0.01
25g	26.2 ± 2.5	12.4 ± 0.3	7.8 ± 0.1	0.4 ± 0.04	0.02 ± 0.01
45g	25.8 ± 2.5	12.1 ± 0.3	7.8 ± 0.1	0.3 ± 0.04	0.03 ± 0.01
Day 29 - 56					
0g	27.0 ± 2.3	11.4 ± 0.3	7.8 ± 0.04	0.8 ± 0.04	0.04 ± 0.01
3g	27.0 ± 2.3	13.0 ± 0.3	7.7 ± 0.1	0.8 ± 0.04	0.03 ± 0.01
13g	27.1 ± 2.3	11.7 ± 0.3	7.7 ± 0.1	0.8 ± 0.04	0.07 ± 0.01
25g	27.0 ± 2.3	10.6 ± 0.3	7.8 ± 0.1	0.6 ± 0.04	0.03 ± 0.01
45g	27.3 ± 2.3	11.0 ± 0.3	7.5 ± 0.1	0.6 ± 0.04	0.03 ± 0.01
Day 57 - 84					
0g	27.4 ± 2.0	10.5 ± 0.7	7.6 ± 0.1	1.2 ± 0.04	0.04 ± 0.01
3g	27.4 ± 2.0	13.3 ± 0.3	7.4± 0.1	1.1 ± 0.04	0.02 ± 0.01
13g	27.1 ± 2.0	11.2 ± 0.3	7.2 ± 0.1	1.2 ± 0.04	0.03 ± 0.01
25g	27.4 ± 2.0	11.1 ± 0.3	7.5 ± 0.1	1.0 ± 0.04	0.03 ± 0.01
45g	27.7 ± 2.0	12.2 ± 0.3	7.2 ± 0.1	1.2 ± 0.04	0.02 ± 0.01
Day 85 - 112					
0g	27.1 ± 2.7	12.4 ± 0.3	7.3 ± 0.1	1.2 ± 0.1	0.04 ± 0.01
3g	27.2 ± 2.7	12.6 ± 0.3	7.3 ± 0.1	1.1 ± 0.1	0.03 ± 0.01
13g	26.5 ± 2.7	12.3 ± 0.3	7.3 ± 0.1	1.3 ± 0.1	0.03 ± 0.01
25g	26.7 ± 2.7	12.5 ± 0.3	7.3 ± 0.1	1.4 ± 0.1	0.02 ± 0.01
45g	26.9 ± 2.7	12.2 ± 0.3	7.2 ± 0.1	1.2 ± 0.1	0.04 ± 0.01
Significance of linear Increase[1,2]	p > 0.05a	p > 0.05a	p < 0.01a	p < 0.01a	p < 0.01a
Overall Mean[1,2]					
0 g	27.8 ± 2.8a	11.7 ± 0.3a	7.6 ± 0.02a	0.9 ± 0.03a	0.04 ± 0.01a
3 g	26.7 ± 2.8a	12.9 ± 0.2a	7.6 ± 0.04a	0.8 ± 0.04a	0.03 ± 0.01a
13 g	26.5 ± 2.8a	12.5 ± 0.3a	7.6 ± 0.04a	0.8 ± 0.04a	0.03 ± 0.01a
25 g	26.7 ± 2.8a	12.1 ± 0.3a	7.5 ± 0.04a	0.8 ± 0.06a	0.02 ± 0.01a
45 g	26.5 ± 2.8a	12.3 ± 0.2a	7.6 ± 0.04a	0.8 ± 0.04a	0.02 ± 0.02a

1. Linear trend p > 0.05 were not significantly different from zero.
2. Values with the same letter were not significantly different within the same column.

Table 3.2. Continued.

	Temperature (°C)	Dissolved O_2 (mg/L)	pH	TAN (mg/L)	NH_3-N (mg/L)
Day 113 - 142					
0g	26.5 ± 0.3	14.3 ± 0.5	7.5 ± 0.1	0.8 ± 0.1	0.02 ± 0.01
13g	26.4 ± 0.3	13.0 ± 0.5	7.3 ± 0.1	1.4 ± 0.1	0.02 ± 0.01
25g	26.2 ± 0.3	14.1 ± 0.5	7.3 ± 0.1	1.2 ± 0.1	0.02 ± 0.01
Day 143 - 174					
0g	24.2 ± 0.4	13.7 ± 0.5	7.7 ± 0.1	1.0 ± 0.1	0.03 ± 0.01
13g	24.1 ± 0.4	13.1 ± 0.5	7. 4± 0.1	1.5 ± 0.1	0.02 ± 0.01
25g	24.4 ± 0.4	13.9 ± 0.5	6.9 ± 0.1	1.2 ± 0.1	0.02 ± 0.01
Day 175 - 204					
0g	24.05 ± 0.31	13.58 ± 0.44	7.6 ± 0.11	1.37 ± 0.12	0.03 ± 0.01
13g	24.04 ± 0.31	13.84 ± 0.44	7.3 ± 0.11	1.35 ± 0.12	0.02 ± 0.01
25g	23.98 ± 0.31	13.17 ± 0.44	7.2 ± 0.11	1.45 ± 0.12	0.02 ± 0.01
Day 205 - 236					
0g	24.0 ± 0.3	14.5 ± 0.4	7.3 ± 0.1	1.3 ± 0.04	0.02 ± 0.01
13g	24.0 ± 0.3	13.8 ± 0.4	7.1 ± 0.1	1.4 ± 0.1	0.01 ± 0.001
25g	24.2 ± 0.1	13.5 ± 0.4	7.0 ± 0.1	1.6 ± 0.1	0.01 ± 0.001
Significance linear increase[1,2]	$p > 0.05$a	$p > 0.05$a	$p < 0.01$b	$p < 0.01$b	$p < 0.01$a
Overall Mean[1,2]					
0 g	25.6 ± 0.1a	13.0 ± 0.2a	7.6 ± 0.04a	0.9 ± 0.3a	0.03 ± 0.01a
13 g	25.7 ± 0.1a	12.4± 0.2a	7.5 ± 0.04b	1.1 ± 0.3a	0.02 ± 0.01a
25 g	25.3 ± 0.2a	12.9 ± 0.2a	7.5 ± 0.04b	1.2 ± 0.4a	0.02 ± 0.01a

1. Linear trend $p > 0.05$ were not significantly different from zero.
2. Values with the same letter were not significantly different within the same column.

