

**A COMPARATIVE ANALYSIS OF THREE BIOFILTER  
TYPES TREATING WASTEWATER PRODUCED IN  
RECIRCULATING AQUACULTURE SYSTEMS**

By

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## **ABSTRACT**

Nine recirculating systems at the Virginia Tech Aquaculture Center were placed on line and stocked with yellow perch, *Perca flavescens*, fingerlings. Fish were stocked at a density of approximately 455 fish m<sup>-3</sup>. Biofilter types were the only factor differing among system designs and were an upflow pulsed bed bead filter, packed tower trickling filter and a rotating biological contactor (RBC). After stocking, systems were allowed to acclimate using ammonia excreted by the yellow perch. Following acclimation, a comparative analysis on biofilter performance began. To evaluate filter performance, water quality parameters tested were temperature (°C), pH, dissolved oxygen (DO), total ammonia-nitrogen (TAN), nitrite-nitrogen (NO<sub>2</sub><sup>-</sup>-N), nitrate-nitrogen (NO<sub>3</sub><sup>-</sup>-N), alkalinity (as CaCO<sub>3</sub>), water hardness (as CaCO<sub>3</sub>), carbonaceous biochemical oxygen demand (cBOD<sub>5</sub>), dissolved organic carbon (DOC), and total suspended solids (TSS). Basic water quality analysis encompassed samples drawn at 8 AM. TAN mass removal analysis encompassed water quality samples drawn at 8 AM and over 24 hours. Higher TAN mass removal rates were achieved in trickling and RBC filters than in bead filters for 8 AM (0.037, 0.14, and 0.004 g/m<sup>2</sup>/d, respectively) and diurnal sample periods. Analysis of areas under mass removal curves depicted RBC filters as surface area limited. Trickling filters proved most effective at carbon dioxide stripping and pH maintenance and also effectively removed TSS from the culture water. The study did not show filter type as having a significant effect on median organic water quality parameter values.

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## CHAPTER 1

### BIOFILTER DYNAMICS IN RECIRCULATING AQUACULTURE SYSTEMS

#### RECIRCULATING AQUACULTURE

Because capture fisheries have overharvested many of the world's natural fisheries stocks (Youngs and Timmons, 1991), a large proportion of fish and shellfish for research and food are produced by aquaculture, with the majority being produced for food (Youngs and Timmons, 1991). Due to concerns regarding detrimental impacts of aquaculture production on the environment (Rosenthal, 1994), increased regulations on aquaculture effluents, and the need to conserve water resources (Klontz, 1979) and energy, the aquaculture industry is focusing on development and refinement of water recycling technologies. This is evident in the emergence of conferences (e.g., the International Conference on Recirculating Aquaculture, hosted by Virginia Polytechnic Institute and State University) and scientific journals (International Journal of Recirculating Aquaculture) dedicated to coverage of recirculating aquaculture systems. Consequently, the number of recirculating aquaculture systems employed in production facilities is increasing (Malone et al., 1993).

As defined by Libey (1996), a recirculating aquaculture system is an assemblage of parts used for the culture of aquatic organisms where water is continuously cleaned and reused. Water is cleaned via mechanical and biological filtration. Mechanical filtration removes particulate wastes, while biological filtration removes dissolved wastes via biochemical reactions that occur during bacterial metabolism. These processes allow water to be cleaned and reused several times prior to discharge. These processes conserve water by reducing the amount of water needed (from an external source) to maintain a biologically suitable culture environment for the crop. Water recycling allows the majority of recirculating systems to exchange approximately 10 % of total system volume per day while recycling 90 % of the culture water. Owsley (1993) reported five of six aquaculture facilities maintaining a daily exchange rate of approximately 10 %,



while one facility reported a 5 % value. Westerman et al. (1996) reported 9 to 11 % exchange rates for four recirculating systems employed in a filter evaluation study.

In addition to water conservation, recirculating systems allow large fish yields to be obtained in a relatively small area and provide year-round production (Van Gorder, 1994). Both attributes increase economic growth potential of the industry. Although recirculating water aids in natural resource conservation and allows consistent production of high quality protein in fish and other aquatic food crops, development and use of recirculating technologies also present substantial challenges to fish culturists.

## **SYSTEM WATER QUALITY AND MANAGEMENT PRACTICES**

A major challenge to aquaculturists is maintaining system water quality suitable for the crop throughout the culture process. System water quality is a result of several factors, but primarily can be attributed to source water quality, culture management practices, and system unit processes. Assuming that source water quality is suitable for satisfactory fish health, management practices and system unit processes can be viewed as the dominant factors affecting system water quality.

Management practices range from stocking and feeding fish, to daily water quality analysis, adding new water to tanks, discharging organic wastes from biofilters and settling sumps, and maintaining motors and pumps. Best management practices should be devised prior to operation of a recirculating system. Feed administration is a major aspect of management practice. Because feed is regarded as the major source of metabolic wastes generated within recirculating systems (Drennan et al., 1993), feeding regimens should optimize availability of feed for rapid growth while minimizing the amount of feed waste. According to Kolsäter (1995), ammonia and nitrate wastes in culture effluents largely are determined by feed protein levels, protein digestibility, amino acid balance (quantity and quality), and the feed protein to total energy ratio.

Ammonia is the major by-product of deamination of protein in aquaculture feeds (Spotte, 1979; Lystad and Selvik, 1991), and is released primarily through fish's gills, but also can be released from decaying feed and feces. Total ammonia nitrogen (TAN) is comprised of ammonium ion ( $\text{NH}_4^+\text{-N}$ ) and un-ionized ammonia ( $\text{NH}_3\text{-N}$ ). Un-ionized

ammonia is highly toxic to aquatic organisms and can detrimentally affect fish growth and health (Colt and Armstrong, 1981; Wheaton et al., 1991b). Excess TAN can be removed from the culture system by exchanging a percentage of tank volume with clean unused water, ionic exchange processes (e.g., use of clinoptilolite) or nitrification where nitrifying bacteria oxidize ammonia to nitrate, the latter being most used (Parker and Davis, 1979; Wheaton, 1985; Wedemeyer, 1996).

## BIOFILTRATION

Like all living organisms, fish require a clean environment for optimal growth and survival. As fish respire and metabolize feed, toxic metabolites are released into the water column. Metabolite accumulation increasingly degrades system water quality. If inorganic or organic toxins within the water surpass biologically critical levels, fish growth may become inhibited and mortality increased. To maintain a clean environment in recirculating systems, a combination of mechanical and biological filtration techniques must be employed. Although nitrification can occur throughout the culture system (e.g., in biofilms on pipe and tank walls) (Losordo, 1991), the majority of biochemical reactions pertaining to heterotrophic and autotrophic bacteria occur within biofilters. Biofilters are specifically designed for concentrated bacterial attachment and nitrification via fixed-film processes.

Autotrophic bacteria are credited for performing nitrification (Wedemeyer, 1996). Nitrification is a two-step process, where *Nitrosomonas sp.* oxidize ammonia to nitrite, and *Nitrobacter sp.* oxidize nitrite to nitrate. Although less toxic than ammonia, nitrites also are considered toxic to fish, while nitrates ( $\text{NO}_3^-$ -N), the final oxidized form in nitrification, are considered relatively nontoxic to fish unless high concentrations are sustained for an extended period of time (Spotte, 1979). Since biofiltration is the principal unit process used for treating fish metabolites, biofilters can be considered major components in intensive recirculating aquaculture systems (Libey and Miller, 1985).

To ensure prolonged fish survival, high levels of sustained nitrification must be achieved. Therefore, ecological requirements of the bacteria (Malone et al., 1993) must be met within biofilters for effective nitrification to occur. System water quality and

filter design characteristics affect filter environmental conditions. Although a larger number of water quality parameters affect nitrification kinetics, Kaiser and Wheaton (1983) stated that dissolved oxygen, pH, water temperature, ammonia-N concentrations, and filter flow rate are the dominant factors affecting a filter's nitrification efficacy.

## **ORGANIC CONSTITUENTS IN RECIRCULATING SYSTEMS**

The majority of organics in recirculating aquaculture production systems are derived from uneaten feed, sloughed biofilm, and fecal wastes (Libey, 1993; Piedrahita et al., 1996). The growth rate in organic-laden waters is faster for heterotrophs than for nitrifiers (Grady and Lim, 1980). Heterotrophs and nitrifiers compete for available surface area in biofilters. High organic loadings to the biofilter can result in establishment of large heterotrophic populations on the filter media, enabling them to outcompete nitrifiers for available surface area, potentially decreasing nitrification efficacy of the filter (Manem and Rittmann, 1992). Wheaton et al. (1994) reported on a study by Pano and Middlebrooks (1983), where ammonia removal in a rotating biological contactor (RBC) was reported to decrease as organic loading increased.

Organic wastes and their effects on system water quality can be quantified in several forms. They may be characterized as total suspended solids (TSS), carbonaceous biochemical oxygen demand (cBOD<sub>5</sub>), and dissolved organic carbon (DOC). Organic wastes also may be quantified in other forms (e.g. total Kjeldahl nitrogen, TKN), but TSS, cBOD<sub>5</sub>, and DOC are the primary measures of interest to this study.

### **Total Suspended Solids**

TSS refers to matter suspended within the water column and the fraction of total solids retained by a filter during sample analysis (APHA et al., 1985). The majority of suspended solids within a culture system are of an organic nature, consisting of uneaten feed, feces and unattached biofloc (Libey, 1993; Piedrahita et al., 1996). Because suspended solids can degrade water quality if not rapidly removed (Piedrahita et al., 1996), several studies have focused on strategies for removal of these solids from aquaculture effluents (Chen and Malone, 1991; Cripps, 1991; Libey, 1993; Piedrahita et

al., 1996; Summerfelt, 1996). Particulates generally are removed via settling or straining processes immediately following tank discharge (Rosenthal and Black, 1993). If not promptly removed, high concentrations of suspended solids may clog biofilters and act as physical gill irritants to fish (Chen et al., 1991). In a sludge characterization study by Chen et al. (1993), TSS concentrations were shown to increase as daily feed input increased. The linear relationship shown characterized feed as a major source of organic wasteloading and suspended solids as a byproduct of the wasteloading.

High TSS concentrations also can increase a system's BOD. As the amount of suspended solids increases, heterotrophic bacteria have more organic material to oxidize. Oxygen demand increases as a result of increased activity of heterotrophic metabolism. A study by Mathieu and Timmons (1993) showed that BOD significantly increased over time if organic solids were not removed rapidly. They reported a 5-day limit to discharge solids without significantly increasing system BOD. High BOD may cause ambient dissolved oxygen levels to fall below concentrations critical for optimal fish metabolism and nitrification. A minimum DO of 4 mg/L is recommended for warmwater fish (Wedemeyer, 1996), while 2 mg/L is recommended for nitrifying bacteria (Wheaton et al., 1994).

### **Carbonaceous biochemical oxygen demand**

cBOD<sub>5</sub> accounts for the oxygen demand exerted during biochemical degradation of organics (APHA et al., 1985). Nitrification can be inhibited during cBOD<sub>5</sub> incubation; therefore, the test may be used to primarily measure heterotrophic oxygen consumption. This test provides an indirect measure of the effects of organic degradation on the culture system.

### **Dissolved Organic Carbon**

Dissolved organic carbon (DOC) quantitatively accounts for organically bound carbon dissolved within the culture water that can pass through a 0.45 µm filter (APHA et al., 1985). This carbon accounts for a fraction of total organic carbon (TOC). TOC is the summation of dissolved and particulate organic carbon. Organic carbon represents an energy source to heterotrophic populations (Kaiser and Wheaton, 1983). Abeysinghe et

al. (1996) observed decreased nitrification efficiency as a function of increased total organic carbon (TOC) levels. This suggests that elevated organic carbon levels may induce a large increase in heterotrophic populations, decreasing chemoautotrophic populations and filter nitrification efficiency.

## **BIOFILTER DESCRIPTIONS AND OPERATIONAL CHARACTERISTICS**

Several types of biofilters have been developed for use in the aquaculture industry, each with its own design and operational characteristics. Biofilter types range from submerged media and fluidized bed reactors to trickling filters, rotating biological contactors and rotating drums. However, descriptions given below are of biofilter types that pertained to the filters used in this study, a rotating biological contactor (RBC), packed tower trickling filter and an upflow pulsed bed bead filter (Figure 1.1).

### **Rotating Biological Contactor**

A rotating biological contactor (RBC) is arranged as a cylindrical drum designed to rotate perpendicular to water flow in the filter vessel while providing biochemical treatment to fish culture wastewater. Drum rotation allows the media and fixed biofilm to be alternately submerged in and emerged from the wastewater for waste treatment and oxygenation purposes, respectively (Wheaton et al., 1991a). Proper rotational speed must be maintained to ensure maintenance of viable bacterial populations on the media. If rotated too slowly, the biofilm may become oxygen starved, while rotating too rapidly may shear large portions of the biofilm from the drum (Wheaton et al., 1991b). Either situation has the potential to decrease the filter's nitrification efficiency.

RBC designs submerge approximately 40 % (Lawson, 1995) of the drum at any one time during operation. Grady and Lim (1980) reported an optimal drum submergence range of 35 to 50 percent. Water turbulence created by drum rotation simultaneously aerates the biofilm and water column (Wheaton et al., 1994). These forces also enable the RBC to be self-cleaning and to sustain relatively clog-resistant conditions (Rogers and Klemetson, 1985). Self-cleansing involves constant removal of

dead microbes, allowing an active biofilm to be sustained (Wheaton et al., 1994). According to Hess (1979), biofilm activity controls filter effectiveness.

Historically, RBCs have been mechanically powered by motor and shaft designs. Despite adequate filtration performance, these designs have been known to fail, primarily due to mechanical malfunctions (Wheaton et al., 1994). Problems often entailed gear motor malfunction and media detachment from the drum shaft. These failures are regarded as a major problem with RBC filters. As a result, some of the more recently designed RBC filters use air to drive drum rotation as opposed to mechanical operation.

### **Trickling Filter**

Trickling filter designs are numerous, and media range from rock to many types of plastic materials. Depending on the filter's design, the medium either is dumped or fixed inside the filter vessel. Water then is pumped to the top of the medium, where it is dispersed via a spray-bar and allowed to trickle by gravity throughout the medium. As water trickles downward through the filter, nitrifying bacteria oxidize nitrogenous wastes while simultaneous agitation of the water occurs. Agitation increases gas exchange through the water-air interface that exists inside the filter, allowing oxygen to dissolve into the water while carbon dioxide is released. These properties allow this filter type to be regarded as self-aerating and aiding pH buffering. However, pumping cost is one drawback associated with the operation of this filter.

