THESIS ABSTRACT

Espey, James Lee. Acute Toxicity of Ammonia and Nitrite to Yellow Perch, *Perca flavescens* (Under the direction of Harry V. Daniels.)

Yellow perch (*Perca flavescens*) is a commercially valuable fish in the Midwestern U.S. There is growing interest in the culture of yellow perch to compliment the dwindling commercial harvest. However, there is little information about the water quality parameters needed to optimize yellow perch growth; especially in indoor tank systems. This information is needed to develop water reuse systems and filters to treat culture water. A series of static toxicity tests were conducted to determine the tolerance of yellow perch to ammonia and nitrite on larvae, juveniles and adult fish. Toxicity test on adults were conducted at 18, 22, and 25 C. The tests on larvae showed highly variable results and were ultimately inconclusive. The 96 h LC_{50} for ammonia and nitrite to juveniles were 0.77 mg/L as NH_3-N and 78.24 mg/L as NO_2-N, respectively. Adult and juvenile yellow perch had similar tolerance levels. LC_{50} values for ammonia and nitrite varied with temperature and were 0.64 mg/L as NH_3-N and 65.8 mg/L as NO_2-N, respectively at 22 C. Toxicity values at 25 C were similar to those reported at 22 C. Lower water temperature reduced the toxicity of ammonia and nitrite to yellow perch adults. At 18 C, the LC_{50} values for ammonia and nitrite were 59% higher (1.02 mg/L as NH_3-N) and 34% higher (88.5 mg/L as NO_2-N), respectively than the values at 22 C.

Based on the results obtained from the above studies, a theoretical biological filter for removal of ammonia and nitrite was designed. The filter is larger than those currently in use for culture of *Tilapia sp.* The filter would need a surface area of 6.68 m^2, and a diameter of 2.92 meters. It would also need 14.1 kg per day of oxygen added to the

system, and have an estimated 199.0 lpm flow rate. This would support a fish biomass of 3,623 kg, with a fish weight of 150 g.

Acute Toxicity of Ammonia and Nitrite to
Yellow Perch, *Perca flavescens*

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It has been a long road....

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Acute Toxicity of Ammonia and Nitrite to Yellow Perch

1.1   Introduction

Ammonia and nitrite accumulation in aquaculture can lead to several problems for fish, including increased susceptibility to disease and death (Reviewed by Lewis and Morris 1986). The primary sources of ammonia are from fish feeds and fish wastes, which contain large amounts of nitrogen (Reviewed by Lewis and Morris 1986). Excess nitrogen excreted by the fish is converted to ammonia, and then nitrite, through bacterial processes. In culture systems with low water exchange rates, or inadequate filters, ammonia and nitrite can rapidly build up to lethal levels.

Ammonia exists in water in both an ionized (NH_4^+) and un-ionized form (NH_3). This equilibrium depends on pH, temperature, and salinity, with the un-ionized form making up a much smaller percentage. The gill membrane epithelium is very effective in preventing the ionized form from entering the bloodstream of the fish. However, it is not an effective barrier to the un-ionized form, allowing it to enter the bloodstream (Wilkie 1997). A small increase in either temperature or pH will cause an increase in the amount of un-ionized ammonia by several percent.

Nitrite (NO_2^-) is formed from ammonia through bacterial nitrification. Nitrite enters the fish's red blood cells, changing the hemoglobin molecule in the cell to methemoglobin, reducing its ability to carry oxygen. This results in a condition commonly referred to as Brown Blood Disease, where the gills actually turn a brownish color. Chloride levels can affect the toxicity of nitrite (Reviewed by Lewis and Morris 1986). It has been shown that in water with higher chloride levels, a higher concentration

of nitrite is required to cause mortality. Chloride cells in the gills of fish take up nitrite. A higher chloride concentration in the water makes it more likely that chloride is taken in at these sites, effectively blocking nitrite uptake (Reviewed by Lewis and Morris 1986).

The median lethal dosage is expressed as an LC_{50} value, which is the concentration one could expect 50% of a given population of fish to die from exposure to the toxicant. From the LC_{50} value, a NO Effect Level (NOEL) can be obtained. The NOEL is the concentration of the toxicant where one can expect the fish to live with no long-term adverse effects. Studies conducted on a variety of species have indicated that a safe NOEL is 9% of the LC_{50} value (Ashe et al. 1996, Tomasso 1994). Toxicity to ammonia and nitrite is highly variable across different species of fish. Shortnose sturgeon fingerlings kept at 18 C, in water with less then 1.0 g/L Cl^-, had LC_{50} values of 0.58 mg/L as NH_3-N for un-ionized ammonia, and 11.3 mg/L as NO_2-N for nitrite (Fontenot, et al. 1998). Hybrid striped bass fingerlings kept at 23 C, had LC_{50} values of 0.68 mg/L as NH_3-N for un-ionized ammonia and 18.8 mg/L as NO_2-N for nitrite (Harcke 1999). Large mouth bass have been found to have an LC_{50} for nitrite of 140 mg/L as NO_2-N (Palachek and Tomasso 1984). The toxicity can even vary by age of the fish, the LC_{50} of fingerling hybrid striped bass to nitrite was found to be 18.8 mg/L as NO_2-N, and for adults, was 23.5 mg/L as NO_2-N (Harcke 1999).

Yellow perch, (*Perca flavescens*), are in the family Percidae, order Perciformes. Perciformes is the largest order of vertebrates. The family Percidae includes the perches and darters. There are at least 162 species in Percidae, of which 150 are found in North America. The yellow perch's natural range is mid-Canada to Missouri, and along the Atlantic coast to South Carolina. They can also be found isolated along the gulf coast

from Louisiana to west Florida. Yellow perch typically live in schools in open waters in cool and temperate streams, lakes, ponds and reservoirs, and can reach a size of 38 cm and 1.9 kg.

Yellow perch are commercially valuable fish in the mid west and Canada. However, due to over fishing of natural stocks, the fishing industry can no longer keep up with the demand, increasing the interest in the culture of yellow perch on farms. Because of their natural range and preference for schooling in cool open waters, yellow perch on North Carolina farms will likely show best results in indoor water reuse systems.

Recirculating systems, as an identifying feature, reuse the same water. This is accomplished by taking water from the culture tanks, passing it through a filtering system, and returning the water to the culture tanks. In ponds and flow-through systems, where water is used then discarded into ditches and settling ponds, ammonia and nitrite are quickly flushed from the system. In recirculating systems, over feeding or inadequately sized filters can allow ammonia and nitrite to build to lethal concentrations.

The purpose of this study was to determine the median lethal dosage of ammonia and nitrite to yellow perch at different life stages: larval, juvenile, and adults. Additionally, the adults were tested at several different temperatures, to better relate the data with the juveniles, and to examine the effect temperature has on the lethal concentrations.

This information would be useful in forming guidelines or benchmarks for the management of water quality in reuse systems for yellow perch, such as what size filter is needed for the stocking densities and feeding rates required. It will also help farm and

hatchery managers in determining if there is a danger of fish mortality due to ammonia or nitrite levels present in their systems.

1.2 Materials and Methods

Static renewal tests were done on 4-month-old juveniles and adult yellow perch according to Standard Methods (APHA et al. 1995) to determine the 96 h median lethal concentrations of un-ionized ammonia and nitrite. The tests were conducted at the North Carolina Department of Agriculture's Tidewater Research Station in Plymouth, North Carolina. Juveniles and adults were obtained from Onley the Best Fish Farm in Hertford, NC and the Edenton National Fish Hatchery, Edenton, NC. Eggs were collected from ponds and rivers where adult females used branches along the shores to anchor the strips of eggs. The eggs were then placed in vats, which used flow through filtration, with a fry collector placed in line between the vat and the drain. The larvae were raised in 70-liter containers that also used flow through filtration.

General Experimental Protocol

The well water used for these tests was drawn from the Castle Hayne aquifer and had a total hardness of 225 mg/L as $CaCO_3$, calcium hardness of 50 mg/L as $CaCO_3$, a chloride level of 90 mg/L, total alkalinity of 300 mg/L as $CaCO_3$, and conductivity of 1500 umhos/cm (Harcke and Daniels 1999).

In each case, the control containers had only well water. The test containers were dosed with ammonium chloride for the un-ionized tests, or sodium nitrite for the nitrite tests. All tests for the juveniles were done in triplicate. The tests for the adult stages

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were done in duplicate. Salinity (Yellow Springs Instrument Co., Inc., Model 33-S-C-T, Yellow Springs, OH), temperature, and pH (Hanna Instruments, Model HI 9023, Ronchi di Villatranco, Italy) were measured daily. The water temperature was allowed to fluctuate with the ambient air temperature, which was controlled with a commercial grade air conditioner.

Water samples were taken daily from each container. Ammonia was measured using the Phenate method. Nitrite was determined using the diazotization method (APHA et al. 1995). Percent un-ionized ammonia was calculated based on the temperature and pH. Water was removed from the containers once daily, and replaced with fresh well water. The containers were then re-dosed as appropriate to achieve target concentrations. Test containers were checked at the following intervals: 1.5, 3, 6, 12, 24, 36, 48, 60, 72, 84, and 96 hours post initial dosing (APHA et al. 1995). Dead fish were removed and counted at each interval. A fish was determined to be dead if it did not respond to stimulus and had an opaque color. Test duration for both juvenile and adult fish was 96 hours.

The 96 h LC_{50} values were calculated using statistical analysis software (SAS 8.0, see Appendix 1). The NOEL was calculated as 9% of the 96 h LC_{50} for ammonia and nitrite at each life stage and each temperature (Ashe et al. 1996).

