

# International Journal of Recirculating Aquaculture

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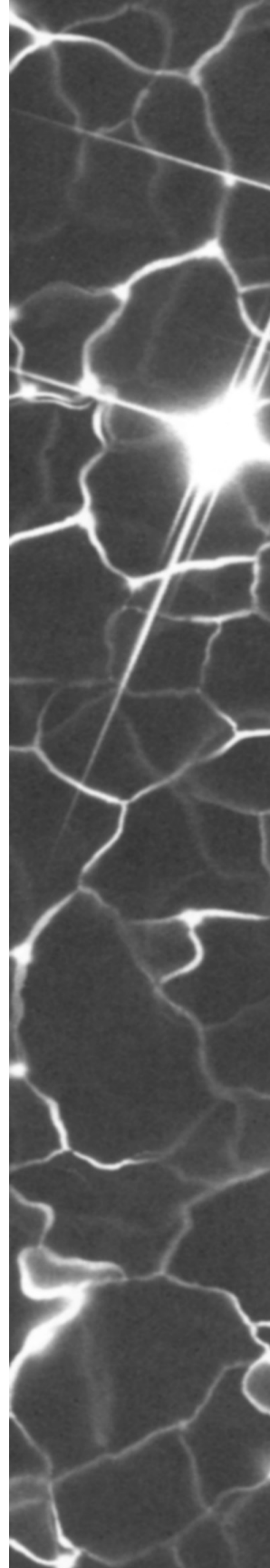
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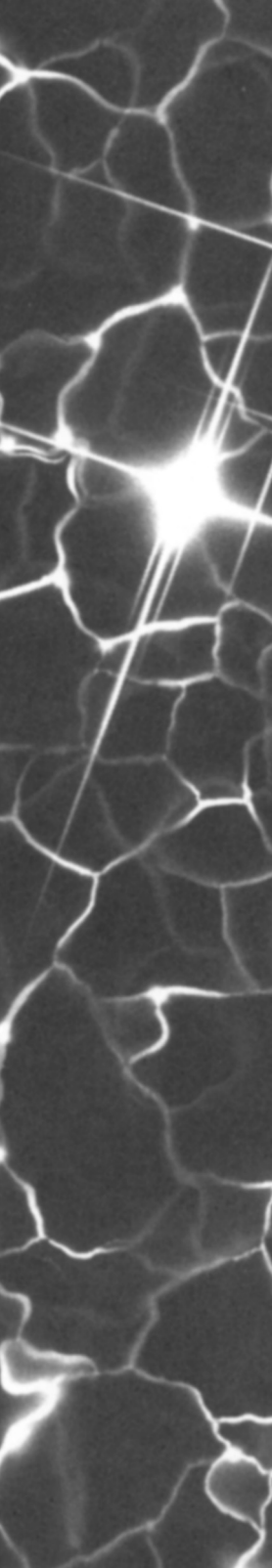
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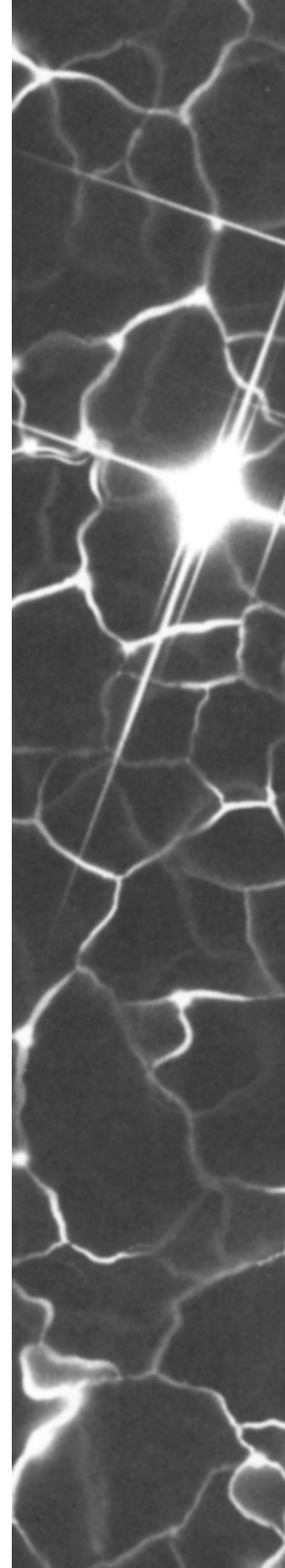


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## Dear Reader:

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This issue features three manuscripts -- the first explores the feasibility of intensive tilapia aquaculture in an area of the world where water resources are scarce. The second and third articles delve into specific questions of recirculating aquaculture systems' and species' management, seeking to optimize both fish health and the characteristics of effluents discharged from a recirculation facility.

We continue to solicit high-quality manuscripts and reports on all topics relating to recirculating aquaculture, and encourage our readers to contact our production office with any comments, suggestions, or ideas they may have concerning the IJRA and its content. The journal is listed by various U.S. and international citation and abstracting services. This means that the journal is fully searchable by author, title, and/or subject. The journal's website, [www.ijra.com](http://www.ijra.com), contains archives of past and current abstracts of all papers and book reviews published in the journal, as well as instructions for authors. In addition, numerous university and aquaculture/agriculture libraries worldwide are now receiving issues of the journal for inclusion in their collections. It is our goal to make the journal widely available to aquaculturists, academicians, and researchers around the world.

We would like to thank our readers, our reviewers, and our authors for their continued support of the journal.

Sincerely,

Stephen A. Smith  
Executive Editor

# **Design and Performance of an Indigenous Water Recirculating Aquaculture System for Intensive Production of Nile Tilapia, *Oreochromis niloticus* (L.), in Saudi Arabia**

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Keywords: Nile tilapia, recycling, biofilter, waste, Saudi Arabia, nitrification

## **ABSTRACT**

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The increasing demand for fish, and the scarcity of fresh water in Saudi Arabia both press the need to adapt new technologies for intensive water recirculating aquaculture to maximize water recycling and increase fish production. Using locally available materials, a commercial-scale recirculating aquaculture system was developed in triplicate to produce Nile tilapia. The system was operated to produce more than 50 kg fish/m<sup>3</sup>/cycle, and the filters were evaluated for their efficiency in removing organic wastes from the effluent. Each replicate consisted of a culture tank, two mechanical filters with sand/gravel medium, two submerged biofilters with plastic media, a sump and two pumps. Mixed sex tilapia with an average size of 76.4 g were stocked at a density of 188 fish/m<sup>3</sup> and fed a 34% protein diet at 3% body weight per day (initially). Water temperature was maintained at 28 ± 1°C, water flow rate was adjusted to 300 liters/min and the culture tank and biofilter were aerated. Water

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samples were collected from the inlet and outlet of each component and were analyzed for important parameters.

Values ( $\pm$  SE) of total ammonia nitrogen (TAN) ( $0.98 \pm 0.1$  ppm) and nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ) ( $0.48 \pm 0.02$  ppm) in the fish culture tanks were within the acceptable limits, while the other water quality parameters were also maintained under normal range by the filtration system. Removal rates ( $\pm$  SE) of  $186.7 \pm 31.59$  g TAN/ $\text{m}^3$ /day and  $66.53 \pm 16.9$  g  $\text{NO}_2\text{-N}$ / $\text{m}^3$ /day, respectively, as well as TAN and  $\text{NO}_2\text{-N}$  removal efficiencies ( $31.45 \pm 2.32\%$  and  $21.05 \pm 3.8\%$ , respectively) were measured across the PVC biofilter medium. The area specific TAN and  $\text{NO}_2\text{-N}$  removal rates ( $\pm$  SE) or nitrification rates ( $0.34 \pm 0.06$  g/ $\text{m}^2$ /day and  $0.15 \pm 0.05$  g/ $\text{m}^2$ /day) for the biofilter were comparable with the performance of other commercial intensive recirculation systems. Mean final weight ( $\pm$ SE), final biomass, growth rate, SGR, FCR, and percent survival for the mixed-sex tilapia were 277.21 (1.76) g/fish, 50.21 kg/ $\text{m}^3$ , 1.34 g/fish/day, 0.86%, 1.89, and 96.53%, respectively, whereas the average water use was 0.4  $\text{m}^3$ /kg of fish production. Locally available materials were found to be appropriate for solid and organic waste removal. More than 85% of the system water volume could be recycled daily, while fish production per unit space was also multiplied 3-6 fold compared to the traditional culture practice.

## **INTRODUCTION**

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The development of freshwater aquaculture in Saudi Arabia has been slow because of water scarcity coupled with harsh, arid climatic conditions. Flow-through systems consisting of ponds or tanks made of concrete or fiberglass use excess groundwater and space to achieve only limited production (El-Gamal 2001). Increasing demand for fresh fish and the scarcity of fresh water press the need to adapt new technologies of intensive water-recirculating aquaculture to facilitate more fish production by maximum recycling of water.

Recirculating aquaculture systems represent a relatively new and expanding technology with a wide variation in system design and quality available. Compared with ponds and flow-through aquaculture, a recirculating system generally occupies very little area, requires less water than conventional aquaculture and provides a predictable and constant

environment for the culture species (Dunning *et al.* 1998). Designed to conserve both land and water resources, recirculating systems can be located in areas not conducive to open pond culture, where water is of poor quality, ambient temperatures are outside the optimum range for the species to be cultured, or if the species is exotic. Because of these advantages, interest in water recirculating systems for fish production continues to grow, despite the lack of consensus available for their design and operation.

Recirculating aquaculture systems recycle and reuse water with mechanical and biological treatment between each use; thus, they require wastewater treatment and filtration techniques for continuous waste removal. These techniques have traditionally been relatively expensive and require skilled personnel to operate (Losordo *et al.* 1992). Imported aquaculture wastewater treatment systems can be installed, but due to particular local farming and operating conditions, lack of expertise, and the high price tag, these treatment systems may not be suitable. The design of the wastewater treatment and water reuse system needs to be efficient, cost-effective, and simple to operate. The design needs to consider the use of local materials under specific fish culture and environmental conditions. The development of an efficient wastewater treatment system that takes into consideration culture conditions necessitates testing of various mechanical and biological filters.

Establishment and testing of recirculating aquaculture technologies using local resources under specific climatic and culture conditions is, therefore, one of the most significant approaches for maximizing water reuse and intensifying fish production in Saudi Arabia.

Although few farms are mechanized, water aeration or oxygenation is a common practice in Saudi Arabia. Fish feeds are made locally, formulated according to the dietary requirements of farmed fish. Food conversion ratios (FCR) are normally 2-2.5 for tilapia species. Annual productivity rates for most tilapia farms range from 5 to 25 kg/m<sup>3</sup> (El-Gamal 2001). These yields are not satisfactory despite the incentives provided by the government of Saudi Arabia, such as interest-free loans to farmers, appropriate technical support, inexpensive energy, subsidized costs for fish feed, etc. (Al-Thobaity and James 1994, Al-Sahli and Dass

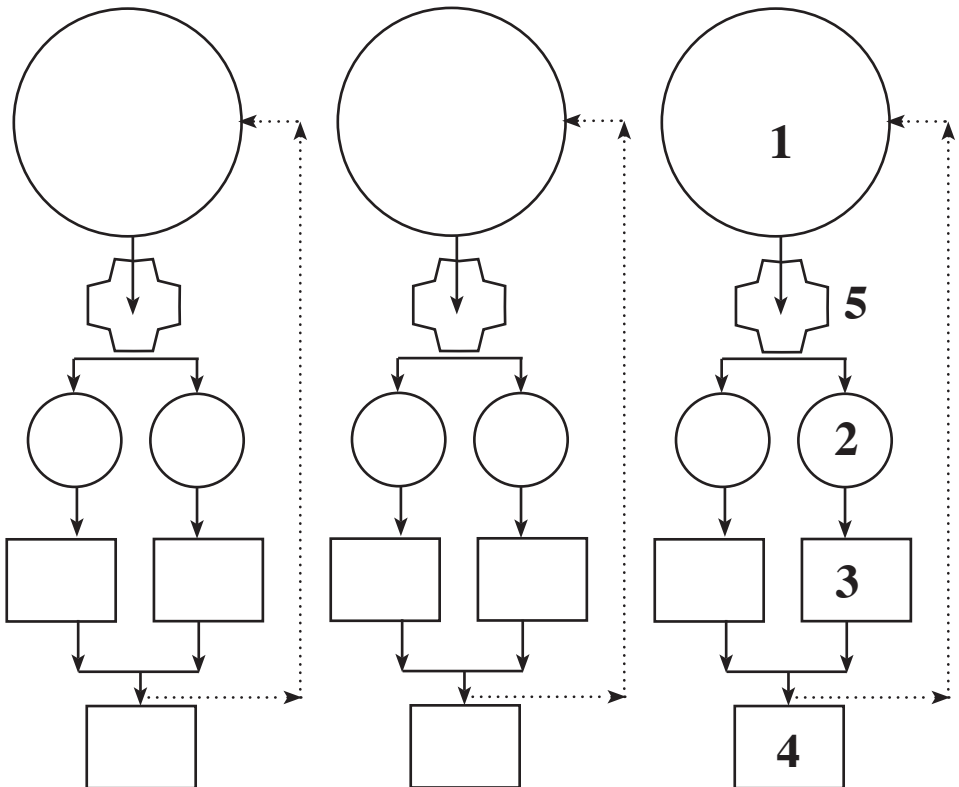
1995). Objectives of this research, therefore, were to use locally-available filter media in the development of a closed aquaculture system to achieve a better FCR (<2), an annual tilapia productivity of 100 kg/m<sup>3</sup> when producing 2 crops/year, and to cut average water requirements per kg fish production from several m<sup>3</sup>, common in flow-through systems, to less than 0.5 m<sup>3</sup>.

