8-1991

The evaluation of an ozone purification system in a recirculating aquaculture system

Brian L. Brazil

To the Graduate Council:

I am submitting herewith a thesis written by Brian L. Brazil entitled "The evaluation of an ozone purification system in a recirculating aquaculture system." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

J. Larry Wilson, Major Professor

We have read this thesis and recommend its acceptance:

David A. Etnier, Thomas K. Hill, Norman L. Betz

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Vice Provost
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THE EVALUATION OF AN OZONE PURIFICATION SYSTEM IN A RECIRCULATING AQUACULTURE SYSTEM

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Brian L. Brazil

August 1991

ACKNOWLEDGEMENTS

I would like to convey thanks to my committee members, Dr. David A. Etnier, Dr. Thomas K. Hill, and Dr. Norman L. Betz for their expertise and recommendations during this study. A special thanks goes Dr. J Larry Wilson who served as chairperson without his leadership, suggestions, friendship my time at the University of Tennessee and this research would not have been as meaningful. A special thanks goes out to Joyce Quickel and Tommy Burch for their behind the scenes help. During my time in graduate school, I was lucky to have the aide of Rob Lindbom (Chief), Jim Negus, Paul Stodola, and Chris Skelton, Marcia Sossamon, John Taylor, and Roger Petrie. I also want thank my friends who have given me support many times without truly understanding my reasoning for staying in school. And finally to my mother and father, sister and grandfather, a simple thanks does not really convey the appreciation and gratitude that I have for your support, encouragement, and understanding.

ABSTRACT

An Ozone Purification System (OPS,Robert B. Hill Co., Minneapolis, MN) was evaluated to determine its potential as a reconditioning agent for the water within an intensive culture system using original cross hybrid striped bass. In the first of two 36-day trials, two stocking densities (3.5 g/L (1X)), and 7.1 g/L (2X)) in the recirculating systems were compared to a 3.5 g/L (1X) stocking rate in the typical flow through system (i.e., the water is used only once). At stocking, mean weight and length per system averaged 11.6 g / 100 mm, 12.6 g / 102 mm, and 11.2 g / 101 mm for the flow-through system and single and double density systems, respectively. Final weights ranged from 33.8 g in the flow-through system to 30.0 g in the single density system and 21.8 g in the double density system. The second trial evaluated only the recirculating systems using stocking densities of 4.8 g/L (1X) and 9.5 g/L (2X). Stocking measurements for the 1X system were 34.0 g and 135 mm and 34.0 g and 137 mm for the 2X system. Final weights were 47.5 g and 44.0 g for the 1X and 2X systems, respectively. A photoperiod was kept on a 12 hour dark/12 hour light cycle during the study. An economic analysis on the cost effectiveness of use the Ozone Purfication System within the recirculating system was significantly more effective at growing fish than the flow-though system stocked at the same density.

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CHAPTER I

INTRODUCTION

Aquaculture is the propagation of fish under controlled conditions for either recreational use or human consumption. As a source for recreational fish, both state and federal hatcheries in this country have incorporated intensive culture techniques to increase annual yields. Only in the last 40 to 50 years has there been a concerted effort in the development of aquaculture in the United States, but its history in the United States can be traced back to the artificial propagation of rainbow trout (*Oncorhynchus mykiss*) in the late 1890's when it was more of an art than a science (Leitritz and Lewis 1976; Stroud 1986).

In more recent times, many species of fish including *Tilapia* sp., rainbow trout, brook trout (*Salvelinus fontinalis*) , and various species of salmon (*Oncorhynchus* sp.), along with some shellfish, shrimp, and crawfish, have been the subject of aquacultural ventures, but none have gained the popularity nor had the success of the channel catfish (*Ictalurus punctatus*). One species that appears to have the potential to be as successful as the channel catfish is the hybrid striped bass (HSB) (Dr. Thomas Hill, personal communication). The hybrid is a cross between the striped bass, *Morone saxatilis* (Walbaum), and the white bass, *M. chrysops* (Rafinesque). Using the female striped bass and the male white bass in the hybridization procedure gives the "original" while using a female white bass and male striped bass produces the "reciprocal" cross (Hodson 1989; Hodson and Hayes 1989). In the mid-1960's, the "original" cross was first produced in South Carolina. Crosses between the striped bass and other *Morone* and non-*Morone* species have been produced, but none have gained the acceptance of the cross with the white bass.

The striped bass is an anadromous species, but with the implementation of reservoir stocking, landlocked populations are found throughout the Southeast. Original distribution of the striped bass ranged along the East Coast from New Brunswick to Florida and to the Gulf Coast from Florida to Texas. Reproducing populations, established in the 1890's, are found on the West Coast from British Columbia to the Mexican border (Hodson 1989).

The white bass, along with yellow bass (*M. mississippiensis*) (Jordan and Eigenmann), are freshwater species (Hodson 1989). The white bass was initially located within the Mississippi River basin and along the Gulf Coast. Since being stocked in reservoirs for recreational fishing, the white bass can be found throughout the country (Hodson 1989).

Both striped bass and white bass have great sportfishing value. Striped bass may reach 32 kilograms in weight where as the white bass only reaches 2-2.5 kilograms (Hodson 1989). Because of declining populations in its native waters, strict regulations on the harvest of the striped bass are being enforced. This has played a major role in the development of artificial propagation methods of the striped bass/white bass hybrid.

A frequent limiting factor in the production of aquatic species is water quality. In aquaculture, the success of an operation is determined by total fish weight produced; water quality can adversely affect fish growth. The term water quality in reference to aquaculture refers to those parameters that have been found to affect growth (e.g., dissolved oxygen, ammonia, nitrogenous waste, alkalinity, temperature, etc.). Maintaining water quality that promotes optimum growth can be achieved in a number of ways: by limiting density of stocking, by limiting the number of times that the effluent is used, or by reconditioning the effluent. Water in recirculating systems has distinct problems associated with it. Dissolved oxygen, ammonia, pH, nitrates, and temperature should be closely monitored for evidence of water quality degradation (Bonn et al. 1976). It is the goal of the aquaculturalist to keep the water quality parameters within the ranges for optimum growth.

Dissolved oxygen (DO) is of primary importance in all types of production systems used by aquaculturalists; Moss and Scott (1961) reported that low DO concentrations affected growth, tolerance to infections, and behavior. Dissolved oxygen requirements for optimum growth are for the most part not species dependent. It has been reported that the fathead minnow (*Pimephales promelas*) and the channel catfish have reduced growth rates at oxygen concentrations below saturation (Brungs 1971; Andrews et al. 1973; Tucker et al. 1979). Oxygen saturation in water is dependent on both temperature and atmospheric pressure. The efficiency at which fish remove oxygen from water is dependent on the oxygen tension. During fish respiration, oxygen is taken into the blood and delivered to tissues while carbon dioxide (CO_2) is removed from the body. Water with an oxygen tension of about 70 mm Hg, corresponding to an oxygen concentration of 3 ppm at a temperature of

30 C at standard pressure, is not adequate for optimum growth in trout (Downey and Klontz 1981). DO ranges for optimum growth of fish have been established (Burrows and Combs 1968; Buss and Miller 1971; Carlson et al. 1980) and are used by aquaculturalists. Various methods are used to keep DO concentrations above a suggested minimum level of 3 ppm, such as reduction of biomass, splashboards, mechanical aerators, and oxygen diffusion (Soderberg 1982).

In water re-use systems, nitrogenous wastes accumulate if steps are not taken to remove them. Nitrogenous waste refers to: (1) ammonia--NH_3-N, (2) nitrite--NO_2-N, and (3) nitrate--NO_3-N. Nitrogen generally enters the water in the form of ammonia. Ammonia is a by-product of food metabolism and must be excreted from the body because of its toxicity. It can also be formed by bacterial action on waste products. Once excreted, ammonia is converted to nitrite by *Nitrosomonas* and then to nitrate by *Nitrobacter*. Both the ammonia and the nitrite phases of the bacterial conversion processes are toxic to fish at high concentrations. Extended exposure to relatively high but sublethal concentrations of ammonia and nitrites can result in decreased growth rates and increased susceptibility to disease and parasites (Larmoyeaux and Piper 1973; Robinette 1976; Burkhalter and Kaya 1977).

Ammonia production and excretion are controlled by several factors including protein content of the feed, body weight, and water temperature (Jobling 1981). It was reported by Porter et al. (1987) that ammonia excretion in gilthead seabream (*Sparus aurata*) showed some diurnal variation. Rychly and Marina (1977) reported similar findings for rainbow trout. This is of extreme importance since nitrite production is dependent on ammonia production.