### **Bead Filter**

Bead filters are classified as expandable granular filters (Wheaton et al., 1994), which employ a submerged bed of small plastic beads. Most designs utilize beads that are less dense than water (e.g., propeller-washed bead filters, Armant Aquaculture Inc., Vacherie, Louisiana). However, some employ beads slightly denser than water. Granular filters can be desirable due to their high specific surface area and ability to capture solids while performing nitrification (Chen et al., 1993; Losordo and Timmons, 1994; Westerman, et al., 1996). Because the beaded bed is submerged, all oxygen to the nitrifiers must be supplied as dissolved oxygen in the culture water (Wheaton et al., 1994). System carrying capacity and productivity may be limited if ambient air is the

sole oxygen source. System carrying capacity refers to the maximum amount of fish biomass a recirculating system is designed to support during a production cycle, while productivity refers to the amount of fish biomass actually produced during that cycle.

Accumulation of solids within bead filters also may limit system carrying capacity and productivity. Excessive solids capture may lead to media biofouling, where the bed may experience clogging and channelization of water flow due to solids degradation and biofilm hyper-productivity. Intermittent cleaning of the bed should help to prevent such occurrences, although consequent biofilm shearing may decrease the filter's nitrification efficacy (Malone et al., 1993). Cleaning frequency also may present problems to sustaining proper maintenance of the filter.

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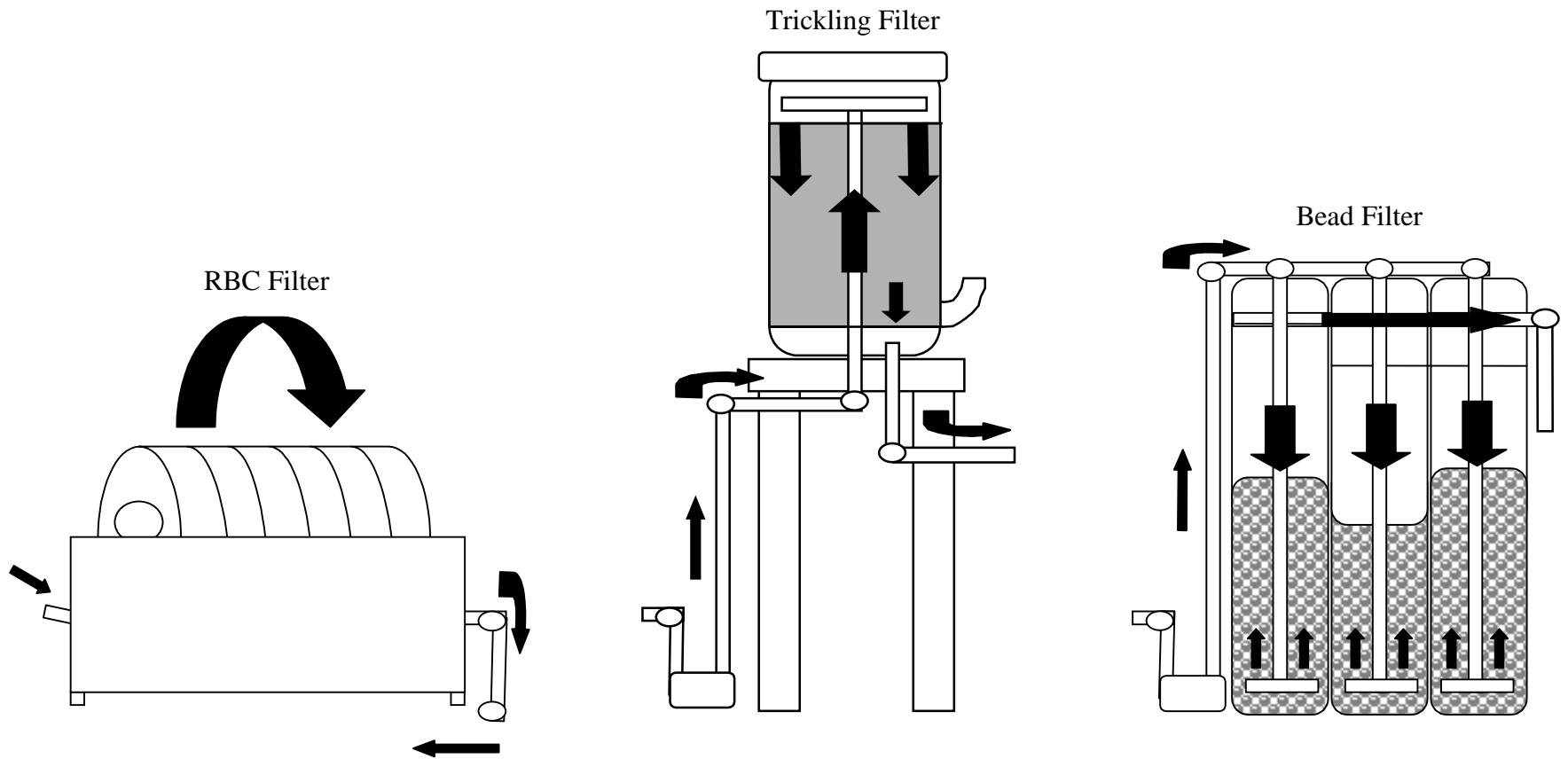


Figure 1.1. Schematic diagrams of a rotating biological contactor (RBC), trickling filter and pulsed bed bead filter. Arrows depict RBC drum rotation and water flow through the filters.

## CHAPTER 2

# COMPARATIVE ANALYSIS OF BIOFILTER PERFORMANCE IN RECIRCULATING AQUACULTURE

### INTRODUCTION

Biofilters are an integral part of recirculating aquaculture systems (Libey and Miller, 1985; Wheaton et al., 1991) and maintain chemoautotrophic bacteria, including those that oxidize ammonia to nitrate in a two-step process known as nitrification. Excess unionized ammonia (as  $\text{NH}_3\text{-N}$ ) concentration can detrimentally effect fish growth and health, and ultimately lead to mortality (Colt and Armstrong, 1981). Mortality results from gill hyperplasia (Colt and Armstrong, 1981), a condition which decreases gill surface area and thereby leads to inadequate transfer of toxic metabolites from the fish to the culture water. Although acute ammonia toxicity values vary between fish species (Rogers and Klemetson, 1985), Colt and Armstrong (1981) reported that most aquatic organisms experience significant growth reductions at concentrations between 0.05-0.20 mg/L. Because fish growth rate is a significant profit-determining factor in production aquaculture, ammonia concentrations must be maintained consistently below toxic levels.

Nitrification biochemically oxidizes total ammonia ( $\text{NH}_4^+\text{-N}$  and  $\text{NH}_3\text{-N}$ ) to nitrate, allowing culture water to be recycled many times prior to discharge from the system. Recycling reduces the volume of effluent discharged on a day-to-day basis. Although nitrification has been found to exist throughout the culture system (Rogers and Klemetson, 1985; Losordo, 1991), high levels of sustained nitrification could not be attained without use of a biofilter.

Mechanical filtration also must be employed to ensure consistent removal of particulate matter and organic wastes. Organic degradation within the culture environment can significantly deteriorate system water quality and increase biofilter

clogging (Lucchetti and Gray, 1988). The majority of organic wastes stem from uneaten feed, sloughed biofilm, and fecal matter (Libey, 1993; Piedrahita et al., 1996).

Biofilter types range from submerged bead and fluidized sand bed reactors to trickling filters, rotating biological contactors and rotating drums. Several of these designs are suitable for use in production aquaculture (Miller and Libey, 1985; Rogers and Klemetson, 1985; Malone et al., 1993; Honeyfield and Watten, 1996; Summerfelt, 1996; Westerman et al., 1996). However, no configuration has been found best suited for treatment of aquaculture effluents. This raises the question of which configuration expresses the greatest number of positive attributes regarding treatment effectiveness, filter operational characteristics and filter management needs when confronted with wasteloading conditions normally encountered in production aquaculture. This study evaluated three types of biofilters used for the production of yellow perch in recirculating aquaculture systems at Virginia Polytechnic Institute and State University. The biofilter designs evaluated were an upflow pulsed bed bead filter, packed tower trickling filter and a rotating biological contactor (RBC). These filters were selected because of their current use in production aquaculture. Objectives of this study were:

- 1.) To evaluate filter acclimation time as a function of filter type employed to a recirculating aquaculture system,
- 2.) To evaluate system water quality as a function of filter type employed to treat wastewater produced in a recirculating aquaculture system,
- 3.) To relate treatment efficiencies for each filter type as a function of filter wasteloading rates ( $\text{g}/\text{m}^2/\text{d}$ ), and
- 4.) To evaluate filter performance as a function of filter design and operational characteristics.

## METHODS

### Culture Methods

**Stocking and System Characterization.** Nine recirculating systems at the Virginia Tech Aquaculture Center were placed on line and stocked with yellow perch, *Perca flavescens*, fingerlings measuring approximately 9 cm total length. Fish were stocked at a density of approximately 455 fish m<sup>-3</sup>. Four systems were stocked with a mono-sex stock of female perch, while the remaining five were stocked with mixed-sex populations (Schmitz, 1999). Mono-sex females averaged 4.4 g, while mixed-sex stocks averaged 5.6 g.

Each system consisted of an 8,330 L rectangular culture tank (6.1m x 1.5m x 1.2m), micro-screen drum filter, biofilter, U- tube with pure oxygen injection and three 0.75 kW pumps. The drum filter employed a 120-micron mesh screen and a vacuum device for solid waste removal, and was also the site for new water additions to the system. Biofilter types were the only factor differing among system designs (Figures 2.1-2.3). Three culture systems for each filter type provided treatment replication. Biofilters were randomly assigned to culture systems to avoid any bias of position effects. System flowrates were adjusted to obtain similar flows between all filter types. An average flow of 379 Lpm was chosen to obtain approximately two system turnovers per hour. Average flow for all systems equaled 370 Lpm.

The systems were located in an aluminum frame building (33.5m x 15.2m x 4.8m), where low lighting conditions were maintained to minimize algal growth and perch fright responses to activity around the tanks. An automatic timer producing a 16-hour light: 8-hour dark photoperiod controlled lighting. Throughout the study, an exhaust fan and four propane gas heaters were used to regulate ambient air temperature.

**Biofilter Characterization.** The filters used in this study were an upflow pulsed bed bead filter, a packed tower trickling filter, and an RBC. Media characteristics for each filter type are given in Table 2.1.



The upflow pulsed bed bead filters were separated into three stages (Figure 2.1) where each column (0.74 m diameter x 2.11 m height) represented one stage. Each stage employed a bed of 2 × 3 mm ABS (acrylonitrile, butadiene and styrene) plastic beads with a specific gravity of 1.04 (International Polymer Corp., Allentown, Pennsylvania). Water was pumped to the stages and alternately expanded the beds with an upwelling flow. Expansion allowed for bed turnover and agitation of the biofilm, and was induced every 3 minutes. Each bed was expanded for approximately 1 minute. Two minutes then were allotted for bed tumbling and contraction (Honeyfield and Watten, 1996). Bed expansion was controlled with a timed electric ball valve assembly.

Packed tower trickling filters (Aqua-Manna Inc., Ladoga, Indiana) utilized a fixed medium design. Each filter consisted of a cylindrical vessel packed with a single-face corrugated plastic medium (0.76 m diameter x 0.76 m height) positioned parallel to water flow. Water was pumped approximately 2.4 m through a center pipe to the top of the medium and was distributed by a rotating spray bar. As water trickled downward throughout the medium, CO<sub>2</sub> was stripped from the water while simultaneous oxygenation of the biofilm occurred.

The RBC filter (Fresh-Culture Systems, Inc., Breinigsville, Pennsylvania) consisted of an air driven cylindrical drum (1.22 m diameter x 1.52 m length) where air was injected below a “waterwheel” located in the center of the drum. Drum rotation was approximately 1 rpm and water was gravity fed to the filter. Alternate emergence of the biofilm from the water column partly fulfilled some of the biofilm's oxygen requirements.

**Biofilter Acclimation.** After stocking, systems were allowed to acclimate using ammonia excreted by the yellow perch. This was done to observe whether one biofilter type acclimated faster than another type. Concentrations of total ammonia-nitrogen (TAN) and nitrite-nitrogen (NO<sub>2</sub><sup>-</sup>-N) were monitored daily to assess nitrifier establishment and activity. Water exchanges were used to decrease the risk of high fish mortality resulting from prolonged exposure to elevated TAN and NO<sub>2</sub><sup>-</sup>-N concentrations. Biofilters were considered fully acclimated when TAN and NO<sub>2</sub><sup>-</sup>-N

levels consistently remained below 0.5 mg/L and water exchanges were no longer necessary to aid concentration control. Following acclimation, studies on biofilter and fish growth performance began on December 17, 1997. Schmitz (1999) reported data on fish growth performance.

**Daily Operations and Water Quality Parameters.** All systems were filled initially with well water. Municipal water was utilized for daily water replacements. New water was introduced into the systems each morning following water sampling. Well water also was used for emergency water exchanges. Sodium bicarbonate ( $\text{NaHCO}_3$ ) additions were made as needed to maintain pH and alkalinity at desired levels within the culture systems. The targeted ranges for basic water quality parameters throughout the study were chosen to optimize environmental conditions for both fish and nitrifiers (Table 2.2).

**Feed Administration.** Fish were fed a 42 % crude protein, 12 % fat, 3 % crude fiber and 13 % moisture floating pelleted diet (Rangen, Inc., Buhl, Idaho) two to three times daily. Rations were recorded to monitor fish feed conversion ratios (Schmitz, 1999) and system feed input (Figure 2.4).

### **Water Quality Monitoring**

**Nitrogenous Wastes and Physical Characteristics.** Daily water sampling commenced at 8 AM, prior to the first fish feeding, to monitor levels of nitrogenous wastes. Samples were taken prior to mechanical and biofilter treatment (sample point 1) (Figures 2.1-2.3). These samples represented concentrations experienced by the fish prior to water exiting the tank. Grab samples were taken periodically from biofilter influents and effluents (sample points 2 and 3) to monitor filter performance. Filter performance also was monitored during analysis of diurnal system dynamics, when samples were drawn at 4-hour intervals.