## **MATERIALS AND METHODS**

### **System Components**

The water recirculating system was a large-scale fish culture unit that was built in triplicate. Each set contained one fish tank, two mechanical filters, two biological filters, and a sump tank. All fish culture tanks, filters, and sump tanks were made of fiberglass (SKAFCO, Riyadh, Saudi

*Figure 1. Schematic diagram of the water recirculating aquaculture system.  
1. Fish Rearing Tank; 2. Mechanical Filters; 3. Biofilters; 4. Sump Tank; 5. Pump*



*Table 1. Specifications for the water recirculating aquaculture system.*

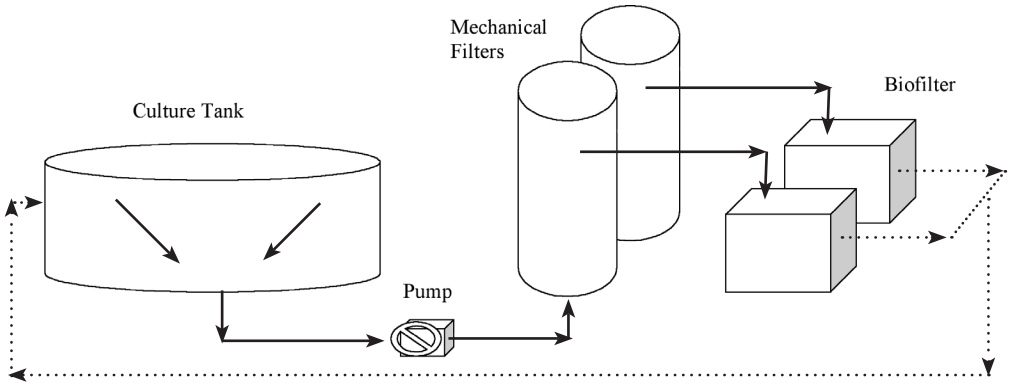
<b>Specifications of Tanks</b>	<b>Number &amp; Shape</b>	<b>Dimensions Meters (m)</b>	<b>Total Volume m<sup>3</sup></b>	<b>Water Volume m<sup>3</sup></b>
Fish Rearing Tanks	3, Circular	4.0 Dia, 1.00 H	12.6/Tank	8.0/Tank
Solid Filters	6, Cylindrical	1.0 Dia, 1.50 H	1.18/Tank	--
Biofilters	6, Rectangular	1.25 L, 1W, 1 D	1.25/Tank	1.0/Tank
Sump	3, Rectangular	1 L, 1 W, 0.7 D	1.00/Tank	--

Arabia). The fish culture tanks (12.5 m<sup>3</sup>) were circular, 4 m in diameter. The mechanical filters were also circular, 1 m diameter, 1.5 m height. The biological filters were rectangular, 1 m x 1 m x 1.25 m (Table 1, Figure 1).

### **Operation**

Effluents from fish culture tanks were pumped into the two up-flow cylindrical filters containing a 15-cm layer of coarse gravel and a 35-cm layer of sand (number 20) as filter media for solid particle removal. Water was introduced from the bottom of the cylindrical filters through a 7.62-cm diameter inlet. Backwash from the filters was carried out using a perforated 25-mm diameter circular brass pipe which was connected to a compressor. Compressed air routed through the pipe agitated the sand medium once daily while water back flowed through the filter under the drain. Backwash was collected at the bottom of the cylinder and discharged through an opening to a drain. The outflow of each of the two cylinders was connected to one submerged biofilter where the water filled in by gravity. Two biofilters in a replicate contained locally available plastic media (PVC pipes, 2.5 cm diameter cut into 5-cm pieces). Each of the two biofilters in a replicate had a surface area of 200 m<sup>2</sup> for bacterial growth. Thus, total biofilter surface area for a replicate was 400 m<sup>2</sup>, which was based on the calculations that 400-450 kg of fish fed at 2% body weight would produce 240-270 g TAN/day and the ammonia removal rate was expected to lie between 0.50-0.70 g TAN/m<sup>2</sup>/Day (Lawson 1995). Two water pumps (0.75 kw each) located outside the

Figure 2. Connections and flow pattern in a replicate of the water recirculating aquaculture system.



fish tank were used alternatively to pump water from each fish tank to the solids-removing filters while the water was recirculated back by gravity from the biofilters to the fish culture tank (Figure 2). A sump tank (2.25 m<sup>3</sup>) was used to collect filter backwash, drains, and overflows. Wastewater from the sump could either be pumped back into the system through the mechanical filters or discharged outside the system.

### Experimental Conditions

Rearing tanks were stocked at a density of 188 fish/m<sup>3</sup> with mixed sex Nile tilapia (*Oreochromis niloticus*) weighing (on average) 76.41 g/fish. Floating pellets (4 mm) containing 34% protein were used to feed the fish at a rate of 3% body weight per day. Feeding rate was decreased to 2% after the first two months. Daily ration was equally divided for a morning (0800 h) and evening (1600 h) feeding. The water temperature was maintained at 28 ± 1°C by installing heaters with thermostats. Water flow rate was maintained at 300 liter/min throughout the experiment and 10% of the total water in each rearing tank was exchanged daily in the morning for the first two months. For next two months, water exchange was increased to 12% of the total water in the rearing tank, and in the last month it was raised to 15%. Details of the mechanical and biofiltration components are given in Table 2.

Each fish rearing tank and biofilter was aerated by 22 (15.2 cm x 3.8 cm x 3.8 cm) and 8 (7.6 cm x 2.5 cm x 2.5 cm) air diffusers, respectively. Two foam fractionators were used in each rearing tank to remove fine

solids, reduce dissolved organics like proteins, and to control carbon dioxide. Performance of the filters was evaluated by measuring filter reductions (in and out samples) of TAN (total ammonia nitrogen), NO<sub>2</sub>-N (nitrite-nitrogen) and accumulation rates of NO<sub>3</sub>-N (nitrate-nitrogen). The operational goals included maintaining the DO (dissolved oxygen) above 5.0 ppm and TAN and NO<sub>2</sub>-N below 1.0 ppm. At the end of the experimental period all the fish were sexed and weighed to get the final biomass and total weight gain.

*Table 2. Physical parameters of the water recirculating aquaculture system.*

<b>Unit</b>	<b>Parameter</b>	<b>Description</b>
<b><i>Culture tanks</i></b>	Flow rate	300 L/min
	Water volume	8 m <sup>3</sup> /tank
	Number of culture tanks	3 (1/replicate)
	Water level control	Central stand pipe
	Residence time	26.67 minutes
<b><i>Mechanical filter</i></b>	Type	Upflow; submerged
	Total number of mechanical filters	6 (2/replicate)
	Filter medium characteristics	Dual Sand/Gravel (2 mm particle size)
	Filter bed depth	Two layers 15 + 35 cm
	Filter bed volume	0.39 m <sup>3</sup>
	Mechanical filter water volume	0.97 m <sup>3</sup>
	Specific hydraulic loading rate	553.85 m <sup>3</sup> /m <sup>3</sup> /day
	Residence time	2.6 minutes
<b><i>Biofilters</i></b>	Types	Downflow; submerged
	Total number of biofilter tanks	6 (2/replicate)
	Filter volume/biofilter tank	0.364 m <sup>3</sup>
	Specific hydraulic loading rate	594 m <sup>3</sup> /m <sup>3</sup> /day
	Residence time	2.42 minutes
	Filter medium characteristics	
		1. Type
	2. Specific surface area	550 m <sup>2</sup> /m <sup>3</sup> respectively
	3. Total surface area/biofilter	200 m <sup>2</sup>
	4. Total surface area/replicate	400 m <sup>2</sup>

## **Water Quality Determination**

Water samples were collected weekly from the inlet and outlet of each culture tank, mechanical and biofilter unit of the system. Samples were analyzed for TAN (total ammonia nitrogen), NO<sub>2</sub>-N (nitrite-nitrogen) and NO<sub>3</sub>-N (nitrate-nitrogen) using a Hach DR 4000 spectrophotometer (Hach, Loveland, CO, USA). Water temperature, pH, DO (dissolved oxygen), total alkalinity and free CO<sub>2</sub>, (carbon dioxide) were recorded twice a week following standard methods (APHA 1998).

## **Calculations**

Daily weight gain (DWG) expressed as g/fish/day, percent specific growth rate (SGR) and feed conversion ratio (FCR) were calculated by using the following formulae:

$$\text{DWG} = (\text{final weight} - \text{initial weight}) / \text{No. of fish} / \text{time (days)}$$

$$\text{SGR (\%)} = (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days} \times 100$$

$$\text{FCR} = \text{total dry feed weight} / (\text{final fish biomass} - \text{initial fish biomass})$$

The efficiency of the biofilters in nitrogenous waste removal was determined using the estimated waste loading and removal rates as follows:

$$\text{Waste loading rate (g/m}^3\text{/day)} = C_i \times Q$$

$$\text{Waste removal rate (g/m}^3\text{/day)} = (C_i - C_e) \times Q$$

$$\text{Removal efficiency (E)} = \text{Waste removal rate} / \text{waste loading rate} \times 100$$

where:  $C_i$  = influent waste concentration (g/m<sup>3</sup>)

$C_e$  = effluent waste concentration (g/m<sup>3</sup>)

$Q$  = water flow rate (m<sup>3</sup>/m<sup>3</sup>/day)

*Table 3. Water quality data recorded in the fish culture tanks of the water recirculating aquaculture system.*

<b>Parameters</b>	<b>Range</b>	<b>Mean</b>	<b>± SE</b>
Water Temperature (°C)	27.5 – 28.5	27.81	0.02
Dissolved Oxygen (mg/L)	3.51 - 4.71	4.33	0.21
pH	8.25 - 8.91	8.37	0.14
Total Ammonia-N (mg/L)	0.75 - 1.1	0.98	0.1
Nitrite-N (mg/L)	0.41 - 0.54	0.48	0.02
Nitrate-N (mg/L)	6.32 - 9.73	8.02	0.54
Free CO <sub>2</sub> (mg/L)	6.43 - 6.98	6.75	0.09
Total Suspended Solids (mg/L)	9.14 – 16.7	13.24	0.61
Total Dissolved Solids (mg/L)	1233 - 1268	1255.2	6.03

*Table 4. Waste loading rates, waste removal rates, waste removal efficiencies and specific waste removal rates of the biofilter utilizing plastic-pipe medium.*

<b>Waste</b>	<b>Plastic pipes (± SE)</b>
<b><i>Ammonia</i></b>	
Loading Rate (g/m <sup>3</sup> /Day)	580.93 (61.23)
Removal Rate (g/m <sup>3</sup> /Day)	186.7 (31.59)
Specific Removal rate (g/m <sup>2</sup> /Day)	0.34 (0.06)
Removal Efficiency (%)	31.45 (2.32)
<b><i>Nitrite</i></b>	
Loading Rate (g/m <sup>3</sup> /Day)	286.31 (13.6)
Removal Rate (g/m <sup>3</sup> /Day)	66.53 (16.9)
Specific Removal rate (g/m <sup>2</sup> /Day)	0.15 (0.05)
Removal Efficiency (%)	21.05 (3.8)

## **RESULTS AND DISCUSSION**

Values of critical metabolic wastes like total ammonia nitrogen (TAN) ( $0.98 \pm 0.1$  mg/L) and nitrite-nitrogen (NO<sub>2</sub>-N) ( $0.48 \pm 0.02$  mg/L) were well within the acceptable limits, while other quality parameters in the culture water were also maintained under normal ranges by the filtration system (Table 3). Removal rates (± SE) were calculated to be 186.7 (± 31.59) g TAN/m<sup>3</sup>/day and 66.53 (± 16.9) g NO<sub>2</sub>-N/m<sup>3</sup>/day, whereas TAN

and NO<sub>2</sub>-N removal efficiencies ( $\pm$  SE) were 31.45% ( $\pm$  2.32) and 21.05% ( $\pm$  3.8) respectively for the biofilter. Specific TAN and NO<sub>2</sub>-N removal rates ( $\pm$  SE) were 0.34 ( $\pm$  0.06) g/m<sup>2</sup>/day and 0.15 ( $\pm$  0.05) g/m<sup>2</sup>/day, respectively for the PVC hose biofilter medium (Table 4).

Mean weight ( $\pm$  SE) for the mixed sex tilapia population (49.88% males and 50.12% females) produced in the recirculating system was calculated to be 277.21 (1.76) g/fish. A final biomass of 50.21 kg/m<sup>3</sup> was harvested after the experiment finished at a growth rate of 1.34 g/fish/day. The FCR averaged to 1.89 and the SGR was 0.86% while the survival was recorded to be 96.53% (Table 5).