Larvae

One-liter glass containers were used to hold the larvae, without supplemental aeration. Oxygen levels in the jars were measured daily and never fell below 7.0 mg/L. The jars were placed in water baths to maintain a constant temperature of 19 C. Larvae

were removed from the stock tank with a beaker then counted onto a Petri dish. Ten to fifteen larvae were placed in each jar.

Juveniles

One hundred and fifty juvenile fish, approximately four months old, with a mean weight of 6 g, were divided randomly among 10 tanks, each with a 15 L capacity. The weight of the fish was determined by placing a 150 ml beaker on a scale and taring it. Then a random selection of fish was placed in the beaker, and it was weighed again. The weight was then divided by the number of fish in the beaker. This was done 20 times. The fish were not fed for the 24 hours prior to being placed in the test containers. Once in the test containers, they were allowed to acclimate for 24 hours with supplemental aeration. After the acclimation period was over, the containers were dosed to the appropriate concentration that had been randomly assigned to it. Each day, 10 L of water was removed, and fresh well water was added, then re-dosed to the appropriate concentration.Adults

Adult yellow perch, approximately one year old, were used for these tests. One hundred fish were randomly selected out of the population, weighed and measured. The mean total length of the population was 17.4 centimeters, and the mean weight was 55.7 grams. The fish were placed in a bath with a few drops of Eugenol to knock them out. The fish were not placed back into the population. For the tests, 10 fish were randomly selected from the population, placed in each test container, and allowed to acclimate for 24 hours. Black plastic gridding was placed on the top of each container to minimize outside disturbances, lighting levels, and prevent jumpers. In all tests, 40 L of water was removed daily, and replaced with fresh water. The container was then re-dosed to the

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appropriate concentration. The ammonia test for adult fish at 22 C was run twice. In the initial run, the concentrations used had a high mortality in the higher concentrations, and no mortality in the low end. A second run was conducted, using more concentrations in the middle, and the data were combined.

To obtain the three temperatures for the adults, the water was heated first if needed, and then heaters were placed in the test containers to keep the water at the given temperature. For the 18 C water, the water from the well was already at the desired temperature. For the 22 C and the 25 C tests, water was placed into two 1000 liter tanks, and heated to the desired temperature the day before it was used. Once used, the tanks were refilled and heated to be used for the next day.

1.3 Results

The target concentrations varied from test to test, depending on the results of range-finding tests. The target concentrations were then chosen to bracket the most likely true toxicity value. Measured concentrations never varied from the target concentrations by more than 15%. In all tests, the pH ranged between 8.4 and 8.7, and the dissolved oxygen never fell below 7.0 mg/L. The target temperatures for the temperature effects on toxicity experiments were 18 C, 22 C, and 25 C.

Larvae

Several attempts were made to determine LC_{50} values for the larval stage of yellow perch. However, meaningful results were not obtained. In each case, after 96 hours had passed, all larvae in a treatment were either alive or dead. There were no intermediate mortalities at the concentrations chosen. Apparently, the toxic range is so narrow that we

were unable to properly achieve intermediate mortalities using these methods. Due to the short breeding season for yellow perch, more larvae could not be obtained for that year for repeated tests with different concentrations

Juveniles

For the tests on juvenile fish, the water temperature ranged between 21.3 C and 22.7 C, the pH ranged between 8.4 and 8.75, and the oxygen levels exceeded 7.0 mg/L when tested each day. The control containers for the nitrite tests never exceeded 0.29 mg/L as NO_2-N and for the ammonia tests concentrations never exceeded 0.3 mg/L as NH_3-N. There were no mortalities in any of the control treatment.

Ammonia

The 96 h LC_{50} (95% confidence intervals) for juveniles at 22 C was 0.77 mg/L (0.54, 1.0) as NH_3-N (Table 1). Fish, as they approached mortality, became very dark and excitable, with a few jumping out of their containers. These deaths were not counted, even though the ammonia concentration was the likely reason for the jumping. Once placed in fresh water, the fish seemed to recover very quickly.

Nitrite

The 96 h LC_{50} (95% confidence intervals) for nitrite was found to be 78.2 mg/L (67.4, 88.6) as NO_2-N. Fish, as they approached mortality, became very light and lethargic. Once placed in fresh water, the fish seemed to recover very quickly.

Adults

For the adult stages, three temperatures were used: 18 C, 22 C, and 25 C, and the temperature never exceeded ± 1C from the target temperatures. The pH ranged between 8.4 and 8.7 across all the tests, and the oxygen levels exceeded 7.0 mg/L when tested daily. The control containers for the nitrite tests never exceeded 0.29 mg/L as NO_2-N

and for ammonia they never exceeded 0.3 mg/L as NH_3-N. There were no mortalities in any of the control containers.

Ammonia

The 96 h LC_{50} (95% confidence interval) for adults at 18 C was 1.02 mg/L (0.96, 1.08) as NH3-N. For the 22 C series of tests, it was 0.64 mg/L (0.57, 0.69) as NH_3-N. For the 25 C series of tests, it was 0.66 mg/L (0.58, 0.80) as NH_3-N. There were four concentrations with intermediate mortalities. These were used to calculate the 96 h LC_{50} values. In all temperatures, those in concentrations of less then 0.3 mg/L unionized ammonia suffered no mortalities. In concentrations of 1.0 mg/L and higher, full mortality was realized in all temperatures except 18 C. Fish, as they approached mortality, became very dark and excitable, with a few jumping out of their containers. These deaths were not counted, even though the ammonia concentration was the likely reason for the jumping. Once placed in fresh water, the fish seemed to recover very quickly.

Nitrite

The 96 h LC_{50} (95% confidence interval) for the adult nitrite test at 18 C was 111.8 mg/L (87.1, 141.0) as NO_2-N. For the 22 C series of tests, it was 65.8 mg/L (52.7, 77.0) as NO_2-N. For the 25 C test, it was 58.4 mg/L (42.1, 71.4) as NO_2-N. Other than the controls, all concentrations suffered some mortality. Fish, as they approached mortality, became very light and lethargic. Once placed in fresh water, the fish seemed to recover very quickly.

1.4 Discussion

Ammonia and nitrite are toxic to fish. Ammonia and nitrite are related in that ammonia becomes nitrite through bacterial processes. Ammonia and nitrite can build up

to toxic levels in a recirculating system through the use of fish feed and waste products of the fish. Therefore, knowing the threshold of toxicity to a species of fish is very important to controlling mortality of yellow perch in culture systems.

The toxicity of ammonia and nitrite are affected by a myriad of other factors, such as temperature, salinity, and pH (Reviewed by Lewis and Morris 1986). These factors can either affect the toxicity of ammonia and nitrite directly, or the effect can be indirect through stress placed on the fish, lessening its ability to cope with toxicants.

In a recirculating system, factors such as temperature can be controlled fairly easily. However, the only real method of preventing the build up of ammonia and nitrite to toxic levels is through the use of effective biological filtration. A filtration system can be an expensive consideration to any farming enterprise. To help control these costs, and make the farming enterprise more profitable over all, a filtration system that can just meet the demands of the intended species is used. This is based on the level of sensitivity of the species to these toxicants. To determine the level of filtration one needs, one must discover how much of the toxicant the species can withstand. This is typically found through LC_{50} studies. This gives the concentration where a farmer could reasonably expect to see half his stock die due to ammonia or nitrite.

However, in real terms, this is not very practical to the fish farmer, as any enterprise that has a system where half the stock dies will fail. Therefore, a no effect level, or NOEL, is determined from the LC_{50}. At this concentration, the farmer could reasonably expect to see no effect on his stock from the toxicant. The biological filtration size will be based on the NOEL value. By EPA standards, this is usually calculated as approximately 9% of the LC_{50} concentration.

Each species has a different LC_{50} and NOEL, and this can be the determining factor in both the suitability of the fish for a farming enterprise, and how that enterprise is set up because of filtration needs.

Rainbow trout, *Salmo gairdneri*, have been found to be fairly sensitive to ammonia and nitrite, with a 96 h for ammonia of about 0.44 mg/L as NH_3-N. the 96 h LC_{50} for nitrite, with a chloride level of 20 mg/L and 12 C, was found to be 6.6 mg/L as NO_2-N (Thurston, R.V., R.C.Russo 1983). Trout are typically farmed in raceways, with a river diverted into the raceways, to provide constant flushing of the toxicants. Blue tilapia, *Oreochromis aureus*, on the other hand, is on the other end of the spectrum of tolerance, handling extremely high concentrations of ammonia. They have been found to have an ammonia LC_{50} of over 2 mg/L, allowing them to be packed into containers, one on top of the other, with little filtration. They have been found to have a nitrite LC_{50} of 15.0 mg/L as NO_2-N with a chloride level of 20 mg/L as NO_2-N at 23 C (Reviewed by Lewis and Morris 1986). The hybrid striped bass, (*Morone chrysops* X *M. saxatilis*, has an intermediate tolerance to ammonia and nitrite, with an ammonia LC_{50} of approximately 0.65 as NH_3-N. The nitrite LC_{50} for four month old fingerlings, in the same well water used for the yellow perch was 18.8 C (Harcke and Daniels1999). Hybrid striped bass are cultured in both recirculating systems and ponds. Finally there is the channel catfish, *Ictalurus punctatus*, which has been found to have an ammonia LC_{50} value of 3.8 mg/L as NH_3-N and a nitrite LC_{50} value of 44 mg/L as NO_2-N (Holt 1976). However, this study was done in the mid-70's, before it was understood that the chloride level would have an affect, and so it was not reported. Another study, in which the chloride level was

reported at 20 mg/L, the LC_{50} was found to be 6.4 mg/L as NO_2-N (Reviewed by Lewis and Morris 1986).