Temperature ( $^{\circ}\text{C}$ ), pH, dissolved oxygen (DO) and TAN were measured daily, while nitrite-nitrogen ( $\text{NO}_2^-$ -N), nitrate-nitrogen ( $\text{NO}_3^-$ -N) and alkalinity (as  $\text{CaCO}_3$ ) were measured weekly. Water hardness (as  $\text{CaCO}_3$ ) was tested periodically. All tests followed protocols presented in the Standard Methods handbook (APHA et al., 1995). A YSI model 58 dissolved oxygen meter (YSI Co., Yellow Springs, Ohio) was used for temperature and DO measurements, and a Hanna Instruments model HI 1270 pH probe (Hanna Instruments, Woonsocket, Rhode Island) was used to monitor pH. TAN,  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N were analyzed using a Hach DR/2000 spectrophotometer (Hach Co., Loveland, Colorado). Total alkalinity and hardness both were analyzed via Hach titrations. Calculations of  $\text{NH}_3$ -N were made using equations presented by Emmerson et al. (1975).

**Organic Wastes.** Carbonaceous biochemical oxygen demand ( $\text{cBOD}_5$ ), dissolved organic carbon (DOC), and total suspended solids (TSS) analysis began on days 126, 259 and 108 of the study, respectively and levels were monitored for the remainder of the production cycle.

$\text{cBOD}_5$  samples were drawn from sample points 1 and 3 for each system. Samples were drawn in triplicate and immediately analyzed for initial DO concentrations. Final DO concentrations were measured following a 5-day incubation period (APHA et al., 1995). A YSI model 5905 BOD probe (YSI Co., Yellow Springs, Ohio) was used to obtain both initial and final DO concentrations.

DOC samples were drawn from sample points 1 and 3 for each system. Samples were immediately filtered through 0.45 micron membrane filters (Gelman Sciences Inc., Ann Arbor, Michigan) and stored at  $4^{\circ}\text{C}$  until analysis (APHA et al., 1995). A Dohrmann model DC-80 TOC Analyzer (Rosemount Analytical Inc., Lansdowne, PA) and Horiba model PIR-2000 Infrared Gas Analyzer (Horiba Instruments Inc., Irvine, CA) were used for analysis.

TSS were estimated using the filtration method (APHA et al., 1995). Grab samples were collected from all system sample points and stored at 4°C until analysis. Samples were analyzed within 7 days of sampling (APHA et al., 1995).

### **Statistical Analysis**

All statistical tests were performed using the Minitab statistical software package Release 10 Xtra (Minitab, 1995). Data for all test parameters were tested for normality. Because the majority of test parameters were not normally distributed, nonparametric statistical analysis was applied to the data. Mood's median analysis tested for equality of the medians between all filter types for the test parameter being analyzed. If a significant difference was detected ( $p \leq 0.05$ ), a Mann-Whitney two-sample rank test was applied to the data to determine which filter types were statistically significantly different ( $p \leq 0.05$ ). If while applying a Mood's median test, a significant difference between filter types was not detected, further statistical analysis was not performed for the test parameter.

## **RESULTS and DISCUSSION**

### **Biofilter Acclimation**

In all biofilters, TAN and NO<sub>2</sub>-N levels increased to a peak prior to decreasing to steady state conditions (Figures 2.5a and 2.5b). The crests and the troughs of curves before the peaks depict the effects of water flushing on the acclimation process. TAN concentrations for all filters peaked between days 22 and 25. Bead and RBC filters peaked at concentrations of 3.68 and 2.92 mg/L, respectively, with trickling filters peaking at a concentration of 1.60 mg/L. Similar dynamics occurred in NO<sub>2</sub>-N concentrations, where peaks were observed between days 40 and 43. RBC filters peaked at 4.06 mg/L, while the bead and trickling filters peaked at 2.41 and 2.03 mg/L, respectively. The rate of decline to steady state conditions in nitrogenous waste levels was similar among all filter types. Time to TAN and NO<sub>2</sub>-N stabilization exceeded the

typical 20 to 35 day stabilization period for a new biofilter reported by Wheaton et al. (1991). All filters reached TAN steady state conditions around day 42, with  $\text{NO}_2\text{-N}$  stabilization occurring around day 52. Based on inspection of these curves, filter type did not affect biofilter acclimation time.

### **Water Quality Analysis**

Data for all water quality test parameters were analyzed by filter type. Data were analyzed for systems that proved viable throughout the entire 292-day growth study (December 17, 1997 to October 5, 1998). Systems 3 (RBC) and 8 (bead) were not included in the analysis due to massive fish mortalities that occurred prior to the study's end. Data from system 3 mortalities resulted from a break in the aquaculture facility's main water distribution pipe, where excess water entered the culture tank, killing all fish. System 8 mortalities resulted from an unknown cause, resulting in a > 60 % population reduction within the system (Schmitz, 1999). Data from system 7 (trickling) also was excluded from final analysis due to concerns of initial understocking or high rates of perch cannibalism (Schmitz, 1999). Therefore, data in the final analysis encompassed two replicates for each filter type.

Filter flow rates also were analyzed by filter type and accounted for systems that proved viable throughout the entire growth study. Flow rates were not statistically different ( $p = 0.37$ ) (Table 2.1).

**Basic Water Quality Analysis.** TAN,  $\text{NH}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations increased in all systems throughout the study. TAN and  $\text{NO}_3\text{-N}$  steadily increased to about days 98 and 182, respectively; water flushing then was practiced to manage their concentrations.  $\text{NO}_3\text{-N}$  concentrations were directly reduced via water exchanges. TAN fluctuations were probably more a function of  $\text{NO}_2\text{-N}$  control, and also were directly reduced via water exchanges when microbial oxidation was not sufficient for nitrite reduction. Maximum TAN and  $\text{NH}_3\text{-N}$  values experienced by the filters are presented in Table 2.3.  $\text{NH}_3\text{-N}$  rarely reached levels considered harmful to fish health,

and was used as an indicator of excessive TAN or elevated pH and temperature. Median  $\text{NH}_3\text{-N}$  (Table 2.4) was highest in trickling filters, and was significantly different between systems with RBC and bead filters ( $p < 0.00001$ ); however, all medians were well below the upper limit of the target range set for this study (Table 2.2).

$\text{NO}_2^-$ -N accumulation was observed throughout the study for all filter types (Figure 2.6). Concentrations for all systems never reached steady state conditions following filter acclimation. This suggests that *Nitrobacter* populations may have had difficulty adjusting to increasing  $\text{NO}_2^-$ -N levels, resulting in a lag between loading and microbial community response. This phenomenon has been regarded as one of the problems associated with biofiltration (Lucchetti and Gray, 1988) in recirculating aquaculture systems. The greatest  $\text{NO}_2^-$ -N fluctuations were observed in trickling filter systems, where a maximum value of 2.24 mg/L was observed on day 201 of the study. These large fluctuations may have resulted from the high rate of feed administration to the trickling filter systems instead of inability to oxidize ammonia to nitrite to nitrate. Median daily rate of feed administered to trickling filter systems was significantly higher than that to bead ( $p = 0.0003$ ) and RBC ( $p = 0.01$ ) systems, while those to bead and RBC systems were not different ( $p = 0.10$ ) (Table 2.1).  $\text{NO}_3^-$ -N also was greatest in the trickling filter systems, with a maximum value of 143 mg/L, where bead and RBC filters both had maximum values of 118 mg/L. Nitrate levels in trickling filters were significantly different from those in bead filters ( $p = 0.04$ ), but were not different from those in RBC filters; levels in bead and RBC filters were not statistically different ( $p = 0.07$ ). Differences in  $\text{NO}_3^-$ -N levels are indicative of each filter type's ability to effectively carry out both ammonia and nitrite oxidation.  $\text{NO}_3^-$ -N levels were considered non-problematic until levels surpassed 100 mg/L. This did not occur until around day 182 of the study (Figure 2.7). Thereafter, water exchanges were used for nitrate concentration control.

Temperature and pH values for all systems typically ranged from 22-24 °C and 7.0-7.4, respectively. These ranges were considered biologically suitable for both fish and nitrifiers. pH levels in trickling and RBC systems typically were higher than in bead

systems. Higher values most likely resulted from the carbon dioxide stripping capabilities of the trickling and RBC filters. This is further evidenced by alkalinity medians (Table 2.4), where  $\text{NaHCO}_3$  additions were made when pH and alkalinity levels dropped below 7.0 and 100 mg/L (as  $\text{CaCO}_3$ ), respectively. As bicarbonate ions ( $\text{HCO}_3^-$ ) are destroyed during microbial nitrification, the bicarbonate-carbonate chemical equilibrium shifts to the left. This shift will increase dissolved  $\text{CO}_2$  concentrations within the water column. Unless mechanisms are in place to effectively strip the excess dissolved  $\text{CO}_2$  from the water column, prolonged conditions of elevated dissolved  $\text{CO}_2$  potentially can cause fish mortality. Mortality can result by way of disturbing osmoregulation ion balances through excessive  $\text{Na}^+$  losses to the external environment (Moyle and Cech, 1988, pp. 82-84). Elevated dissolved  $\text{CO}_2$  also can suppress pH below optimal levels for nitrifiers (Grace and Piedrahita, 1994), potentially decreasing nitrification rates. Due to observed pH suppression, around day 156 of the study surface agitators were placed in all bead systems to aid in carbon dioxide stripping and maintenance of pH above 7.

Higher  $\text{NH}_3\text{-N}$  observed in trickling filter systems over RBC and bead filter systems also can be attributed to elevated pH levels. Again, this is likely due to the effective carbon dioxide stripping abilities of the trickling filters.

DO typically ranged from 8.5-10.5 mg/L, with 5.2 mg/L being the lowest concentration observed among all systems. DO was not a limiting factor for fish or biofilter performance at these concentrations (Kaiser and Wheaton, 1983; Losordo, 1991). Alkalinity and hardness values typically ranged from 70-180 and 150-280 mg/L in all systems, respectively. Hardness remained above levels considered critical for fish growth. Alkalinity fluctuations resulted from nitrification effects and repeated  $\text{NaHCO}_3$  additions to maintain concentrations above 100 mg/L (as  $\text{CaCO}_3$ ).

**Organic Water Quality Analysis.** All median organic water quality values were not significantly different between filters (Table 2.5). Fitting trend lines to the data

allowed determination of approximate changes in organic levels during the course of the study.

The greatest cBOD<sub>5</sub> increase was observed in bead filter systems, where levels increased by approximately 34 mg/L (Figure 2.8). cBOD<sub>5</sub> values in trickling and RBC systems increased approximately 28 and 29 mg/L, respectively. Since function of nitrifiers was inhibited using a nitrification inhibitor during cBOD<sub>5</sub> analysis, heterotrophic bacteria should have exhibited all of the oxygen demand. These high values indicated that heterotrophs were consuming a lot of dissolved oxygen, so much that they likely were impacting the activity of nitrifying bacteria.

Bead and RBC systems displayed the greatest increases in TSS levels (Figure 2.9). TSS in both bead and RBC systems increased approximately 7 mg/L, while TSS in trickling systems showed almost no increase (1 mg/L). This suggests that systems employing trickling filters were most effective in suspended solids control. This was unexpected, since trickling filters are not designed to maximize on solids removal. Bead filters would be expected to be most efficient in terms of suspended solids control. In a filter comparison study by Westerman et al. (1996), floating-bead biofilters were the only filter type found capable of significantly reducing suspended solids levels (5-6 kg SS/m<sup>3</sup> day<sup>-1</sup>). Delos Reyes and Lawson (1996), reporting on performance of a floating-bead filter and RBC operating in series, also found that the bead filter captured a large portion of the solids in the filter influent.

DOC levels were observed to decrease over the remainder of the study period for all filter types (Figure 2.10). Bead filter systems showed a DOC reduction of approximately 5 mg/L. Levels in trickling and RBC systems remained relatively stable, decreasing approximately 1 and 2 mg/L, respectively.

### **Mass Removal Analysis**

**TAN Mass Removal.** Influent TAN was highest in bead filter systems and was significantly higher than TAN in trickling ( $p = 0.004$ ) and RBC ( $p = 0.03$ ) filter units (Table 2.6). Influent TAN was lowest in RBC systems, and was not significantly



different from TAN in trickling filters ( $p = 0.48$ ). Higher influent TAN to bead filters was most likely a function of lower nitrification rates in bead as compared to trickling and RBC filters. Median mass loading values ( $\text{g}/\text{m}^2/\text{d}$ ) were greatest in RBC and trickling filter systems (Table 2.6). The highest TAN mass removal rate ( $\text{g}/\text{m}^2/\text{d}$ ) was observed in RBC systems, followed by trickling filter systems (Table 2.7). Bead filters exhibited the lowest removal rate, which was found to be significantly different from those in both RBC ( $p = 0.05$ ) and trickling ( $p = 0.01$ ) filters. Tan removal rates in trickling and RBC filters were not significantly different ( $p = 0.13$ ). Tan removal efficiencies were not significantly different for all filter types ( $p = 0.82$ ) (Table 2.7).

The findings in this portion of the study agree with those in other filtration studies, where RBC filters have been noted to provide the best or most consistent nitrification performance when compared to other filter types. Miller and Libey (1985) compared three biofilter types (RBC, packed tower trickling filter, and a fluidized bed reactor) at three channel catfish stocking densities, and reported the RBC to have yielded the greatest TAN mass removal rates for all stocking densities. Rogers and Klemetson (1985) found TAN removal efficiency of more than 90% for an RBC and 50% for a trickling filter. Westerman et al. (1996) reported mean TAN removal rates in grams removed per unit filter volume ( $\text{g}/\text{m}^3/\text{d}$ ) for a combination of biofilters, including floating-bead filters and an RBC. TAN removal rates were 120-160  $\text{g}/\text{m}^3/\text{d}$  and 101  $\text{g}/\text{m}^3/\text{d}$  for the bead filters and RBC, respectively. However, converting these rates into grams removed per unit filter surface area, yielded 0.10-0.13  $\text{g}/\text{m}^2/\text{d}$  and 0.27  $\text{g}/\text{m}^2/\text{d}$  for the bead filters and RBC, respectively. Also, the system employing an upflow sand filter and RBC in combination maintained TAN levels below 4 mg/L throughout the majority of the study. Concentrations up to 40 mg/L were observed in all other systems. The RBC was reported to be the most reliable nitrifying filter in the study. Malone et al. (1993) also compared TAN mass removal rates for various filter types, but found that a mechanical washed bead filter performed slightly better than an RBC configuration (0.291 and 0.280  $\text{g}/\text{m}^2/\text{d}$ , respectively). However, Delos Reyes and Lawson (1996) found that an RBC yielded higher nitrification performance than a mechanical washed bead

filter when operated in series. Bead areal TAN mass removal rate was  $0.056 \text{ g/m}^2/\text{d}$ , with the RBC yielding a removal rate of  $0.257 \text{ g/m}^2/\text{d}$ . Removal efficiencies were 5 and 52 % for the bead filter and RBC, respectively.