*Table 5. Growth data of Nile tilapia in the water recirculating aquaculture system.*

<b>Parameters</b>	<b>Tank 1</b>	<b>Tank 2</b>	<b>Tank 3</b>	<b>Average</b>
<i>Period</i>	01 Aug-30 Dec	01 Aug-30 Dec	01 Aug-30 Dec	01 Aug-30 Dec
<i>Days</i>	150	150	150	150
<i>Initial weight (g <math>\pm</math> SE)</i>	79.13 (1.61)	72.77 (2.07)	77.33 (2.15)	76.41(1.94)
<i>Total Initial Biomass (Kg)</i>	118.7	109.16	116.3	114.72
<i>Initial Biomass (kg/m<sup>3</sup>)</i>	14.84	13.65	14.54	14.34
<i>Final Weight (g <math>\pm</math> SE)</i>	282.47 (1.79)	281.94 (1.63)	267.22 (1.85)	277.21(1.76)
<i>Total Final Biomass (Kg)</i>	416.92	414.17	373.84	401.64
<i>Final Biomass (kg/m<sup>3</sup>)</i>	52.12	51.77	46.73	50.21
<i>Growth rate (g/Fish/Day)</i>	1.36	1.39	1.27	1.34
<i>Net Yield (g/m<sup>3</sup>/Day)</i>	248.53	254.13	214.6	239.09
<i>FCR</i>	1.85	1.86	1.96	1.89
<i>SGR (%/Day)</i>	0.85	0.9	0.83	0.86
<i>Final density (number/m<sup>3</sup>)</i>	184	183	175	181
<i>Survival (%)</i>	98.4	97.93	93.27	96.53

### **Solids in Water Recirculating System**

Performance of solid upflow sand and gravel filters in the system during the experiment was found to be satisfactory as reflected from the values of total suspended solids (TSS) in the outflow water going to the biofilters. The average TSS value was found to be 13.24 mg/L in the rearing tanks of the system; that is below the allowable limit of TSS in recirculating systems (Lawson 1995) while total dissolved solids (TDS) averaged to 1255.2 mg/L. Reported solids production values (uneaten feed and excreta) in fish culture systems range from 11 to 38% of the applied feed (McLaughlin 1981, Chen *et al.* 1993). The large variation can be attributed to difference in feed, species management, and variability in the rates of decay of organic matter within the culture units.

### **Nitrogen in Water Recirculating System**

Total Ammonia Nitrogen (TAN) is the most critical water quality parameter in intensive recirculating systems. According to Lawson (1995) and Van Rijn and Rivera (1990), TAN should be kept at less than 1 mg/L in intensive recirculating systems. TAN consists of two fractions, un-ionized ammonia ( $\text{NH}_3$ ) and ionized ammonia ( $\text{NH}_4^+$ ). The former is extremely toxic to fish. The proportion of TAN in the un-ionized form is dependent upon the pH and temperature of the water. The higher the pH and temperature of the water, the higher the percentage of toxic un-ionized ammonia. During the operation of the recirculating system, an average TAN value of 0.92 mg/L was recorded in the rearing tanks. Based on the mole fraction values of un-ionized ammonia for different temperature and pH (Huguenin and Colt 1989), the concentration of  $\text{NH}_3\text{-N}$  was calculated to range between 0.009 and 0.06 mg/L in the rearing tanks, and averaged 0.034 mg/L. Levels of un-ionized ammonia, which may adversely affect growth in tilapia, ranged from 0.24 mg/l to 0.5 mg/l (Balarin and Haller 1982, Daud *et al.* 1988). At a level of 0.25 mg/l un-ionized ammonia, the health and growth of the fish may be impacted.

Nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ) and nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) are the products of ammonia oxidation. Nitrate-nitrogen is not generally of great concern to aquaculturists as it has been shown that aquatic species can tolerate extremely high (greater than 100 mg/L) concentrations of  $\text{NO}_3\text{-N}$

(Ebeling *et al.* 1993). Nitrite-nitrogen is also not as toxic as ammonia-nitrogen, however, it is harmful to aquatic species and must be removed from the system. According to Ebeling *et al.* (1993), concentrations of nitrite-nitrogen should not exceed 0.5 mg/L in culture water whereas the level of concern was reported to be 0.45 mg/l of nitrite-nitrogen by Balarin and Haller (1982). Average concentration of NO<sub>2</sub>-N in commercial scale operation of the recirculating system during this study was recorded to be 0.58 mg/L in the rearing tanks.

### **Nitrification and Biofiltration Efficiency**

Removal rates ( $\pm$  SE) of the biofilter medium consisting of pieces of PVC pipe were 186.7 (31.59) g TAN/m<sup>3</sup>/day and 66.53 (16.9) g NO<sub>2</sub>-N/m<sup>3</sup>/day, respectively, whereas TAN and NO<sub>2</sub>-N removal efficiencies were 31.45% and 21.05%, respectively (Table 4). Miller and Libey (1985) have reported the best TAN removal efficiencies of rotating biological contactor (RBC) to be 74-82% compared with a trickling filter (23-52%) and a fluidized sand bed filter (8-32%).

Overall nitrification rates (specific removal rates) for the biofilter medium averaged 0.34 g TAN/m<sup>2</sup>/day and 0.15 g NO<sub>2</sub>-N/m<sup>2</sup>/day. Lekang and Kleppe (2000) have reported a nitrification rate between 0.1 and 0.2 g TAN/m<sup>2</sup>/day for trickling biofilters with plastic media while Van Rijn and Rivera (1990) reported a removal rate of 0.43 g TAN/m<sup>2</sup>/day for trickling filters. In their study of biofilters, Westerman *et al.* (1993) found nitrification rates of 0.25 g TAN/m<sup>2</sup>/day for the rotating biological filters and 0.1-0.15 g TAN/m<sup>2</sup>/day for upflow sand and bead filters. Various biofilter media configurations are commercially available with maximum ammonia (TAN) removal rates between 0.28 and 0.55 g TAN/m<sup>2</sup>/day (Kruner and Rosenthal 1983, Nijhof and Bovendeur 1990, Van Rijn and Rivera 1990, Kikuchi *et al.* 1994). According to Van Rijn (1996), maximum ammonia removal rates are sometimes misleading as they are often measured at unrealistically high ammonia levels. Ammonia oxidation follows a hyperbolic trend with increasing ammonia concentration (Michaelis-Menten Kinetics) and maximum ammonia removal rates are found at very high ambient ammonia concentrations (Brune and Gunther 1981).

## **Dissolved Gases**

A minimum DO concentration of 5 mg/L is required for proper functioning of the recirculating system (Greiner and Timmons 1998). When DO levels drop below 2 mg/L, the biological filters start to show negative effects on the activities of *Nitrobacter* and *Nitrosomonas* because the rate of oxygen diffusion into the bacterial film begins to limit the nitrification process (Malone 1995). During this experiment, the aeration was maintaining optimum DO in the system, keeping the average DO content at 4.3 mg/L, within a range of 3.5 to 4.7 mg/L.

Accumulation of free CO<sub>2</sub> lowers the pH in recirculating aquaculture systems, especially when alkalinity is low. In recirculating systems, this gas becomes problematic and can adversely affect fish health under high stocking densities, where fish consume large amount of oxygen and liberate large amounts of CO<sub>2</sub> as a result of respiration. A free CO<sub>2</sub> concentration greater than 50 mg/L is reported to be toxic for most fish species (Heinen *et al.* 1996). During the operation of the recirculating system in this study, free CO<sub>2</sub> averaged 6.75 mg/L with a range from 6.4 to 7.0 mg/L.

## **pH**

pH is an important water quality parameter in recirculating systems because various processes such as nitrification and fish health are related to the range of pH in the water. As pH decreases, ammonia is converted into a less toxic ammonium form, therefore, an increase in pH will lead to the accumulation of ammonia in the system (Lawson 1995). The optimum pH range for nitrifying bacteria is between 7.0 and 8.0. If pH drops below 6.8, nitrifying bacteria are inhibited and do not remove the toxic nitrogenous wastes (Michael *et al.* 1995), though nitrifying bacteria can adapt to pH values outside this range if given enough time (Wheaton *et al.* 1994). Basically, two factors are responsible for lowering pH: the nitrifying bacteria that produce acid as a result of nitrification; and respiration of fish and bacteria, producing carbon dioxide which is converted into carbonic acid. During this study, pH was found to occur in its optimum range, averaging 8.4 in the system.

## **Fish Production**

Overall, growth of mixed sex tilapia in all three replicates of the recirculating system was satisfactory (Table 5). Among the three replicates, there was no significant difference ( $p > 0.05$ ) in the feed conversion ratio, daily weight gain, net yield and total weight gain of the fish. The mean daily growth rate of 1.34 g/fish/day in our study was similar to the 1.2 g per day reported by Ridha and Cruz (2001) for Nile tilapia in the recirculating system in Kuwait. The mean FCR of 1.89 recorded in this study is comparable with the 1.86-2.04 reported by Rakocy *et al.* (1992) and the 1.98 and 2.04 reported by Ridha and Cruz (2001) for Nile tilapia. Average net yield (239.09 g/m<sup>3</sup>/day) showed little variation between the system replicates.

## **Water Conservation**

Compared to semi-intensive culture practices in Saudi Arabia where 20-25% of total water is exchanged daily to produce 8-15 kg fish/m<sup>3</sup> of water, only 10-15% of total water was exchanged daily in the intensive recirculating aquaculture system to produce 50 kg fish/m<sup>3</sup>/crop. Calculating from these figures, there is a requirement of approximately 3 to 4.5 m<sup>3</sup> of water to produce one kg fish in semi-intensive aquaculture. In contrast, the production of one kg fish required approximately 0.4 m<sup>3</sup> of water in the water-recirculating intensive aquaculture.

## **Socio-economics of Aquaculture in Saudi Arabia**

Inland aquaculture is mostly practiced indoors in Saudi Arabia, and farm raised tilapia weighing > 200 g are well received (Siddiqui and Al-Najada 1992). Under good conditions and a nutritious feeding regime, Nile tilapia usually attain 250-500 g in 6-8 months in tank systems. Owing to the shortage of fresh water, integrated and closed culture systems that allow water to be reused need to be developed more extensively. Recirculating systems have two disadvantages: they are expensive to establish, and require expertise to operate. These problems are minor, however, compared to the many advantages Saudi Arabia has created to promote aquaculture development. The government supports the industry with research and development, as well as extension programs that provide brood stock, hatchery-reared seeds, essential technical and commercial

information, and training (Al-Thobaity and James 1994, Al-Sahli and Dass 1995). Land in Saudi Arabia is inexpensive, and because aquaculture can be practiced on non-arable land, competition with the nation's important agriculture industry is reduced. Furthermore, credit is readily available (interest-free loans are provided by the government to farmers for purchase of machinery and other facilities), energy is inexpensive (aquaculture is somewhat energy intensive), and fish feed is subsidized by the government (Ming-Hsien 2004). The nation's climate, as noted, can be harsh, but aquaculture systems technology makes it possible to raise fish even in these conditions.

## **CONCLUSION**

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To save maximum water and increase fish production per unit volume of water in a large scale water recirculating system, locally available sand/gravel and small pieces of plastic electrical hose were found to be an appropriate media for solid (mechanical filtration) as well as organic waste (biological filtration) removal, respectively, from the fish culture effluents. Accordingly, only 10-15% of total system water was exchanged daily to sustain 50.21 kg fish/m<sup>3</sup>, compared to extensive and semi-intensive traditional culture practices in Saudi Arabia where 20-25% water is exchanged daily to produce only 8-15 kg fish/m<sup>3</sup> of water. The implementation of water recirculation aquaculture technology in Saudi Arabia can effectively check water waste. More than 85% of water may be recycled using local materials in the design of water filtration components. Fish production per unit space can also be multiplied by more than 3-6 fold in the water recirculating fish culture systems. The remarkable reduction in water use associated with recirculation technology, and the availability of local materials for establishing the filter components would encourage local farmers to embrace the new techniques and enable a rapid transfer of the technology to the private sector.

## **ACKNOWLEDGMENTS**

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# Using Oxygen Gas Transfer Coefficients to Predict Carbon Dioxide Removal

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## ABSTRACT

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The purpose of this research was to determine if oxygen gas transfer coefficients, as reflected by overall mass transfer coefficient ( $K_La$ ) values, could be used to predict carbon dioxide ( $CO_2$ ) removal by degassing in aquaculture production systems. The motivation for this approach was that while there is ample literature related to oxygen gas transfer, there is limited information on  $CO_2$  removal. A series of tests was conducted to determine the ratio ( $\phi_E$ ) of  $K_La$  for  $CO_2$  to that of oxygen for two commonly used surface aerators and then compare  $\phi_E$  to the theoretical

ratio,  $\phi_T$ , which is 0.90 based upon gas molecular diameters. Experiments were conducted in a 10,000 L circular tank aerated by means of two different surface agitators. The two aerators were selected to represent aeration patterns with high and moderate water to gas interface exposures or breakup patterns (photos supplied, Figures 2 and 3). The results showed that  $\phi_E/\phi_T$  ratios were 96% (for high air exposure) and 74% (for moderate air exposure) for water with an alkalinity of ~130 mg/L as CaCO<sub>3</sub>. The  $\phi_E/\phi_T$  ratio decreased to 0.84 and 0.51 for the high and moderate air exposures, respectively, when higher alkalinity waters (~1,000 mg/L as CaCO<sub>3</sub>) were used.

## INTRODUCTION

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Oxygen is essential for the production of fish in aquaculture systems. Adding oxygen or air to culture water can dramatically increase the system carrying capacity when dissolved oxygen is the limiting factor (Lawson 1995). Carbon dioxide (CO<sub>2</sub>) can pose serious risks to fish health in intensive aquaculture and could be the limiting water quality factor in some cases. Increased CO<sub>2</sub> levels in water result in a lowering of culture water pH. Similarly, increased CO<sub>2</sub> decreases the pH of a fish's blood, which reduces the amount of oxygen their blood hemoglobin can carry (Eddy *et al.* 1977). Elevated levels of CO<sub>2</sub> in blood cause a drop in blood pH and produce a condition known as hypercapnia (Berg and Tandstat 1995). Despite the presence of adequate dissolved oxygen in the culture water, elevated blood CO<sub>2</sub> levels may result in respiratory distress due to a decrease in hemoglobin's affinity for oxygen (the Bohr effect) or a decrease in the maximum oxygen binding capacity of hemoglobin (the Root effect) (Lawson 1995, Wedemeyer 1996).