Table 3.3. Harvest results obtained at day 236 for hybrid striped bass (Morone chrysops (f) x M. saxatilis (m)) reared in pilot-scale ozonated recirculating aquaculture systems. Total biomass figures were obtained through summation of individual batch weights (25 individuals). FCR values represent overall utilization efficiency.

Treatment ($g O_3$ /kg feed)	Day	Biomass (kg)		Density (kg / m^3)		% Survival	FCR
		Intial	Final[1]	Intial	Final	Final[1]	Final[1]
0 g	236	22.49	524.2a	2.7	63.3	98	1.91a
3 g	113	23.33	(316.0)[2]	2.8	(38.0)[2]	99	(1.50)[2]
13 g	236	22.42	671.7b	2.7	80.8	98	1.58b
25 g	236	21.69	690.2b	2.6	82.9	98	1.59b
45 g	106	22.37	(341.2)[2]	2.7	(41.3)[2]	99	(1.26)[2]

1. Harvest data reported for treatment ratios 3 and 45 g O_3 / kg of feed reflects production through the days 84 and 112, respectively. Mechanical failure resulted in the premature termination of these two treatment.
2. Values with the same letter within the same column are not significantly different.

Table 3.4. Arithmetic mean ± standard error (with n ≥ 60)for growth characteristics for hybrid striped bass (Morone chrysops (f) x M. saxatilis (m)) reared in pilot-scale recirculating aquaculture systems receiving different ozone treatment rates (g O3 / kg feed / day).

Time Treatment	Mean weight (g)	Mean length (mm)	Specific growth (g / day)	Relative growth (% body weight)	Condition factor
Day 0	18.7 ± 1.0	132.8 ± 2.3	--	--	--
0g	19.4 ± 1.0	127.7 ± 1.9	--	--	--
3g	18.7 ± 1.0	132.2 ± 2.4	--	--	--
13g	18.1 ± 1.5	129.3 ± 3.2	--	--	--
25g	18.6 ± 1.3	131.7 ± 3.2	--	--	--
45g					
Day 0 - 28					
0g	43.2 ± 1.0	153.5 ± 1.2	0.8	129.8	1.2 ± 0.01
3g	49.7 ± 1.6	158.4 ± 1.6	1.0	155.4	1.2 ± 0.01
13g	45.2 ± 1.1	155.4 ± 1.2	1.2	150.4	1.2 ± 0.01
25g	52.1 ± 1.2	172.3 ± 1..2	0.9	178.4	1.2 ± 0.02
45g	47.5 ± 1.2	157.3 ± 1.2	1.0	154.9	1.2 ± 0.01
Day 29 - 56					
0g	106.7 ± 2.6	197.5 ± 1.5	2.2	147.3	1.4 ± 0.01
3g	119.9 ± 2.7	199.0 ± 1.4	2.4	141.5	1.5 ± 0.01
13g	112.2 ± 2.3	199.0 ± 1.2	2.1	147.9	1.4 ± 0.01
25g	114.0 ± 2.4	199.1 ± 1.3	2.3	119.3	1.4 ± 0.01
45g	117.0 ± 2.2	197.7 ± 1.2	2.4	146.3	1.5 ± 0.01
Day 57 - 84					
0g	205.2 ± 3.7	237.3 ± 1.3	3.4	92.3	1.5 ± 0.01
3g	197.6 ± 4.9	231.1 ± 1.8	2.7	64.9	1.6 ± 0.01
13g	207.8 ± 4.9	237.3 ± 1.6	3.5	85.3	1.5 ± 0.01
25g	215.3 ± 4.6	237.8 ± 1.6	3.3	88.8	1.6 ± 0.01
45g	212.9 ± 5.1	237.0 ± 1.9	3.3	82.0	1.6 ± 0.01
Day 85 - 112					
0g	234.3 ± 5.0	253.6 ± 1.5	1.0	14.2	1.4 ± 0.01
3g	265.1 ± 5.5	256.4 ± 1.7	2.3	34.1	1.6 ± 0.02
13g	280.0 ± 5.1	263.6 ± 1.4	2.0	34.8	1.5 ± 0.01
25g	278.3 ± 4.8	260.2 ± 1.4	2.5	26.9	1.6 ± 0.01
45g	287.6 ± 7.6	257.1 ± 1.8	2.6	35.1	1.7 ± 0.03
Day 0 - 112[1]					
0g	147.3 ± 5.2a	210.5 ± 2.6a	2.3 ± 0.2a	95.9 ± 29.5a	1.4 ± 0.01a
3g	158.1 ± 5.6a	211.2 ± 2.5a	2.1 ± 0.3a	99.0 ± 29.4a	1.5 ± 0.01a
13g	161.2 ± 6.1a	213.8 ± 2.7a	2.2 ± 0.2a	103.4 ± 27.7a	1.4 ± 0.01a
25g	166.0 ± 5.9a	217.3 ± 2.9a	2.3 ± 0.2a	104.5 ± 31.6a	1.4 ± 0.01a
45g	166.3 ± 6.4a	212.3 ± 2.6a	2.5 ± 0.4a	104.6 ± 28.3a	1.5 ± 0.01a

1. Values with the same letter within the same row and column are not significantly different.

Table 3.4. Continued.