Nitrite affects the blood hemoglobin. An oxidizing reaction takes place converting the hemoglobin to methemoglobin, giving a brown color to the blood (Tomasso et al. 1979) and resulting in what is commonly referred to as brown blood disease. Fish exposed to continuously high concentrations of nitrites may die from asphyxiation. Functional anemia syndrome is the first mechanism to kill the fish. A study with sea bass (*Dicentrarchus labrax*) held at a lethal concentration for 96 hours showed that 75 to 80% of the total blood hemoglobin converted to methemoglobin. Functionally, the fish is unable to extract oxygen from the water and essentially suffocates. The addition of chloride results in a relatively lowered nitrite toxicity (Smith and Russo 1975; Eddy et al. 1983; Scarano and Saroglia 1984; Scarano et al. 1984). This is important to HSB production because of the implications of reduced growth and even larger problems with fish kills (Burrows 1964). Thus the emphasis is placed on prevention rather than treatment. Controlling nitrite reverts back to alleviation of an ammonia problem.

According to Reeves (1972) and Marking and Bills (1982), the three potential ways of removing ammonia from water include air stripping, ion exchange, and bio-filtration. Air stripping is the process where agitation and aeration are utilized to remove ammonia in gaseous form at pH levels exceeding 10. Air stripping has high efficiencies of > 80% but several drawbacks exist. A large air-stripping tower is required to obtain the correct air to water ratio needed for high efficiency, ammonia can quickly redissolve, and chemicals are necessary to manipulate the pH level for can quickly redissolve, and chemicals are necessary to manipulate the pH level for effective ammonia removal. A second treatment is ion exchange. Ion exchange utilizes clinoptilolite, a natural zeolite having a high affinity for ammonia ions. Using clinoptilolite has its limitations. When the zeolite becomes saturated with ammonia it must be regenerated by flushing it with 20 to 30 times the bed filter volume of brine (NaCl) solution. Storage and disposal of the large volumes of brine solution required for regeneration was the major problem (Semmens et al. 1977). The third and probably most widely used method of ammonia removal is bio-filtration. Bio-filters use nitrifying bacteria to oxidize ammonia to nitrite and then to nitrate. Bio-filters are relatively low cost, easy to maintain, very efficient, and can be adapted to closed systems (Lucchetti and Gray 1988).

The nitrifying bacteria colonize substrates that are submerged in ammonia rich water. Substrates used are chosen based on the amount of surface area they provide. Typical bed bio-filters use gravel, expanded shale, and mussel shells. Not only are submerged bed filters used but rotating biological contactors (RBC) are employed. RBC's are rotating vertical disks in a series that serve as substrate for bacteria (Lewis and Buynak 1976).

As with the ion exchange and air-stripping methods, the bio-filter is not without problems. Two primary problems exist: (1) establishing and maintaining the colonies of nitrifying bacteria, and (2) complete nitrification. Sudden changes in water quality or temperature, a reduction in available nutrients, or pH levels below 6 can destroy the bacterial populations. In addition, various chemicals used for the

Combs 1968; Scott and Gillespie 1972; Bowser et al. 1983). Complete nitrification is accomplished by establishing equal populations of *Nitrosomonas* sp. and *Nitrobacter* sp. Von Gorder and Fritch (1982) recommended 32 - 33 days for the establishment of a complete nitrification system with a gradual increase in fish density in the system. The time period given allows for the lag time that *Nitrobacter* sp. have in colonization of substrates.

One of the newest techniques in the maintenance of water quality in closed systems is the utilization of ozone (O_3). Ozone is produced naturally in the atmosphere during electrical storms. Lightning excites oxygen electrons producing the allotrope O_3. Ozone can be produced mechanically in similar fashion by passing oxygen through an electrical field. Ozone is effective in the reducting of biological oxygen demand and helps to lower ammonia and nitrites levels (Colburg and Lingg 1978; Paller and Lewis 1988) and the elimination of pathogens affecting fish (Wedemeyer et al. 1979; Tipping 1988). A distinctive drawback of ozone is that it is highly toxic to fish. Arthur and Mount (1975) reported that concentrations of 0.2 to 0.3 mg/L ozone were lethal for fathead minnows. For this reason careful consideration and constant monitoring must be employed when using ozone.

Until recently, ozone was used primarily to recondition water for human consumption (Schwartz 1989) and as a disinfectant in some hospitals for disease prevention in water systems (Edelstein et al. 1982). Ozone has also been used as a disinfectant in the poultry industry (Sheldon and Brown 1986; Chang and Sheldon 1989; Chang et al. 1989). Whister and Sheldon (1989) reported that ozonation of pre-chiller water was as effective as other methods of disinfection while being more cost-effective.

Using ozone in closed systems offers benefits that no other reconditioning agent provides. Ozone is very unstable and generally has a retention time in water of only 20 minutes (temperature dependent). Unoxidized ozone will revert back to oxygen, thus maintaining high dissolved oxygen levels. It is known to reduce if not eliminate parasite and disease problems that are water borne. All of these problems prove to be the foe of the aquaculturalist and especially those working with recirculating systems.

This study was designed to evaluate a water reconditioning unit employing ozone in a recirculating fish culture system. The objectives were to: (1) evaluate any differences in growth of HSB reared in the recirculating system as compared to those reared in a typical flow-through system (single use of the water), (2) assess water quality in closed versus flow-through systems and varying densities of fish, and (3) identify economic benefits from the utilization of the ozone purification system.

CHAPTER II

METHODS

On July 16, 1990, 3500 two-month old original cross hybrid striped bass averaging 0.77 g were obtained from the Tennessee Wildlife Resources Agency Eagle Bend Hatchery and transported to the fisheries research laboratory at the University of Tennessee in Knoxville, Tennessee. The fish were held in two 850-L circular tanks for 30 days; they were treated with potassium permanganate ($KMnO_4$) at 3 ppm for *Saprolegnia* sp. On August 16, 1990, the fish were randomly allocated to the research tanks for acclimation.

The study was developed in two phases; each phase lasted 36 days. Phase 1 evaluated fish growth between two recirculating systems varying in stocking density and a flow-through system (Figure 1). Phase 2 evaluated stocking density effects on fish growth using only the two recirculating systems (Figure 1).

Recirculating Systems

The recirculating systems utilized two 850-L circular tanks (rearing tanks) containing 250 L of water each. The rearing tanks were elevated over a fiberglass trough (settling tank, 3.1 m X 61 cm X 46 cm) and an Ozone Purification System (OPS) (Robert B. Hill Co., Minneapolis, MN) (Figure 2). Water circulated through the experimental systems via 13-mm PVC pipe (Schedule 40) at a rate of 15.2 L / min. Water entered the rearing tanks at a rate of 7.6 L / min.