Fish densities in recirculating aquaculture systems (RAS) are often above 100 kg/m<sup>3</sup>, which generally require the use of pure oxygen and active CO<sub>2</sub> stripping. There are various methods to remove CO<sub>2</sub>, e.g., surface aerators (Boyd 1998), packed column aerators (Grace and Piedrahita 1994), and bubble columns or airlift pumps (Loyless and Malone 1998). While there is extensive literature that describes oxygen transfer and associated mass transfer coefficients, there is limited information on transfer coefficients for CO<sub>2</sub> removal. Therefore, the objective of this research was

to determine if oxygen gas transfer rates as reflected by an overall mass transfer coefficient ( $K_L a$ ,  $\text{time}^{-1}$ ; e.g.  $\text{hr}^{-1}$ ) value could be used to predict  $\text{CO}_2$  transfer in aquaculture production systems.

### 1.1. Gas transfer theory

The driving force for gas transfer is the difference in gas concentration (or pressure) between the air and water. The gas transfer rate is proportional to this gas pressure difference, the characteristics of the air-water interface, and gas diffusion and convective transport characteristics across the air-water interface. An overall mass transfer coefficient ( $K_L a$ ) is used to predict device performance as described by Equation 1 (Stenstrom 1979):

$$\frac{dC}{dt} = K_L a(C_s - C)V \quad (\text{Equation 1})$$

where:

$dC/dt$  = gas transfer rate ( $\text{mg hr}^{-1}$ )

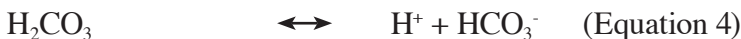
$C_s$  = saturation concentration of the gas ( $\text{mg L}^{-1}$ )

$C$  = measured gas concentration at time,  $t$  ( $\text{mg L}^{-1}$ )

$V$  = volume of water subjected to gas transfer (L)

### 1.2. Carbon dioxide removal

It is only as a dissolved gas,  $\text{CO}_2(\text{aq})$ , that  $\text{CO}_2$  is directly affected by aeration (Berg and Tandstad 1995). Unlike other important dissolved gases, such as nitrogen and oxygen,  $\text{CO}_2$  exists as part of the carbonate chemical equilibrium system (carbon dioxide  $\text{CO}_2$ , carbonic acid  $\text{H}_2\text{CO}_3$ , bicarbonate  $\text{HCO}_3^-$ , and carbonate  $\text{CO}_3^{2-}$ ) (Grace and Piedrahita 1994):



The equilibrium concentration of  $\text{CO}_2(\text{aq})$  in Equation 2 is a function of  $\text{CO}_2$  gas pressure and a solubility constant (Henry's Law constant). The concentration of each component in Equations 2–5 depends on total carbonate carbon ( $C_T$ ) and an ionization fraction:

$$C_T = [\text{H}_2\text{CO}_3] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \quad (\text{Equation 6})$$

The magnitude of the ionization fraction depends upon pH, salinity, and temperature (Grace and Piedrahita 1994). Removal of  $\text{CO}_2$  causes carbonic acid ( $\text{H}_2\text{CO}_3$ ) to disassociate into more  $\text{CO}_2(\text{aq})$  and  $\text{H}_2\text{O}$ . This in turn causes the concentration of the other constituents of the carbonate system to change.  $\text{CO}_2$  removal causes a short-term depletion of  $\text{CO}_2$  until a new equilibrium in the carbonate system is established (Grace and Piedrahita 1994). Alkalinity is conserved during the  $\text{CO}_2$  removal process, where a simplified definition of alkalinity (ALK) is:

$$\text{ALK} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] - [\text{H}^+] \quad (\text{Equation 7})$$

The temporary imbalance in the carbonate system caused by  $\text{CO}_2$  removal will result in larger gas pressure differences for  $\text{CO}_2$  removal existing through an aeration device than what would be predicted based upon equilibrium concentrations.

For practical reasons, the concentrations of  $\text{CO}_2(\text{aq})$  and  $\text{H}_2\text{CO}_3$  are combined and called  $\text{H}_2\text{CO}_3^*$  or free  $\text{CO}_2$  (Stumm and Morgan 1996).

The ratio of the two species  $\frac{\text{CO}_2(\text{aq})}{\text{H}_2\text{CO}_3}$  is  $\sim 650$  and remains both constant and independent of pH (Stumm and Morgan 1996). Thus, from a practical perspective, essentially all measured  $\text{CO}_2$  is  $\text{CO}_2(\text{aq})$ . For the remainder of this paper,  $\text{CO}_2$  will be synonymous with  $\text{H}_2\text{CO}_3^*$  when referring to dissolved  $\text{CO}_2$  in the water column.

### 1.3. Diffusion Theory

Diffusion across the interface between a gas and a liquid represents the rate limiting factor to gas transfer (Tsvoglou *et al.* 1965). Although Einstein's law of diffusion usually is applied to gas transfer in a single, viscous medium, the gas-liquid interface in a turbulent system can also

be considered a viscous resistance to diffusion. Therefore, applying Einstein's law of diffusion to the transfer of gases into or out of a liquid yields that  $K_L a$  values and molecular diameters ( $d$ ) are inversely proportional for any pair of gases (Tsivoglou *et al.* 1965). Using the molecular diameters of oxygen ( $d_{O_2} = 2.92 \cdot 10^{-10}$  m) and  $CO_2$  ( $d_{CO_2} = 3.23 \cdot 10^{-10}$  m), the theoretical ratio of mass transfer coefficients ( $\phi_T$ ) for  $CO_2$  relative to oxygen is (Lide 1992):

$$\phi_T = \frac{d_{O_2}}{d_{CO_2}} = \frac{2.92}{3.23} = 0.90 \quad (\text{Equation 8})$$

This same ratio, determined from physical experiments, can be defined as:

$$\phi_E = \frac{(K_L a)_{CO_2}}{(K_L a)_{O_2}} \quad (\text{Equation 9})$$

Given that the  $\phi_T$  value for  $CO_2$  relative to oxygen is 0.90, it can be assumed that the mass transfer coefficient for  $CO_2$  could only be up to 90% of the oxygen mass transfer coefficient. Experimentally determined  $K_L a$  ratios ( $\phi_E$ ) below the theoretical maximum ( $\phi_T$ ) would suggest that there are factors other than gas molecular diameter differences that are affecting the relative mass transfer.

## MATERIALS AND METHODS

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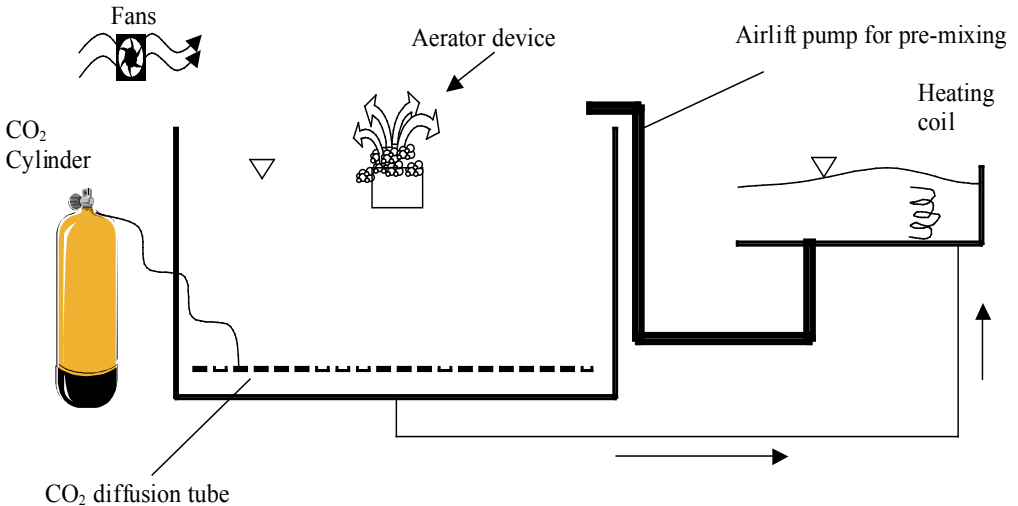
Oxygen and  $CO_2$  mass transfer rates were measured using two types of mechanical aerators. The two aerators are commonly used in aquaculture systems, as described in section 2.2.

### 2.1. Experimental setup

Oxygen and  $CO_2$  mass transfer were measured in a 10,000 L (nominal volume) circular tank (3.7 m diameter by 1.2 m high). The water level was kept constant in the tank at 0.91 m. Well water (14°C) with a pH of

7.5 and an alkalinity of  $130 \text{ mg L}^{-1}$  as  $\text{CaCO}_3$  was used for all tests. The well water was warmed to  $22\text{--}25^\circ\text{C}$  using a tank equipped with heating coils and an air lift pump to recirculate the water prior to any test being performed. The aerators were positioned in the center of the test tank. A schematic of the test system is shown in Figure 1. The elevation at the site was 375 m above sea level.

Figure 1. General schematic of experimental set up using a  $10 \text{ m}^3$  tank.

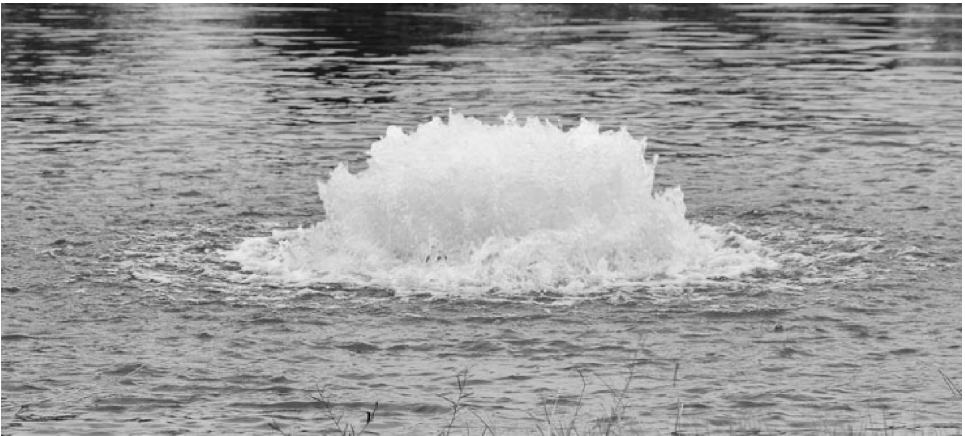


## 2.2. Description of aeration devices

The aerators tested were a Kasco model KA751 and a Sweetwater model HS5 (both supplied by Aquatic Ecosystems, Inc., Apopka, Florida, USA). Both aerators were circular, surface-draw aerators. The Kasco unit was equipped with a continuous duty 0.56 kW (0.75 hp) motor and was supported on a polyethylene float. The manufacturer specified that the unit pumps approximately  $41 \text{ L s}^{-1}$  and draws 6.7 amps. During operation, water agitation in the tank was extremely violent with the entire water plume ejected into the air being whitewater (Figure 2). The pumped water was evenly distributed about the tank in a circular fashion and the aerator was deemed to have a “moderate air exposure” relative to the Sweetwater unit, as described next.

The Sweetwater unit was a much smaller unit designed for small ponds and tanks. It was powered by a 0.12 kW (0.17 hp) motor and was floated

on top of the water by a Styrofoam collar. The manufacturer specified that the unit pumps  $7.6 \text{ L s}^{-1}$  and draws 1.8 amps. During operation, water agitation in the tank was less turbulent than with the Kasco unit, and the plume of water ejected into the air contained almost no whitewater and produced few bubbles on the water surface (Figure 3). However, the Sweetwater unit created a large air-water exposure during operation and was defined as having a “high air exposure” relative to the Kasco unit. These two units were chosen because they represented two levels of water breakup and air exposure. While the Kasco unit broke up more water, the air exposure of the water was not as complete as with the Sweetwater unit.



*Figure 2. Water breakup pattern for Kasco Model KA 751 unit (moderate air exposure, MAE).*



*Figure 3. Water breakup pattern for Sweetwater Model HS5 unit (high air exposure, HAE).*

### **2.3. Determination of oxygen transfer**

Well water (130 mg L<sup>-1</sup> as CaCO<sub>3</sub> alkalinity) was deoxygenated using sodium sulfite catalyzed with cobalt chloride (ASCE 1984). Cobalt chloride was added first to the test tank at a concentration of 0.5 mg L<sup>-1</sup> with the aerator running to ensure uniform mixing. The sodium sulfite was then added at a concentration of 7.88 mg L<sup>-1</sup> for every milligram per liter of dissolved oxygen to be removed. A sufficient quantity of sodium sulfite was added to drop the dissolved oxygen concentration below 0.5 mg L<sup>-1</sup>. The dissolved oxygen content of the water was measured prior to beginning any test to prevent the addition of excess chemical and ensure that the starting concentration remained below 0.5 mg L<sup>-1</sup>. During an oxygenation test, composite water samples were collected as a function of time (ASCE 1984). Four sample points were used for each water composite sample for measurements of dissolved oxygen: one shallow, one mid-depth, one deep, and one chosen by the researchers (ASCE 1984).

For the Kasco unit, water samples in a given test were taken such that two-thirds of the values corresponded to the period during which dissolved oxygen concentration changed rapidly and one-third during the more stationary period as the water moved towards equilibrium oxygen concentration. For the Sweetwater unit, water samples were taken at equal time intervals between the first and last dissolved oxygen readings. Oxygen readings were taken using a dissolved oxygen meter (Model 54A, YSI, Yellow Springs, Ohio, USA) and polarographic oxygen probe (Model 5739, YSI, Yellow Springs, Ohio, USA). The oxygen meter was calibrated prior to each test according to the manufacturer's specifications. Oxygen tests were replicated three times for each of the two aerators and the oxygen  $K_La$  values were calculated as described in section 2.5.