Time Treatment	Mean weight (g)	Mean length (mm)	Daily gain[1] (g / day)	Specific growth[1] (% body weight)	Condition factor
Day 113 - 142					
0g	260.7 ± 6.5	264.6 ± 1.7	0.9	11.3	1.4 ±0.01
13g	354.9 ± 7.3	281.7 ± 1.6	2.4	26.8	1.6 ± 0.01
25g	371.4 ± 6.6	281.8 ± 1.5	2.6	35.9	1.7 ± 0.02
Day 143 -174					
0g	297.5 ± 9.0	275.5 ± 2.1	1.3	14.1	1.4 ± 0.01
13g	413.0 ± 9.1	295.2 ± 2.4	2.2	16.6	1.6 ± 0.06
25g	436.3 ± 8.8	298.7 ± 1.8	2.0	17.5	1.6 ± 0.01
Day 175 - 204					
0g	387.1 ± 10.6	296.8 ± 2.3	3.1	30.1	1.5 ± 0.01
13g	520.5 ± 11.5	317.0 ± 1.9	3.0	25.8	1.6 ± 0.02
25g	524.3 ± 11.5	313.5 ± 1.9	3.7	20.2	1.7 ± 0.02
Day 205 - 236[1]					
0g	443.5 ± 11.6a	310.3 ± 2.1a	2.0	14.6	1.5 ± 0.02
13g	568.8 ± 16.7b	323.9 ± 2.9b	1.7	9.3	1.7 ± 0.03
25g	584.4 ± 16.0b	321.2 ± 2.5b	2.2	11.5	1.8 ± 0.02
Day 0 - 236[1]					
0g	247.3 ± 6.2a	248.7 ± 2.3b	1.8 ± 0.4a	56.7 ± 20.3a	1.4 ± 0.1a
13g	310.1 ± 8.5b	258.5 ± 2.6b	2.3 ± 0.4a	62.1 ± 29.4a	1.5 ± 0.1a
25g	325.8 ± 8.7b	261.4 ± 2.5b	2.4 ± 0.3a	62.3 ± 20.6a	1.6 ± 0.1a

1. Values with the same letter within the same row and column are not significantly different.

Table 3.5. Arithmetic mean ± standard error for hematological values for hybrid striped bass (Morone chrysops (f) x M.saxatilis (m)) reared in pilot-scale recirculating aquaculture systems at each sampling period. Established reference intervals listed for comparison. No treatment effects determined for any cell type. Significant time effect indicated when observed for each cell type.

Analyte	Treatment Ratio (g O_3 / kg / feed / day)					
	0g	3g	13g	25g	45g	Reference Interval[1]
Erythrocytes (x 10^6 / μL)						
Day 0	3.2 ± 0.3	4.0 ± 0.2	4.2 ± 0.4	3.6 ± 0.3	3.2 ± 0.2	2.10-4.32
Day 84	9.9 ± 1.1	6.1 ± 0.6	5.1 ± 1.1	7.6 ± 0.4	1.7 ± 0.9	
Day 236	12.7 ± 0.8	------	14.0 ± 0.6	15.2 ± 0.7	------	
Significant Time Effect						
Leukocytes (x 10^3 / μL)						
Large						
Day 0	64.2 ± 3.1	73.5 ± 4.1	92.1 ± 74.8	103.0 ± 7.4	121.3 ± 17.5	51 - 202
Day 84	69.3 ± 6.1	53.4 ± 1.7	55.8 ± 10.7	79.8 ± 3.9	62.3 ± 25.8	
Day 236	240.4 ± 19.0	------	150.6 ± 6.7	172.2 ± 13.5	------	
Significant Time Effect						
Lymphocytes (x 10^3 / μL)						
Small						
Day 0	41.9 ± 2.5	11.2 ± 2.0	15.8 ± 3.1	32.5 ± 10.1	25.8 ± 5.0	31 - 154
Day 84	25.2 ± 3.8	19.4 ± 1.3	28.2 ± 4.1	29.0 ± 5.1	46.3 ± 1.8	
Day 236	50.5 ± 4.9	------	51.9 ± 5.4	51.8 ± 10.6	------	
Significant Time Effect						
Large						
Day 0	4.4 ± 0.7	3.3 ± 0.7	5.8 ± 0.9	16.9 ± 3.8	14.0 ± 2.5	
Day 84	9.6 ± 1.0	6.6 ± 0.8	8.7 ± 1.3	10.9 ± 3.4	14.6 ± 2.7	0 - 40
Day 236	27.2 ± 4.5	------	15.2 ± 2.1	14.9 ± 2.2	------	
Significant Time Effect						
Monocytes (x 10^3 / μL)						
Day 0	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	1.0 ± 0.4	
Day 84	1.3 ± 0.3	0.7 ± 0.3	1.0 ± 0.5	2.4 ± 0.7	1.3 ± 0.3	0 - 22
Day 236	2.9 ± 0.7	------	3.4 ± 0.7	4.7 ± 1.2	------	
Significant Time Effect						
Neutrophils (x 10^3 / μL)	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	1.0 ± 0.3	1.0 ± 0.4	
Day 0	1.1 ± 0.3	0.1 ± 0.1	0.5 ± 0.2	2.2 ± 0.7	1.3 ± 0.2	0 - 2
Day 84	2.6± 0.7	------	2.5 ± 0.6	3.2 ± 1.0	-------	
Day 236						
Thrombocytes (x 10^3 / μL)						
Day 0	27.5 ± 2.0	39.0 ± 2.3	46.6 ± 4.4	32.3 ± 6.1	44.9 ± 6.7	
Day 84	18.7 ± 1.91	15.8 ± 1.6	18.8 ± 2.2	19.5 ± 3.1	14.0 ± 4.4	34 - 128
Day 236	98.6 ± 12.4	------	45.8 ± 5.1	60.5 ± 10.3	-------	
Significant Time Effect[#]						

1. Reference interval data for hybrid striped bass (Hrubec et al. 1995a,b).

Table 3.6. Arithmetic means of liver weight (L_w) and liver somatic index (I_L) for hybrid striped bass (Morone chrysops (f) x M.saxatilis (m)) reared at different ozone treatment ratios in pilot-scale recirculating aquaculture systems. Values calculated across the entire trial period, 236-days, and shown under six weight categories (W_f). Liver somatic indices were not significantly different between ozone treatments or weight categories.