Water flowed from the rearing tanks and into the settling tank via a 5 cm

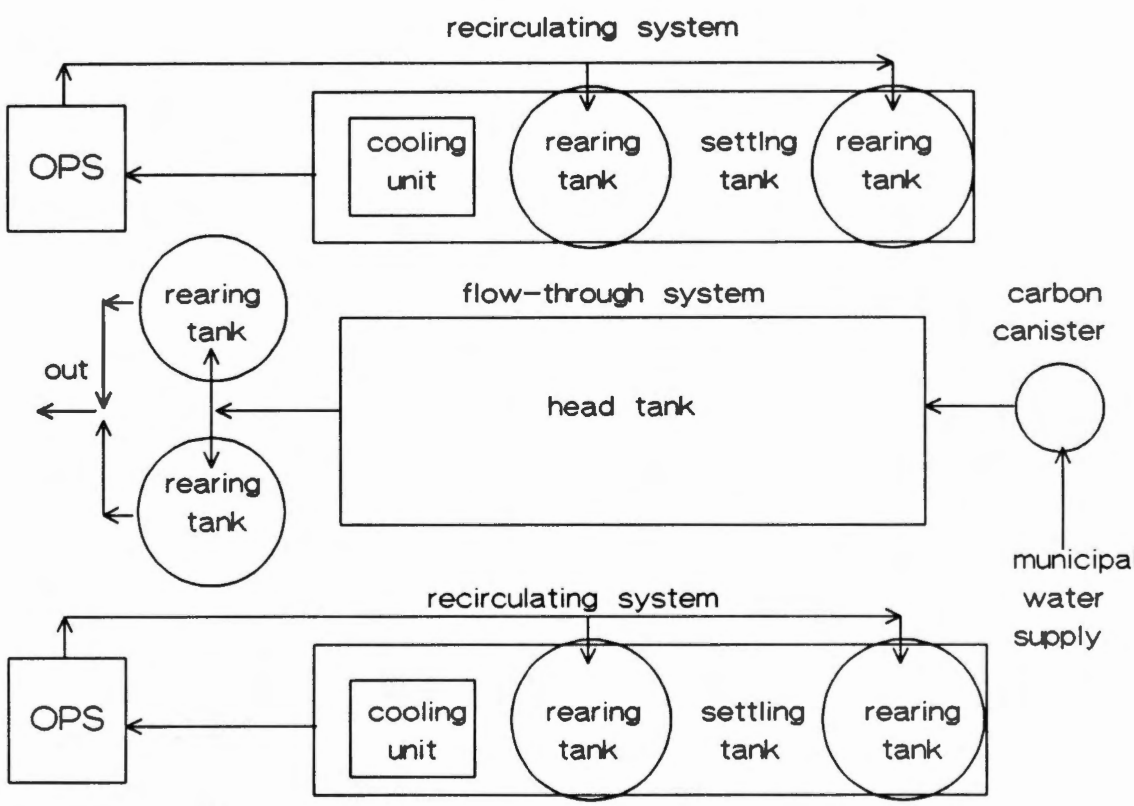


Figure 1. The experimental design established utilizing two recirculating systems with Ozone Purification Systems (filters) and a flow-through system. All systems operated at a water flow rate of 15.2 L/min.

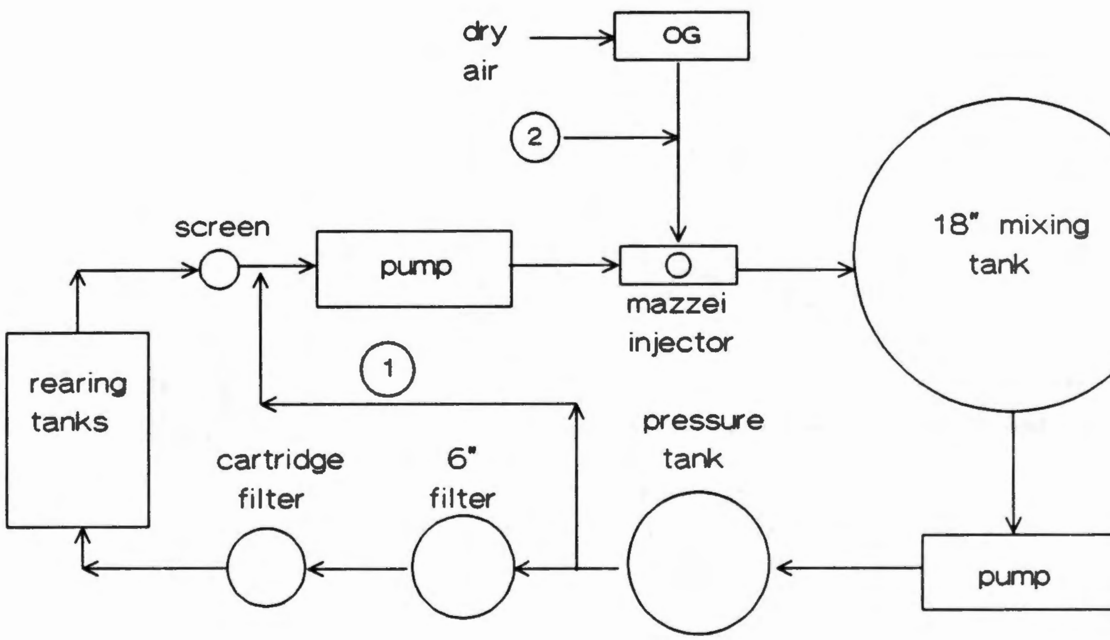


Figure 2. Schematic diagram of the Ozone Purification System (OPS) used in the experimental design of the project. OPS operated between 25-35 psi during the study. (1) Cross-back line transported ozonated water to the circulation pump to keep organics from building up within the pump. (2) Air line used to draw ambient air into the Mazzei injector when ozone was not generated. OG represents the corona discharge generator.

PVC (Schedule 40) venturi drain system. In the settling tank particulate matter was allowed to collect at the bottom. Water was drawn from a level 15.2 cm above the bottom which helped to keep the filter within the OPS relatively free of particulate matter. After leaving the settling tank, water was pumped into the OPS by way of 13-mm PVC pipe.

Mounted on the settling tank was a Coolerator unit (Frigid Units, Inc., Toledo, Ohio). This unit was employed to keep the water temperatures of the recirculating systems equal to those within flow-through system. The Coolerator unit ran until the temperature of the water within the settling tank was lowered to the desired temperature. It was used on three separate occasions for a total of 13.5 hours when water temperatures within the recirculating systems rose above 30 C; water temperatures were subsequently lowered to 25 C.

The OPS had a wire mesh screen that kept large particulate matter from entering the system. After the screen, a pump was used to circulate the water through the Mazzei injector. At this point ozone was injected into the water at a rate of 3 ft^2 per hour at one atmosphere of pressure.

Ozone was produced using a Corona Discharge Generator (American Ozone Technologies, Marion, IA) drawing ambient air though an electrical field generated by this unit. Ambient air was drawn through a canister (30.48 cm X 45.72 cm) containing Drierite (Anhydrous Hammond, $CaSO_4$). The canister had two 32 mm holes in the bottom for ambient air to enter. A cap covering a 7.6 cm opening on the top had a 32 mm plastic hose connected to it for the dried air to exit. Air was then drawn up the hose to an air flow indicator where the flow rate was regulated. After the flow regulator, air proceeded through another drying chamber (45.72 cm X 10.16 cm column) which had dual purposes: (1) it indicated when the primary drying canister was exhausted, and (2) it served as a backup until the primary canister was replaced. There was a color change to indicate when the primary drying canister was exhausted. Dry air was then delivered to the Corona Discharge Generator. Ozone was then moved by solenoid valve, through a back-flow check valve, and injected into the water via the Mazzei injector (Figure 3). Ozone was generated for 12 minutes out of every hour. This injection rate was chosen based on recommendations of the supplier.

Following ozone injection, water entered the mixing tank. While in the mixing tank, ozone either converted back to oxygen or dissipated. In the top of the mixing tank, a portal (7.2 cm) was cut and fixed with a column of activated carbon to keep ozone from entering the atmosphere.

Within the mixing tank, two float switches were used to coordinate the pumps. A lower float switch was located 15 cm from the bottom of the tank. This controlled the repressurization. If the water level fell below this point the pump would shut off. The upper switch was 70 cm above the lower switch. This switch controlled the circulation pump and would shut down if the water level exceeded the desired level. Water was then pumped with a second pump (repressurization) to a pressure tank. This tank contained a pressure switch that was connected to the repressurazation pump to help maintain a constant pressure throughout the OPS. The system

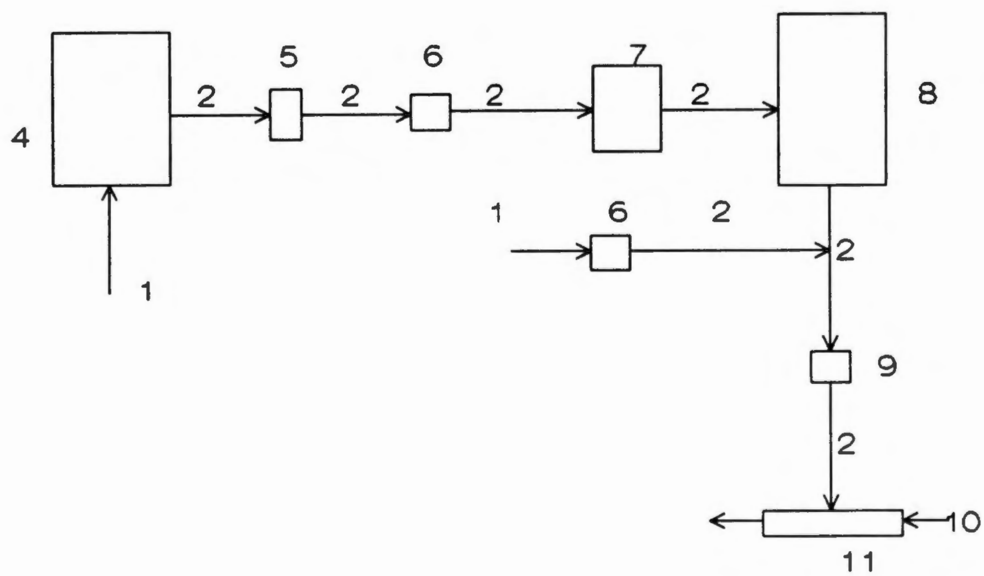


Figure 3. Ozone generation began with ambient air (1) drawn through the primary drying agent, Drierite (4). Air then went from the flow indicator (5), through a solenoid (6), through the secondary drying agent (7), through the ozone generator (8), and to the Mazzei injector (11) via 1/8" plastic air line (2). Water (10) entering the OPS was injected with ozone at this point.

operated between pressures of 25 and 35 pounds per square inch. After the pressure tank, water flowed through an activated carbon canister (15 cm X 70 cm). A back-flushing timer was attached to the canister. This self-cleaning feature flushed any particulate matter that managed to get through the first screens. Back-flushing took place every 24 hours. Water then flowed through a 30.5 cm cartridge filter which served as a back-up while the activated carbon canister was back flushing, and then flowed back to the rearing tanks.