Results in this study pertaining to trickling filter performance also were similar to results in a study by Singh et al. (1999), where trickling and bead filter configurations were compared. Systems employing trickling filters maintained lower TAN and  $\text{NO}_2\text{-N}$  levels over the course of the study and were regarded to have performed better than bead filter units. Performance variations were attributed to operational differences existing between filter types. TAN mass removal rates were not presented.

TAN mass removal rates in this study were somewhat lower than removal rates in the filtration studies mentioned above. Results for this portion of the study were derived from data acquired at 8 AM, sampling periods before the fish were fed. Relatively low TAN conditions existed in the culture systems prior to the first fish feeding and may have contributed to the low removal rates and nitrification efficiencies observed.

System  $\text{cBOD}_5$  levels ranged from 12-75 mg/L, with median values around 45 mg/L (Table 2.5). Compared to allowable discharge levels in the wastewater treatment industry, median  $\text{cBOD}_5$  values in this study were relatively high. The maximum federal wastewater  $\text{cBOD}_5$  discharge limit is 30 mg/L as a thirty day average (EPA, 1999). Figueroa and Silverstein, 1992 showed that nitrification rates decreased at  $\text{cBOD}_5$  levels  $> 20 \text{ mg/L}$ . Abeysinghe et al. (1996) observed that nitrification efficiency dropped below 10% once total organic carbon (TOC) levels were 12 mg/L; this TOC level corresponded with a BOD level of about 20 mg/L. Based on these findings, I believe that the high  $\text{cBOD}_5$  levels in this study also may have been responsible for the relatively low TAN removal rates and nitrification efficiencies exhibited by all filter types.

**Organic Mass Removal.** Influent organic levels were not significantly different between filter types (Table 2.6). Organic mass loading was highest in RBC filters, lowest in bead filters, and was significantly different among all filter types.

Organic mass removal rates were determined (Table 2.7), and RBC filters were found to have the highest removal rates among all filters for all organic parameters tested. Significant differences between filter types were observed for cBOD<sub>5</sub> and TSS removed, but not for DOC removal rates ( $p = 0.47$ ) (Table 2.7).

Bead and RBC cBOD<sub>5</sub> mass removal rates were significantly different ( $p = 0.03$ ). RBC cBOD<sub>5</sub> removal rate was approximately 17 times greater than bead removal rate with total grams removed approximately 5 times greater than that observed in bead filters. cBOD<sub>5</sub> removal rate in trickling filters did not differ from those in bead ( $p = 0.09$ ) or RBC ( $p = 0.09$ ) filters.

TSS removal rate in RBC filters did not differ from those in bead ( $p = 0.13$ ) or trickling ( $p = 0.33$ ) filters. Bead and trickling filter TSS removal rates were significantly different ( $p = 0.003$ ), with trickling filter removal rate approximately 21 times greater than bead filter removal rate. Total grams removed in trickling filters were approximately 9 times greater than that in bead filters. As previously noted, trickling filters are not intended for solids removal. However, the data showed that trickling filters in this study performed effective solids removal from the culture effluent. This also is evident in the observation that the system 2 trickling filter clogged within the last two months of the study. To clean the filter medium, the filter was taken offline and pressure washed. Excess solids and biofloc were discharged to the aquaculture facility's central drain. The filter then was placed back online and operated normally through the remainder of the study.

A net increase in DOC was observed across bead filter beds, although DOC levels decreased in all systems for all filter types over the course of the study. Organic matter was observed to accumulate in the beaded beds throughout the study. Degradation of this matter was most likely responsible for the net increase in DOC concentrations across the filter beds. However, dissolution and degradation processes may not have occurred fast enough to cause system concentrations to increase over the course of the study.

Percent removal values were not significantly different ( $p = 0.35$ ) between filter types for any organic test parameter.

### **Diurnal TAN Analysis**

Because nitrification rates are known to vary over the course of a day, due to fish feedings and associated ammonia production, diurnal fluctuations in TAN mass removal rate and percent removal values were investigated. Fish normally were fed in the morning around 9 or 10 AM and again in the evening around 5 or 6 PM.

TAN mass removal rates were not significantly different between filter types for hr 0 ( $p = 0.51$ ) and hr 4 ( $p = 0.51$ ) (Figure 2.11). However, removal rates increased in all filter types between these periods. By hr 8, bead systems exhibited the lowest TAN mass removal rate, which differed significantly from those in trickling ( $p = 0.03$ ) and RBC ( $p = 0.03$ ) filters, where removal rates among trickling and RBC filters were not statistically different ( $p = 0.94$ ). This also occurred at hr 12. TAN mass removal rates in trickling and RBC filter systems were not significantly different ( $p = 0.41$ ). After peaking between hr 12 and hr 16, filter mass removal rates declined in all filter types, but did not decline to levels observed at hr 0. Twaroska et al. (1997) and Westerman et al. (1996) observed similar results in their filtration studies, where 24 hr analyses of various biofilters showed TAN removal to increase with increasing TAN concentrations before peaking and declining. These studies also did not observe TAN mass removal rates at hr 24 to have declined to levels observed at hr 0.

Increases in TAN mass removal rates were most likely in response to increased ammonia production and associated influent TAN due to fish feeding activity. Nitrification efficiencies also increased once adequate feed induced ammonia was present (Figure 2.12), where efficiency values as high as 45, 39, and 42% were observed in bead, trickling and RBC filters, respectively, by hr 4 in the analysis.

Analysis of the area under the concentration curve showed that trickling filters removed both the greatest amount of TAN mass per unit filter surface area ( $\text{g/m}^2$ ) and TAN mass (g) over the 24 hr sampling periods. TAN mass removed per unit surface area was 0.04, 0.11, and 0.10  $\text{g/m}^2$  for bead, trickling and RBC filters, respectively. Total mass removed for the 24 hr sampling periods was 40, 50, and 33 g for bead, trickling and

RBC filters, respectively. TAN mass removed for bead filters was higher than that for RBC filters, although TAN mass removed per unit surface area was higher for RBC filters. The data show that RBC surface area limited total TAN mass removed during the course of 24 hours. The following formula was used to estimate the additional RBC surface area needed to compensate for this removal difference.

$$\text{Additional SA (m}^2\text{)} = (\text{Bead}_{\text{TMR}} - \text{RBC}_{\text{TMR}}) / \text{RBC}_{\text{M RSA}}$$

where: TMR = Total TAN mass removed (g)

M RSA = Mass removed per unit surface area (g/ m<sup>2</sup>)

It was estimated that RBC drums would have needed an additional 70 m<sup>2</sup> of surface area.

### **Performance vs. Filter Design and Operational Characteristics**

Filter design and operational characteristics were believed to have been the dominant factors affecting performance of the filter types employed in this study.

RBC filters yielded the highest TAN mass removal rate followed by trickling filters, with the lowest removal rate observed in bead configurations. One reason why this was observed may have been due to RBC and trickling filter beds being partly exposed to the air, where atmospheric oxygen was capable of satisfying some of the oxygen demands of the exposed biofilms. Due to bed submergence, bacterial oxygen requirements in bead filters could only be met by oxygen available within the water column. Continuous submergence of the bed may present problems for a biofilm, problems that may not exist if the filter bed is alternately or partly exposed to the atmosphere. The source of the problem lies in the diffusion properties controlling oxygen transfer to the bacteria in the biofilm. Bacterial cells in the biofilm are contained in a cell membrane, a slime layer, and a static water layer (Wheaton et al., 1991). A static water layer can exist if water flow, relative to the bacteria, is fairly non-turbulent and allows for laminar conditions to exist within the immediate vicinity of the bacteria cells. The static

layer together with the slime layer and cell membrane increases resistance to diffusion of oxygen from the water to the bacteria cells. If the rate of oxygen diffusion is slowed enough, it may potentially limit nitrification rates, as inferred based on theory from Frick's Law. Wheaton et al. (1991) stated that increasing water velocity around the bacteria would decrease static water layer thickness and increase the oxygen or ammonia substrate diffusion rate into the cells. Increased diffusion rates subsequently would allow greater utilization of oxygen and ammonia substrate by the bacteria.

Exposing the biofilm to the atmosphere also should produce a thinner static water layer. While emerged from the water column, water drains from the biofilm leaving only a thin water film in contact with the biofilm's surface. During this time, static water depth should be reduced and diffusion rates increased. Oxygen diffusion pressure in air (20 parts per hundred) is much greater than in water (several parts per million). In this study, biofilms in the trickling filters were exposed to the atmosphere as water droplets entrapped air while descending through the filter beds. Biofilms on the RBC filters were exposed to the atmosphere as the drums rotated out of the water column. Beaded beds were never emerged from the water column.

Another reason for lower TAN mass removal rates observed in the bead filters as compared to trickling and RBC filters may have been due to the bead filters' solids capturing capabilities. Periodic upwelling for bed expansion was intended to alleviate the need for manual backwashing of the filters. During each upwelling cycle a portion of aggregated solids were to be released from the filter bed back to the main water flow allowing the mechanical filter to intercept the solids and discharge them from the system. As organic wasteloading to the filters increased, resulting from increases in fish feeding levels, periodic upwelling of the filter beds became inadequate at effectively controlling organic material in the filter beds. Organic matter accumulated in several of the beaded beds. This organic matter most likely accounted for the 23 % TSS reduction observed in the bead filter systems (Table 2.7). Water channelization also occurred in several bead filter columns, indicating filter bed clogging. Intermittent and gentle agitation of the filter beds via upwelling water were most likely responsible for these occurrences.

Several studies (Bovendeur et al., 1990; Figueroa and Silverstein, 1992; Manem and Rittman, 1992 and Malone et al., 1993) have shown nitrification efficiency to decrease with increasing organic concentrations. Competition between heterotrophic and nitrifying bacteria, primarily for space and oxygen, has been credited for the observed nitrification decrease. Heterotrophs exhibit faster growth rates than nitrifiers, especially in organic-laden waters. According to Manem and Rittman (1992), heterotrophs will grow on top of existing biofilm layers, forcing nitrifiers to reside at deeper biofilm depths. Malone et al. (1993) attributed inhibition of oxygen and nutrient transport to deeper biofilm depths as the cause of nitrification reductions. Although direct measurement of the biofilm was beyond the scope of this study, the data suggest that organic accumulation may have facilitated development of reasonably large heterotroph populations in the bead filters.

During diurnal analysis, analysis of areas under removal curves showed that trickling filters removed the greatest amount of TAN mass over the 24 hr sampling periods. TSS removal and intermittent foam fractionation observed in trickling filter systems 2 and 4 are believed responsible for the boost in nitrification rates. Foam condensate was first observed around day 217 of the study and was intermittently observed thereafter. Condensate would emerge from the water column in the degassing chambers (Figure 2.2) following discharge from the trickling filters. Although foam production was not measured, at times condensate production was great enough that prompt removal of the foam was necessary to prevent spillage onto the surrounding floor area. As previously mentioned, high organic levels (in dissolved and particulate forms) can decrease nitrification efficiency of a biofilm. Therefore, it is imperative to remove organic matter from the culture water as quickly as possible. Foam fractionation has been reported to remove suspended as well as dissolved solids from aquaculture effluents (Timmons, 1994). Solids < 30  $\mu\text{m}$  are characterized as fine solids, and are not easily removed by conventional solids removal mechanisms (e.g., settling tanks and microscreen filters). Poor removal of fine solids allows them to accumulate in recirculating aquaculture systems with the passage of time. Findings by Chen et al.

(1991) and Easter (1992) confirmed that fine solids predominate in aquaculture effluent particle size distributions. Findings of a study where foam fractionation was used to treat particulate waste in aquaculture effluents (Chen et al., 1993) foam fractionation provided effective solids removal. Solids removed were  $< 30 \mu\text{m}$  in diameter and primarily organic in nature. Weeks et al. (1992) found that foam fractionation concentrated volatile solids (VS), total suspended solids (TSS) and nitrogen as total Kjeldahl nitrogen (TKN). VS, TKN, and TSS condensate concentrations, on average, were 2.7, 44 and 25 times higher than their respective concentrations found in the culture water, respectively. Based on these findings and the observed nitrification performance of trickling filters in this study, it can be assumed that foam fractionation proved beneficial to nitrifiers in trickling filters by removing fine solids that would normally not have been removed by microscreen filtration.

The high percentage of TSS removed by trickling filters (Table 2.7) confirms that these filters were effectively removing solids from the culture water. Small opening sizes of the vertical passages in the trickling filter medium, single face corrugated plastic, were most likely responsible for the observed foam fractionation and TSS removal. Passage openings were roughly semi-circular in shape and had a cross-sectional area of approximately  $16 \text{ mm}^2$ .

As previously stated, each system utilized three 0.75 kW pumps with system flowrates being adjusted to achieve similar flows between all filter types. Adjusting the main 7.6 cm gate-valve controlling water flow in each system restricted pump output. RBC pumps were restricted the most, followed by trickling filters, with bead filter pumps being almost non-restricted. This indicates that all of the energy output from the three pumps was needed for bead filter system operation and that trickling and RBC filter systems required less energy to function properly under the set conditions. The fact that RBC system pumps were restricted the most and that water was gravity fed to the RBC filters, also indicates that it may have been possible that removal of one pump from each RBC filter system still would have rendered similar performance results observed in this study. Additionally, air driven rotation of RBC drums rendered these filters less prone to



mechanical failure than either bead or trickling filters. Bead filter operation was highly dependent upon relatively complex timer devices, which were subject to failure. Both bead and trickling filters were highly susceptible to pump failure. Lastly, RBC rotational speed was independent of system flowrate potentially allowing the filters to retain performance at substantially higher flowrates. Rogers and Klemetson (1985) found an RBC to have sustained higher nitrification efficiencies over a wider range of increasing hydraulic loadings than biodrum and trickling filter configurations.