Treatment ratio g O_3 / kg feed	Weight Categories											
	W_f < 100 g		100 g < W_f < 200 g		200 g < W_f < 300 g		300 g < W_f < 400 g		400 g < W_f < 500 g		W_f > 500 g	
(n)	L_w	I_L	Liver	I_L	L_w	I_L	L_w	I_L	L_w	I_L	L_w	I_L
0 g (48)	2.5	0.042	5.8	0.044	7.5	0.032	12.5	0.037	13.8	0.030	18.5	0.035
3 g (18)	2.0	0.038	6.2	0.042	8.8	0.042	------	------	------	------	------	------
13 g (48)	2.5	0.043	6.3	0.051	7.7	0.032	8.7	0.025	11.8	0.026	17.5	0.028
25 g (48)	2.7	0.041	5.9	0.038	6.6	0.027	4.6	0.013	13.7	0.029	18.3	0.033
45 g (12)	1.8	0.037	6.1	0.043	7.8	0.031	-----	-----	------	------	------	------

Table 3.7. Percentages of individual hybrid striped bass (Morone chrysops (f) x M.saxatilis (m)) given gill damage rankings (1= mostly excess mucus production, 2 = approximately 10 - 20 % of gill lamella examined possessed telangiectasia, 3 = telangiectasia observed in 30 - 50 % of the lamella, 4 = three quarters of the gill tissue possess telangiectasia and discoloration, and 5 = the removal of mucus from the lamella) after exposure to water quality in pilot scale recirculating aquaculture systems receiving ozone injection. No statistically significant treatment differences.

Treatment $g O_3$	Gill Damage Ranking					
	n	Good 1	2	3	4	Poor 5
0 g	0.0	4.0	46.0	33.1	14.8	2.1
3 g	18	76.4	22.2	1.4	0.0	0.0
13 g	48	73.3	18.3	6.3	0.0	2.1
25 g	48	69.3	19.5	7.0	2.1	2.1
45 g	12	94.1	5.9	0.0	0.0	0.0

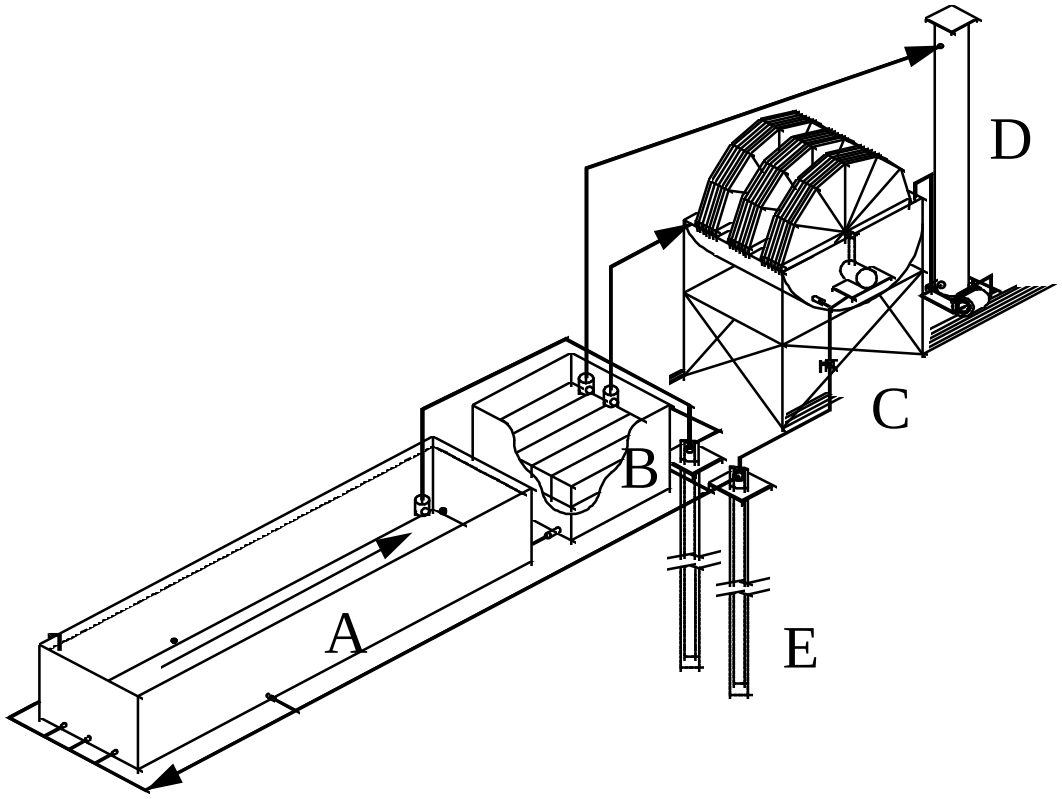

Figure 3.1. Pilot scale recirculating aquaculture system used to reared hybrid striped bass under ozonated and unozonated conditions. Water flowed from the rearing tank (A) up through a multi-tube clarifier (B). Water then was pumped directly to the rotating biological contactor (C) or through the ozone contactor (D) before biological filtration. All water exiting biological filter gravity flowed down the U-tube aeration device (E) receiving gaseous oxygen injection before reentering the rearing tank.