### **2.4. Determination of carbon dioxide transfer**

Three trials at low alkalinity (well water) were conducted for each aerator with three different initial levels of tank water CO<sub>2</sub>. In addition, tests for both aerators were conducted at elevated levels of both alkalinity (~ 1,000 mg L<sup>-1</sup> as CaCO<sub>3</sub>) and CO<sub>2</sub> to see if there was a noticeable effect on measured  $K_La$  values. Initial CO<sub>2</sub> values were selected to cover an expected range of concentrations that would be experienced in commercial RAS. The high alkalinity levels were created by adding

sodium bicarbonate directly to the water. Alkalinity was verified by titrating a 100 ml sample with 1.600 N sulfuric acid to a pH of 4.8. A Hach Co. (Loveland, CO, USA) titrator and reagents were used.

Compressed CO<sub>2</sub> gas (CO<sub>2</sub> > 99%) was added to the main tank water using a tube-diffuser hose. The diffuser assembly was connected to a CO<sub>2</sub> tank with a flow meter to control the rate of application. For each test, after injection of CO<sub>2</sub> gas brought the dissolved level to the desired concentration, the water was allowed to equilibrate for five minutes. This procedure was repeated until the desired CO<sub>2</sub> level remained constant. Alkalinity does not change due to the addition or removal of CO<sub>2</sub> (APHA 1995, Stumm and Morgan 1996), hence it was measured prior to the beginning and at the end of each test and the average of these two values was used in all calculations of the CO<sub>2</sub> concentration for that particular run.

Concentrations of CO<sub>2</sub> were calculated from measurements of temperature, alkalinity and pH according to Standard Methods 4500-CO<sub>2</sub> D (APHA, 1995). The pH measurements were obtained using an Ion Analyzer (Model 250, Corning Inc., Corning, NY, USA) and a sealed, gel filled, combination pH probe (Model 910600, Orion Research, Beverly, MA, USA). The pH meter was calibrated using a two-point method prior to each test run with standard buffer solutions of pH 4.00 and pH 7.00.

Measurements of CO<sub>2</sub> for the Kasco unit were taken at four minute intervals for the first hour and at eight minute intervals thereafter. Measurements for the Sweetwater unit were taken at five minute intervals for one hour and at ten minute intervals thereafter. In all cases, a water sample of approximately 200 ml was taken from the test tank and immediately tested for pH. The pH meter stabilized in approximately 30 seconds for each reading. Water samples were taken from various positions and depths in the test tank using closed flasks and a siphon hose in order to reduce sampling position bias.

### 2.5. Determination of the Mass Transfer Coefficient, $K_L a$

The overall mass transfer coefficient in Equation (1) was determined by using the log-deficit model in integrated form (Stenstrom 1979):

$$\ln|C_s - C| = -K_L a \cdot t + \ln|C_s - C_{t=0}| \quad (\text{Equation 10})$$

The equilibrium values ( $C_s$ ) for oxygen and  $\text{CO}_2$  were calculated using the gas solubility equations as presented by Weiss (1970, 1974) and ASHRAE (1972); effects of barometric pressure, gas partial pressures, and temperature were included in these calculations. A semi-log plot of the gas deficit versus time yields a straight line with a slope equal to  $K_L a$ . A linear least squares regression of the data was performed using Microsoft® Excel to obtain  $K_L a$  and  $R^2$  values. The same log-deficit method was used to determine the  $K_L a$  values for oxygen and  $\text{CO}_2$ . For ease of comparison,  $K_L a$  values were standardized to a reference temperature of 20°C by (ASCE 1984):

$$(K_L a)_T = (K_L a)_{20} \Theta^{(T-20)} \quad (\text{Equation 11})$$

where :

$(K_L a)_T$  = value from Equation 10

$\Theta$  = temperature correction factor, 1.024 in fresh water

T = temperature, °C

## RESULTS

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The results of the oxygen transfer tests for the Kasco and Sweetwater units yielded mean  $(K_L a)_{\text{O}_2,20}$  values of 7.71 hr<sup>-1</sup> (sd = 0.04) and 1.23 hr<sup>-1</sup> (sd = 0.15), respectively. For the low alkalinity tests, the  $(K_L a)_{\text{CO}_2,20}$  values for the Kasco and Sweetwater units yielded average values of 5.17 hr<sup>-1</sup> (sd = 0.64) and 1.06 hr<sup>-1</sup> (sd = 0.04), respectively. The  $K_L a$  values for  $\text{CO}_2$  in the high alkalinity water obtained from a single test for each aerator were 3.58 hr<sup>-1</sup> and 0.93 hr<sup>-1</sup> for the Kasco and Sweetwater units, respectively. All regression curves used to determine  $K_L a$  values for either oxygen or  $\text{CO}_2$  had  $R^2$  values greater than 0.90 (Aitchison 1999).

Mean values for  $\phi_E$  were 0.67 (sd = 0.08) and 0.86 (sd = 0.03) for the Kasco (moderate air exposure) and Sweetwater (high air exposure) units, respectively, for the low alkalinity trials. For these trials, the  $\phi_E/\phi_T$  ratios were 0.74 and 0.96 for the Kasco and Sweetwater units, respectively. For the high alkalinity trials,  $\phi_E/\phi_T$  ratios were 0.51 and 0.84 for the Kasco and Sweetwater units, respectively. Mean  $K_La$  values for oxygen and CO<sub>2</sub> and associated ratios of  $\phi_E$  and  $\phi_E/\phi_T$  are given in Tables 1 and 2. Representative graphs of the change in oxygen and CO<sub>2</sub> over time for the two aerators are shown in Figures 4 and 5.

## DISCUSSION

The major objective of this research was to determine whether mass transfer coefficients ( $K_La$ ) for oxygen could be used to predict CO<sub>2</sub> transfer for the same device by using the theoretical adjustment  $\phi_T$  factor, which is based upon the ratio of gas molecular diameters. If the theoretical correction proved to be valid, then the  $K_La$  for CO<sub>2</sub> gas transfer

Table 1. Oxygen and carbon dioxide testing results for Kasco Model KA751 Aerator (Moderate Air Exposure, MAE)

	Trial #1	Trial #2	Trial #3	Mean	Trial #4*
Water Temp (°C)	22.3	24.5	25.0	—	25.0
Initial CO <sub>2</sub> (mg L <sup>-1</sup> )	27	56	104	—	143
Alkalinity (mg L <sup>-1</sup> )	108	137	133	—	1,046
( $K_La$ ) <sub>CO<sub>2</sub>,20</sub> (hr <sup>-1</sup> )	5.90	4.73	4.87	5.17	3.58
Mean ( $K_La$ ) <sub>O<sub>2</sub>,20</sub> (hr <sup>-1</sup> )**	—	—	—	7.71	—
$\phi_E$	0.76	0.61	0.63	0.67	0.46
$\phi_E/(\phi_T = 0.90)$	0.84	0.68	0.70	0.74	0.51

\* Carbon dioxide stripping test at elevated alkalinity; results not averaged with other trials;  $K_La$  value for oxygen assumed to be the average of the low alkalinity trials.

\*\* The oxygen  $K_La$  value is the mean of three separate tests and was used for all  $\phi_E$  calculations.

Table 2. Oxygen and Carbon Dioxide Testing Results for Sweetwater Model HS5 Aerator (High Air Exposure, HAE)

	Trial #1	Trial #2	Trial #3	Mean	Trial #4*
Water Temp (°C)	26.0	25.8	25.1	—	25.0
Initial CO <sub>2</sub> (mg L <sup>-1</sup> )	28	59	102	—	121
Alkalinity (mg L <sup>-1</sup> )	128	128	125	—	920
(K <sub>L</sub> a) <sub>CO<sub>2</sub>,20</sub> (hr <sup>-1</sup> )	1.01	1.09	1.08	1.06	0.93
Mean (K <sub>L</sub> a) <sub>O<sub>2</sub>,20</sub> (hr <sup>-1</sup> )**	—	—	—	1.23	—
φ <sub>E</sub>	0.83	0.89	0.88	0.86	0.76
φ <sub>E</sub> /(φ <sub>T</sub> = 0.90)	0.92	0.99	0.98	0.96	0.84

\* Carbon dioxide stripping test at elevated alkalinity; results not averaged with other trials; K<sub>L</sub>a value for oxygen assumed to be the average of the low alkalinity trials.

\*\* The oxygen K<sub>L</sub>a value is the mean of three separate tests and was used for all φ<sub>E</sub> calculations.

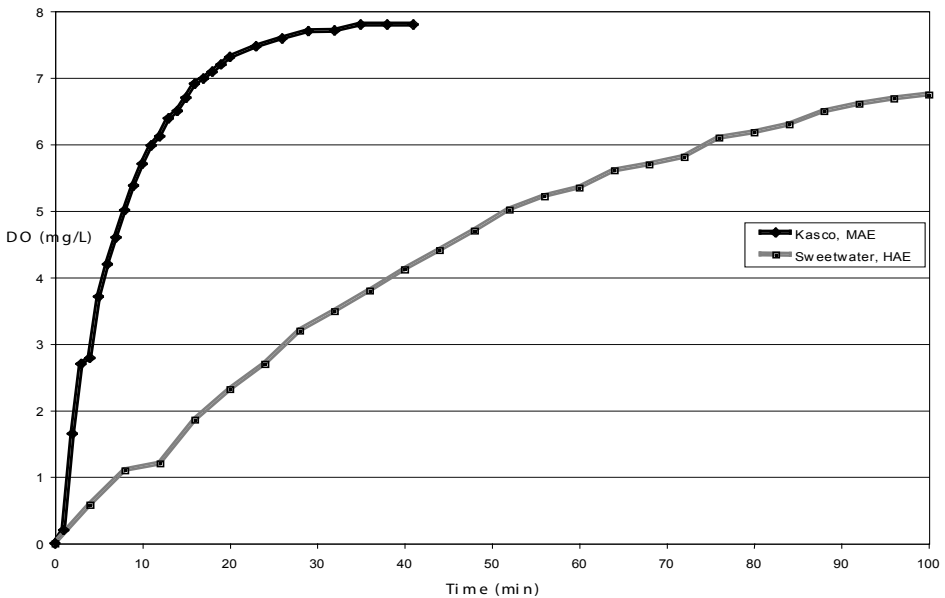


Figure 4. Representative data from an oxygen transfer trial for both aerators showing dissolved oxygen (DO) versus time (water temperature 22°C for both aerators).

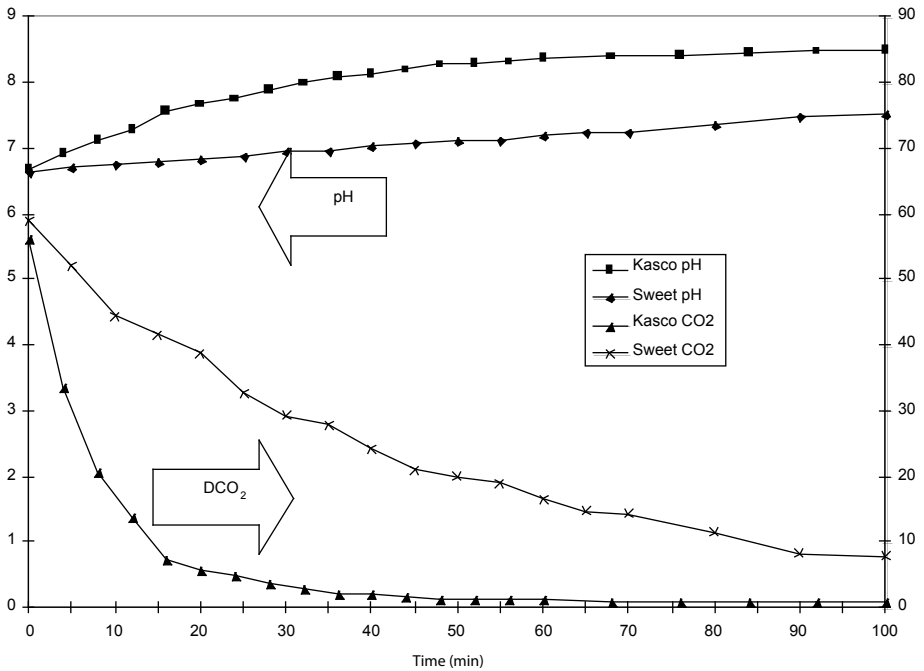


Figure 5. Representative data from a carbon dioxide transfer trial for both aerators showing dissolved (free) carbon dioxide ( $\text{DCO}_2$ ) and pH versus time; water temperature  $22^\circ\text{C}$  and alkalinity of  $137 \text{ mg L}^{-1}$  as  $\text{CaCO}_3$  for Kasco (MAE) unit and water temperature of  $25^\circ\text{C}$  and  $127 \text{ mg L}^{-1}$  alkalinity as  $\text{CaCO}_3$  for Sweetwater (HAE) unit.

would be 90% of the  $K_L a$  for oxygen in all cases. The experiments described here were performed using two types of surface aerators, one representing a moderate (Figure 2, Kasco Unit) and one a high air exposure pattern (Figure 3, Sweetwater Unit). The data for the Sweetwater unit (high air exposure) indicates that the  $\phi_T$  correction (0.90) may be used ( $\phi_E/\phi_T = 0.96$ ) in waters with low alkalinity ( $\sim 130 \text{ mg L}^{-1}$  as  $\text{CaCO}_3$ ) and a broad range of dissolved  $\text{CO}_2$  concentrations ( $\sim 30\text{-}100 \text{ mg L}^{-1}$ ). However, results for the Kasco unit (moderate air exposure) showed that the  $\phi_E/\phi_T$  ratio was only 0.74 for the same water quality conditions.