This study was performed on a pilot-scale; however trickling and RBC filters of the designs used in this study have been employed in commercial aquaculture facilities. According to operators, of the commercial facility, three trickling filters were employed for each culture tank, where two operated simultaneously, with the third one offline. Due to frequent filter clogging, filter operation was rotated every 24 hours. An operating filter would be taken offline for cleaning and the previously cleaned filter would be placed back online. Thus, filter cleaning was considered the major drawback to operation of these filters (J. Bradley, Aqua-Manna, Inc., personal communication). Performance data for the RBC filters depicts this filter configuration as relatively maintenance free, less prone to mechanical failure, and capable of sustaining TAN at approximately 3 mg/L when feeding up to 272 kg feed/day (D. Prillaman, Blue Ridge Aquaculture, personal communication). Bead filters in this study have not been used on a commercial scale (B. Watten, US Fish and Wildlife Services, personal communication).

## **SUMMARY and CONCLUSIONS**

All filter types proved able to effectively treat the wastewater generated during the course of this study, where the majority of water quality parameters were within ranges considered biologically suitable for both fish and nitrifiers. Although differences in nitrification performance and certain water quality parameters were observed between filter types, this study could not conclude that one filter type should be considered most effective at treating wastewater produced in a recirculating aquaculture system. Each

filter type expressed certain positive and negative attributes related to filter performance. Filter design and operational characteristics were regarded as the main factors in controlling overall performance of each filter type.

The following statements summarize the major findings of this study.

1. Filter bed emergence was responsible for effective carbon dioxide stripping, pH maintenance and nitrification performance, where trickling filters proved most effective at carbon dioxide stripping and pH maintenance.
2. Higher TAN mass removal rates were achieved in trickling and RBC filters than in bead filters, where bed submergence and accumulation of organic material within the bead filter beds were believed to have affected the filters' nitrification performance.
3. Low TAN mass removal rates and nitrification efficiencies for all filters resulted from relatively high cBOD<sub>5</sub> values at issue in this study.
4. Analysis of areas under mass removal curves depicted RBC filters as surface area limited.
5. Trickling filters effectively removed TSS from the culture water.
6. The study did not show filter type as having a significant effect on median organic water quality parameter values.

It is noteworthy that findings may differ when examining these filter types with different design or under different system conditions than those in this study. This may especially be true concerning bead filter performance, since sinking beads were employed in this configuration. The majority of filtration studies has encompassed floating bead biofilters, which have not been regarded as self-cleaning.

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Table 2.1. Media characteristics and median system flow rates (95% CI) for each biofilter type employed.

	Media Surface Area (m <sup>2</sup> )	Media Volume (m <sup>3</sup> )	Specific Surface Area (m <sup>2</sup> /m <sup>3</sup> )	Median Flow Rate (Lpm)
Bead	1044	0.379	2757	269 (223-329)
Trickling	465	0.277	1681	327 (303-394)
RBC	325	1.78	184	340 (318-390)

Table 2.2. Target ranges for basic water quality parameters.

Parameter	Unit	Target Range	Reference
NH <sub>3</sub> -N	(mg/L)	< 0.05	Colt and Armstrong 1981
NO <sub>2</sub> <sup>-</sup> -N	(mg/L)	< 1.0	Losordo 1991
NO <sub>3</sub> <sup>-</sup> -N	(mg/L)	< 100	Losordo 1991
Dissolved Oxygen	(mg/L)	> 5	Kaiser and Wheaton 1983; Losordo 1991
pH		6.5 – 8.0	Meade 1989
Temperature	°C	22 – 23	Schmitz, 1999
Alkalinity	(mg/L)	> 100	Meade 1989; Losordo 1991
Hardness	(mg/L)	> 100	Meade 1989



Table 2.3. Maximum TAN and NH<sub>3</sub>-N values experienced by systems with each filter type during the course of the study.

Filter Type	TAN (mg/L)	NH <sub>3</sub> -N (mg/L)
Bead	1.89	0.015
Trickling	1.82	0.018
RBC	1.74	0.071

Table 2.4. Median values (95% CI) for basic water quality parameters. Parameters in each column with same superscript are not significantly different at the  $p < 0.05$  level.

Filter Type	TAN (mg/L)	NH <sub>3</sub> -N (mg/L)	NO <sub>2</sub> <sup>-</sup> -N (mg/L)	NO <sub>3</sub> <sup>-</sup> -N (mg/L)	DO (mg/L)	pH	Temp (° C)	Alkalinity* (mg/L)	Hardness* (mg/L)	Feed (kg/d)
Bead	1.06 <sup>a</sup> (1.00-1.09)	0.006 <sup>a</sup> (0.0051-0.0063)	0.390 <sup>a</sup> (0.340-0.435)	66 <sup>a</sup> (62-71)	9.5 <sup>a</sup> (9.3-9.7)	7.12 <sup>a</sup> (7.09-7.14)	23.2 <sup>a</sup> (23.2-23.4)	136 <sup>a</sup> (124-148)	217 <sup>a</sup> (192-253)	1.95 <sup>a</sup> (1.92-2.53)
Trickling	0.90 <sup>b</sup> (0.85-0.94)	0.008 <sup>b</sup> (0.0077-0.0084)	0.400 <sup>a</sup> (0.345-0.472)	77 <sup>b</sup> (69-83)	9.2 <sup>a</sup> (9.0-9.4)	7.28 <sup>b</sup> (7.26-7.33)	23.2 <sup>a</sup> (23.1-23.3)	112 <sup>b</sup> (104-123)	202 <sup>a</sup> (178-237)	1.99 <sup>a</sup> (1.78-2.37)
RBC	0.85 <sup>b</sup> (0.82-0.90)	0.006 <sup>a</sup> (0.0055-0.0061)	0.381 <sup>a</sup> (0.355-0.405)	77 <sup>ab</sup> (67-85)	9.5 <sup>a</sup> (9.2-9.8)	7.19 <sup>c</sup> (7.16-7.23)	22.3 <sup>b</sup> (22.1-22.4)	128 <sup>a</sup> (118-137)	223 <sup>a</sup> (192-253)	2.22 <sup>a</sup> (1.92-2.53)

\* as CaCO<sub>3</sub>

Table 2.5. Median values (95% CI) for organic water quality parameters. None of the values in a given column are significantly different at the  $p < 0.05$  level.

Filter Type	CBOD <sub>5</sub> (mg/L)	DOC (mg/L)	TSS (mg/L)
Bead	48 (32-64)	14 (12-20)	13 (10-19)
Trickling	45 (31-58)	13 (10-14)	11 (8-16)
RBC	44 (33-55)	15 (14-19)	8 (5-13)

Table 2.6. Median influent and mass loading values for TAN and organic water quality parameters (95% CI). Corresponding values in same row with different superscripts are significantly different at  $p < 0.05$ .

Test Parameter	Bead		Trickling		RBC	
	Influent (mg/L)	Mass Loading (g/m <sup>2</sup> -d <sup>-1</sup> )	Influent (mg/L)	Mass Loading (g/m <sup>2</sup> -d <sup>-1</sup> )	Influent (mg/L)	Mass Loading (g/m <sup>2</sup> -d <sup>-1</sup> )
TAN	1.10 <sup>a</sup> (0.10-1.23)	0.14 <sup>a</sup> (0.13-0.15)	0.91 <sup>b</sup> (0.83-0.99)	0.96 <sup>b</sup> (0.87-1.04)	0.88 <sup>b</sup> (0.79-1.02)	8.17 <sup>c</sup> (7.41-9.48)
cBOD <sub>5</sub>	48 <sup>a</sup> (32-64)	6 <sup>a</sup> (4-8)	45 <sup>a</sup> (31-58)	48 <sup>b</sup> (35-58)	44 <sup>a</sup> (33-55)	409 <sup>c</sup> (298-560)
DOC	14 <sup>a</sup> (12-20)	1.8 <sup>a</sup> (1.5-2.5)	13 <sup>a</sup> (10-14)	13.6 <sup>b</sup> (10.4-15.0)	15 <sup>a</sup> (14-19)	136.5 <sup>c</sup> (126.8-174.7)
TSS	17 <sup>a</sup> (10-21)	2.1 <sup>a</sup> (1.3-2.7)	13 <sup>a</sup> (9-25)	13.3 <sup>b</sup> (9.0-26.5)	10 <sup>a</sup> (6-15)	96.9 <sup>c</sup> (54.4-138.2)

Table 2.7. Median mass removal rate and percent removed values (95% CI) for TAN and organic parameters tested over the course of study. Corresponding values in same row with different superscripts are significantly different at  $p < 0.05$ .

Parameter	Bead		Trickling		RBC	
	Mass Removal (g/m <sup>2</sup> -d <sup>-1</sup> )	Percent Removed (%)	Mass Removal (g/m <sup>2</sup> -d <sup>-1</sup> )	Percent Removed (%)	Mass Removal (g/m <sup>2</sup> -d <sup>-1</sup> )	Percent Removed (%)
TAN	0.004 <sup>a</sup> (0.003-0.009)	4.3 <sup>a</sup> (2.4-10.2)	0.037 <sup>b</sup> (0.006-0.062)	5.2 <sup>a</sup> (0.6-9.4)	0.14 <sup>b</sup> (-0.18-0.33)	1.9 <sup>a</sup> (-1.7-5.5)
cBOD <sub>5</sub>	0.5 <sup>a</sup> (0.2-1.0)	9.7 <sup>a</sup> (4.4-12.8)	1.4 <sup>a,b</sup> (-2.9-7.7)	5.6 <sup>a</sup> (-6.5-15.2)	8.3 <sup>b</sup> (-2.9-127.7)	3.3 <sup>a</sup> (-0.9-30.1)
DOC	-0.02 <sup>a</sup> (-1.02-0.41)	-0.9 <sup>a</sup> (-69.0-16.4)	0.33 <sup>a</sup> (-0.40-1.11)	2.7 <sup>a</sup> (-3.8-8.6)	2.68 <sup>a</sup> (-53.28-22.27)	1.7 <sup>a</sup> (-43.7-11.8)
TSS	0.4 <sup>a</sup> (0.1-1.1)	22.5 <sup>a</sup> (4.4-41.5)	8.3 <sup>b</sup> (2.1-15.4)	54.6 <sup>a</sup> (19.6-66.9)	12.5 <sup>a,b</sup> (-15.3-54.0)	9.6 <sup>a</sup> (-14.8-51.9)

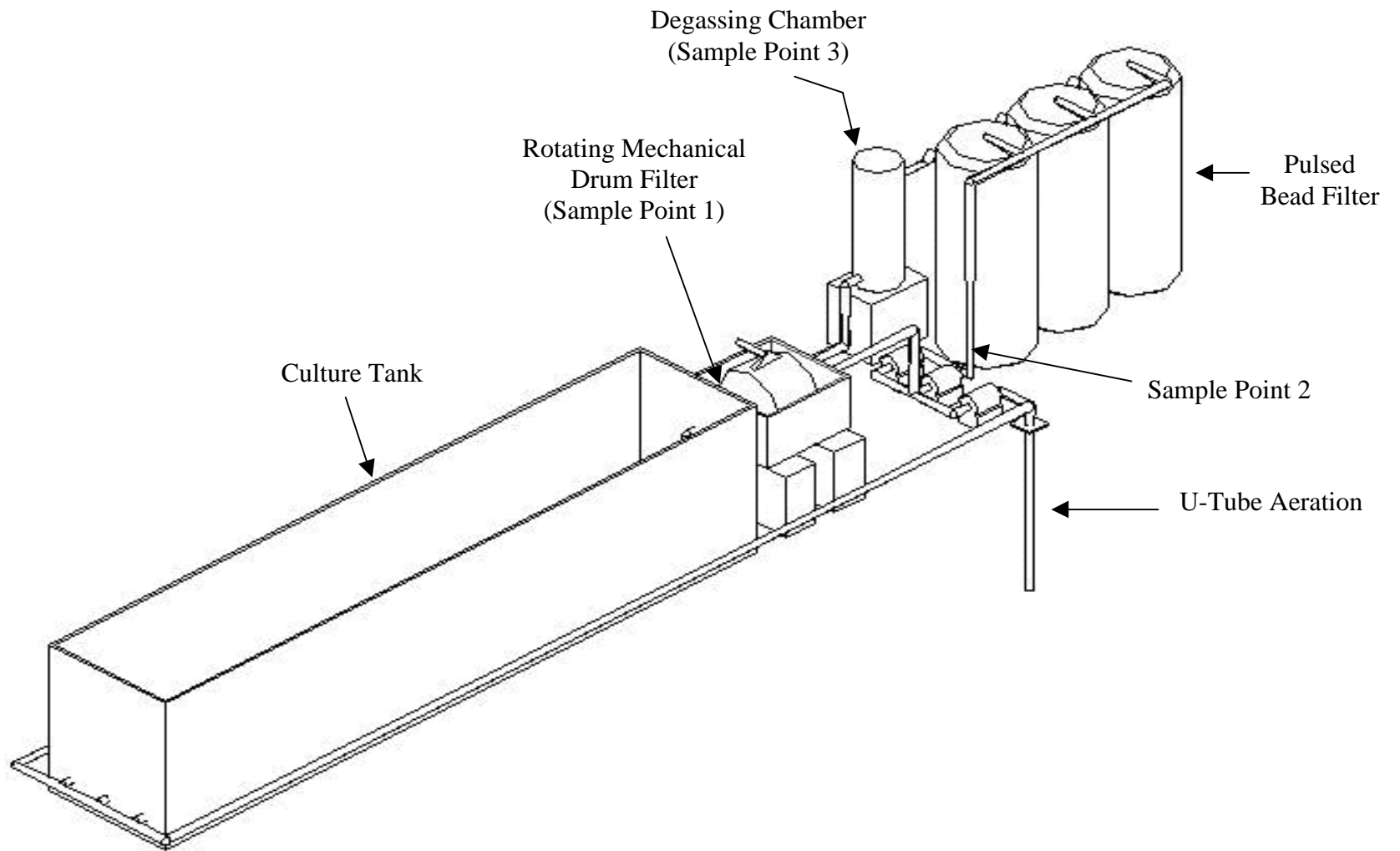


Figure 2.1. Schematic diagram of recirculating aquaculture system employing an upflow pulsed bed bead filter.