Because the tests done on the hybrid striped bass were conducted using the same methods and well water as the yellow perch, and they are farmed in both ponds and recirculating systems, it is a very good comparison fish for the yellow perch.

Yellow perch have an LC_{50} of about 0.75 mg/L as NH_3-N, making it very similar in tolerance to hybrid stripped bass in practical terms. In our experiments, temperature also played a roll, with lower temperatures making yellow perch somewhat less sensitive to ammonia and nitrite. At 18 C, the 96 h LC_{50} for ammonia in regards to un-ionized ammonia was found to be about 1 mg/ L as NH_3-N. This implies that yellow perch, kept at about 18 C, would make a good candidate for fish farming.

It may also be worthy of note that the geographical region of origin of yellow perch may affect the ability of the species to cope with ammonia and nitrite. There is some evidence now that the population in North Carolina is less heat sensitive than the populations of the northern mid west, allowing them to spend less energy dealing with heat stress and more energy on dealing with toxicity. This could also imply that yellow perch from the North Carolina region would make better candidates for fish farming than the northern populations, since less money would have to be spent on controlling the temperature, and for any given temperature, less money spent on filtration. More research is needed in this area to evaluate the effect of temperature on tolerance to environmental toxicants such as ammonia and nitrite.

1.5 List of References

Ashe, D., J. R. Tomasso, and A. G. Eversole. 1996. Toxicity of Ammonia to Fingerling White Bass: Effect of Selected Environments on Uptake Dynamics. The Progressive Fish- Culturist, 58:277-280.

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Federation. 1995 Standard Methods for the Examination of Water and Wastewater, 19th Edition. APHA, Washington, DC, USA.

Bartlett, F., D. Neumann. 1998. Sensitivity of Brown Trout Alevins (*Salmo trutta* L.) to Nitrite at Different Chloride Concentrations. Bulletin of Environmental Contamination and Toxicology, 60:340-346.

Bower, C. E., J. P. Bidwell. 1978. Ionization of Ammonia in Seawater: Effects of Temperature, pH, and Salinity. Journal of Fisheries Research Board of Canada, 35:1012-1015.

Calamari, D., R. Marchetti and G. Vailati. 1981. Effects of Long Term Exposure to Ammonia on the Developmental Stages of Rainbow Trout (*Salmo Gairdneri* R.). Rapp. P.-v. Reun. Cons. Int. Explor. Mer, 178: 81-86

Daniels, H. V., C. E. Boyd, and R. V. Minton. 1987. Acute Toxicity of Ammonia and Nitrite to Spotted Seatrout. The Progressive Fish-Culturist, 49:260-263.

Fontenot, Q. C., and J. J. Isely, J. R. Tomasso. 1998. Acute Toxicity of Ammonia and Nitrite to Shortnose Sturgeon Fingerlings. The Progressive Fish-Culturist, 60:315-318.

Frances, J., B. F. Nowak, G. L. Allan. 2000. Effects of Ammonia on Juvenile Silver Perch (*Bidyanus bidyanus*) Aquaculture, 183: 95-103.

Guillen, J. L., M. Endo, J. F. Turnball, H. Kawatsu, R. H. Richards, and T. Aoki. 1993. Depressed Growth Rate and Damage to the Cartilage of Red Sea Bream Larvae Associated with Exposure to Ammonia. Nippon Suisan Gakkaishi, 59:1231-1234.

Harcke, J. E., H. V. Daniels. 1999. Acute Toxicity of Ammonia and Nitrite to Reciprocal Cross Hybrid Striped Bass. Masters Thesis.

Hargreaves, J. A., S. Kucuk. 2001. Effects of Diel Un-Ionized Ammonia Fluctuation on Juvenile Hybrid Striped Bass, Channel Catfish, and Blue Tilapia. Aquaculture, 195:163-181.

Holt, J., G. Tchobanoglous. 1976. Evaluation of the Short Term Toxicity of Nitrogenous Compounds to Channel Catfish, *Ictalurus Punctatus*. Aquaculture, 8:209-224.

Lewis, William M., Donald P. Morris. 1986. Toxicity of Nitrite to Fish: A Review. Transactions of the American Fisheries Society. 115:183-195.

Person-Le Ruyet, J., H. Chartois, L. Quemener. 1995. Comparative Acute Ammonia Toxicity in Marine Fish and Plasma Ammonia Response. Aquaculture, 136: 181-194.

Soderberg, R. W., J. B. Flynn, H. R. Schmittou. 1983. Effects of Ammonia on Growth and Survival of Rainbow Trout in Intensive Static-Water Culture. Transactions of the American Fisheries Society, 112:448-451

Thurston, R.V., R. C. Russo, R. J. Luedtke, C. E. Smith, E. L. Meyn, C. Chakoumakos, K. C. Wang, C. J. D. Brown. 1984. Chronic Toxicity of Ammonia to Rainbow Trout. Transactions of the American Fisheries Society, 113: 56-73.

Thurston, R. V., R. C. Russo. 1983. Acute Toxicity of Ammonia to Rainbow Trout. Transactions of the American Fisheries Society, 112:696-704

Tudor, M., I. Katavic, J. Marsic-Lucic. 1994. Acute Toxicity of Ammonia to juvenile Sea Bass (*Dicentrarchus labrax* L.) at Different Aeration Levels. Aquaculture, 128:89-95.

Vedel, N. E., B. Korsgaard, F. B. Jensen. 1998. Isolated and Combined Exposure to Ammonia and Nitrite in Rainbow Trout (*Oncorhynchus mykiss*): Effects on Electrolyte Status, Blood Respiratory Properties and Brain Glutamine/Glutamate Concentrations. Aquatic Toxicology, 41: 325-342.

Weirich, C. R., J. R. Tomasso. 1993. Toxicity of Ammonia and Nitrite to Sunshine Bass in Selected Environments. Journal of Aquatic Animal Health, 5: 64-72.

Wilkie, M. P. 1997. Mechanisms of ammonia Excretion Across Fish Gills. Comparitive Biochemistry and Physiology. 118A:39-50.

Design of a Biological Filter for Removal of Ammonia and Nitrite Water used to Culture Yellow Perch

2.1   Introduction

Water reuse systems are an efficient means of reducing the amount of water needed to culture fish. Although the specific components of a reuse system are highly variable, the principles (unit processes) of physically removing the solids (i.e. fish feces and uneaten feed) and biologically removing ammonia-nitrogen and nitrite-nitrogen are common to all systems. Physical removal of solids is accomplished through the use of various screen technologies, granular media filters or gravity separators (settling basins or swirl separators). Biological removal of potentially toxic substances (un-ionized ammonia-nitrogen and nitrite-nitrogen) requires the use of bacterially-based biological filters.

Biological filters are designed to optimize the conditions for autotrophic bacteria to convert toxic metabolites to less toxic compounds. These filters are basically containers with added substrate that provide surface area for the bacteria to grow. There are a wide variety of biofilter media and flow schemes for these filters. For purposes of this study, a general formula that applies to all types of filters will be used.

Because biological filters are designed to promote autotrophic bacterial growth, environmental conditions that influence bacterial growth are important to filter design. The main factors are: 1) dissolved oxygen for respiration, 2) temperature for optimum growth, 3) pH, 4) ammonia-nitrogen and nitrite-nitrogen concentration, and 5) organic

waste concentration. The feed that is given to the fish is typically the sole source of nutrients in a water reuse system.

Protein in the feed is broken down and assimilated by the fish. Unfortunately, fish are only able to convert about 20% of the protein into fish flesh. The rest is excreted via the gills or in feces into the water. The nitrogen in the protein in feces and uneaten food is broken down and turned into ammonia by heterotrophic bacteria (ammonification). Thus, high feed rates can result in high levels of ammonia in the culture water if it is not removed at a rate equal to production. Ammonia is further processed by nitrifying bacteria into nitrite and nitrate (nitrification). Nitrate is not very toxic to the fish but nitrite can cause methemoglobinemia or brown blood disease. The bacteria responsible for nitrification are highly sensitive to ammonia-nitrogen concentration and somewhat sensitive to temperature. Low temperatures will slow nitrification, however high temperatures are stressful on the fish. In most cases, it is not yet economically practical to biologically remove nitrate-nitrogen so the water must be periodically discharged and replaced with new water.

It is imperative to have a biological filter large enough for the demands of the expected feeding rates within the facility. However, because initial investment cost is a large concern for any farm, it is equally important to ensure the filter is not unnecessarily large. This requires careful planning even before the first tanks are put in place.

In the past, the design of the filtration system has been largely a trial and error process. This has been because either the engineers have not been interested in aquaculture, or the designers missing important engineering principles (Losordo 1991;

Losordo and Westers 1994). For purposes of this paper, a modeling approach using mass balance analysis as described by Losordo and Westers, 1994 will be utilized.

. This approach follows a series of steps:

1)   Determine and define the system boundaries, in the case of a recirculation system; these are the production system (tank) and the biological filter.

2)   Isolate and identify the flow streams that cross these boundaries. These include the water input, the water output, the wastewater removing the nitrates, and the ammonia production and consumption rates for the production system. Identify the material to be balanced. For the purposes of this paper, it will be the TAN. TAN, or total ammonia nitrogen, is a combination of both ionized and unionized ammonia. Even though the unionized ammonia is lethal to the fish, its concentration depends on many variables, including temperature, pH, and salinity. Because the ionized and unionized ammonia are in equilibrium, as these factors change, the percentage of unionized ammonia varies, but the TAN remains the same.

3)   Identify the processes in the system that affects nitrification. In this situation, the bacteria are causing ammonia to be transformed into nitrate.