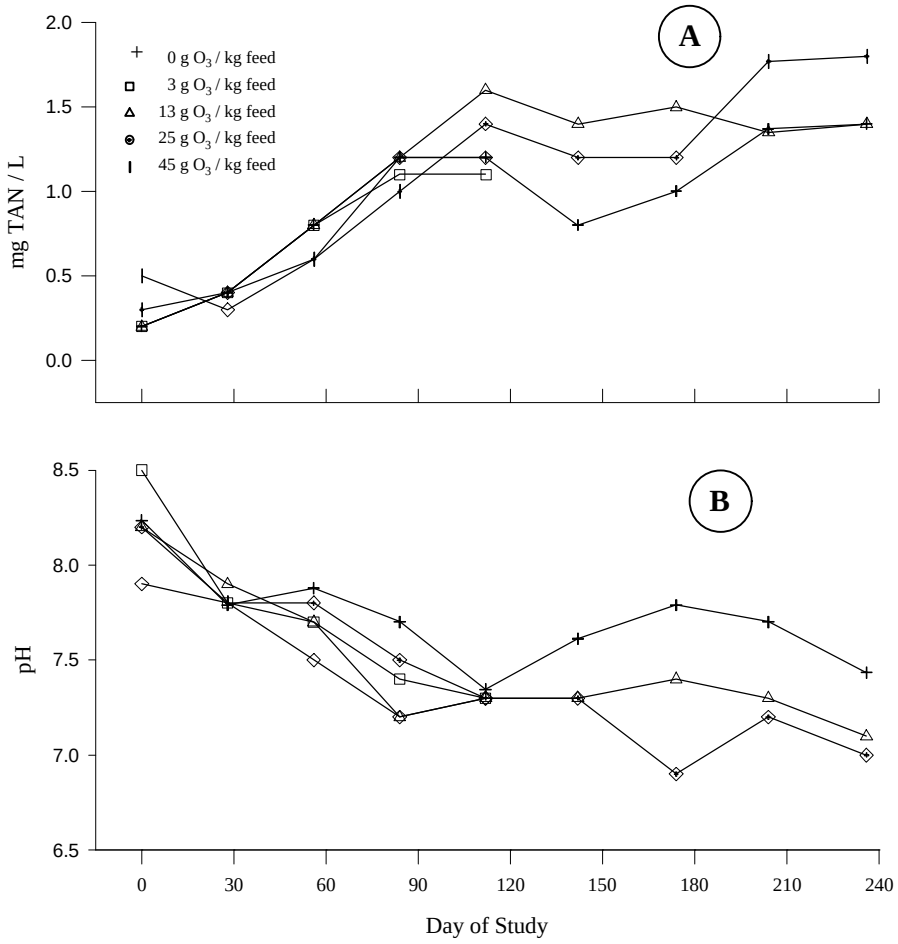

Figure 3.2. Monthly TAN (A) and pH (B) measurements from pilot scale recirculating aquaculture systems rearing hybrid striped bass under ozonated and unozonated conditions. Plots demonstrate declining environmental quality at all ozone treatments.

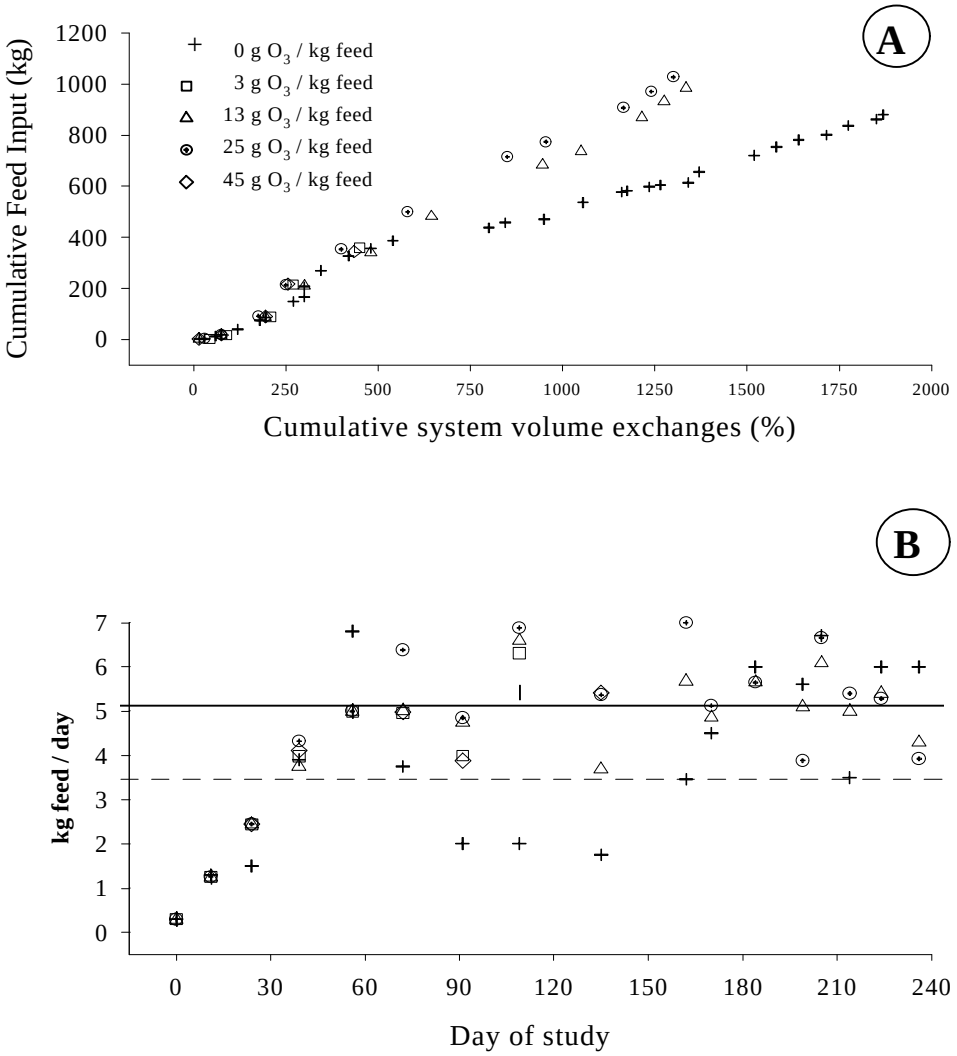

Figure 3.3. (A) Cumulative feed input plotted against the cumulative water volume exchanged in pilot scale recirculating aquaculture systems rearing hybrid striped bass. (B) Weekly feed consumed by hybrid striped bass reared. Solid and dashed horizontal lines denote daily mean feed consumed by hybrid striped bass (Morone chrysops (f) x M. saxatilis (m)) reared in ozonated and unozonated pilot-scale recirculating systems.