Flow-through System (FTS)

This system contained two rearing tanks with venturi drains; and a head tank (Frigid Units, Inc., Toledo, Ohio) holding 314 L of water that was elevated 1.5 m above the floor. Dechlorinated municipal water was directed at a constant flow rate of 15.2 L/min. into the head tank. Water was gravity fed to the rearing tanks via 5-cm PVC pipe (Schedule 40) where it was divided, sending 7.6 L/min to each tank. Water entered the tank at an angle creating a current within the tank to aid in removal of feces and uneaten food. The water exited the tank and flowed out to the municipal waste water system.

Municipal water, prior to reaching the head tank, was directed through an activated carbon canister (Culligan Water Conditioning of Knoxville, TN) for the removal of chlorine (Cl_2). Municipal water temperatures were on the average 5 C cooler than those temperatures of the recirculating systems; thus, the head tank was used to store the water so that ambient air could increase the water temperature equal to that of the recirculating systems.

Sampling

The day prior to sampling the fish were not fed to ensure that all food had been digested. On sampling day, 20 fish were randomly netted out of each tank and placed into a bucket containing 7.6 L of tank water containing tricaine methanesulfonate (MS-222) at a concentration of 50 ppm. Once fish began to exhibit the effects of the MS-222, they were weighed to the nearest milligram and measured to the nearest millimeter. After each fish was worked up, it was put into 15.2 L of fresh water to recover. After all fish had recovered, they were returned to their tank. All rearing tanks were sampled on the same day.

Feeding

The fish were fed commercial trout feeds. During Phase 1, Trout Starter #4 and Trout Grower 1/8" (C. R. Brown Enterprises, Inc., Andrews, NJ) containing a crude protein level of 50% and crude fat content level of 15% were used. During Phase 2 of the study, Trout Grower 3/32" (Zeigler Bros., Inc., Gardners, PA) was used with crude protein and fat content of 38 and 8%, respectively.

At the onset of the study, feeding was conducted three times daily. The daily ration given was 3% of the total body weight for each tank. As daily feeding allotments increased so did the number of feeding times during the day. By the end of Phase 2, a minimum of 5 feedings a day were necessary. The addition of feeding times was based on the ability of the fish to eat all the feed at that time. During each feeding several passes were made delivering feed to each tank. The feed was cast evenly over the surface of the water. If it appeared that an excess amount of feed was reaching the bottom, then an additional feeding time was added. Feeding periods generally lasted for 15 minutes during which time observations of fish health, appearance, and activity were recorded.

Regular feeding schedules were maintained on all days with the exception of the sampling day and the day before sampling was to take place. On sampling day, fish received half of the new calculated ration for the day. This feeding technique was used during both phases of the study.

Water Quality

Water quality parameters were checked on a weekly basis, although temperature was recorded daily in the morning. Water lost to evaporation and back-flushing of the activated carbon canister within the OPS was replaced as needed. In addition to replacing lost water, particulate matter that had settled to the bottom was removed by siphoning. This was done to help maintain water quality by removing material that would decompose producing additional ammonia and other by-products.

Those parameters checked weekly included dissolved oxygen (DO), alkalinity (Alk), pH, ozone (O_3), ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3). These parameters were tested using HACH titration tests; tests were run every seven days.

Stocking

At the beginning of Phase 1, the hybrids were 120 days old. The fish averaged 11.6 g in weight and measured 100 mm in the flow-through system (TMT 1), 12.6 g and 102 mm in the single density recirculating system (TMT 2), and 11.2 g and 101 mm in the double density recirculating system (TMT 3). The system stocking data are shown in Table 1. For Phase 2, the fish weighed an average of 34.0 g and measured an average 135 mm in length in the single density recirculating system (TMT 4) and 34.0 g and 137 mm in the double density recirculating (TMT 5) (Table 2). Water quality parameters were checked one day prior to stocking for both Phases 1 and 2 in each tank so that any degradation of water quality could be identified. Water quality data are shown in Table 3 (Phase 1) and Table 4 (Phase 2).

The municipal water supply was used to fill the recirculating systems for both phases of the study. The water was run through an activated charcoal canister for dechlorination and allowed to age for three days prior to stocking.

Evaluation

The effectiveness of the OPS was determined by the average total weight gain of fish in each system and water quality conditions. Statistical analyses on the data were performed on an IBM PS/2 using PC-SAS (SAS Institute Inc., Cary, NC) and on an Apple Macintosh SE/30 using STAT-VIEW 512+ (Brain Power, Inc., Calabasas, CA) for both phases. Statistical comparisons were made to determine differences in flow-through verses recirculating systems as well as the effects of different stocking densities (Phase 1). In Phase 2, differences in growth (weight gain) of fish due to the influence of density were compared. The statistical model used for these tests was:

Table 1. System stocking rates for the three treatments (TMT) for Phase 1 of the study. Each rearing tank contained 250 L of water. TMT 1 = flow-through, TMT 2 = single density recirculating, TMT 3 = double density recirculating.

TMT	No. Stocked	Ave. Weight per Fish (g)	Tot. Weight per Tank (g)	Stocking Density (g/L)
1	150	11.6	1740.0	3.5
2	150	12.6	1890.0	3.7
3	300	11.2	3360.0	7.1

Table 2. System stocking rates for the two treatments (TMT) for Phase 2 of the study. Each rearing tank contained 250 L of water. TMT 4 = single density recirculating, TMT 5 = double density recirculating.

TMT	No. of Fish	Ave. Weight per Fish (g)	Tot. Weight per Tank (g)	Stocking Density (g/L)
4	70	34.0	2380.0	4.8
5	140	34.0	4760.0	9.5

Table 3. Phase 1 water quality parameters of the three treatments (TMT) sampled 1 day prior to stocking of HSB in each system. Shown are system averages for each parameter monitored. TMT 1 = flow-through, TMT 2 = single density recirculating, TMT 3 = double density recirculating.

TMT	DO (ppm)	Temp (C)	NH_3 (ppm)	NO_2 (ppm)	NO_3 (ppm)	pH	Alkalinity (ppm)
1	6	23	0	0	0	7.0	119
2*	6	24	0	0	0	7.0	119
3*	6	24	0	0	0	7.0	119

*Indicates parameters checked prior to utilization of ozone. Ozonation started on day 1 of the study.

Table 4. Phase 2 water quality parameters for the two treatments (TMT) sampled 1 day prior to stocking of HSB in each system. Shown are system averages for each parameter monitored. TMT 4 = single density recirculating, TMT 5 = double density recirculating.

TMT	DO (ppm)	Temp (C)	NH_3 (ppm)	NO_2 (ppm)	NO_3 (ppm)	pH	Alkalinity (ppm)
4*	6	18	0	0	0	7.0	85
5*	6	18	0	0	0	7.0	85

*Indicates parameters checked prior to utilization of ozone. Ozonation started on day 1 of the study.

$$weight = x + TMT_j + TANK(TMT)_{j(i)} + DAY_k + TMT * DAY_{ik} + DAY * TANK(TMT)_{kj(i)} + E_{l(ijk)}$$

where $i = 1, 2, 3, 4, 5, 6$

$j = 1, 2, 3,$

$k = 1, 13, 27, 36$

Length-weight calculations for the hybrid striped bass used during this study were made using a linear regression model as described by Nielsen and Johnson (1983). The least squares method was used to calculate a and b for the fish of each treatment:

$$\log W = \log a + b * \log L$$

Water quality parameter measurements were used as indications of the efficiency of the OPS as a reconditioning agent of the water relative to stocking density. Economical assessments were made on the flow-through and the recirculating systems over the 72 days of Phases 1 and 2. Comparisons were made on cost of municipal water and sewage treatment for the flow-through system against the cost of electricity to run the corona discharge generator and the pumps of the OPS. Rate schedules used to determine cost per hour for the systems were supplied by Knoxville Utility Board (KUB, Knoxville, TN). The cost per hour for one recirculating system was calculated because the systems differed only in density.