For our purpose, the following model can be used:

Accumulation = Input – Output + Generation – Consumption

There are three considerations for designing the biological filter. The first is its capacity to convert ammonia to nitrite and then nitrate. This capacity is determined by the surface area (larger the surface area, the larger the population of bacteria in the

process of transformation) of the media and the flow rate of water through the filter. If the water is moving too fast, the bacteria do not have sufficient time to convert the ammonia. If the water flow is to slow, ammonia and nitrite will build up in the system.

The second consideration is the removal of nitrate from the system. Though the end product of the process, nitrate, is not highly toxic to fish, if the levels get sufficiently high, it will have some deleterious effects (slow growth or mortality) on the fish. Often, removal of nitrate is accomplished by discharging the water from the system. Bacterial breakdown of nitrate (denitrification) is an anaerobic process and low cost denitrifying filters have not yet been developed.

The last consideration is the dissolved oxygen demand of the biological filter. Though bacteria are very small, such a large population will dramatically lower the oxygen levels of the system, possibly bringing levels of dissolved oxygen in the system down to the point that the fish die. Therefore, the system as a whole must be designed in such a way that there is enough oxygen in the system for both the population of fish being cultured, and the bacteria of the biological filter.

2.2 Assumptions

Using a mass balanced approach requires that certain assumptions must be made. These assumptions must also be realistic for them to be of any use in a practical situation. The following are the assumptions made in the designing of a filtration system:

1)   The feed protein is approximately 16% nitrogen. Nitrogen is the primary limiting element in the production of ammonia, as the only other elements involved in ammonia

are hydrogen and oxygen, the very elements of water. Therefore, this is the primary flow stream controlling the formation of ammonia.

2)   80% of the nitrogen is assimilated into the physiological processes of the fish.

3)   Non-assimilated nitrogen in fecal matter is removed from the tank in a timely manner.

4)   80% of the assimilated nitrogen is nitrogen is excreted in the form of either urea or TAN.

5)   10% of the nitrogen is excreted as urea and 90% as TAN.

6)   All of the TAN is excreted during t hours following a feeding. There is evidence that the metabolic processes of fish increases in activity from 1 to 4 hours after feeding

7)   The time interval between feedings can not be less than t hours, to allow the TAN to be excreted by the fish and utilized by the biological filter. From this, one can deduce that many small evenly spaced feedings are a better strategy than a few large ones.

2.3   Designing the Filter

Designing a filter for a particular species is a complicated series of calculations, each one used to insure the filter is capable of maintaining the desired TAN concentration. Therefore, deciding on the TAN concentration is of utmost importance. In the previous chapter, the NOEL for yellow perch was found to be 0.06 mg/L as NH_3-N. Ammonia is found in water in one of two forms, ionized and unionized, both in balance, with the ionized form generally being the greater concentration. Together, these make up the concentration of TAN in the holding tank. This balance is influenced by

many factors, mostly temperature and pH. This is important in that what the TAN concentration of the holding tank is not really important, but the unionized ammonia cannot exceed 0.06 mg/L.

The water in the experiments in Plymouth had a pH of 8.5 and a temperature of 22 C. However, in a recirculation system, the pH rarely exceeds 7.5, and temperature control is not usually a problem. This is means there is a magnitude of more hydrogen ions in the water to push the ammonia balance in the ionized ammonia direction. With a pH of 8.5, the unionized ammonia makes up 12.9% of the TAN. This would indicate a TAN concentration of 0.465 mg/L. However, a pH of 7.5 changes the percentage of unionized ammonia to 1%. This would allow us to have a TAN up to 6 mg/L. This provides an upper limit for a TAN concentration with which to base our filter calculations on.

In actuality, we will use a TAN concentration of 2 mg/L. The reason for this comes from the ability of bacteria to remove the ammonia from the water. It has been found that the bacteria increase the rate of consumption of ammonia up to a certain concentration (Nijhof 1995). The concentration range for this is up to 2 mg/L. After this transition point, the bacteria will consume the same amount of ammonia no matter the concentration. The equation for ammonia removal is :

$$R_{TAN} = (a) (sqrt [TAN]) - (b) \ (g/m^2/d) \tag{1}$$

R_{TAN} = Removal rate of TAN

TAN = concentration of ammonia-nitrogen

a and b are constants

In this equation, both a and b are constants, when the concentration is below the transition point of 2 mg/L. The constant a depends on external factors such as temperature, salinity, pH. The constant b deals with such things as abundancy of the bacteria, and biofilm thickness. Under conditions similar to what we will be dealing with, a = 0.55, and b = 0.11(Ninjhof 1995). Above the transition point, the following equation is used:

$$R_{TAN} = C \ (g/m_2/d) \tag{2}$$

Where C is mainly determined by the concentration of O_2 available. Under similar conditions as the one we are dealing with, above the transition point of 2 mg/L, the removal rate is between 0.55 and 0.69 $g/m^2/d$.

In using a TAN concentration of 2 mg/L, we will easily have an unionized ammonia concentration below the NOEL. The draw back for using this lower concentration is that the filter will likely be larger then it has to be. Therefore, the removal rate of ammonia by the bacteria in the filter is:

$$R_{TAN} = (0.55)(sqrt \ 2.0 \ mg/L) - (0.11) \tag{3}$$
$$R_{TAN} = 0.667817 \ g/m^2/d$$

The next step in designing the filter is to determine the flowrate of water from the holding tank to the filter. According to Losordo et al. (1991), this can be found with the equation :

$$Q_f = (QC_{tanin} - QC_{tanout} + P_{tan}) / (C_{tan} * E) \qquad (4)$$

Qf = flow rate of water from tank to filter

Q = the flow rate of water in the tank

C_{tanin} = TAN concentration of water added to the system daily from a well (mg/L)

C_{tanout} = TAN concentration of water leaving tank to the filter (mg/L)

P_{tan} = the production rate of ammonia in the tank (g/d)

C_{tan} = the desired TAN concentration (mg/L)

E = the filter efficiency

Q_f is the flow rate (lpd) of recycled water to the filter from the tank. C_{tanin} is the water being added to the tank daily to replace the water that is lost to evaporation and to the water waste to keep nitrate at a manageable level. When situating an aquaculture facility, it is desirable to locate it in a place with a source of water that is very low in ammonia. This would allow us to assume C_{tanin} = 0.

C_{tanout} is the TAN concentration of the water leaving the tank and going to waste. In a well-mixed tank, the TAN concentration will be the same in all parts of the fish tank, including the effluent pipes to the filter. P_{tan}, the production rate of ammonia becomes the major player in the TAN concentration. P_{tan} can be estimated with the equation:

Ptan = (FA * PC * 0.092) / t (5)

FA = the amount fed daily (kg)

PC = the protein content of the feed (percentage)

t = numbers between feeding

FA is used to represent the amount of feed fed to the fish daily. Conventionally, when fish are at adult size, 1% of the total biomass is fed per day. The system we are designing should hold 1000 kg of fish mass, so FA = 10 kg.

PC is the protein content of the feed. This is variable among feed types and manufacturers. It is printed on the outside of a bag of feed. In our case, we are using a protein content of 45%.

The constant 0.092 represents several assumptions. These assumptions are based on studies performed on a variety of species, and they are:

1) Nitrogen makes up about 16% of the protein in the feed.

2) 80% of the nitrogen will be assimilated by the fish

3) 80% of the assimilated nitrogen will be excreted

4) 90% of the excreted nitrogen will be in the form of TAN.

(0.16) (0.8) (0.8) (0.9) = 0.092 (6)

The last variable is t, for time. In our case, we are dealing with amount produced in a day, so t = 1 day. The calculation of the rate of ammonia-nitrogen production in equation 5 becomes:

$P_{tan} = [(10kg) (0.45) (0.092)] / 1$ (7)

$P_{tan} = 0.414$ kg/day

Putting this back into the original flow rate equation 4, we can estimate the biofilter flow rate as:

$Q_f = (414$ g/d$) / (C_{tan} * E)$ (8)

C_{tan} is the desired concentration of TAN we want to achieve. As stated earlier, we will use 2.0 mg/L. We will convert this to grams, giving 0.002 g/L. E, the last variable, represents the efficiency of the filter. This is influenced by many factors, including the temperature, salinity, and depth of the filter. However, 50% is fairly common, and so will be used for this purpose (Personal communication, Tom Losordo, North Carolina State University).

$Q_f = (414$ g/d$) / (0.002$ g/L $* .50)$ (9)

$Q_f = 414000$ L/d

Converting to liters per minute, we get 287.5 L/min. The spreadsheet developed by Losordo and Hobbs (2000) gives 180 L/min. This difference can largely be explained by the constant in finding the P_{tan}. The spreadsheet uses 0.065 instead of 0.092, which is a calibrated constant that approximates a system that yields ammonia nitrogen at a rate of

2.5% of the input feed rate. When this constant is used instead, a flow rate of 203 L/min. is found.

Now the dimensions of the filter can be determined. The first step is to find the surface area needed to allow enough bacteria to remove enough ammonia to keep the holding tank at a TAN concentration of 2 mg/L:

Surface area needed = P_{TAN} / R_{TAN} (10)

Surface area needed = (414 g/day) / (0.667817 g /m^2)

Surface area needed = 619.93m^2

The surface area of filter media is dependant on the type of media used. The media used in the NC Fish Barn has a surface area of approximately 200 m$^{2/}$m$^{3.}$ This would mean that 3.10 m^3 of filter media would be needed. A filter with a depth of 1.00 meters, would have a diameter of 2.92 meters, and this would contain the 3.10 m^3 of media needed.

2.5 References

Losordo, T.M. 1991. Introduction to recirculating production systems design. Pages 32-47 in: Engineering Aspects of Intensive Aquaculture. Proceeding from the Aquaculture Symposium, Cornell University, Ithaca, NY, April 4-6, 1991. Northeast Regional Agricultural Engineering Service, No. NRAES-49.