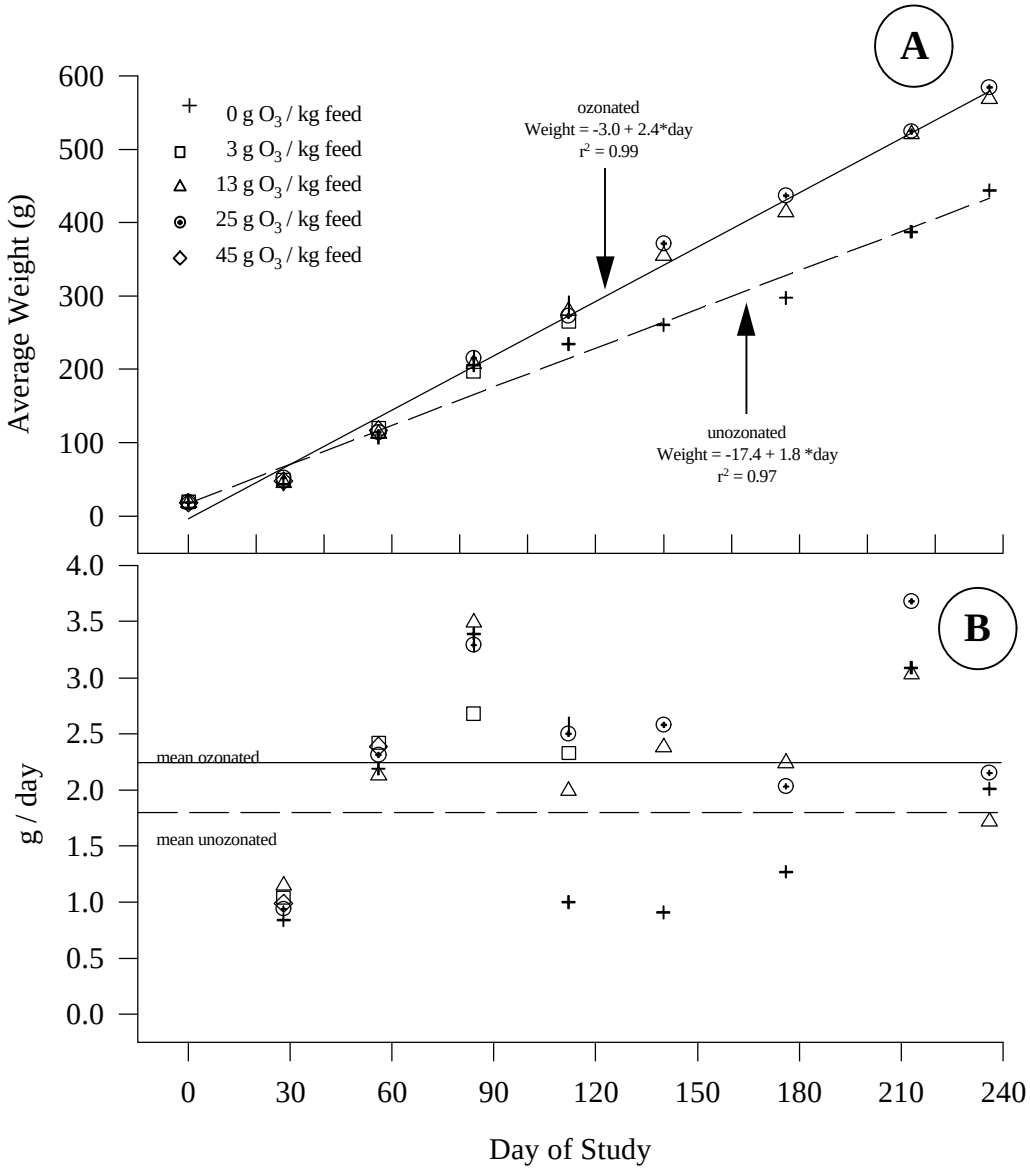

Figure 3.4. (A) Mean and (B) daily weight gain (B) profiles observed in hybrid striped bass in reared in ozonated and unozonated pilot scale recirculating systems. Solid and dashed lines represent overall means calculated for fish reared under ozonated and unozonated environments, respectively.