CHAPTER III

RESULTS

All statistical comparisons were made at the 95% confidence level ($p < .05$). For statistical comparisons, rearing systems (TMT) are discussed separately unless otherwise denoted.

Phase 1

The analysis of growth indicated differences in weight gain among the three treatments tested. The fish reared in the flow-through system (TMT 1) exhibited the greatest growth over the 36-day period, gaining an average of 22.1 g. Average weight gain of fish in the Single Density Recirculating System Phase 1 (TMT 2) was 17.4 g. The least growth was exhibited by fish in the Double Density Recirculating System Phase 1 (TMT 3) which averaged only 10.6 g. Figures 4 and 5 describe the weight gain of fish during Phase 1. The analysis revealed significant differences in growth between the TMT 3 and both the TMT 2 and the TMT 1. There was no statistical difference between growth of fish in the TMT 1 and TMT 2. Average food conversion ratios during the testing period for TMT 1, TMT 2, and TMT 3 were 1.5:1, 2.5:1, and 7.4:1, respectively.

After 30 days into Phase 1, the activated charcoal canister failed, allowing Cl_2 to enter the flow-through system. Therefore, the last sampling weight for TMT 1 was based on the remaining 65 of the original 150.

Data analysis confirmed that greater size variation existed in fish in the flow-

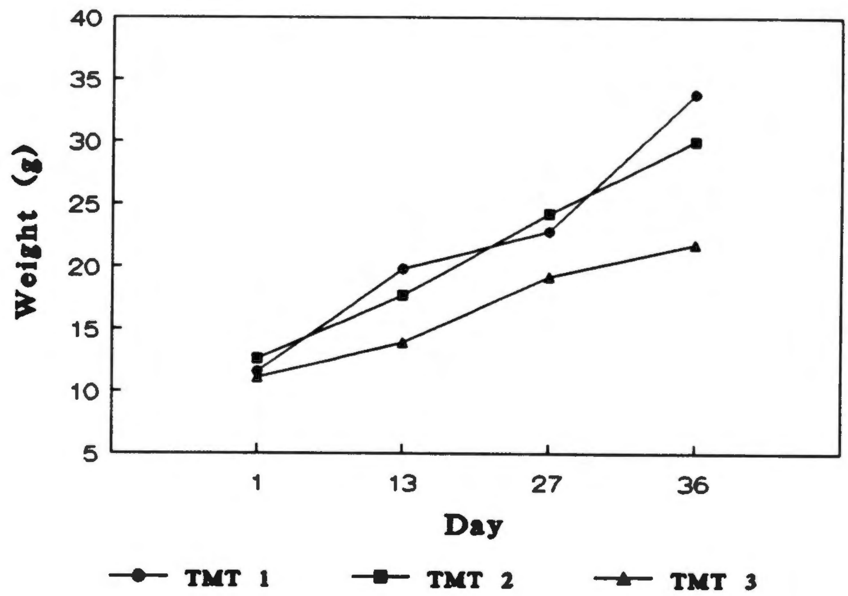


Figure 4. Average fish weight per treatment during Phase 1. Samples taken every 14 days. TMT 1= flow-through, TMT 2 = single density recirculating, TMT 3 = double density recirculating.

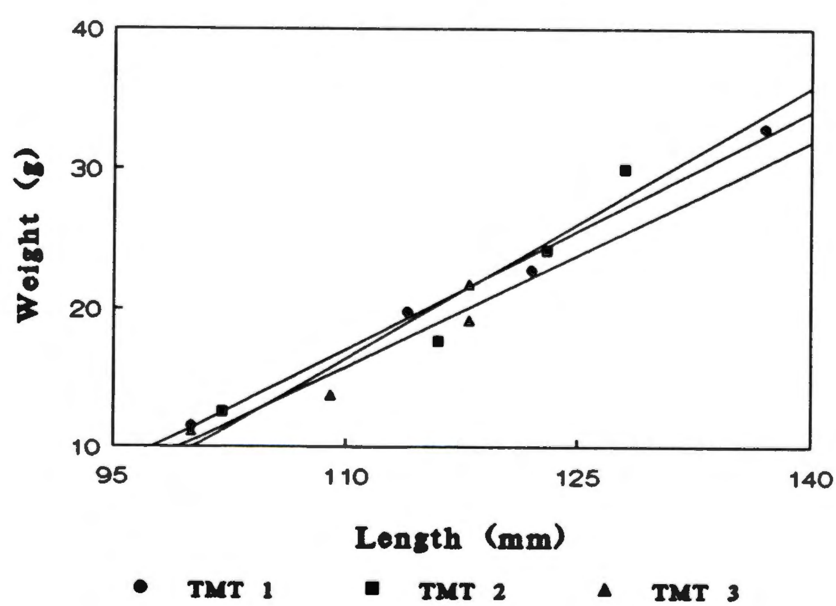


Figure 5. Length/weight relationships plotted using mean weight and length for each sampling day by treatment. TMT 1 = flow-though r^2 = 0.9964, TMT 2 = single density recirculating r^2 = 0.9624, TMT 3 = double density recirculating r^2 = 0.9534.

through and single density recirculating systems than in the double density recirculating system (Table 5).

The length/weight relationships developed for the three treatments were: TMT 1 : W = -13.13 + 3.38 * L, TMT 2 : W = -13.55 + 3.47 * L, TMT 3 : W = -14.13 + 3.58 * L. Contrasts conducted on the length/weight relationships between TMT 1 and TMT 2 and TMT 2 and TMT 3 showed no significant differences. (Figure 5).

Water Quality. During Phase 1, water temperatures ranged from 21 to 30 C, averaging 24 C for both TMT 2 and TMT 3. TMT 1 water temperatures ranged from 21 to 25 C averaging 24 C.

At no time during Phase 1 were there detectable levels of nitrogenous wastes in the FTS. In TMT 2, NH_3-N levels ranged from 0.50 to 2.5 mg/L. TMT 3 NH^3-N levels ranged from 1.0 to 2.5 mg/L (Figure 6). The trend for TMTs 2 and 3 was to start relatively high, drop somewhat, and then rise. Nitrite (NO_2-N) levels ranged from 0.18 to 2.0 mg/L in TMT 3 and 0.06 to 1.0 mg/L in TMT 2 (Figure 7). The trend for TMTs 2 and 3 was to start high, then decline, and remain relatively low. Nitrate levels ranged from 9.0 to 13.0 mg/L and 9.0 to 12.0 mg/L in TMT 2 and TMT 3, respectively (Figure 8). There was no definite trend in either TMT 2 or 3. Levels of nitrates fluctuated throughout Phase 1.

Other water quality parameters monitored (pH, DO, and alkalinity) remained within acceptable limits for fish growth. Dissolved oxygen never fell below 6 mg/L. Alkalinity (expressed as $CaCO_3$) did not fall below 85 mg/L, which helped to