The photographs of the water breakup caused by the two aerators (Figures 2 and 3) show that even though the Kasco unit creates a large degree of turbulence there is less exposure of the water to air than in the Sweetwater unit. The Kasco unit churns and bubbles water up, but does not create a fountain-like pattern, as does the Sweetwater unit. There

is no visible airspace behind the plume of water created by the Kasco unit while the Sweetwater unit created more of a fountain-type spray. A fountain-type spray may result in a higher renewal rate of the gas phase around the aerator, resulting in an effect analogous to the high gas flow to liquid flow (G/L) that is necessary for effective CO<sub>2</sub> removal in a packed column aerator (Grace and Piedrahita 1994). Other aerator types, such as true fountain aerators, may create a higher exposure of the water to air than the Sweetwater unit. However, that degree of water breakup may not be necessary to achieve effective CO<sub>2</sub> removal, as the Sweetwater unit achieved nearly 100% of the  $\phi_E/\phi_T$  ratio. Eschar *et al.* (2003) presented  $K_La$  values for a paddle wheel aerator and a submerged aerator. As in the present research, these two devices created very different water-breakup and air exposure patterns. Using the data presented by Eschar *et al.* (2003), the  $\phi_E/\phi_T$  ratios were 0.89 and 0.65 for the paddle wheel and submerged aerator, respectively. This supports the results observed in this research.

Results from the tests conducted at high alkalinity (alkalinity ~ 1,000 mg L<sup>-1</sup> as CaCO<sub>3</sub>), showed that the  $K_La$  coefficient was reduced 31% in the Kasco unit from the average  $K_La$  value obtained for the low alkalinity tests and 12% in the Sweetwater unit. These results highlight the difference between oxygen and CO<sub>2</sub> removal as CO<sub>2</sub> concentrations are dependent upon the carbonate chemical equilibrium system (see Equations 2–5), which is pH driven, while oxygen concentrations are not. In short, removal of CO<sub>2</sub> causes changes in the concentrations of the other components of the carbonate system such that a chemical equilibrium is re-established. Whereas some of the carbonate system reaction rates are essentially instantaneous, the dissociation of H<sub>2</sub>CO<sub>3</sub> and HCO<sub>3</sub><sup>-</sup> to CO<sub>2</sub> is not, resulting in a lag in the re-establishment of chemical equilibrium. As a result, the CO<sub>2</sub> concentrations used to determine  $K_La$  coefficients, which are measured in samples in which equilibrium has been reached after collection from the aeration tank, overestimate the instantaneous CO<sub>2</sub> concentration in the aeration tank. The consequence is that the mass of CO<sub>2</sub> gas removed from the water due to gas transfer is larger than the mass change that is reflected by a change in dissolved CO<sub>2</sub> concentration (Grace and Piedrahita 1994, Summerfelt *et al.* 2000). The magnitude of the consequence increases as alkalinity increases, and hence would be more noticeable in high alkalinity waters.

Given that the purpose of this paper is to provide a practical means of predicting CO<sub>2</sub> transfer based on oxygen transfer data for fish culture applications, the equilibrium dissolved CO<sub>2</sub> concentration in the water column is the more relevant parameter, because the equilibrium concentration is what impacts a fish physiologically. Thus, from an engineering perspective,  $K_La$  coefficients for CO<sub>2</sub> removal should be based upon equilibrium concentrations of CO<sub>2</sub> as well. Caution should be applied when using  $K_La$  values determined from oxygen transfer experiments to predict CO<sub>2</sub> mass transfer coefficients, as the  $\phi_E/\phi_T$  ratio is lower at high alkalinities (~ 1,000 mg L<sup>-1</sup>) or as air exposure becomes less complete. For low and moderate alkalinities (< 150 mg L<sup>-1</sup>),  $K_La$  values for CO<sub>2</sub> mass transfer can be assumed to be 0.90 of an established  $K_La$  value for oxygen transfer for a specific device when the device causes high air exposure as depicted by Figure 3. Lower values for the  $K_La$  for CO<sub>2</sub> relative to that for oxygen should be used for high alkalinity waters and for aerators with moderate air exposure as shown in Figure 2.

$$\phi_E = \frac{(K_L a)_{CO_2}}{(K_L a)_{O_2}} \quad \text{(Equation 9)}$$

### Example Problem

A recirculating tank system has a volume of 100 m<sup>3</sup>. Culture tank water is maintained at 20°C and the maximum dissolved carbon dioxide concentration is 30 mg/L. The CO<sub>2</sub> saturation concentration ( $C_{s,CO_2}$ ) is 0.5 mg/L. The following two surface aerators are proposed to maintain the target maximum dissolved carbon dioxide concentration in the culture tank:

	Moderate Air Exposure (MAE) Aerator	High Air Exposure (HAE) Aerator
Mean ( $K_La$ ) O <sub>2</sub> 20 (hr <sup>-1</sup> )	7.71	1.23
Estimated $\phi_E$	0.67	0.86
Power, kW	0.60	0.12
Capital Cost	\$930	\$670

Mass balance analysis shows that the aerators must remove 100 kg/d dissolved carbon dioxide. Calculate the number of aerators required for each choice and the cost effectiveness of the two aerators over their expected life cycle.

**Step 1. Calculate the carbon dioxide transfer rate under operating conditions (CTR):**

$$CTR = \frac{(K_L a)_{CO_2} \cdot (C_{\text{tank}} - C_s) \cdot \text{Vol}_{\text{tank}}}{1,000}$$

Solving the CTR equation for the moderate air exposure (MAE) aerator:

$$CTR_{\text{MAE}} = \frac{(7.71 \cdot 0.67) \text{hr}^{-1} \cdot (30 - 0.5) \text{mg/L} \cdot 100 \text{m}^3}{1,000} = 15.2 \text{kgCO}_2 \cdot \text{hr}^{-1}$$

Solving the CTR equation for the high air exposure (HAE) aerator:

$$CTR_{\text{HAE}} = \frac{(1.23 \cdot 0.86) \text{hr}^{-1} \cdot (30 - 0.5) \text{mg/L} \cdot 100 \text{m}^3}{1,000} = 3.12 \text{kgCO}_2 \cdot \text{hr}^{-1}$$

**Step 2. Calculate the number of aerators (n) that would be needed for the two aerator choices:**

$$n_{\text{MAE}} = \frac{100 \text{kgCO}_2 \cdot \text{day}^{-1}}{(15.2 \text{kgCO}_2 \cdot \text{hr}^{-1} \cdot 24 \text{hr} \cdot \text{day}^{-1}) \text{unit}^{-1}} = 0.27 \text{ units}$$

$$n_{\text{HAE}} = \frac{100 \text{kgCO}_2 \cdot \text{day}^{-1}}{(3.12 \text{kgCO}_2 \cdot \text{hr}^{-1} \cdot 24 \text{hr} \cdot \text{day}^{-1}) \text{unit}^{-1}} = 1.34 \text{ units}$$

In practice, a designer must choose in unit increments and not by fractional units as the above example has shown. Obviously, the choice

of 100 kg/d of dissolved carbon dioxide removal was arbitrary and hence, the resulting fractional unit result. The number of units selected then becomes a design choice but typically will be rounded up (to one MAE unit or two HAE units in this case) to ensure that the dissolved carbon dioxide concentration goal is met as a minimum criteria.

**Step 3. Calculate the aeration efficiency for each aerator unit,  $AE_{CO_2}$ :**

$$AE_{CO_2, MAE} = \frac{15.2 \text{kgCO}_2 \cdot \text{hr}^{-1}}{0.6 \text{kW}} = \frac{25.3 \text{kgCO}_2}{\text{hr} \cdot \text{kW}}$$
$$AE_{CO_2, HAE} = \frac{3.12 \text{kgCO}_2 \cdot \text{hr}^{-1}}{0.12 \text{kW}} = \frac{26.0 \text{kgCO}_2}{\text{hr} \cdot \text{kW}}$$

Although the HAE aerator has a much lower mass removal rate for dissolved carbon dioxide, both aerators operate at similar energy efficiency per unit mass of dissolved carbon dioxide removed. Energy efficiency may be a key factor in a designer's final choice of aerators as well as the initial capital cost. Both must be considered to make a rational selection.

**Step 4. Calculate the life cycle cost of the aerators assuming the aerators operate continuously over the life cycle, which is assumed to be 5 years.**

$$\text{Total Cost} = \text{Capital Cost} + \text{Operating Cost}$$

To keep the example simple, work with the fractional aerator units required to remove the 100 kg/d of dissolved carbon dioxide. Assume electrical energy cost is \$0.10 per kWh.

$$\text{Operating Cost}_{\text{MAE}} = 0.27 \text{units} \cdot \frac{0.60 \text{kW}}{\text{unit}} \cdot \frac{24 \text{hr} \cdot 365 \text{day} \cdot 5 \text{yr}}{\text{day} \cdot \text{yr} \cdot \text{cycle}} \cdot \frac{\$0.10}{\text{kWh}} = \frac{\$709}{\text{cycle}}$$

$$\text{Operating Cost}_{\text{HAE}} = 1.34 \text{units} \cdot \frac{0.12 \text{kW}}{\text{unit}} \cdot \frac{24 \text{hr} \cdot 365 \text{day} \cdot 5 \text{yr}}{\text{day} \cdot \text{yr} \cdot \text{cycle}} \cdot \frac{\$0.10}{\text{kWh}} = \frac{\$704}{\text{cycle}}$$

$$\text{Total Cost}_{\text{MAE}} = 0.27 \text{units} \cdot \frac{\$930}{\text{unit}} + \$709 = \$960$$

$$\text{Total Cost}_{\text{HAE}} = 1.34 \text{units} \cdot \frac{\$670}{\text{unit}} + \$704 = \$1,602$$

In the above example, when fractional aerators are used for the calculations, the MAE unit was more cost effective over the 5 year assumed life of the aerator. If the number of aerators is rounded up to one MAE aerator or two HAE aerators instead of using the fractional aerators (since you cannot purchase a fractional aerator), operating costs (if you chose to run the aerators continuously) and total costs become:

$$\text{Operating Cost}_{\text{MAE}} = 1 \text{unit} \cdot \frac{0.60 \text{kW}}{\text{unit}} \cdot \frac{24 \text{hr} \cdot 365 \text{day} \cdot 5 \text{yr}}{\text{day} \cdot \text{yr} \cdot \text{cycle}} \cdot \frac{\$0.10}{\text{kWh}} = \frac{\$2,628}{\text{cycle}}$$

$$\text{Operating Cost}_{\text{HAE}} = 2 \text{units} \cdot \frac{0.12 \text{kW}}{\text{unit}} \cdot \frac{24 \text{hr} \cdot 365 \text{day} \cdot 5 \text{yr}}{\text{day} \cdot \text{yr} \cdot \text{cycle}} \cdot \frac{\$0.10}{\text{kWh}} = \frac{\$1,051}{\text{cycle}}$$

$$\text{Total Cost}_{\text{MAE}} = 1 \text{unit} \cdot \frac{\$930}{\text{unit}} + \$2,638 = \$3,558$$

$$\text{Total Cost}_{\text{HAE}} = 2 \text{units} \cdot \frac{\$670}{\text{unit}} + \$1,051 = \$2,391$$

When the number of aerators is rounded up, the relative costs are reversed and the HAE aerator option has a lower total cost, even though its initial capital cost is higher due to the need to purchase two aerators. As indicated previously, the choice of equipment depends on technical and economic factors that are specific to a particular operation.

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## **Nutrient Retention by Fish in a Multispecies Recirculating Aquaculture Facility**

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### **ABSTRACT**

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The nutrient content (nitrogen and phosphorus, N and P) of the dry weight gain of fish relative to N and P content of the dry weight of feed was used to determine nutrient retention in five species of fish that were reared in a commercial recirculating aquaculture facility. The culture system had five 39.2 m<sup>3</sup> dual-drain culture tanks, one tank each with largemouth bass (*Micropterus salmoides*), hybrid striped bass (aka sunshine bass, *Morone chrysops* x *Morone saxatilis*), and rainbow trout (*Oncorhynchus mykiss*), and two tanks with walleye (*Sander vitreus*). All fish were exposed to the same water temperature (15.8 - 24.1°C) and water quality. On the first day of the study, most rainbow trout (643 g) and walleye (497 g and 398 g) were at or near market size, whereas the largemouth bass (73 g) and hybrid striped bass (96 g) were fingerlings. Measured for a 56-d interval, the range in nutrient retention was 12.0 to 44.1% for N, and 14.8 to 53.8% for P. Nutrient retention was related to fish species and size; e.g., the larger size-group of walleye had nearly half the retention rates of the smaller size-group of walleye. Highly significant ( $p \leq 0.01$ ) positive correlations occurred between retention of

N and P, protein efficiency ratio, and net protein utilization, but nutrient retention was inversely related to food conversion ratio. Total ammonia nitrogen ( $\text{g kg}^{-1}$  feed fed) in the culture tank was inversely related to nitrogen retention. Values for TAN production ranged from 2.9 to 6.9% of daily feeding rate. This study demonstrated an interaction between nutrient retention with fish species, age or size, growth rates, temperature, feeding rates, nutrient content of the feed, and protein retention, all of which are factors that influence biofilter capacity to handle ammonia production and unit processes to reduce N and P content in the effluent.

## **INTRODUCTION**

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The nutrient composition of fish feed and its utilization by fish species in water reuse aquaculture systems has a major influence on effluent concentrations of nitrogen (N) and phosphorous (P), which may cause eutrophication in the aquatic environment (Cowey and Cho 1991, Tomasso 2002). Nutrient content of the water supply, endogenous loss from fish metabolism, fish feces, and uneaten feed are sources of nitrogen and phosphorus in effluents of aquaculture. Dietary protein supplies amino acids for energy and protein synthesis (i.e., growth), and serves as the major source of nitrogen in fish hatcheries and the effluents they discharge. Likewise, fish feeds with indigestible P (phytate in plant feed stuffs) or more P in the feed than needed for growth contribute P to the effluent, yet there is only limited information available on phosphorus retention in fish (Lall 1991). In recirculating aquaculture systems, indigestible feed ingredients also increase the solids in the discharge. Thus, optimized diet formulation and feeding practices are essential components of Best Management Practices for all types of fish culture operations, but are especially critical for water reuse aquaculture (WRA).