Losordo, T.M. and H. Westers. 1994. Systems carrying capacity and flow estimation. Pages 9- 60 in: Timmons, M.B. and T. M. Losordo (eds). Aquaculture Water Reuse Systems: Engineering Design and Management. Developments in Aquaculture and Fisheries Sciences, 27, Elsevier Science B.V., Amsterdam, The Netherlands,

Nijhof, M. 1995. Bacterial Stratification and Hydraulic Loading Effects in a Plug-Flow Model for Nitrifying Trickling Filters Applied in Recirculating Fish Culture Systems. Aquaculture, 134, Pages 49-64. Wageningen, Netherlands.

Table 1.1: Summary of Results from Acute Un-ionized Ammonia and Nitrite Toxicity Tests Expressed as the Lethal Concentration to 50% of Fish (LC_{50}) to Different Life Stages of Yellow Perch After 96 Hours, Confidence Intervals in Parenthesis.

Life Stage	Mean Water Temp C	Unionized Ammonia 96 h LC_{50} (Lower,Upper)		Nitrite 96 h LC_{50} (Lower,Upper)	
Juvenile	22	0.77	(0.54,1.0)	78.24	(67.4,88.6)
Adult	18	1.02	(0.96,1.08)	88.5	(76.6,80.0)
	22	0.64	(0.57,0.69)	65.8	(52.3,77.0)
	25	0.66	(0.58,0.8)	58.4	(42.1,71.4)

Table 1.2: No Effect Levels (NOEL) for Yellow Perch in mg/L NH_3-N for Ammonia and mg/L NO_2-N for Nitrite, to Different Life Stages and Temperatures. Values are Calculated Based on 9% of the 96-h LC_{50} Determined from Static Toxicity Tests for each Life Stage.

Life Stage	Mean Water Temp C	Ammonia	Nitrite
Juvenile	22	0.069	7.04
Adult	18	0.092	7.97
	22	0.058	5.92
	25	0.059	5.26

Figure 1: Yellow Perch Juvenile Survival (%) After 96 Hour Exposure to Different Concentrations of Nitrite (mg/L NO_2-N)
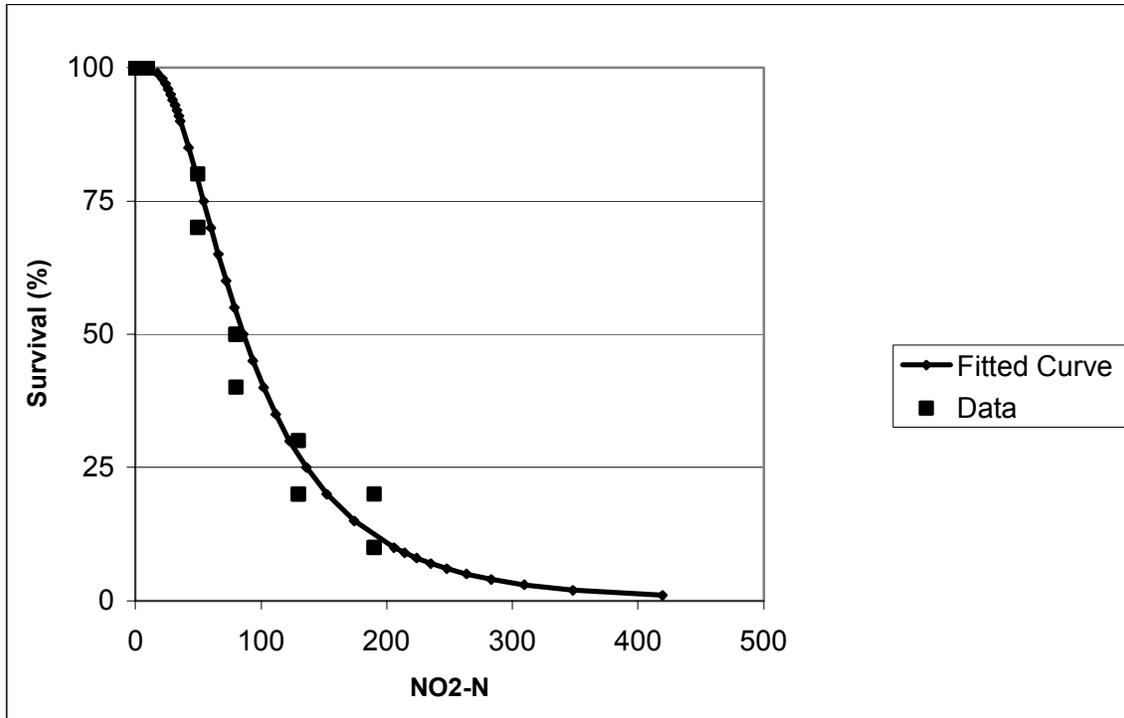

Figure 2: Yellow Perch Adult Survival (%) After 96 hour Exposure to Different Concentrations of Nitrite (mg/L NO_2-N) at 18 C
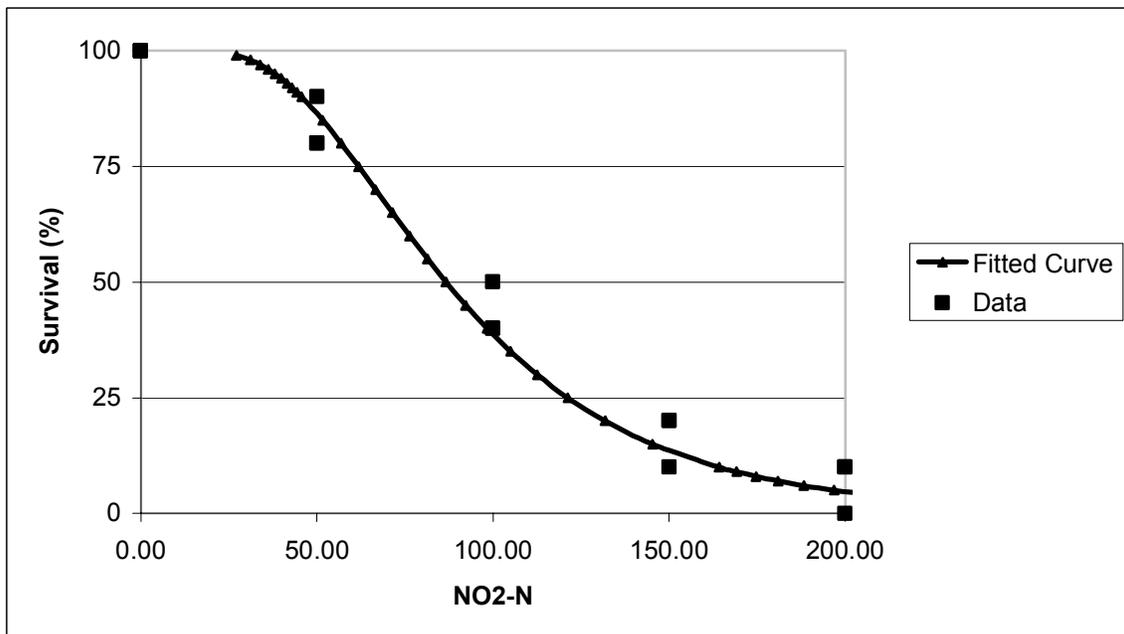
Figure 3: Yellow Perch Adult Survival (%) After 96 Hour Exposure to Different Concentrations of Nitrite (mg/L NO_2-N) at 22 C
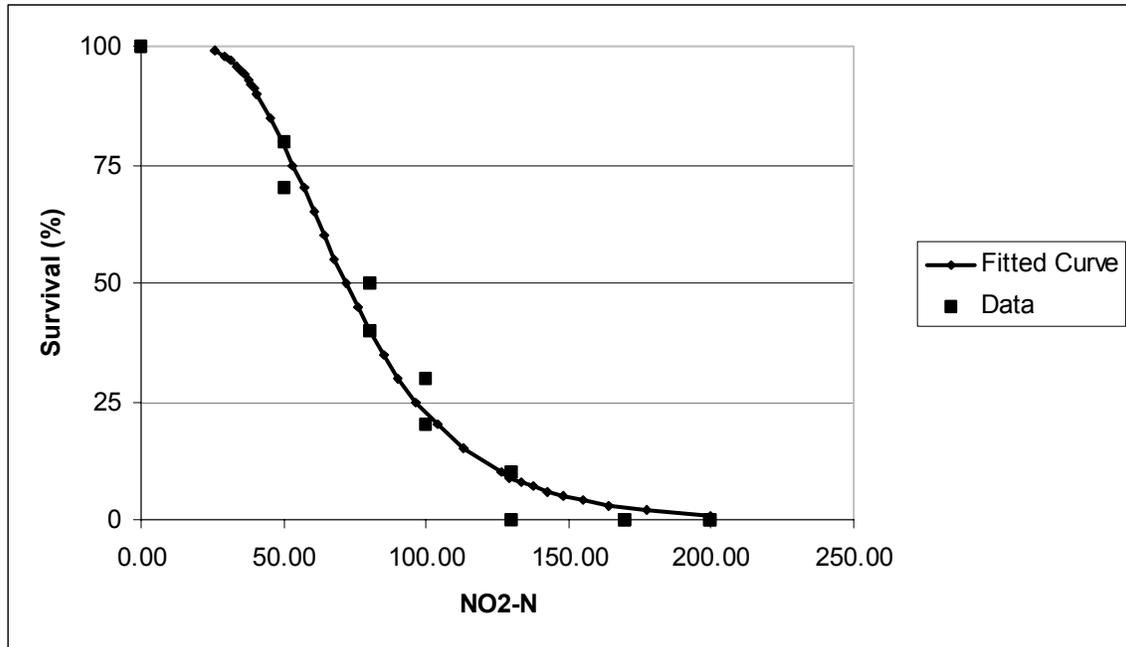