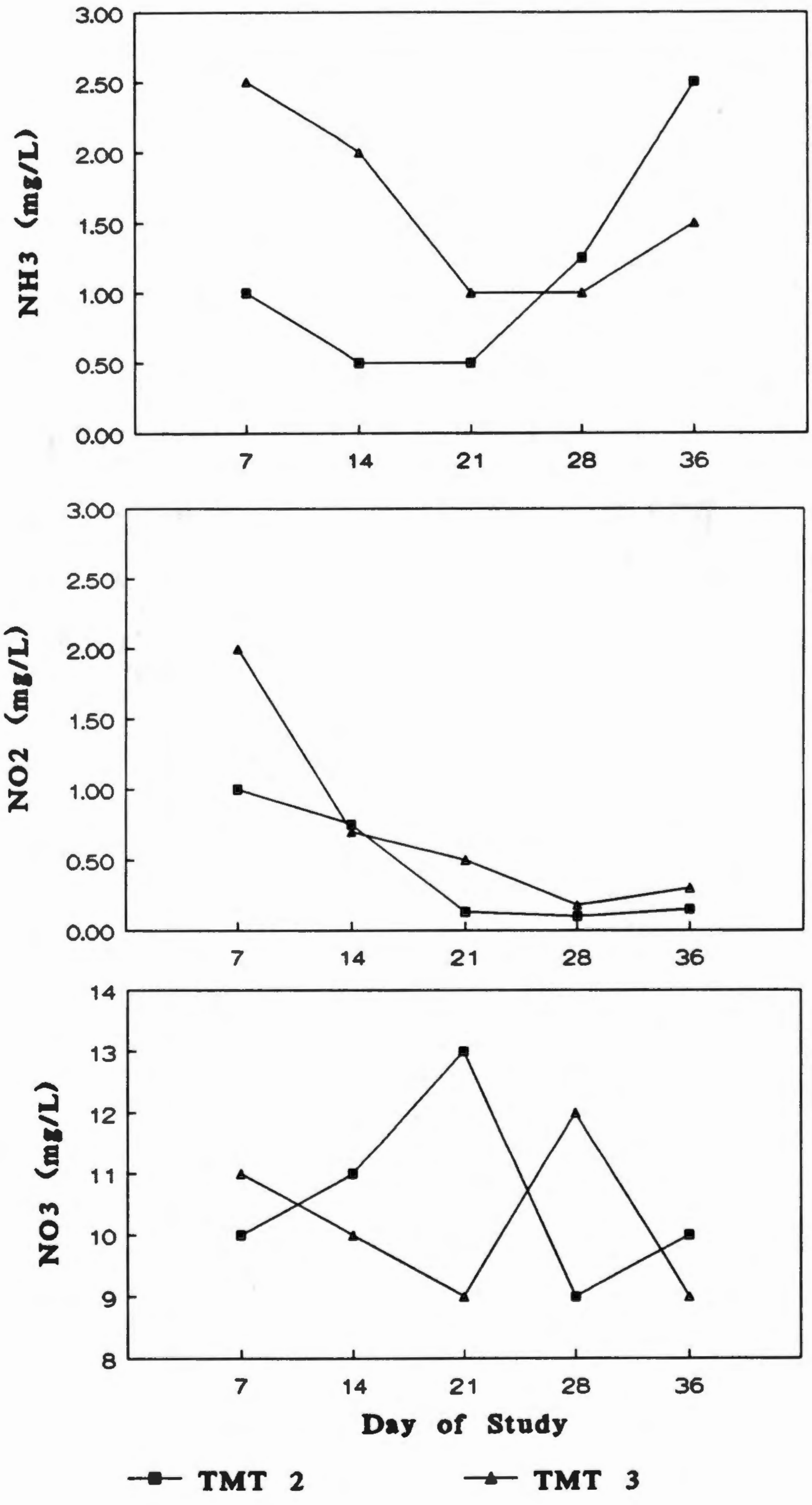
Table 5. Size variation of fish during Phase 1 of the study. Measurements taken from the randomly selected number of fish for sampling (n = 20). TMT 1 = flow-through, TMT 2 = single density recirculating, TMT 3 = double density recirculating.

		Minimum		Maximum		Range	
TMT	Day	L (mm)	W (g)	L (mm)	W (g)	L (mm)	W (g)
1	1	75	6.2	105	15.4	30	9.2
	13	105	14.3	126	26.0	21	11.7
	27	104	15.3	132	32.5	18	17.2
	36	119	15.6	166	54.7	47	39.1
2	1	85	7.5	108	15.4	23	7.9
	13	109	13.3	124	24.5	15	11.2
	27	106	14.2	132	31.5	26	17.3
	36	96	13.7	156	49.5	60	35.8
3	1	91	9.2	106	14.3	15	5.1
	13	105	11.4	115	16.2	10	4.8
	27	113	15.2	128	24.6	15	9.4
	36	104	14.4	146	36.4	36	22.0

Figure 6. TMT 2 (single density recirculating) and TMT 3 (double density recirculating) ammonia levels monitored every 7 days. TMT 1 (flow-through) not shown because there were no detectable levels.

Figure 7. TMT 2 (single density recirculating) and TMT 3 (double density recirculating) nitrite levels monitored every 7 days. TMT 1 (flow-through) not shown because there were no detectable levels.

Figure 8. TMT 2 (single density recirculating) and TMT 3 (double density recirculating) nitrate levels monitored every 7 days. TMT 1 (flow-through) not shown because there were no detectable levels.


3.00
2.50
2.00
1.50
1.00
0.50
0.00
NH3 (mg/L)
7
14
21
28
36
3.00
2.50
2.00
1.50
1.00
0.50
0.00
NO2 (mg/L)
7
14
21
28
36
14
13
12
11
10
9
8
NO3 (mg/L)
7
14
21
28
36
Day of Study
TMT 2
TMT 3

maintain pH levels between 7 and 8. All three systems (TMT 1, TMT 2, TMT 3) exhibited these same characteristics. In the single and double density recirculating systems, the ozone residual was never detected in Phase 1.

Phase 2

During Phase 2, only single density and double density recirculating systems were evaluated. In TMT 4, the single density recirculating system Phase 2, fish gained an average 13.6 g in weight over the 36-day period while fish reared in TMT 5, the double density recirculating system Phase 2, gained an average of 10.0 g in weight during the same period. There was no statistical difference in weight gain between treatments. The average fish weight for both treatments was 34.0 g at the start of Phase 2. On day 36 of the study, the fish reared in TMT 4 weighed an average of 48.0 g while the fish reared in TMT 5 weighed an average of 44.0 g. Figures 9 and 10 describe the weight of the fish during Phase 2. Food conversion ratios for TMT 4 and TMT 5 were 3.3:1 and 5.8:1, respectively. There was also variation in fish length and weight in the treatments of Phase 2 (Table 6). However, there was no apparent trend in one treatment having more variation than the other. It should be noted that measurements were taken on a randomly selected portion of the fish in each tank (n = 20).

The length/weight relationship developed for the two treatments were: TMT 4 : $W = -10.41 + 2.83 * L$, and TMT 5 : $W = -10.78 + 2.90 * L$. Statistical analysis indicated that there was no significant difference between the slopes of the lines (Figure 10).

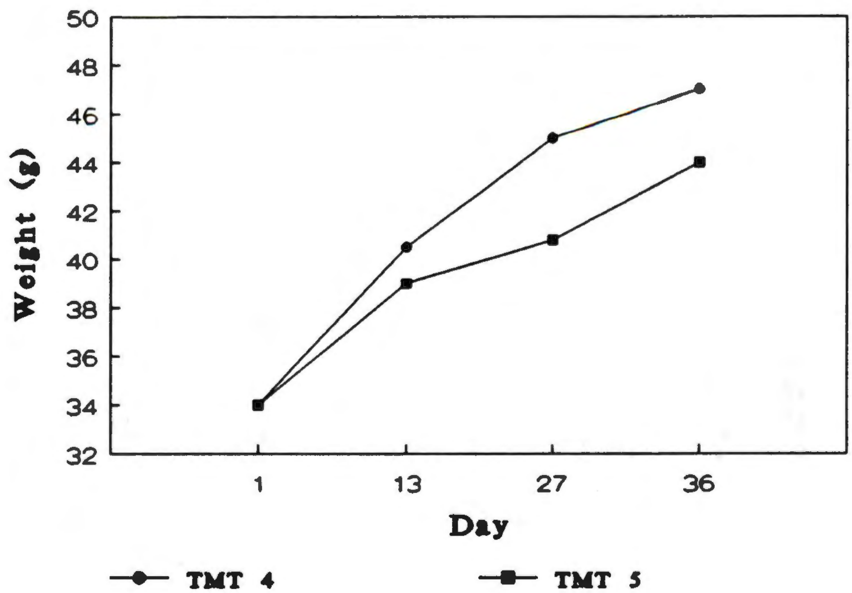


Figure 9. Average fish weight per treatment during Phase 2. TMT 4 = single density recirculating, TMT 5 = double density recirculating.