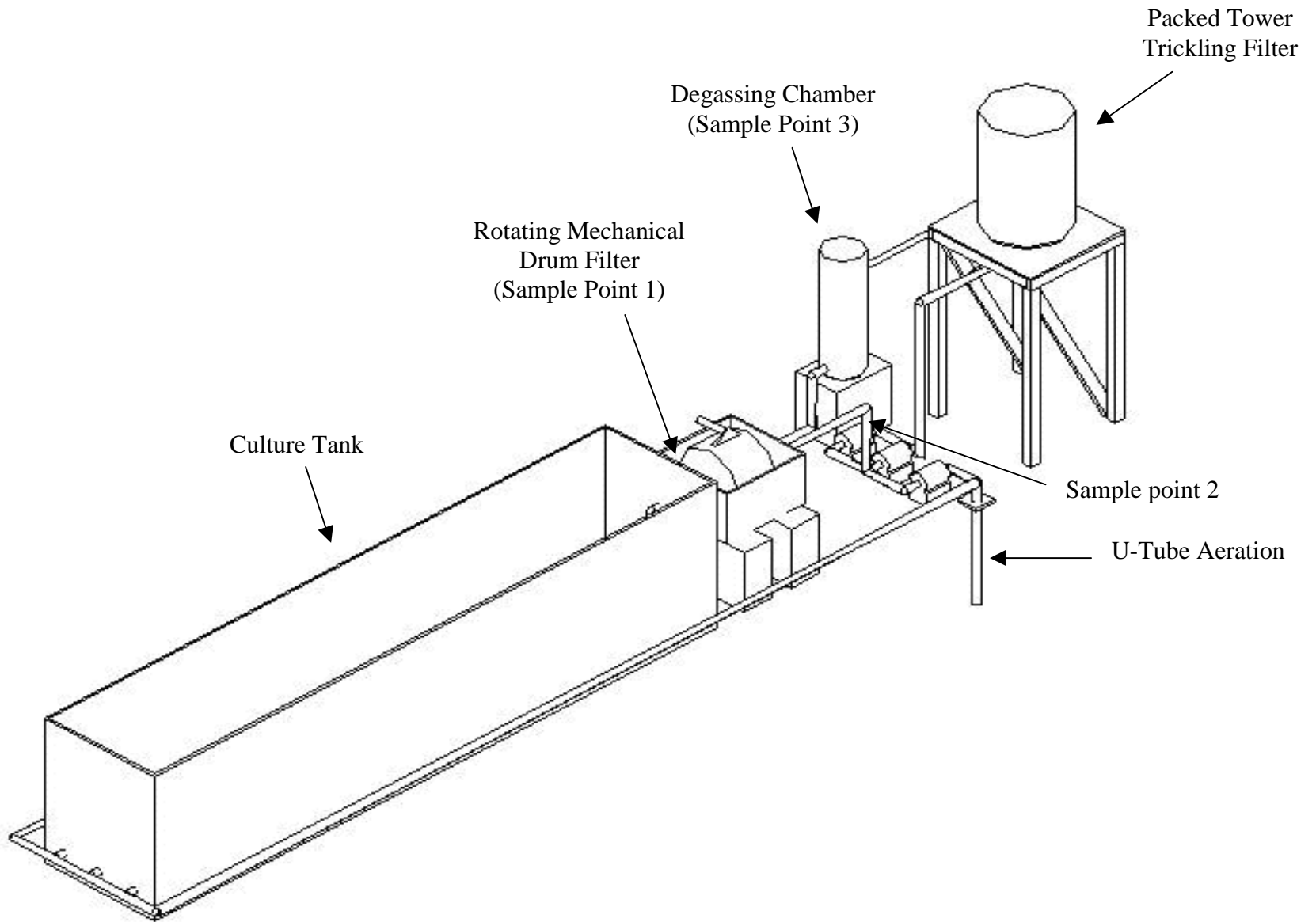


Figure 2.2. Schematic diagram of recirculating aquaculture system employing a packed tower trickling filter.

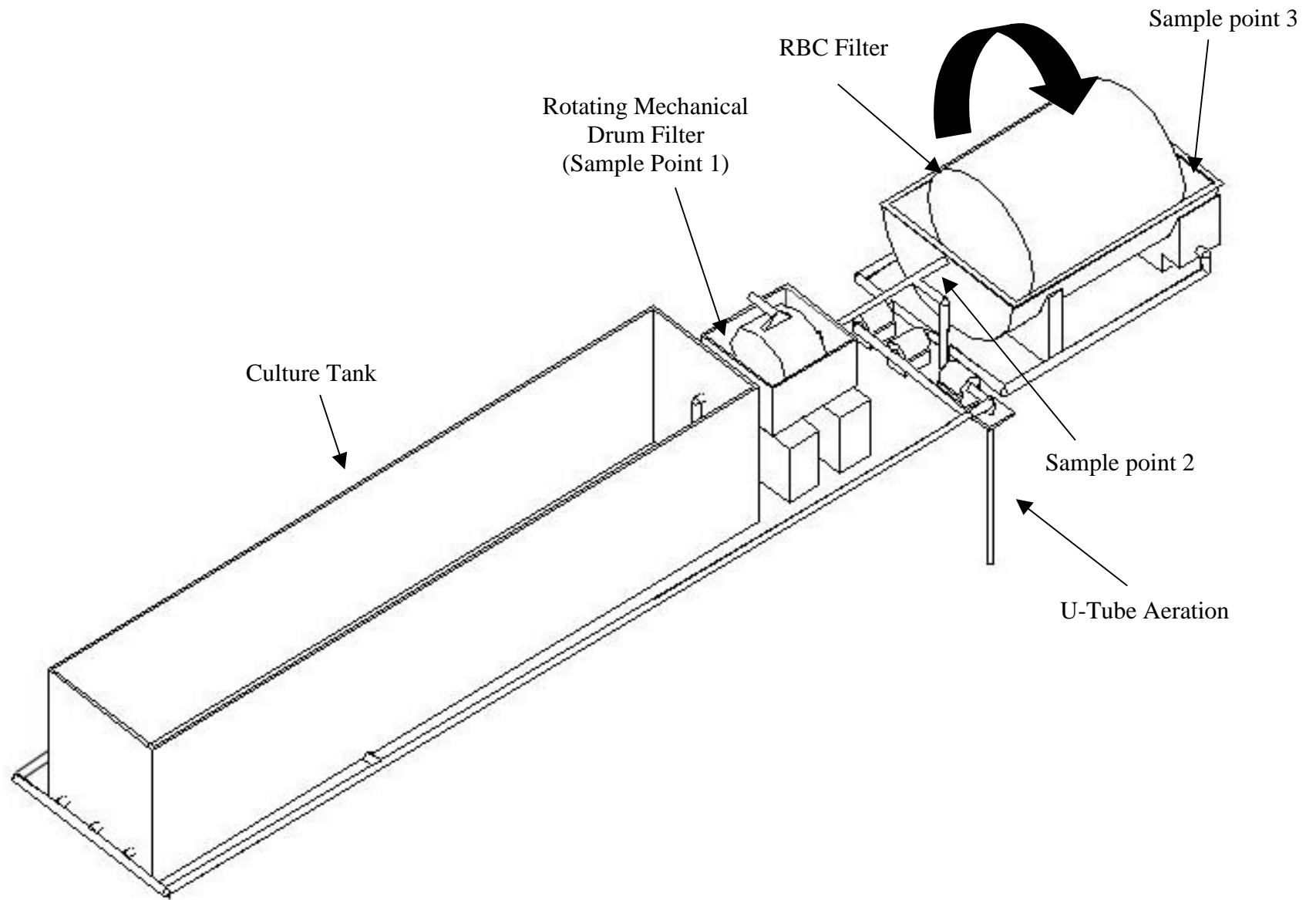


Figure 2.3. Schematic diagram of recirculating aquaculture system employing a rotating biological contactor (RBC) filter.



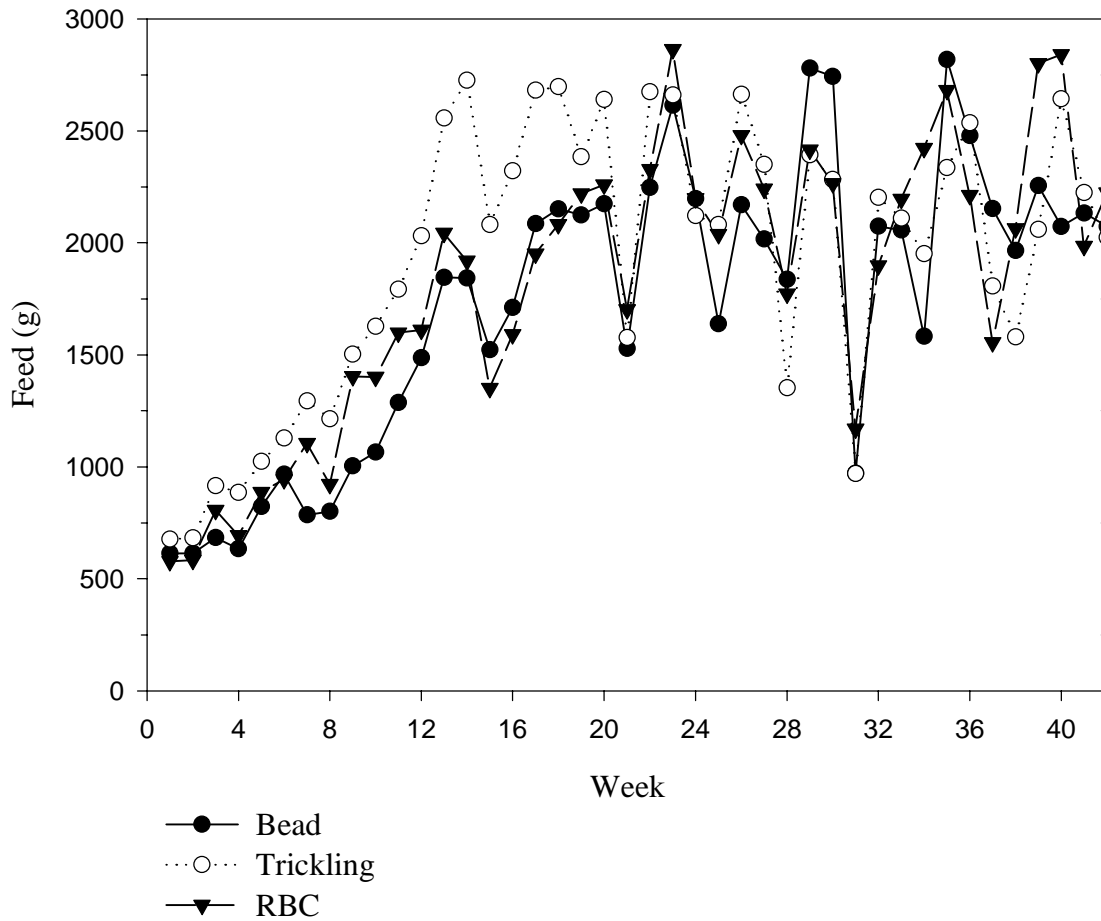


Figure 2.4. Average weekly feed additions during the course of the study.  
 (Source: Schmitz, 1999)

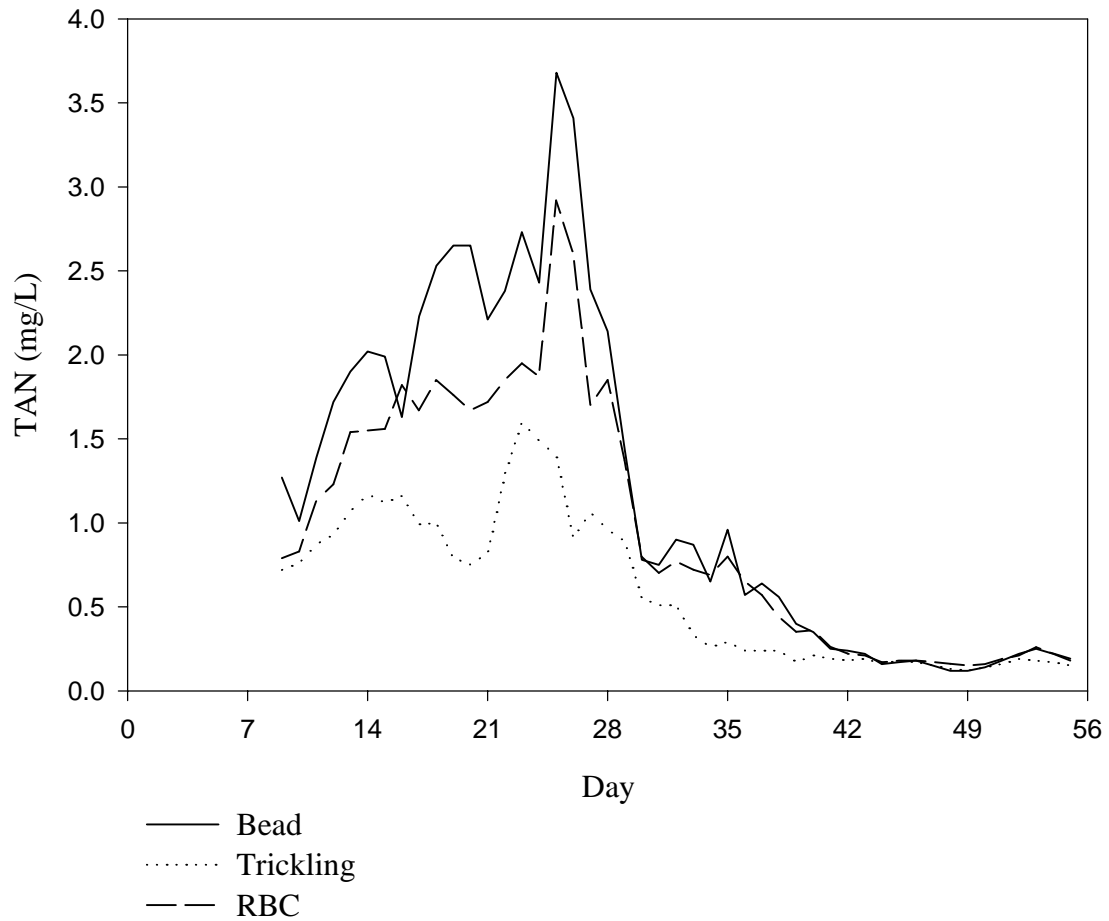


Figure 2.5a. Biofilter microbial acclimation, shown using total ammonia nitrogen (TAN) to indicate first-stage nitrifier population establishment.