Figure 4: Yellow Perch Adult Survival (%) After 96 Hour Exposure to Different Concentrations of Nitrite (mg/L NO_2-N) at 25 C
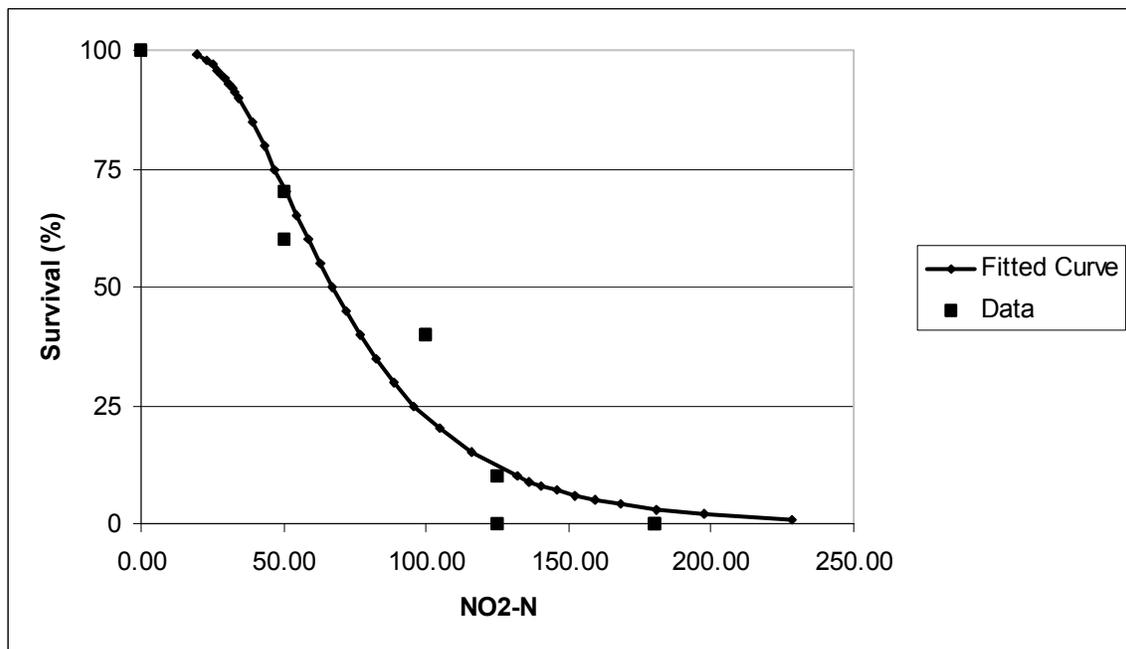
Figure 5: Yellow Perch Juvenile Survival (%) After 96 Hour Exposure to Different Concentrations of Un-ionized Ammonia (mg/L NH_3-N)
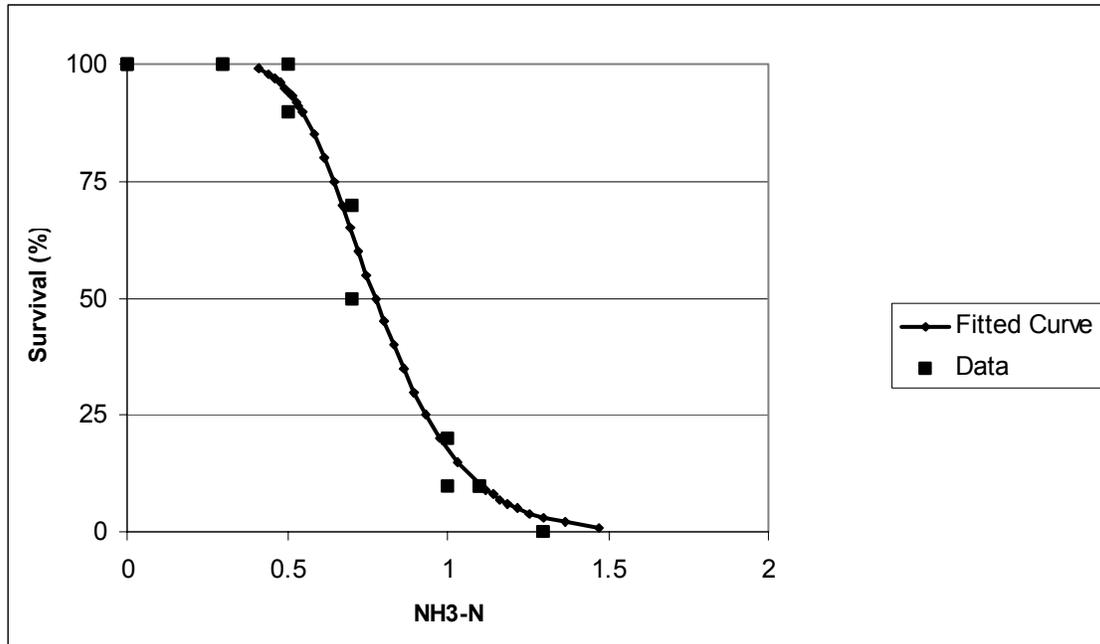

Figure 6: Yellow Perch Adult Survival (%) After 96 Hour Exposure to Different Concentrations of Un-ionized Ammonia (mg/L NH_3-N) at 18 C
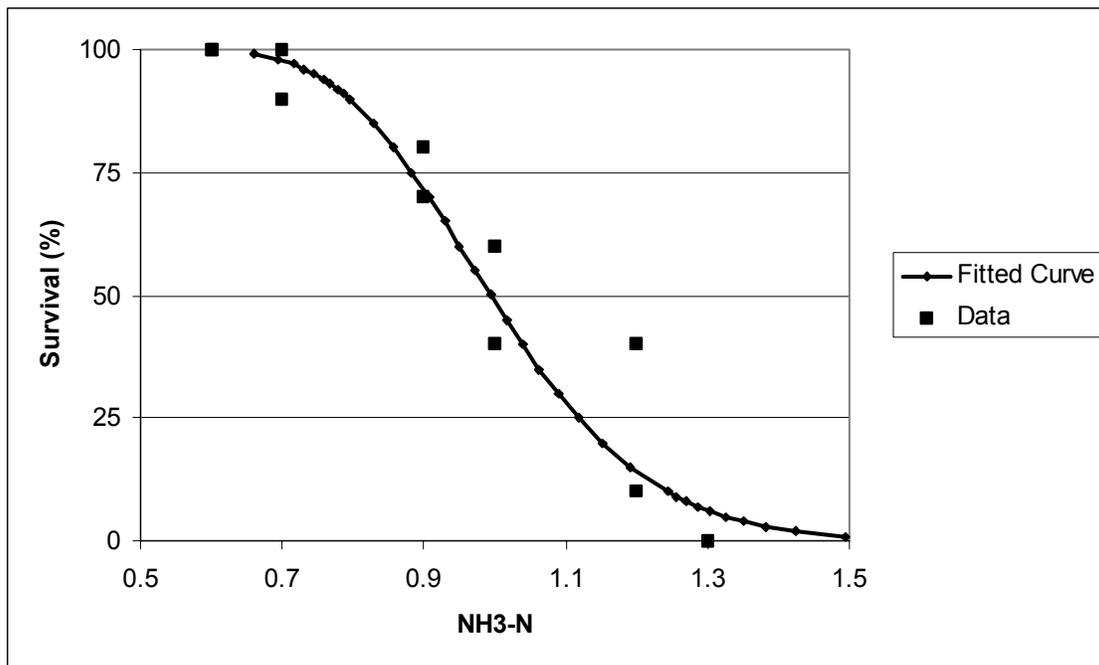
Figure 7: Yellow Perch Adult Survival (%) After 96 Hour Exposure to Different Concentrations of Un-ionized Ammonia (mg/L NH$_3$-N) at 22 C
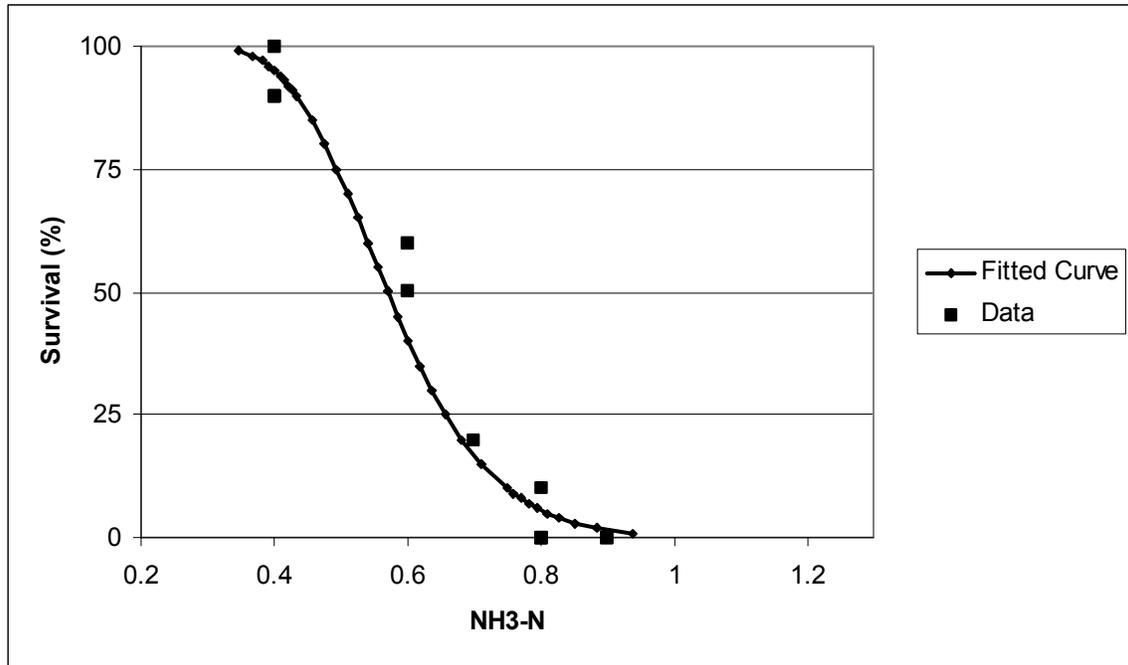