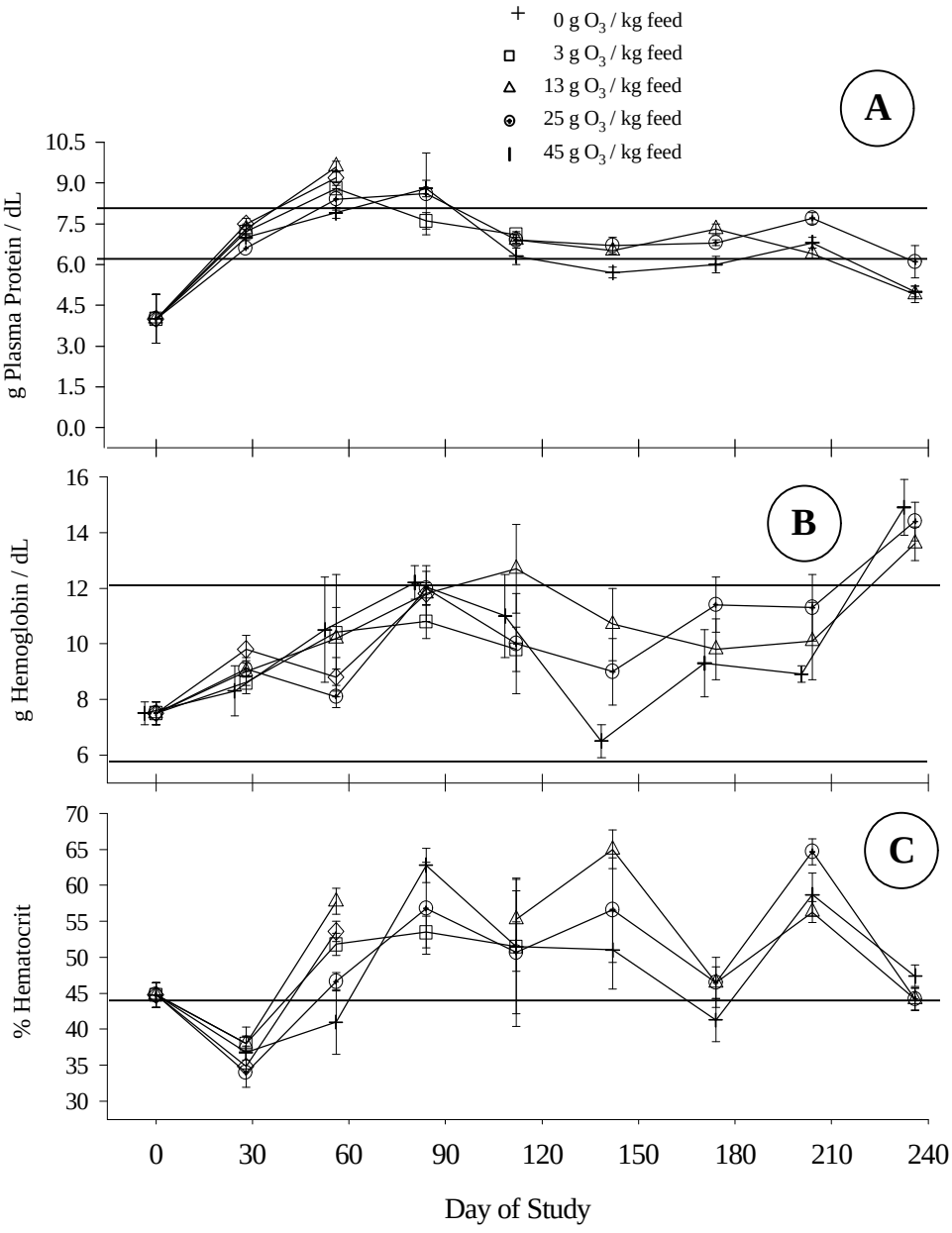

Figure 3.5. (A) Mean plasma protein, (B) hemoglobin, and (C) hematocrit profiles for hybrid striped bass reared in ozonated and unozonated pilot scale recirculating aquaculture systems. Solid horizontal lines represent range of normal values reported for plasma protein, hematocrit (Hrubec et al. 1995a), and hemoglobin (Tisa et al. 1983). Only the upper limit shown for hematocrit.

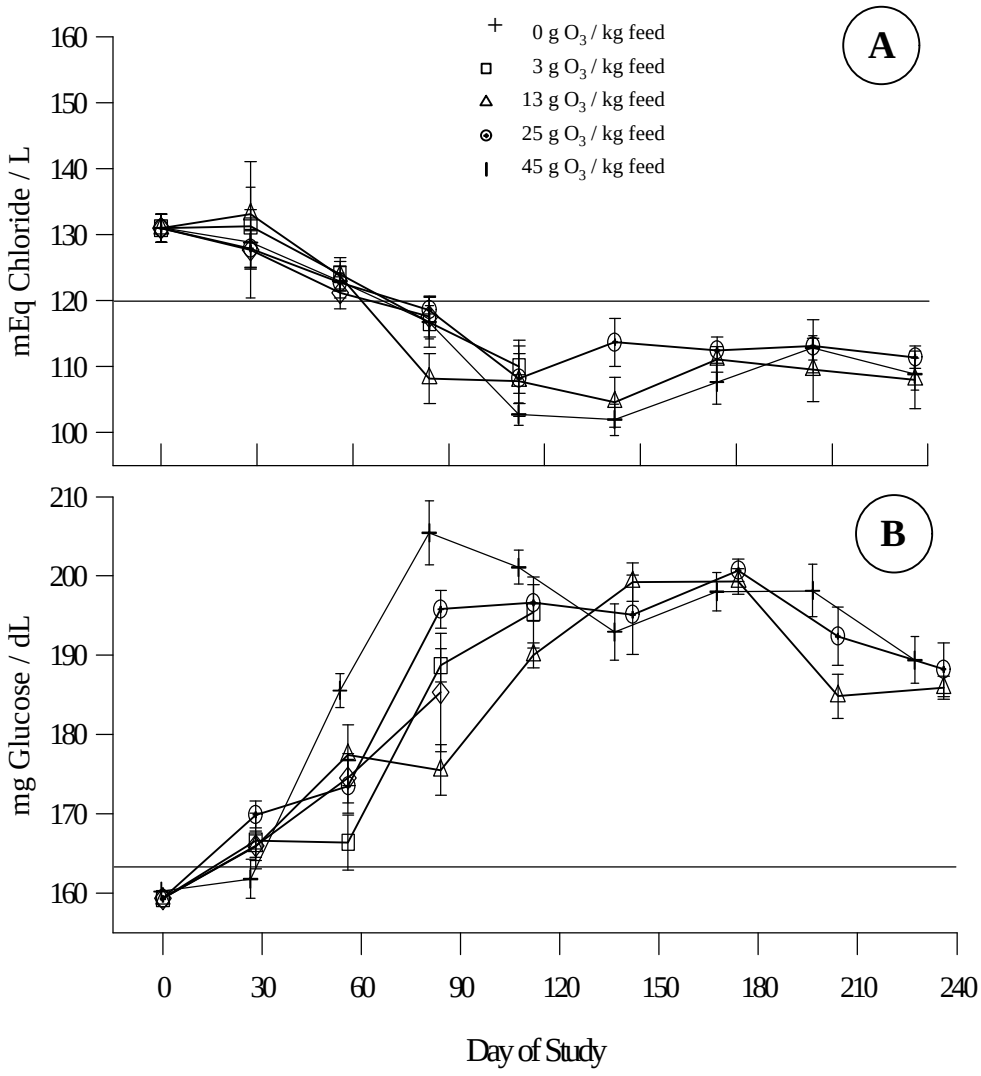

Figure 3.6. (A) Mean chloride and (B) glucose profiles observed in hybrid striped bass reared in ozonated and unozonated pilot scale recirculating aquaculture systems. Solid horizontal lines indicate upper limit of normal range established by Hrubec et al. (1995a) for hybrids striped bass (Morone chrysops (f) x M. saxatilis (m)) maintained in closed systems.