The engineering design of a WRA system depends on characteristics of the fish species: its size and growth, feeding rates and feed conversion, and stocking and harvesting strategies that are related to the production cycle of the fish as well as marketing issues. Realistic design guidelines must include biofilter capacity to handle ammonia production and unit processes to reduce the N and P content of the effluent. Controlling N and P in the effluent demands an understanding of nutrient retention (the amount of nutrients incorporated into fish flesh) relative to nutrients provided in the feed. Maximizing the retention of N and P by cultured

fish can provide numerous benefits for operators. Poor nutrient retention may result from poor feed formulation (i.e., indigestible ingredients, inadequate protein/energy ratio), over-feeding, or reduced feeding activity that may be related to adverse environmental conditions or disease. From a production standpoint, fish that more efficiently utilize nutrients in feed require less feed to reach a marketable size, saving the operator money and increasing the profitability of the enterprise. From a regulatory and ecological standpoint, optimizing nutrient retention assists in the prevention of excessive nutrient loading in culture effluents (Jahan *et al.* 2003).

The objective of this study was to describe nutrient retention in an operating, small-scale commercial WRA facility that had a very low water consumption. The daily inflow of water was only 1.6% of total system volume. The entire system volume was exchanged with makeup water only once every 62 days (Summerfelt and Penne 2007). The operator simultaneously cultured largemouth bass (*Micropterus salmoides*), walleye (*Sander vitreus*), hybrid striped bass (aka sunshine bass, *Morone chrysops* x *Morone saxatilis*), and rainbow trout (*Oncorhynchus mykiss*) in separate tanks, but with a single integrated system with components used in common.

The nutrient content of the dry weight gain of fish relative to the nutrient content of the dry weight of feed added into each tank of fish was used to determine nutrient retention during a 56-d interval. This was not a study of fish nutrition and it was not carried out in a controlled laboratory environment using a single species of fish. Further, we did not control the feeding or stocking of the system; however, we had the advantage of having intensively studied the performance of the system (Summerfelt and Penne 2005, 2007). The findings of this study show the scope of nutrient retention values and suggest relationships to fish age or size, growth rates, temperature, feeding rates, and nutrient content of the feed, which are values useful for design and development of performance-based environmental standards for recirculating aquaculture operations.

## MATERIALS AND METHODS

### Recirculation system

The system had five 39.2 m<sup>3</sup> dual-drain culture tanks as described by Timmons *et al.* (1998) and Summerfelt *et al.* (2000). Each culture tank had a high-volume, low-solids effluent from a side-wall drain (78.7% of flow) and a low-volume, high-solids effluent from the center drain (21.3% of flow). Water leaving the five tanks through their sidewall drain flowed directly to the sump where two 7.5 hp (5.6 Kw) electric centrifugal pumps lifted water to a fluidized sand bed biofilter. From the biofilter, flow was sent through a multi-staged Low Head Oxygenator™ (PR Aqua, Nanaimo, BC, Canada) and then to a head tank before returning to the culture tanks. Flow from the center drain carried most of the suspended solids to an

Table 1. Mean of fish weight, total tank biomass (B, kg/tank), density and loading.

Tank	Species <sup>1</sup>	Fish weight (g)	Number of fish	Biomass kg (%)	Density (kg m <sup>-3</sup> )	Loading <sup>2</sup> (kg m <sup>-3</sup> min <sup>-1</sup> )
<i>May 21, start of study</i>						
1	LMB	73	9,946	726 (13.4)	18.5	926
2	WYE	497	351	174 (3.2)	4.4	222
3	HSB	96	9,865	947 (17.5)	24.2	1,208
4	WYE	398	3,586	1,427 (26.4)	36.4	1,820
5	RBT	643	3,324	2,137 (39.5)	54.5	2,726
		<b>Totals</b>	27,072	5,411 (100)	--	--
				<b>Means</b>	27.6	1,380
<i>July 15, end of study</i>						
1	LMB	92	4,305	396 (8.5)	10.1	529
2	WYE	552	329	182 (3.9)	4.6	243
3	HSB	120	7,404	888 (19.0)	22.7	1,187
4	WYE	441	3,058	1,349 (28.9)	34.4	1,803
5	RBT	660	2,809	1,854 (39.7)	47.3	2,479
		<b>Totals</b>	17,905	4,669 (100)	--	--
				<b>Means</b>	23.8	1,248

<sup>1</sup>Abbreviations: LMB, largemouth bass; WYE, walleye; HSB, hybrid striped bass; RBT, rainbow trout. <sup>2</sup>Loading expresses fish biomass (kg) relative to inflow of water to the culture tank (m<sup>3</sup> min<sup>-1</sup>).

external triple standpipe (TSP) in which a greater part of the flow went to the DF (microscreen drum filter, Water Management Technologies, Inc., Baton Rouge, LA, USA) and a small intermittent flow of heavy solids was diverted to the septic tank. Water cleaned by the DF entered the sump while the backwash was discharged into a septic tank located exterior to the building. The culture tanks comprised 78.5% of the volume, while the plumbing and treatment components made up the balance (21.5%). Solids removal was accomplished by partitioning of solids into the culture tank's center drain flow and the subsequent capture in the quiescent zone of a TSP and by the 60  $\mu\text{m}$  mesh of the DF. Recirculating flow to the culture tanks ( $0.78 \text{ m}^3 \text{ d}^{-1}$ ) provided approximately  $1.2 \text{ exchanges h}^{-1}$ . During this study, daily inflow averaged  $3.9 \text{ m}^3 \text{ d}^{-1}$  or 1.6% of total system volume.

### **Water quality**

Water temperature, pH, and dissolved oxygen (DO) were measured daily with calibrated meters in each culture tank. Alkalinity (as  $\text{CaCO}_3$ ), total ammonia nitrogen,  $\text{NH}_3\text{-N}$  (TAN), total phosphorus (TP), total dissolved solids (TDS), and total suspended solids (TSS) were measured in each culture tank at the start of the study and at bi-weekly intervals. Alkalinity measurements were performed using titrimetric methods with a colorimetric end point (APHA 1998). Biochemical oxygen demand (BOD) was determined by incubation at  $20^\circ\text{C}$  for 5 days (APHA 1998). Samples were analyzed for total dissolved solids (TDS) and total suspended solids (TSS). TAN was determined by the Nessler method and TP by the ascorbic acid method following manual digestion.

### **Fish stocks**

The producer cultured four species of fish in five tanks (Table 1). Largemouth bass and walleye were marketed as stockers for fish enhancement or as food fish. Rainbow trout and hybrid striped bass were marketed exclusively as food fish. On the first day of the study, most of the rainbow trout (RBT, 643 g), and walleye in tank 2 (WYE, 497 g), were considered market size whereas the largemouth bass (LMB, 73 g) and hybrid striped bass (HSB, 96 g) were fingerlings.

Group and individual weights of samples of fish were obtained bi-weekly. Ten fish were collected from each tank in the first two samples, and 20 fish were collected in the last three samples.

Mean standing stock of fish in each tank varied substantially over the course of the study (Table 1). The average of initial and final stock was 5,040 kg, density 25.7 kg m<sup>-3</sup>, and loading 1,314 kg m<sup>-3</sup> min<sup>-1</sup>. The mean standing stock consisted of 561 kg (11.1% of total) largemouth bass, 1,566 kg (31.1%) walleye, 918 kg (18.2%) hybrid striped bass, and 1,996 kg (39.0%) rainbow trout.

A proximate analysis of fish carcass and feed samples was performed by a commercial laboratory (Minnesota Valley Testing Laboratory, New Ulm, MN, USA).

### **Feed and Feeding**

Feeds were commercial feeds, high in total phosphorus (TP) and total protein (TKN x 6.25) (Table 2). Feed types and sizes were selected by the farmer as appropriate for the fish size and species: largemouth bass and hybrid striped bass were fed Silver Cup<sup>TM</sup> brand (Silver Cup Fish Feeds, Murray, UT, USA) steelhead feed (502 g kg<sup>-1</sup> protein, 16 g kg<sup>-1</sup> P); walleye were fed Silver Cup<sup>TM</sup> brand salmon pellets (545 g kg<sup>-1</sup> protein, 18 g kg<sup>-1</sup> P); and rainbow trout were fed Silver Cup<sup>TM</sup> brand trout pellets (467 g kg<sup>-1</sup> protein, 15 g kg<sup>-1</sup> P). Feed samples were analyzed for moisture, total phosphorus (TP), Kjeldahl nitrogen (TKN), and total fat by the same commercial laboratory used for fish carcass analysis (Table 2). The non-protein nitrogen, which was 0.34%, was not added to the total nitrogen content of feed fed as it was not relevant to the measurement of net protein utilization (NPU).

Feed added to each culture tank was recorded by the owner-operator. The tabulated values were used to calculate the total quantity of feed fed. Overall, feeding rates (kg of feed fed per day as a percent of estimated tank biomass on the same day) ranged from 0.15 to 0.82 (Table 4). The lowest feeding rates were for rainbow trout (tank 5) and walleye (tank 4) and the highest rates were fed to hybrid striped bass (tank 3). Differences in feeding rates were related to fish size, market status, and water temperature; thus, rainbow trout (tank 5) and walleye in tank 4 were both at a harvestable size and required only maintenance levels of feeding;

Table 2. Nutrient content of fish feeds.

Tank	Species <sup>1</sup>	Crude Protein (Total N)	Total	Moisture	Lipid <sup>2</sup>	Ash
<i>As-fed basis (%)</i>						
1	LMB	46.0 (7.36)	1.47	8.43	15.1	9.0
2	WYE	48.8 (7.81)	1.61	10.50	12.6	9.1
3	HSB	46.0 (7.36)	1.47	8.43	15.1	9.0
4	WYE	48.8 (7.81)	1.61	10.50	12.6	9.1
5	RBT	42.1 (6.74)	1.36	9.81	12.4	9.0
<i>Dry weight (%)</i>						
1	LMB	50.2 (8.03)	1.61	--	16.5	16.5
2	WYE	54.5 (8.72)	1.80	--	14.1	10.2
3	HSB	50.2 (8.03)	1.61	--	16.5	16.5
4	WYE	54.5 (8.72)	1.80	--	14.1	10.2
5	RBT	46.7 (7.47)	1.51	--	13.8	10.0

<sup>1</sup>See Table 1 for species names.

<sup>2</sup>Ether extract

also, feeding rates for rainbow trout were reduced because of a high relative water temperature.

**Nutrient Retention**

Nutrient retention was based on analysis of the nutrient content of the dry weight of feed added and nutrient content of the dry weight of the biomass gain (growth, B<sub>g</sub>) of the fish. With no mortality, or additions or removal of fish for marketing, biomass dry weight gain (B<sub>g</sub>) in each tank of fish equals:

$$B_g = S_f ( \bar{w}_f - \bar{w}_i ) \tag{Equation 1}$$

where: B<sub>g</sub> = biomass (dry weight) gain during study

S<sub>f</sub> = stock number at end of study

$\bar{w}_f$  = mean biomass (dry weight) of fish at end of study

$\bar{w}_i$  = mean biomass (dry weight) of fish at start of study

*Table 3. Harvest and mortality of fish during 56-d study interval. Percent is of initial stock of each species.*

Tank	Species	Harvest		Mortality	
		Number	(%)	Number	(%)
1	Largemouth bass	5,626	(56.6)	15	(0.2)
2	Walleye	20	(5.7)	2	(0.6)
3	Hybrid striped bass	2,452	(24.9)	9	(< 0.1)
4	Walleye	501	(13.9)	27	(0.8)
5	Rainbow trout	399	(12.0)	116	(3.5)
	<b>Total</b>	8,998	(33.3)	169	(0.6)

Fish population numbers changed as a result of harvest and mortality (Table 3), thus, the final stock ( $S_f$ ) was always expected to be less than the number present at the start of the study. Overall, mortality was trivial; only 0.6% of initial stock. The major changes in fish stock were the result of harvest (33.3%), mainly from a large harvest of largemouth bass (5,626 fish) and hybrid striped bass (2,452 fish).

In most cases, fish that were harvested were weighed at that time, but fish that died were not weighed. To adjust for total biomass gain (dry weight) of fish that died or were harvested required an estimate of their weight on the day they died or were removed. The weight of the fish that died or were removed during the study was estimated from a regression equation of mean fish weight (Y-axis) against day of the study (X-axis).

The biomass gain of fish in each tank from the first day to the day the fish was removed was calculated using equation 2:

$$B_{gr} = N_{rt} ( \bar{w}_i - \bar{w}_{rt} ) \tag{Equation 2}$$

where:  $N_{rt}$  = number of fish that were removed (r) on day (t)

$\bar{w}_{rt}$  = mean weight of fish that died or were removed on day (t)

Table 4. Protein efficiency, net protein utilization, feed conversion, and growth (means ± SE). Means in each row followed by different letters differ at the 0.05 probability level by Fisher's least-significant-difference procedure (Steel and Torrie 1980).