Figure 8: Yellow Perch Adult Survival (%) After 96 Hour Exposure to Different Concentrations of Un-ionized Ammonia (mg/L NH$_3$-N) at 25 C
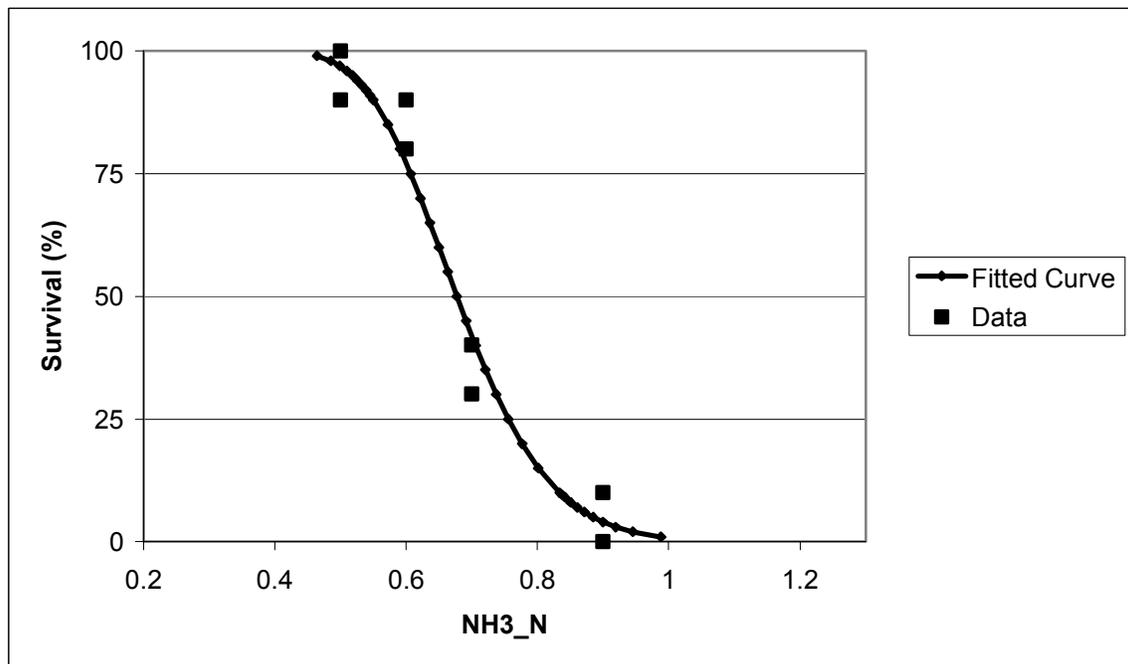
Figure 9: Comparison of Adult Yellow Perch Survival (%) After 96 Hour Exposure to Different Concentrations of Nitrite (mg/L NO$_2$-N) at Different Temperatures.
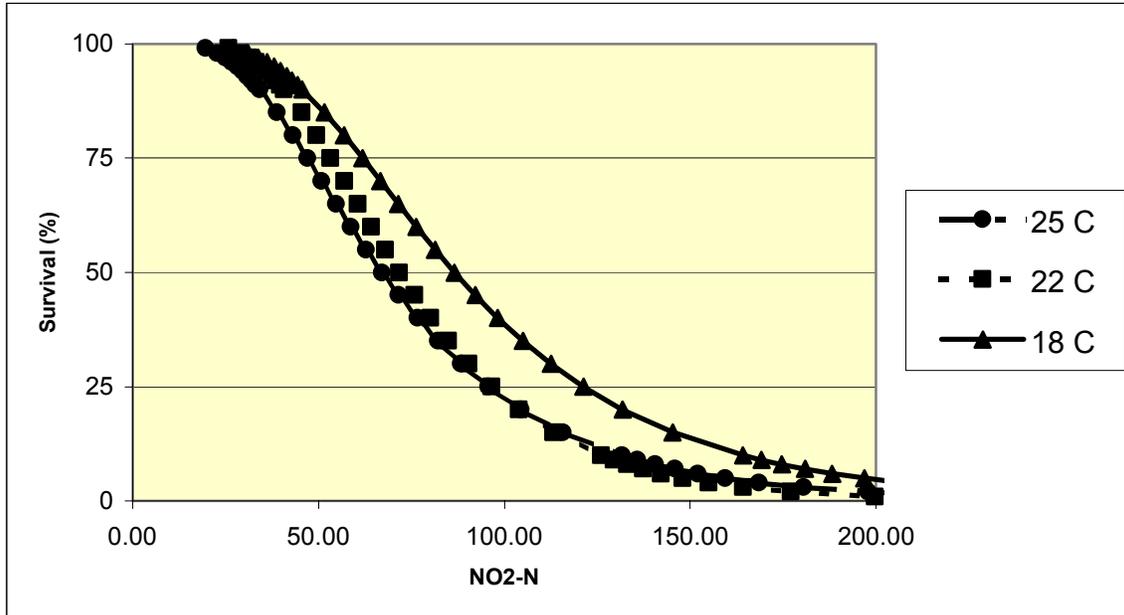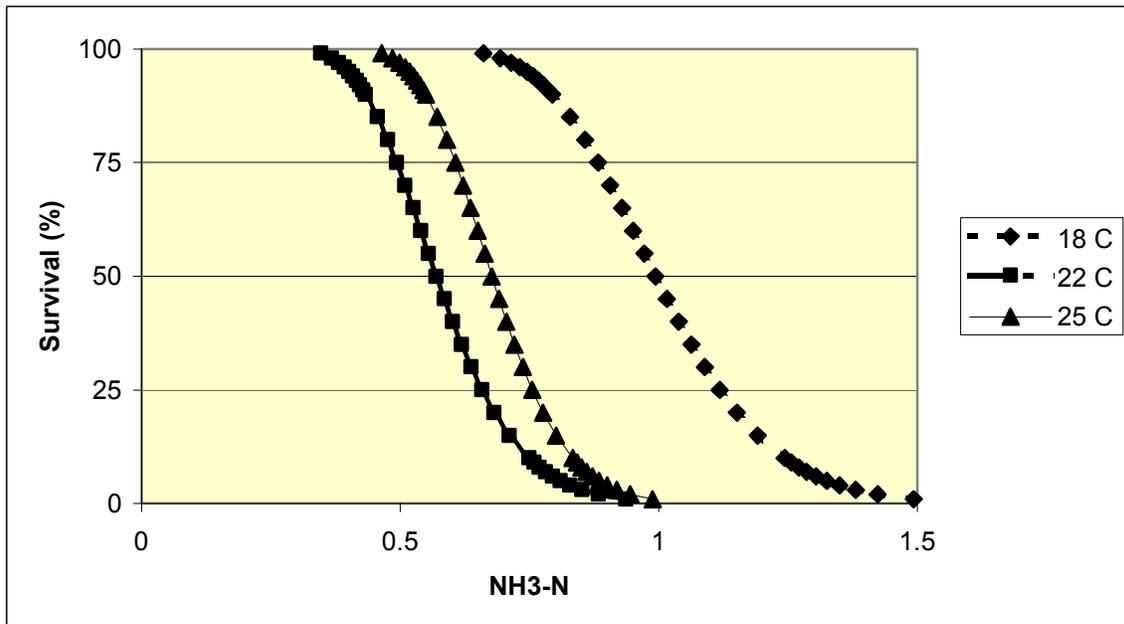

Figure 10: Comparison of Adult Yellow Perch Survival (%) After 96 Hour Exposure to Different Concentrations of Un-ionized Ammonia (mg/L NH$_3$-N) At Different Temperatures.

Appendix A

Results of Larval Experiments

Several attempts were made to estimate an LC_{50} for yellow perch larvae. The results are placed in this appendix as a reference. Due to the sensitive nature of larvae, and their rapid development, the tests were not successful. In both the ammonia and nitrite tests, several larvae in the control containers died, and at intermediate concentrations, the results varied widely, with 70% surviving in one container, 20% surviving in another, yet both containers were the same concentration.

This disparity could be due to several reasons. One possible reason is handling. Morphologically, larvae do not have well formed gills, nor do they have blood, relying on absorbing oxygen through the skin. Nitrite affects the blood's ability to carry oxygen, and since the larvae do not have blood it can lead to a high tolerance of nitrite. However, the rapid development of the larvae can lead to the development of a circulatory system during the test, causing conflicting results as the more developed larvae are sacrificed, and the less developed survive.

Unionized ammonia, however, may be able to pass through the skin easily, and affect the still forming central nervous system. This again may make a given concentration more toxic to larvae born a few hours earlier, which would be further along in its development. This would also allow a "disjointing" of ammonia and nitrite toxicity, a low concentration of ammonia can become toxic, while high levels of nitrite would be needed to reach mortality.