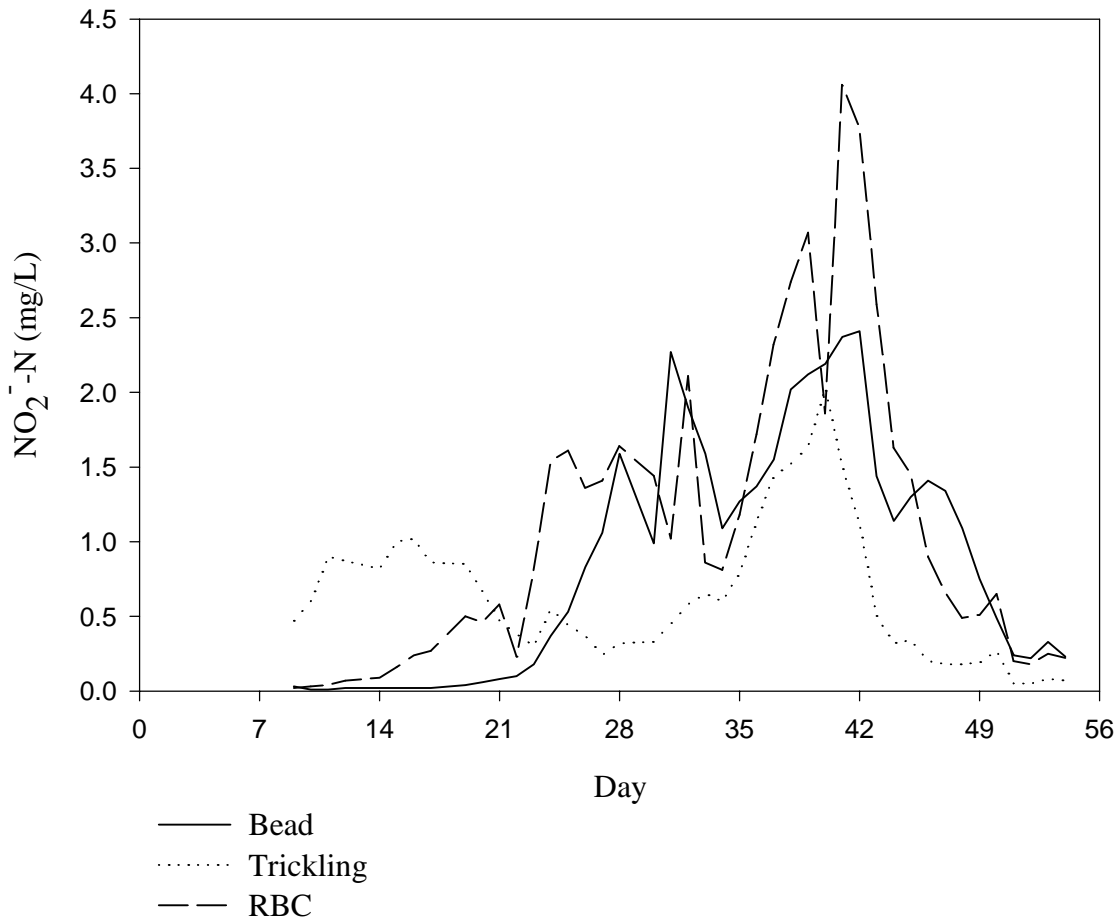


Figure 2.5b. Biofilter microbial acclimation, shown using nitrite nitrogen (NO<sub>2</sub><sup>-</sup>-N) as an indicator of second-stage nitrifier population establishment.

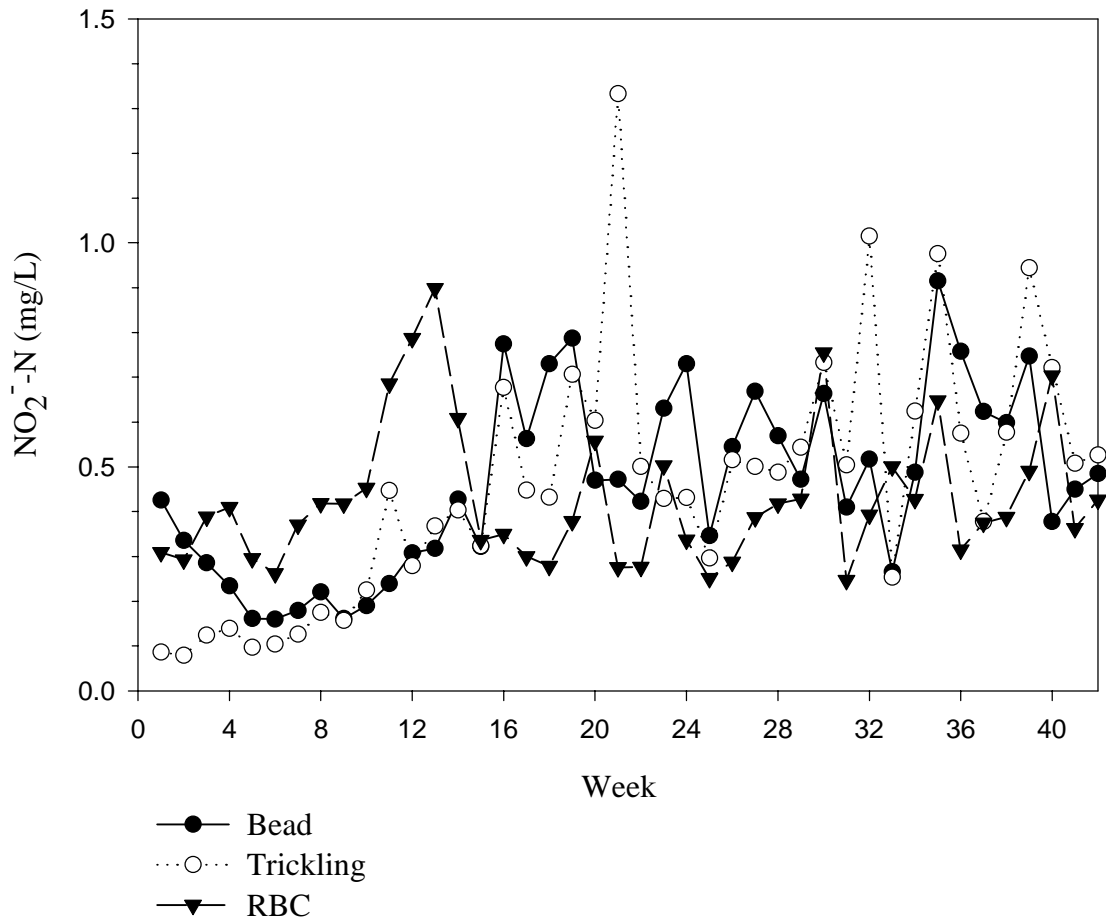


Figure 2.6. Weekly nitrite ( $\text{NO}_2^- \text{-N}$ ) medians during the course of the study.

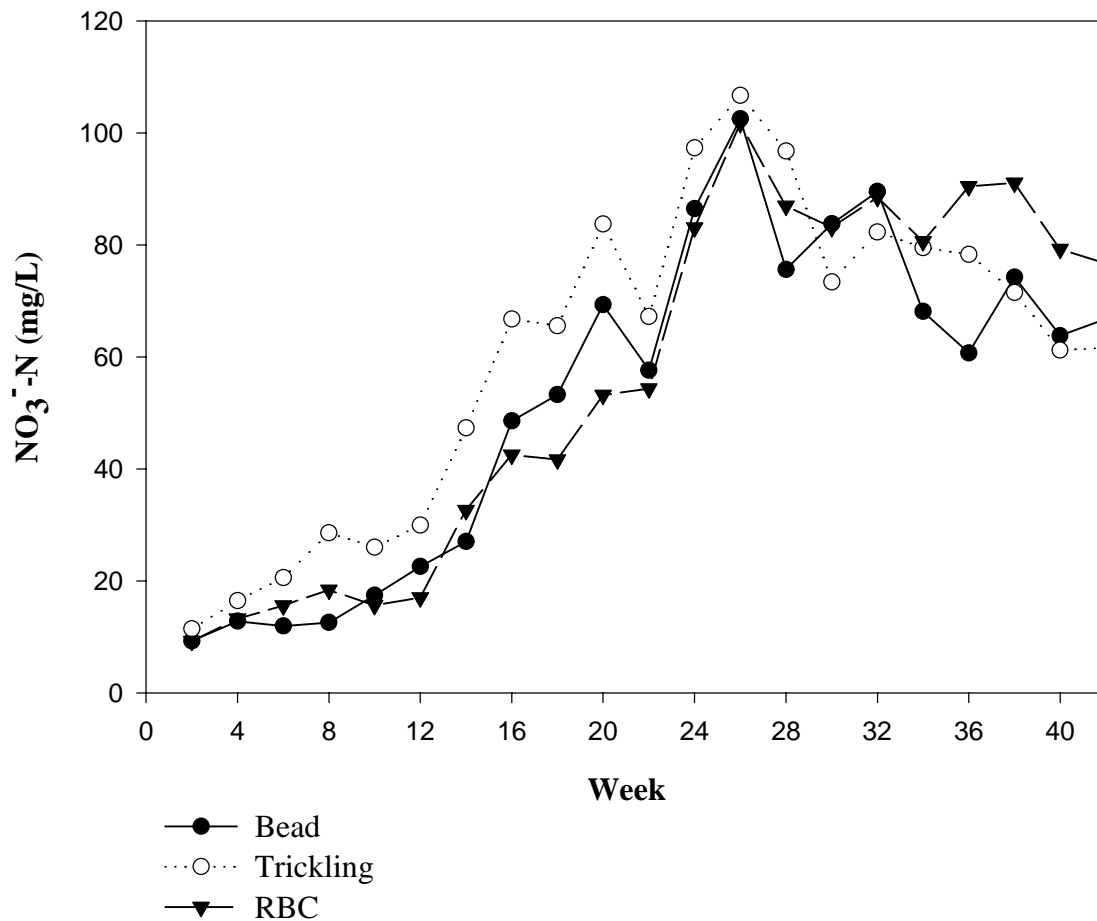


Figure 2.7. Weekly nitrate ( $\text{NO}_3^-$ -N) medians during the course of the study.

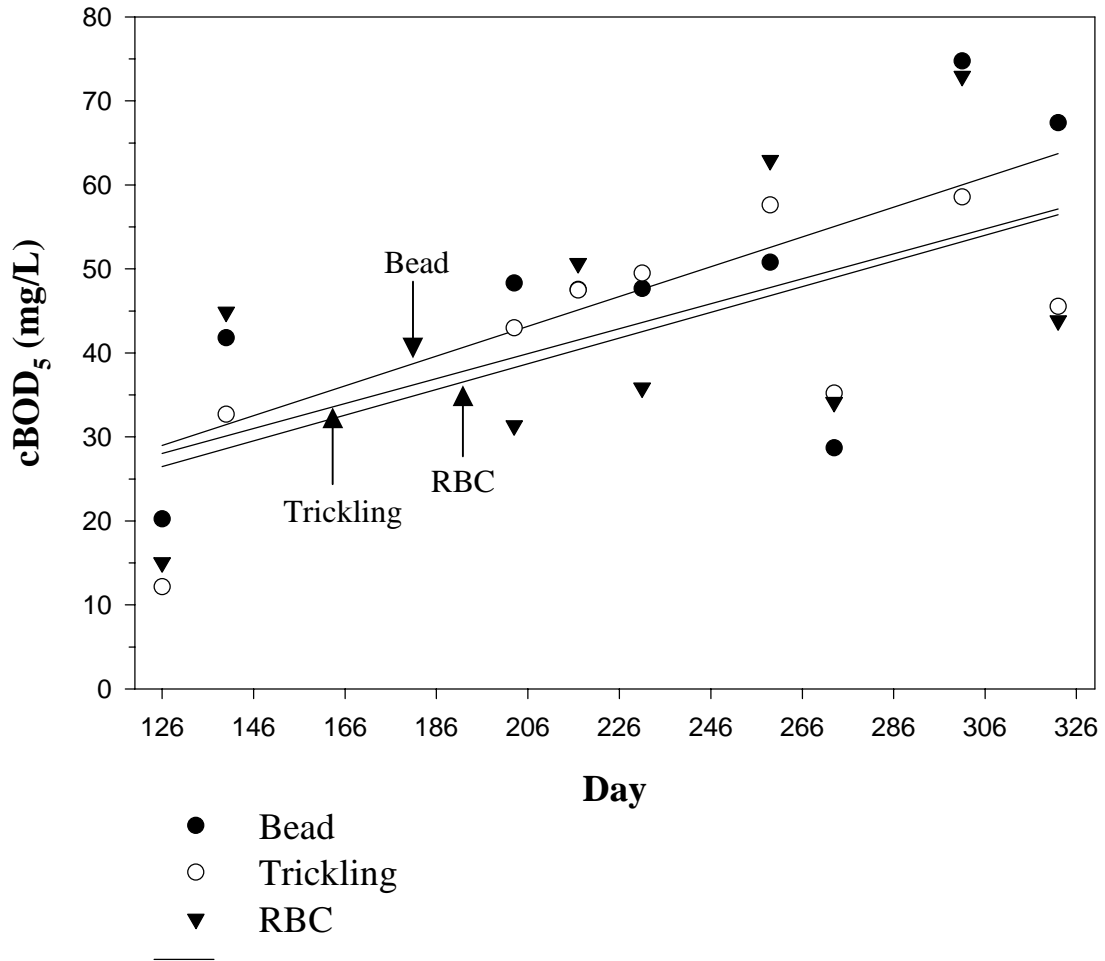


Figure 2.8. Approximate cBOD<sub>5</sub> level increases observed over the course of the study.