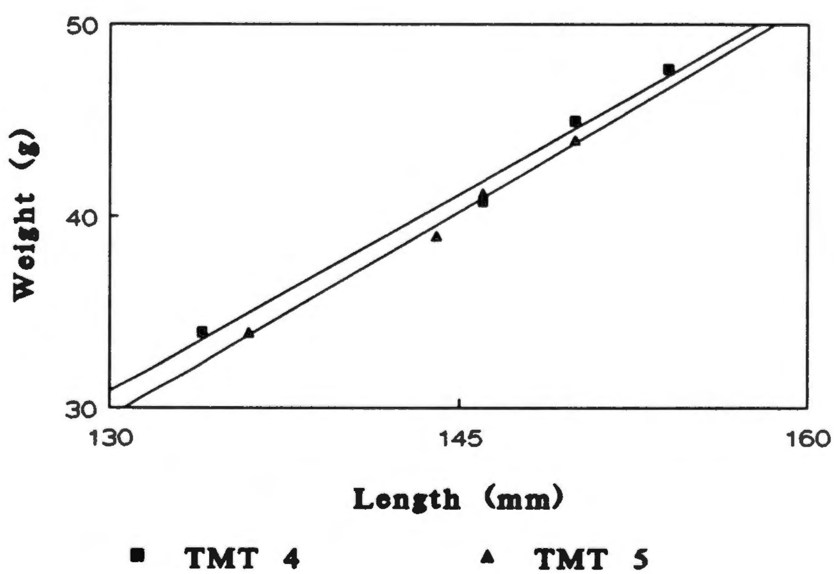


Figure 10. Length/weight relationship plot using the mean weight and length for each sampling day by treatment. TMT 4 = single density recirculating obtained an r^2 = .9928, TMT 5 = double density recirculating obtained an r^2 = .9961.

Table 6. Size variation of fish during Phase 2 of the study. Measurements taken from randomly selected fish for sampling. TMT 4 = single density recirculating, TMT 5 = double density recirculating.

		Minimum		Maximum		Range	
TMT	Day	L (mm)	W (g)	L (mm)	W (g)	L (mm)	W (g)
4	1	114	21.3	156	49.5	42	28.2
	13	124	24.3	178	62.0	54	37.7
	27	112	16.4	183	75.7	71	59.3
	36	116	19.1	184	76.3	68	57.2
5	1	119	22.0	160	54.7	41	32.7
	13	119	22.3	170	77.5	71	55.2
	27	115	18.8	171	61.8	56	43.0
	36	116	19.1	195	88.9	79	69.8

Water Quality. Water temperatures ranged from 11 to 18 C averaging 16 C for both systems. Ammonia, nitrite, and nitrate levels remained lower than those during Phase 1. Ammonia levels ranged from 0.75 to 1.75 mg/L in TMT 5 throughout the study period. The trend was for ammonia to start rising, peak , and then to continually decline. Levels for ammonia in TMT 4 ranged from 0.50 to 0.75 mg/L (Figure 11). The trend was for ammonia to stay relatively constant and then decline. Nitrite levels ranged from 0.07 to 1.25 mg/L and 0.02 to 0.12 mg/L in TMT 5 and TMT 4, respectively (Figure 12). In TMT 5 ammonia declined slightly, rose sharply, and then declined. Again, while in TMT 4 ammonia levels remained relatively constant. Nitrate levels for TMT 5 ranged from 6.0 to 9.0 mg/L and TMT 4 levels ranged from 3.0 to 4.8 mg/L (Figure 13). The trend for TMTs 5 and 4 was to rise slightly and then decline.

Other water quality parameters monitored remained within acceptable limits for fish growth. DO never dropped below 7.0 mg/L. Alkalinity remained at 68 mg/L ($CaCO_3$) while pH remained above 7.0 with a mode of 7.5.

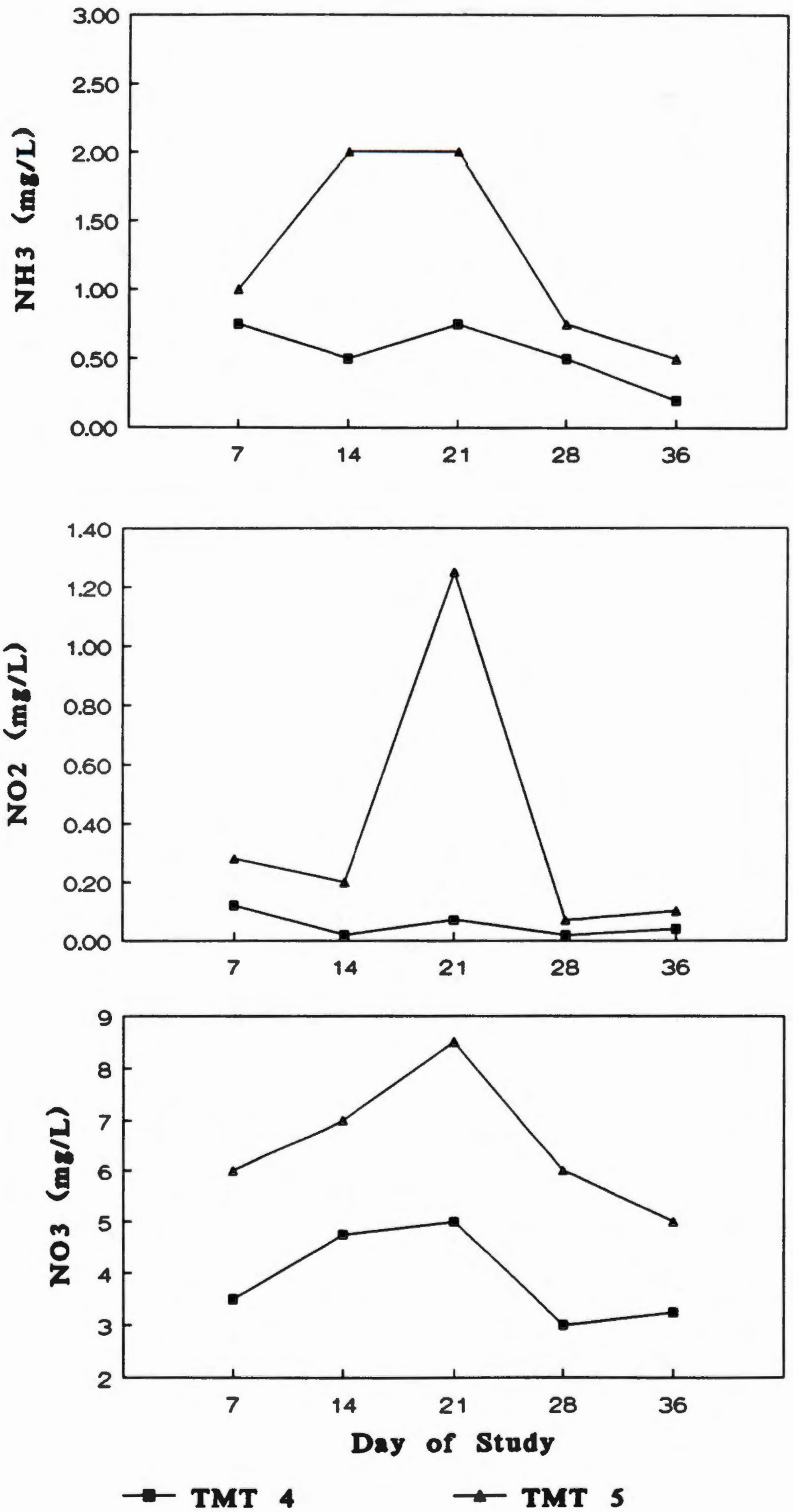
Residual ozone was never found throughout the system, but within the mixing tank (Figure 2) detectable levels of ozone were found. In both systems, concentrations reached a maximum of 0.3 mg/L with a typical residual level of 0.1 mg/L. DO levels in TMT 4 and TMT 5 remained above 7 ppm. Alkalinity held constant at 68 mg/L for TMT 4 and TMT 5 and pH held constant at 7.5 for both TMT 4 and TMT 5.

On day 34 of Phase 2, the ozone generator was inoperable in TMT 5 through

Figure 11. TMT 4 (single density recirculating) and TMT 5 (double density recirculating) ammonia levels monitored every 7 days.

Figure 12. TMT 4 (single density recirculating) and TMT 5 (double density recirculating) nitrite levels monitored every 7 days.

Figure 13. TMT 4 (single density recirculating) and TMT 5 (double density recirculating) nitrate levels monitored every 7 days.


3.00
2.50
2.00
1.50
1.00
0.50
0.00
NH3 (mg/L)
7
14
21
28
36
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
NO2 (mg/L)
7
14
21
28
36
9
8
7
6
5
4
3
2
NO3 (mg/L)
7
14
21
28
36
Day of Study
TMT 4
TMT 5

the end of the study. Water clarity declined the following day accompanied by an earthy, musty odor. Bacteriological tests (American Public Health Association 1985) for *Actinomycetes* were run on water samples and residues on the sides of the rearing tanks and the settling tanks of TMT 4 and TMT 5. Compounds produced by bacteria in the *Actinomycetes* group (i.e., geosmin and 2-methylisoborneol) are known to produce off-flavors in fish (Johnsen and Kuan 1987). Results from these tests indicated that *Actinomyctes* were present in some samples taken from TMT 5 but in not TMT 4. This suggested that ozonation may have prohibited the development of *Actinomycetes* in these recirculating systems.