However, a possible LC_{50} was found. A working LC_{50} for ammonia is 0.191 (0.05, 0.27) and the working LC_{50} for nitrite is 221.83 (97.1,302.2)

Figure A1: Yellow Perch Larvae Survival (%) After 96 Hour Exposure to Different Concentrations of Un-ionized Ammonia (mg/L NH$_3$-N)
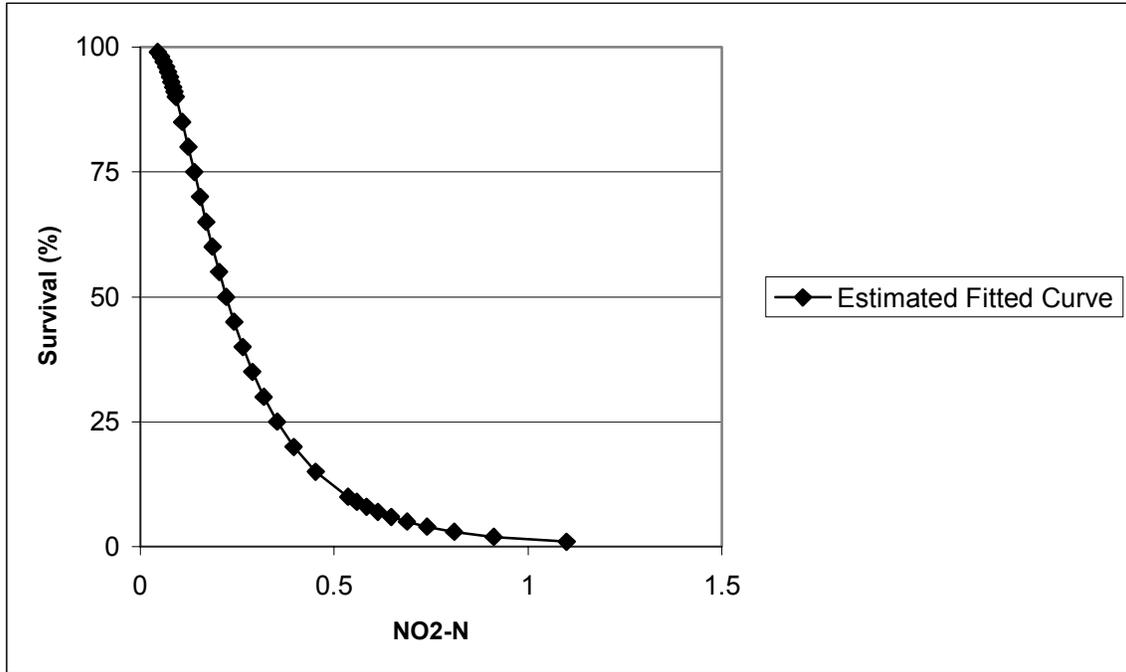
Figure A2: Yellow Perch Larvae Survival (%) After 96 Hour Exposure to Different Concentrations of Nitrite (mg/L NO$_2$-N)
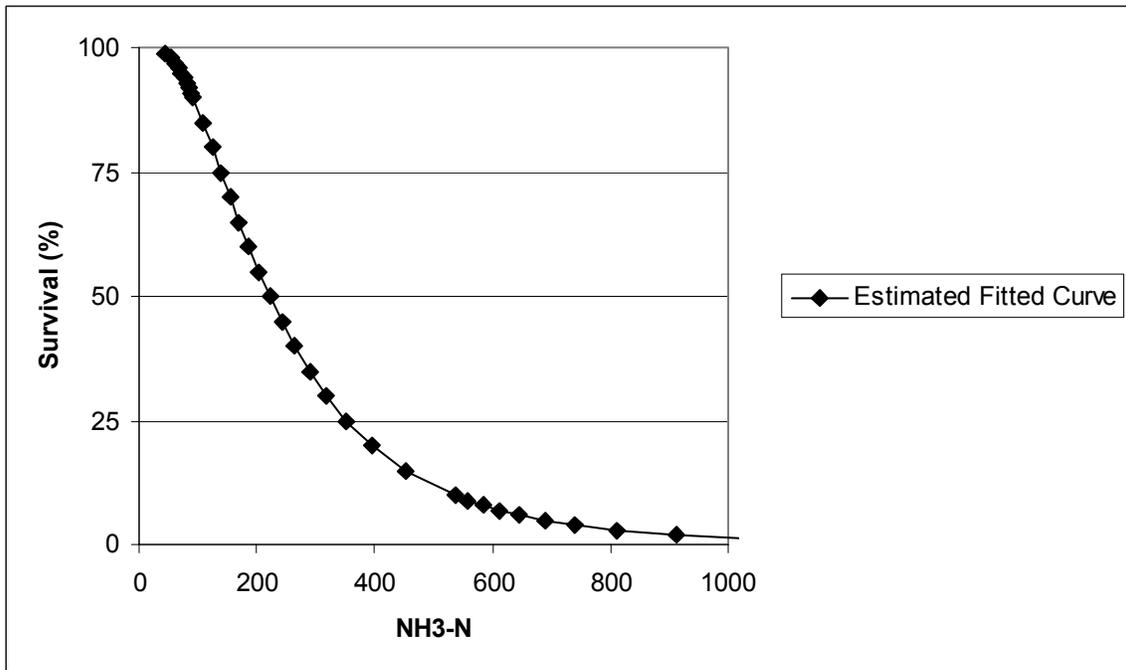
Appendix B

Program Used to Analyze Data

```
data a; input
Treat avConc        conc Begin  mort24      mort48      mort72
     mort96;
mortality=mort24+mort48+mort72+mort96;
logconc=log10(conc);
cards;



proc print;
proc plot; plot mortality*conc mortality*logconc;

proc probit log10 ;
model mortality/begin=conc/lackfit inversecl;

proc probit log10 data=a;
var conc begin mortality;

run;
```

Appendix C:

Spreadsheet for Flow Rate Estimation and Biofilter Sizing

Spreadsheet for Flow Rate Estimation and Biofilter Sizing

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2.1. Tank Size and Biomass	Values	Units	Calculation Formula
Tank water depth	**2.00** m		**2.00**
Tank radius	**3.10** m		**3.10**
Tank volume	60 m^3		=B6*(PI()*(B7*B7))
Maximum culture density	**60** kg/m^3		**60**
Fish biomass	3623 kgs		=B8*B9
Fish count	**6000**		**6000**
Fish weight	603.8 gm		=1000*B10/B11
Feed rate as % of body weight	**1.23%**		**0.0123**
Feed rate	44.6 kg/day		=B10*B13

2.2. TAN Mass Balance Calculations

	Values	Units	Calculation Formula
Feed protein content	**38%**		**0.38**
Total Ammonia Nitrogen (TAN) production rate	1.101 kg/day		=0.065*B14*B18
% TAN from feed	2.47%		=B19/B14
Desired TAN concentration in recirc water	**2** mg/L		**2.0**
Passive nitrification	**10%**		**0.10**
TAN available after passive nitrification	0.991 kg/day		=B19*(1-B22)
Passive denitrification	**0%**		**0.00**
Maximum nitrate concentration desired	**150** mg/L		**150**
New water required maintain nitrate concentration	6604 L/day		=((B23*1000000*(1-B24))/B25)
TAN available to Biofilter after effluent removal	0.977 kg/day		=B23-(B21/1000000)*B26
Biofilter efficiency for TAN removal	**50%**		**0.50**
Flow rate to remove TAN to desired concentration	977425 L/day		=(B27)/(B28*(B21/1000000))
	679 L/min		=B30/1440
	179 gal/min		=B31/3.785

2.3. Biofilter Sizing Calculation

	Values	Units	Calculation Formula
Estimated nitrification rate	**0.45** g TAN/m^2/day		**0.45**
Active nitrification surface required at rate	2172 m^2		=(B27)/(B35/1000)
Surface area of media	**200** m^2/m^3		**200**
Total volume media	10.86 m^3		=B36/B37
Media unit price	**$200** $/m^3		**200**
Media Cost	$2,172.06 media cost		=B39*B38

Media depth	**1.65** m	**1.65**
Volume / depth yields face area	6.58 m^2	=B38/B41
Diameter of biofilter	2.89 m	=2*SQRT(B42/3.1416)

2.4. Solids Mass Balance Calculations

Estimated percentage of feed becoming solid waste	**25%**	**0.25**
Waste solids produced	11.14 kg/day	=B14*B47
Desired SS conc.	**10** mg/L	**10**
Est. % removed by particle trap	**50%** particle trap	**0.50**
Waste solids remaining after particle trap	5.57 kg/day	=B48*(1-B50)
Waste solids remaining solids removal in effluent	5.50 kg/day	=(B51-(B49*B26/1000000))
Settling tank, bead filter, drum filter, etc. efficiency	**50%**	**0.50**
Flow rate to remove SS to desired concentration	1100864 L/day	=(B52)/(B53*(B49/1000000))
	764 L/min	=B55/1440
	202 gal/min	=B56/3.785

2.5. Oxygen Mass Balance Calculations

Submerged filter? (1=yes and 0=no)	**0**	**0**
Oxygen used / kg Feed	**30%**	**0.30**
Oxygen used by feed addition	13.37 kg/day	=B14*B62
Desired oxygen concentration in tank	**5.0** mg/L	**5.0**
Dissolved oxygen concentration supplied to tank	**18.0** mg/L	**18.0**
Oxygen used by passive nitrification	0.50 kg/day	=(B19-B23)*4.57
Oxygen used for nitrification in biofilter	0.00 kg/day	=B61*(B27)*4.57
Total oxygen used	13.87 kg/day	=B63+B66+B67
Estimated flow rate	1067069 L/day	=B68/((B65-B64)/1000000)
	741 L/min	=B69/1440
	195.8 gpm	=B70/3.785