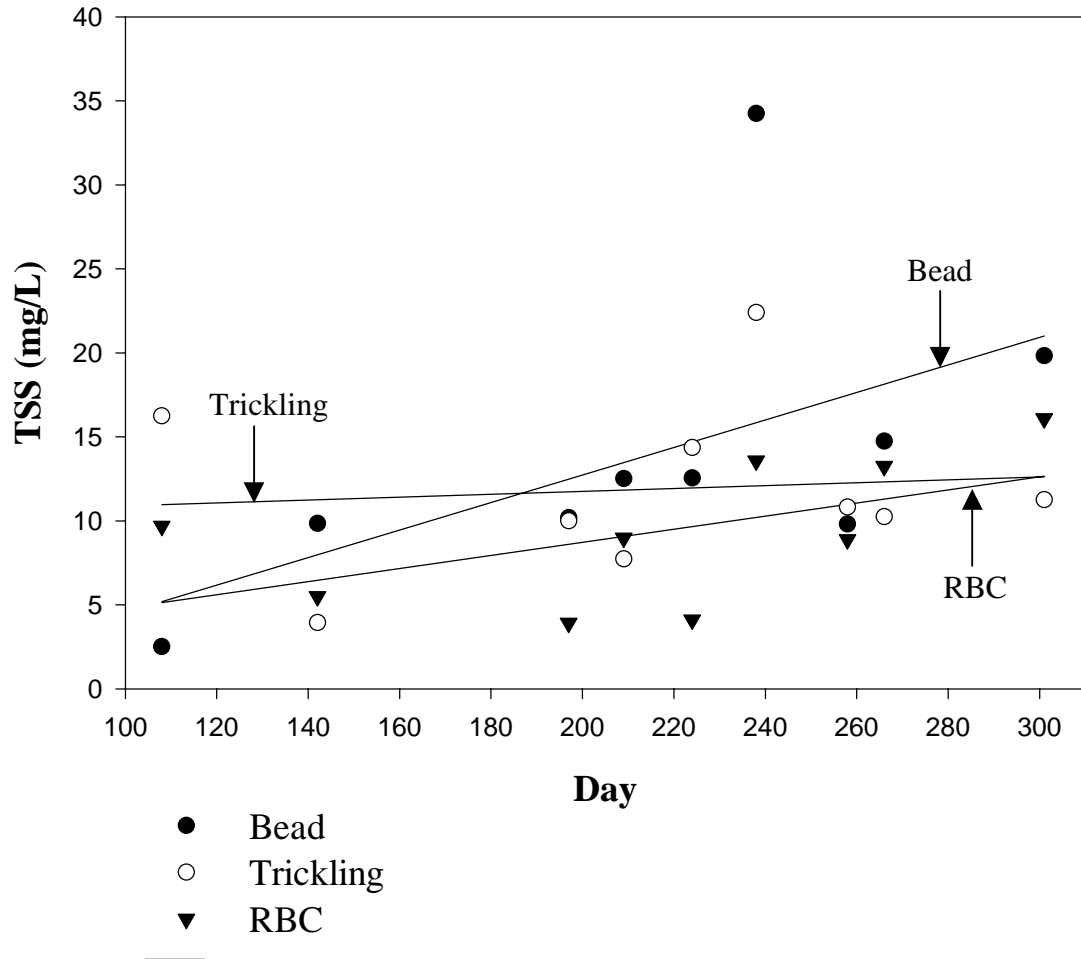


Figure 2.9. Approximate TSS level increases observed over the course of the study.

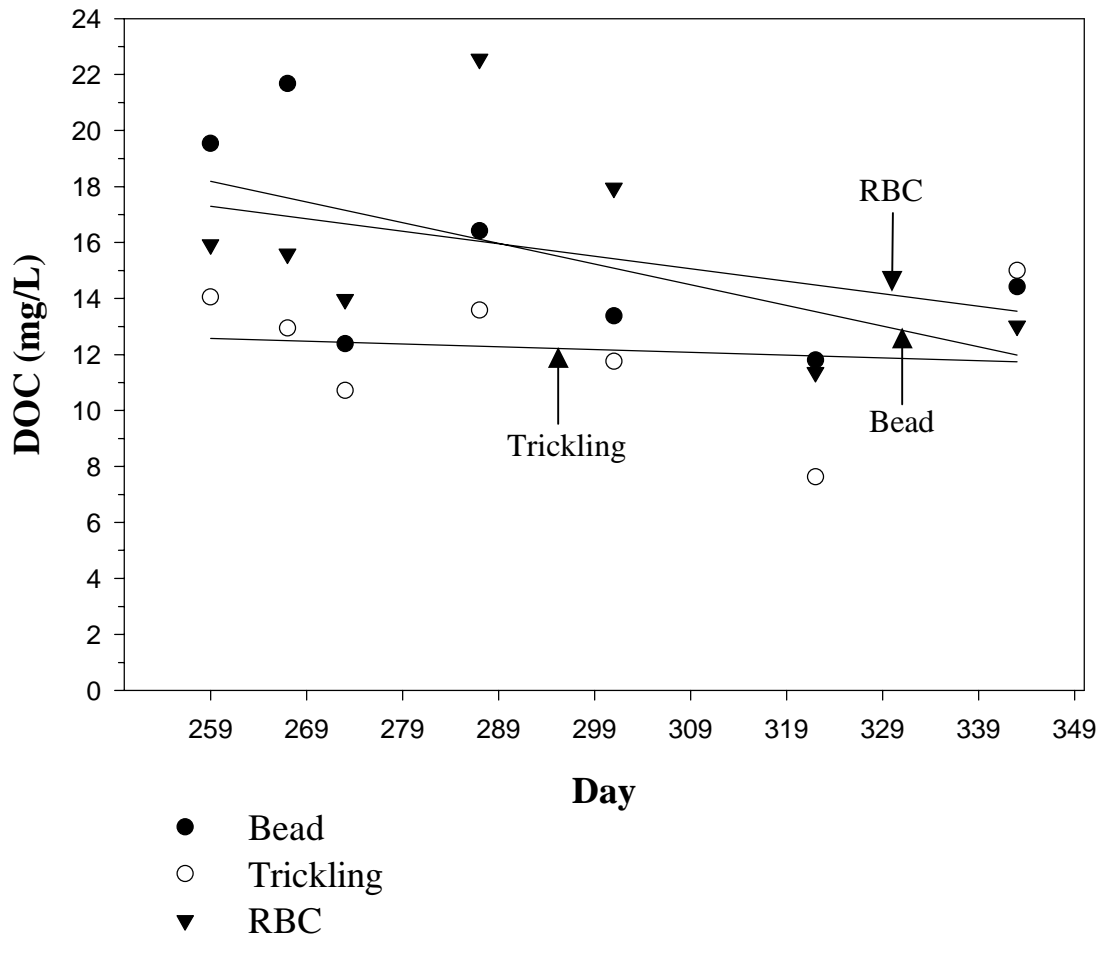


Figure 2.10. Approximate DOC level decreases observed over the course of the study.



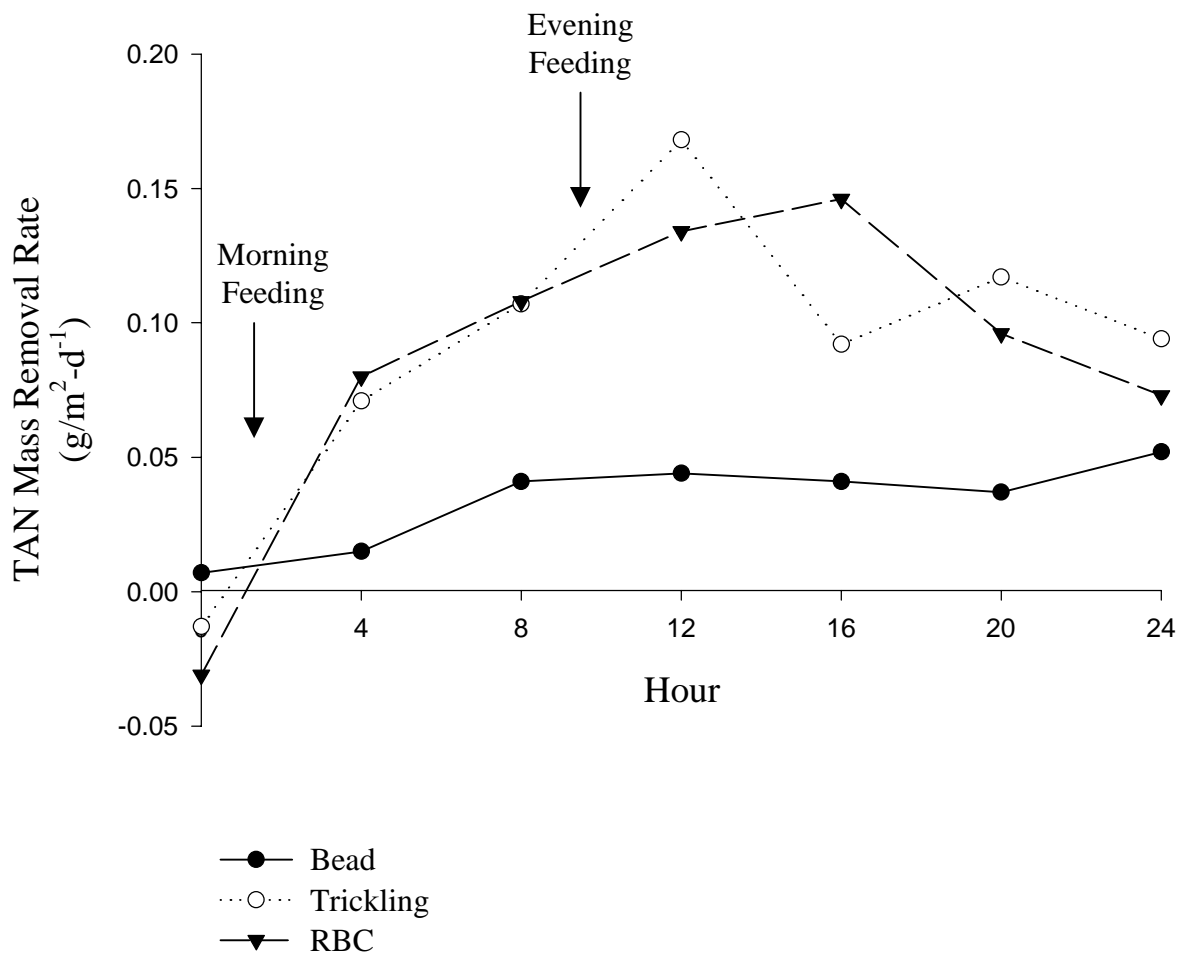


Figure 2.11. Median TAN mass removal rate over 24 hours.

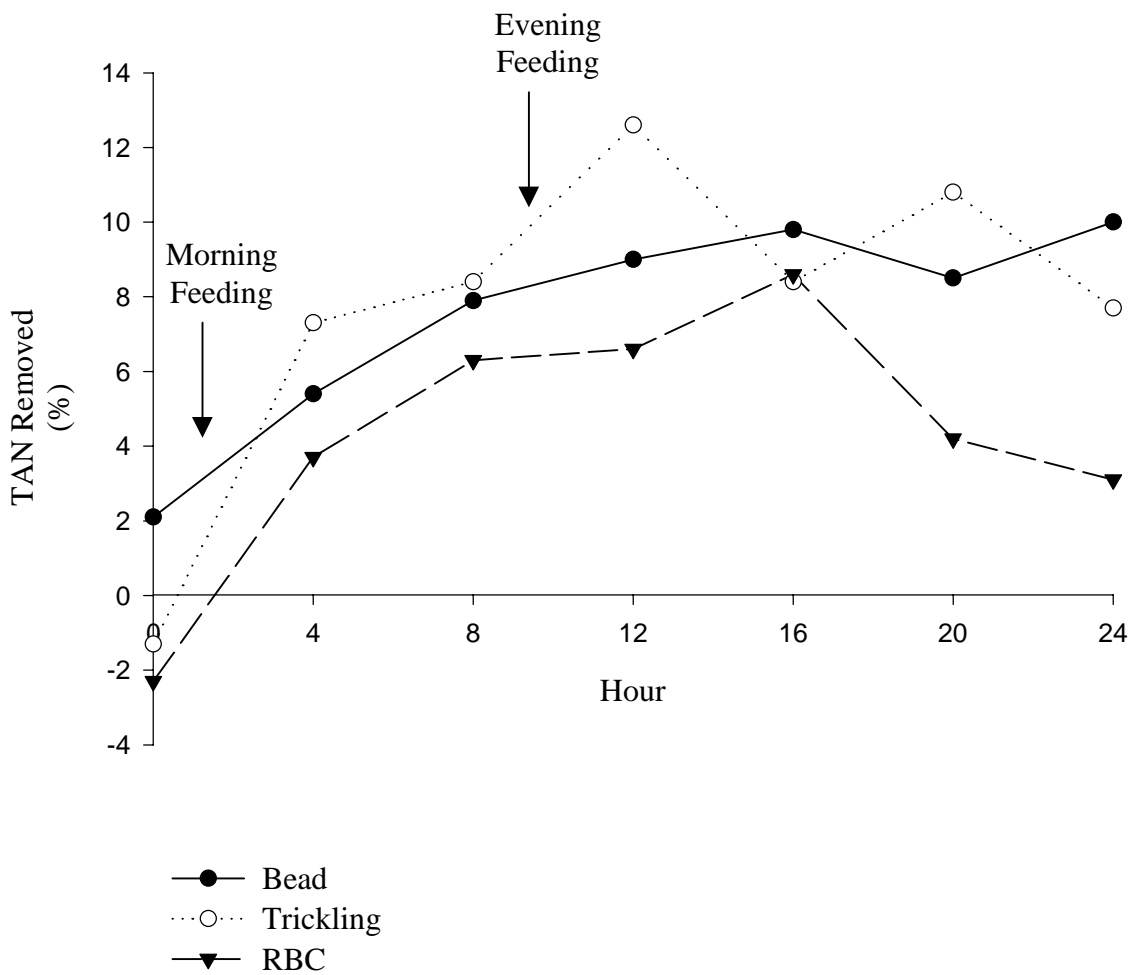


Figure 2.12. Median percent TAN removed over 24 hours.

## VITA

Antar Gamble Hall began his undergraduate career in 1992 at Hampton University where he majored in marine and environmental science. While at Hampton, Antar completed several internships. His first internship was during the summer of 1995 at Mote Marine Laboratory where he was a teacher's aid and camp assistant for the Marine Science II and III summer programs. He fabricated and delivered some of the marine science curricula and participated in snorkeling and scuba activities. The following summer, Antar was awarded an aquaculture cooperative at The Land pavilion in Walt Disney World's EPCOT Center. He and a co-worker were responsible for the daily operation and maintenance of a 27,600-gallon fresh water recirculating aquaculture system. He worked with numerous aquatic organisms such as tilapia, hybrid striped bass, channel catfish, pacu, Gulf of Mexico sturgeon, Australian Redclaw crawfish, American eels and American alligators. He also attended class and information sessions on aquaculture, hydroponics, bio-technology and marine science.

After obtaining his BS from Hampton University (May 1997), Antar was accepted into Virginia Tech's graduate aquaculture program where he conducted biofiltration research. During his time at Virginia Tech he gained a great deal of experience concerning wastewater treatment as it applies to recirculating aquaculture systems. Antar completed the Masters program and graduated December 1999.