CHAPTER IV

DISCUSSION

It is important to note that the OPS used was a modification of a larger model and had never been used under these conditions. Typically, the unit is used for treatment of water for human consumption. Because of this first time use, some modifications were necessary to adapt the unit to this project. In addition, the time schedule that had been established for preliminary testing of the system was changed due to the late arrival of the system itself and availability of the research area. Nevertheless, results obtained from the study give promising indications of potential usefulness of the ozone purification system.

Fish survival in both phases was excellent considering the various problems that were faced. For Phase 1, survival of fish in TMT 1 was 43% while in TMT 2 survival was 96% and 98% in TMT 3. In Phase 2 survival was 97% and 99% for TMT 4 and TMT 5, respectively.

Phase 1

Comparisons conducted between TMT 1 and TMT 2 showed no significant differences in the growth of the fish in either system. Weight gain was slightly greater in the FTS than in the SDRS1 but the difference was not statistically significant. The length/weight relationships developed for the fish reared in TMT 1 and TMT 2 were not significantly different: TMT 1, $W = -13.13 + 3.37 * L$, and TMT 2, $W = -13.55 + 3.47 * L$. Thus, the fish gained more weight as their length in both systems (Nielsen and Johnson 1983).

In TMT 1 and TMT 2, size variation of fish within each system increased as Phase 1 progressed (Table 6). This is an important point of interest to the producer, since uniformity in the final product is preferred. Jenkins et al. (1988) showed that increased stocking density produced similarly-sized stiped bass without significantly reducing weight gain. The data from the present study suggest that stocking density could be increased to decrease size variation and obtain more uniformity in sizes. The optimum density to achieve this situation can be determined with additional studies.

The average weight gain in TMT 3 fish was significantly lower than the total average weight gain for TMT 2 reared fish. The mean difference in weight in the smallest and largest fish in the TMT 2 was 35.8 g and 22.0 g in the TMT 3. Indications from this comparison suggest that density caused the reduction in weight gain. However, that may not be the case. Jenkins et al. (1988) showed that phase II hybrid striped bass (juvenile) can be grown at relatively high densities with no significant reduction in weight gain. By increasing the number of fish reared in that system, there will be an increased nitrogenous waste load and an increased demand on dissolved oxygen. Food conversion ratios showed that TMT 2 fish had a better conversion efficiency (2.5:1) than those fish reared under TMT 3 (7.4:1).

Analysis of the length/weight relationships of TMT 2 and TMT 3 revealed no statistical differences. This indicated that the water purification system handled the increased density effects with no apparent effects on growth. The maximum density increased density effects with no apparent effects on growth. The maximum density which can be supported by the OPS has yet to be determined.

A possible explaination for the decreased weight gain obtained in both recirculating systems (relative to that obtained with the FTS) centers around water quality. In both recirculating systems, nitrogenous wastes were always present. Ammonia concentrations in TMT 2 never fell below 0.5 ppm and in TMT 3 remained at or above 1.0 ppm. The optimum level of ammonia present in the water of recirculating systems for fish growth should not exceed 0.6 ppm (Bonn et al. 1976). Thurston et al. (1986) reported that depressed growth in fathead minnows resulted from exposure to sub-lethal concentrations of ammonia. This would suggest that because ammonia levels remained above 0.6 ppm, the growth of fish in TMT 3 was suppressed. One possible means to reduce the ammonia levels would be to increase the size of the bio-filter (charcoal filter of the OPS) (Figure 2).

Phase 2

During this stage of the study, only the single density recirculating system (TMT 4) and the double density recirculating system (TMT 5) were tested. Those fish reared in the TMT 4 did not gain significantly more weight than those fish reared in TMT 5. Food conversion in TMT 4 was 3.3:1 while in TMT 5 the food conversion efficiency was 5.8:1.

Analysis of size variation of fish in the two systems revealed no apparent trends (Table 7). Both systems resulted in relatively large weight ranges suggesting that the maximum stocking density still had not been reached. The length/weight relationships indicated there were no significant differences in growth in either system. During Phase 2 the increased density and resulting waste load was managed satisfactorily by the OPS.

Weight gain of the fish averaged 0.37 and 0.27 g/day for TMTs 4 and 5, respectively, in Phase 2 and 0.61, 0.48, and 0.29 g/day for TMTs 1, 2, and 3, respectively, in Phase 1. For TMTs 3, 4, and 5, the weight gain was significantly lower than weight gains reported in other studies with hybrid striped bass (Swingle 1972; Allen 1974; Smith et al. 1986).

Economic evaluation. An economic analysis of the flow-through and recirculating systems indicated that utilizing the flow-through system was more costly than employing the recirculating system. It was determined that the FTS required 1585.9 m^3 of water over the 72-day period (22.03 m^3 of water/day); during the same period of time, 878.4 Kw of electricity were required to run the pumps (12.2 Kw of electricity/day) of the recirculating system. The electrical requirements for ozone production was negligible on a per day basis ($ 0.06/day) Municipal water costs were $ 1240.34 ($ 596.34 without waste water service) within the city limits and $ 1513.80 ($ 802.60 without waste water service) outside the city limits. The electrical cost for the recirculating system was $104.76 over the 72-day period with an additional $ 4.32 for the cost of ozone generation. The recirculating system required 125 L of maintenance water costing $ 35.77 (inside the city limits) or $ 53.45 (outside the city limits) bringing the total operating costs to $ 144.85 within city limits and $ 162.54 outside city limits.

A comparison of the two systems (inside the city limits) indicated that the FTS cost $ 17.23/day to operate (with waste water service) as compared to $ 2.01/day for the recirculating system using ozone. This indicated the OPS was 88.3% more effective than the flow-through system. The cost of using the flow-through system can be reduced by eliminating the waste water service. By utilizing waste water lagoons or some other system to reduce the monthly output of waste water the cost can effectively be cut in half.

Present regulations restrict the use of public waters for aquaculture and the discharge of effluents into natural waters as well as the destruction of wetland areas (McCraren 1989). With closed system aquaculture, effluent discharge volumes are considerably lower than those requirred for flow-through systems.

CHAPTER V

SUMMARY

1. The growth of fingerling hybrid striped bass was used as an indicator of a water reconditioning unit employing ozone within a recirculating culture system. In Phase 1, fingerlings averaging 11-12 g were stocked at equal densities (1X) in flow-through and recirculating systems and at twice the normal density (2X) in another recirculating system. In Phase 2, fish averaging 34 g were stocked only in single (1X) and double density (2X) recirculating systems. The recirculating systems in both phases employed an ozone purification system (OPS) for water conditioning.

2. There were no significant differences in growth of fingerlings in the Phase 1 single density systems (flow-through and recirculating); mean weight gain was 0.6 g/day and 0.5 g/day, respectively. Mean weight gain in the double density recirculating system was significantly less at 0.3 g/day.

3. In Phase 2, the larger fingerlings gained 0.4 g/day and 0.3 g/day, respectively, in single and double density recirculating systems. There were no significant differences in growth of fingerlings in these systems.

4 Length/weight relationships developed for fish in Phase 1 indicated similar growth patterns in all three systems; the same situation was found in Phase 2 with fish from the single and double density systems. The smaller fingerlings in Phase 1 studies increased more rapidly in weight when compared

to length than did the larger fingerlings in Phase 2.

5. Alkalinity, pH, and dissolved oxygen remained at acceptable levels for fish growth during the entire study period in all systems. Dissolved oxygen levels never fell below 6.0 mg/L. It is believed that the utilization of ozone helped to maintain dissolved oxygen levels.

6. Nitrogenous wastes were not detected in the flow-through system, but were recorded in the recirculating systems in Phases 1 and 2. Ammonia, nitrite, and nitrate levels were generally lower in Phase 2 than in Phase 1.

7. Raising striped bass hybrids in the recirculating systems using ozone as a water purifying agent was only 11.7% as expensive as raising them at similar densities in a flow-through system.

8. Bacteriological tests on water and residues from TMT 5 tanks which had been without ozone treatment for 3 days indicated the presence of bacteria of the *Actinomycetes* group. This suggests that the ozonation system prevented the development of *Actinomycetes* bacteria which are known to produce off-flavors in fish.

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