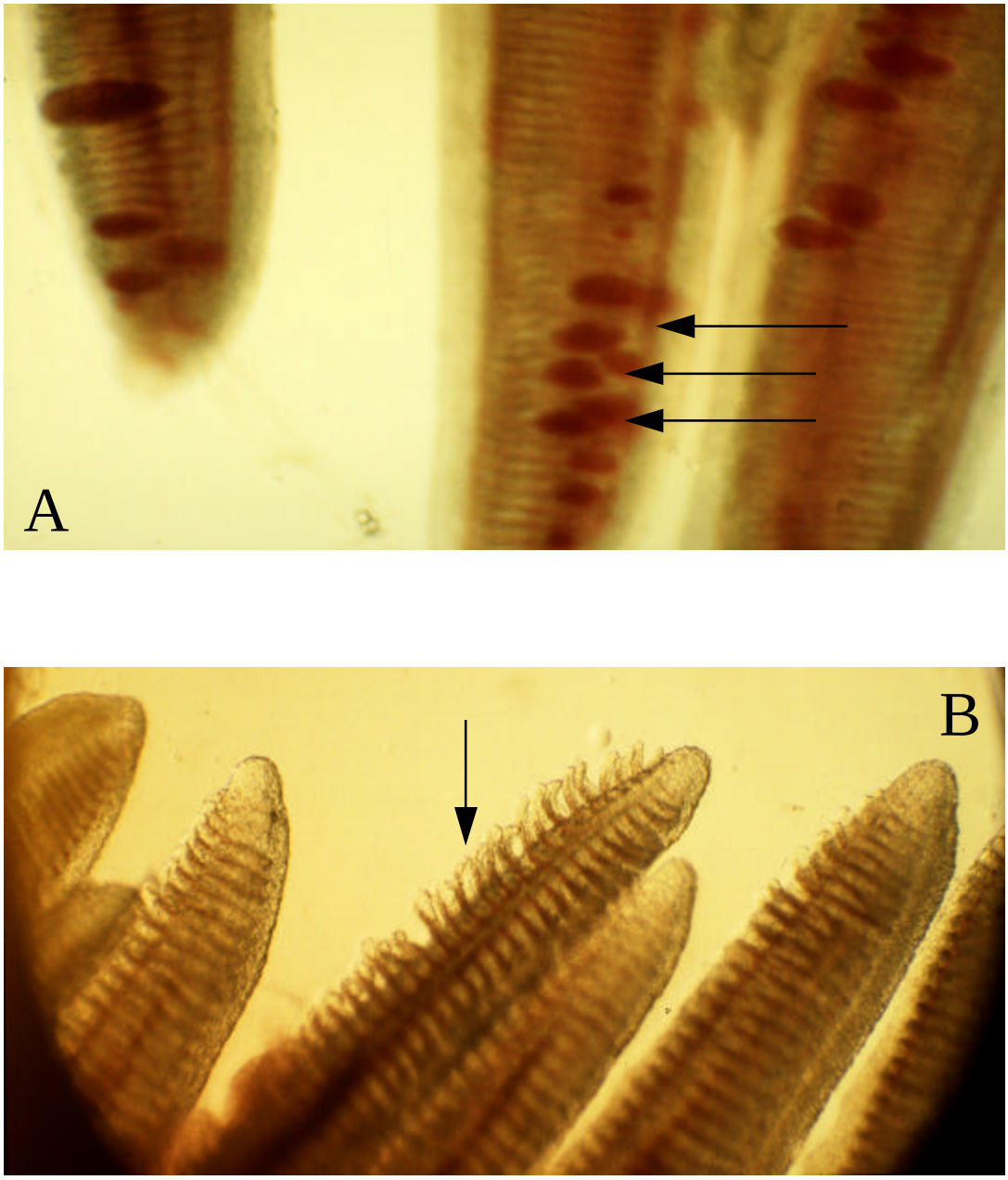

Figure 3.7. Examples of gross gill pathology observed in hybrid striped bass in ozonated and unozonated recirculating aquaculture systems. Pictured are (A) telangiectasis and (B) gill filaments expose directly to the water, as mucus has been completely removed (observed only in fish reared in the unozonated environment).

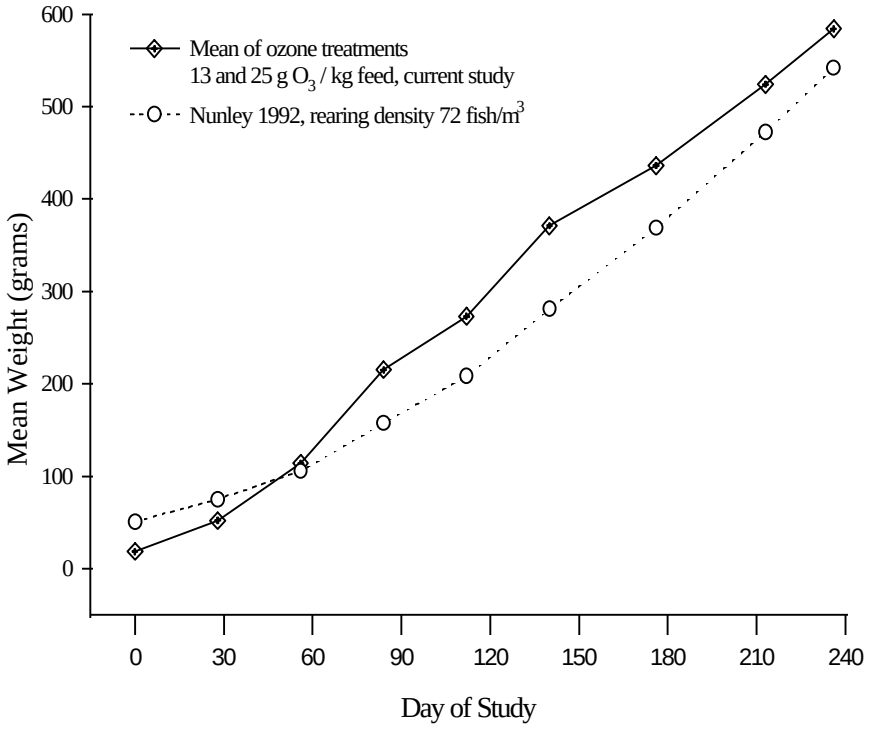

Figure 3.8. Mean weights of hybrid striped bass reared in an ozonated pilot-scale recirculating aquaculture systems at a stocking density of 150 fish/m^3 and hybrid striped bass reared in an unozonated recirculating system (Nunley 1992).