Tank	Species <sup>1</sup>	Total feed fed (kg)	FR <sup>2</sup> (%)	Total biomass gain <sup>3</sup>	PER <sup>4</sup>	NPU <sup>5</sup>	FCR <sup>6</sup>	SGR <sup>7</sup>
1	LMB	97	0.39	108.2	2.43 <sup>a</sup> ± 0.61	39.55 <sup>a</sup> ± 8.85	0.63 <sup>c</sup> ± 0.56	0.41 <sup>a</sup> ± 0.01
2	WYE	49	0.49	18.8	0.79 <sup>b</sup> ± 0.05	16.00 <sup>b</sup> ± 0.81	2.61 <sup>b</sup> ± 0.16	0.18 <sup>b</sup> ± 0.01
3	HSB	394	0.82	185.5	1.03 <sup>b</sup> ± 0.13	15.95 <sup>b</sup> ± 1.65	2.14 <sup>a,b,d</sup> ± 0.27	0.39 <sup>a</sup> ± 0.03
4	WYE	181	0.24	140.6	1.60 <sup>a</sup> ± 0.12	36.35 <sup>a</sup> ± 7.55	1.29 <sup>c,d</sup> ± 0.09	0.18 <sup>b</sup> ± 0.00
5	RBT	161	0.15	51.4	0.76 <sup>b</sup> ± 0.09	13.35 <sup>b</sup> ± 1.65	3.13 <sup>a</sup> ± 0.36	0.04 <sup>c</sup> ± 0.01
<i>P</i> -value of ANOVA					0.04	0.04	0.02	<0.01

<sup>1</sup>See Table 1 for species name

<sup>2</sup>FR, feeding rate = (mean kg feed fed per day/mean kg fish biomass) x 100

<sup>3</sup>Wet weight (kg)

<sup>4</sup>PER, protein efficiency ratio = kg wet weight gain/kg protein in feed as fed.

<sup>5</sup>NPU, net protein utilization = (kg dry weight protein gain by fish/kg dry weight protein fed) x 100.

<sup>6</sup>FCR, feed conversion = kg feed fed in interval/kg gain.

<sup>7</sup>SGR, specific growth rate (%/d) = (lnw<sub>t</sub> - lnw<sub>i</sub>) / t x 100, where lnw<sub>t</sub> = natural log mean fish weight at end of interval, where lnw<sub>i</sub> = natural log mean fish weight at start of interval, and t = length of interval.

Therefore, the adjusted biomass gain of the total tank population was equal to:

$$B_g = S_f (\bar{w}_i - \bar{w}_f) + B_{gr} \quad (\text{Equation 3})$$

Nutrient retention was calculated by dividing the kg of N and P gained (dry weight of fish) by the kg of N and P fed (dry weight of feed) and multiplying by 100. The amount of N and P ( $N_g$ ) gained was derived by multiplying the dry weight of biomass gained by percent N and P in the fish carcass (equation 4):

$$\% \text{ Nutrient retention} = (N_g / N_f) \times 100; \quad (\text{Equation 4})$$

where:

$$N_g = B_g \times B_n$$

$$N_f = FF_t \times F_n$$

$N_g$  = nutrient (N or P) gain in dry weight (kg) of fish

$N_f$  = dry weight (kg) of nutrients (N or P) fed

$B_g$  = biomass gained (dry weight, kg)

$B_n$  = nutrient content of fish (ratio of N or P content per 100 g of fish)

$FF_t$  = total feed fed (dry weight, kg)

$F_n$  = nutrient content of feed (ratio of N or P content per 100 g of feed)

Ammonia excreted by fish per kg of feed fed was calculated using equation 5 (Lawson 1995):

$$\text{TAN}(\text{g kg}^{-1} \text{ feed}) = (1.0 - \text{NPU})(\text{protein content of feed}/6.25)(1000) \quad (\text{Equation 5})$$

Lawson (1995) defined NPU, net protein utilization, as the ratio = kg dry weight protein gain by fish  $\text{kg}^{-1}$  dry weight protein added in feed.

Table 5. Fish carcass composition: Values are means ( $\pm$ SE) of five samples collected on day 1, 15, 28, 41, and 55 (end of study).

Tank	Species <sup>1</sup>	Fresh weight (%)				Dry weight (%)		
		Protein	TN <sup>2</sup>	TP <sup>2</sup>	Moisture	Protein	TN <sup>2</sup>	TP <sup>2</sup>
1	LMB	16.20 <sup>a</sup> $\pm$ 0.46	2.59 <sup>c</sup> $\pm$ 0.07	0.56 <sup>a</sup> $\pm$ 0.10	69.79 <sup>a</sup> $\pm$ 0.60	53.61 <sup>a</sup> $\pm$ 0.92	8.58 <sup>a</sup> $\pm$ 0.15	1.84 <sup>a,c</sup> $\pm$ 0.31
2	WYE	18.08 <sup>b</sup> $\pm$ 0.48	2.89 <sup>b</sup> $\pm$ 0.08	0.76 <sup>a,b</sup> $\pm$ 0.06	72.48 <sup>b</sup> $\pm$ 0.54	65.72 <sup>b</sup> $\pm$ 1.27	10.53 <sup>b</sup> $\pm$ 0.21	2.74 <sup>a,b</sup> $\pm$ 0.16
3	HSB	15.54 <sup>a,c</sup> $\pm$ 0.31	2.48 <sup>c,d</sup> $\pm$ 0.05	0.43 <sup>a</sup> $\pm$ 0.07	64.45 <sup>c</sup> $\pm$ 0.51	43.74 <sup>c</sup> $\pm$ 1.00	6.99 <sup>c</sup> $\pm$ 0.16	1.20 <sup>c</sup> $\pm$ 0.19
4	WYE	18.48 <sup>b</sup> $\pm$ 0.53	2.96 <sup>a</sup> $\pm$ 0.08	1.13 <sup>b</sup> $\pm$ 0.23	71.94 <sup>b</sup> $\pm$ 0.57	65.97 <sup>b</sup> $\pm$ 2.39	10.57 <sup>b</sup> $\pm$ 0.39	4.08 <sup>b</sup> $\pm$ 0.89
5	RBT	16.34 <sup>a,c</sup> $\pm$ 0.65	2.62 <sup>c,d</sup> $\pm$ 0.10	0.71 <sup>a</sup> $\pm$ 0.12	69.24 <sup>a</sup> $\pm$ 1.14	53.25 <sup>a</sup> $\pm$ 1.97	8.52 <sup>a</sup> $\pm$ 0.32	2.30 <sup>a,c</sup> $\pm$ 0.58
	<i>P-value</i> <sup>3</sup>	< 0.01	0.01	< 0.03	< 0.01	< 0.01	< 0.01	< 0.01

<sup>1</sup>See Table 1 for species name

<sup>2</sup>Total nitrogen (TN) and total phosphorus (TP)

<sup>3</sup>*P*-value from Analysis of variance (ANOVA). Column means that have a superscript letter in common are not significantly ( $\leq 0.05$ ) different (Fisher's protected least significant difference multicomparison test).

## RESULTS

### Feeding, feed conversion, and growth rates

Total quantity of feed fed to the four species during the study ranged from 49 to 394 kg (Table 4). Species differences in feeding rate were related to fish size and readiness for market; e.g., the owner reduced the feeding rate for large, market-size rainbow trout and walleye, which were held at a higher density. Larger fish had higher FCR values (i.e., poorer feed conversion); for example, largemouth bass had the lowest mean weight (83 g) and lowest FCR (0.63) and RBT the largest size and highest FCR (3.13). Walleye and HSB had similar FCR values that were between those of RBT and LMB (Table 4). There was also a statistically significant difference in protein efficiency (PER) among tanks of fish. The PER values ranged from 0.76 to 2.43 (Table 4). Largemouth bass and WYE tank 4 had significantly ( $p < 0.05$ ) higher PER than all other tanks.

Table 6. Nitrogen and Phosphorus retention (NR and PR) of cultured fish as percent of N and P fed (kg values are dry weight).

Tank	Species <sup>1</sup>	Nutrient Retention						TAN <sup>3</sup>
		Sum of N fed (kg)	Sum of N gained (kg)	NR (%) <sup>2</sup>	Sum of P fed (kg)	Sum of P gained (kg)	PR (%) <sup>2</sup>	
1	LMB	7.17	3.16	44.07 <sup>a</sup> ± 12.91	1.44	0.68	47.22 <sup>a,b</sup> ± 18.47	28.6 <sup>a</sup> ± 14.71
2	WYE	3.61	0.49	13.57 <sup>b</sup> ± 0.58	0.73	0.13	17.81 <sup>a</sup> ± 1.12	69.2 <sup>b</sup> ± 1.71
3	HSB	28.94	4.55	15.72 <sup>b</sup> ± 1.13	5.40	0.80	14.81 <sup>a</sup> ± 1.64	50.6 <sup>b,c,d</sup> ± 5.03
4	WYE	14.13	4.07	28.80 <sup>a,b</sup> ± 4.74	2.92	1.57	53.77 <sup>b</sup> ± 22.41	47.3 <sup>a,d</sup> ± 10.90
5	RBT	10.85	1.30	11.98 <sup>b</sup> ± 1.27	2.19	0.35	15.98 <sup>a</sup> ± 4.60	57.5 <sup>a,b,c</sup> ± 3.13
<i>P-value of ANOVA</i>				< 0.01			< 0.05	0.06

<sup>1</sup>See Table 1 for long form name of species.

<sup>2</sup>The standard errors ( $\pm$ ) are of calculations for four intervals: 1-15 d, 16-28 d, 29-42, and 43-56.

<sup>3</sup>TAN (g kg<sup>-1</sup> feed) calculated from NPU and protein content of feed with formula 5 in text.

Fingerling hybrid striped bass (HSB) and LMB had the fastest specific growth rates (SGR), and WYE (tanks 2 and 4) and RBT had the slowest growth rates (SGR, Table 4).

**Carcass Composition**

Moisture content among the cultured species ranged from 64.5 to 72.5% (Table 5). The moisture content of WYE was significantly higher than the other species, but on a dry weight basis, both tanks of WYE had higher levels of protein than the other species. HSB had a lower protein content than any other species (Table 5).

**Nutrient retention**

Nitrogen retention by LMB (44.1%) was substantially higher than any other species, although not significantly different from walleye in tank 4 (Table 6). Phosphorus retention for WYE tank 4 (53.8%) was not different from LMB, and both were greater than WYE in tank 2, RBT, and HSB.

*Table 7. Correlation coefficient (r-value) matrix (10 observed values) between nitrogen (NR) and phosphorus (PR) retention with total N and P content in feed fed, FCR, PER, NPU, TAN, RG and SGR<sup>1</sup>. Significance (p-value) of r was calculated with Fisher’s r to Z; \*p-value ≤ 0.05; \*\*p-value ≤ 0.01.*

	<b>Nitrogen retention</b>	<b>Phosphorus retention</b>
Nitrogen fed	-0.22	-0.23
Phosphorus fed	-0.23	-0.23
Nitrogen retention (NR)		0.86**
Phosphorus retention (PR)	0.86**	
Feed conversion ratio (FCR)	-0.96**	-0.80**
Protein efficiency ratio (PER)	0.99**	0.79**
Net protein utilization (NPR)	0.96**	0.88**
Ammonia excretion TAN (g kg <sup>-1</sup> feed)	-0.92**	-0.78**
Specific Growth Rate (SGR)	0.50	0.17

<sup>1</sup>See text for definitions and formulas.

Species	Diet	Feed as-fed		Fish		Reference
		N (%)	P (%)	N ret. (%)	P ret. (%)	
Largemouth bass	Commercial	7.4	1.5	44.1	47.2	Present study
Walleye (tank 2)	Commercial	7.8	1.6	13.6	17.8	
Hybrid striped bass	Commercial	7.4	1.5	15.7	14.8	
Walleye (tank 4)	Commercial	7.8	1.6	28.8	53.8	
Rainbow trout	Commercial	6.7	1.4	12.0	16.0	
Rainbow trout	HP 300 control	7.4	0.6	33.2	36.5	Vielma <i>et al.</i> 2002
	Soycomil P control	7.4	0.6	37.4	28.5	
Atlantic salmon	Fishmeal	8.1	1.8	45.9	27.7	Storebakken <i>et al.</i> 2000
	Soy protein	7.9	1.2	46.1	27.6	
Rainbow trout	Fishmeal	-	1.2	-	34.1	Johnson & Summerfelt 2000
	Fishmeal & spray-dried blood cells	-	0.9	-	42.0	
Turbot	Fishmeal	8.5	1.8	30.4	32.9	Burel <i>et al.</i> 2000
Tilapia hybrid	Commercial tilapia feed	5.4	0.8	21.4	18.8	Siddiqui and Al-Harbi 1999
European sea bass	Pelleted	7.8	2.1	24.7	20.1	Ballestrazzi <i>et al.</i> 1998
	Extruded	7.8	2.1	24.7	19.6	
Rainbow trout	Experimental diets	-	0.85-1.60	-	14-22	Ketola and Harland 1993

*Table 8. Nitrogen and phosphorus retention levels from selected studies.*

There was a highly significant ( $p \leq 0.01$ ) positive correlation between nitrogen retention (NR) and phosphorus retention (PR), and both showed significant correlations (both + and -) with several other variables (Table 7). High positive correlations occurred between nutrient retention and protein efficiency ratio (PER), and nutrient retention and net protein utilization (NPU). There were also strong negative correlations with nutrient retention and both feed conversion ratio (FCR) and ammonia excretion (TAN). The correlations for either NR or PR with nitrogen

and phosphorus fed, or either NR or PR with measures of growth were not significant ( $p \leq 0.05$ ). There is obvious potential for autocorrelation between NR and PER and NR and NPU because of the relationship between N and protein content of the feed, as protein content is 6.25 times TKN. The relationship between NR and TAN reflects the inverse relationship between ammonia excretion and NR.

## DISCUSSION

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Published values for both N and P retention show substantial intra- and interspecies variation (Table 8). For example, three publications cited for RBT fed 0.6 to 1.6% dietary P had PR from 14 to 42% (Table 8), comparable to the results of the present study for RBT. It is likely that differences in bioavailability of phosphorus and phosphorus content of the feed in excess of requirements have a strong influence on relative retention. Nitrogen retention of pond-cultured channel catfish (*Ictalurus punctatus*) fed feeds manufactured from high quality ingredients ranged from 20 to 30% and phosphorus 25-35% of the N and P in the feed (Tucker *et al.* 2008).

Compared with other studies (Table 8), the N and P retention values for LMB were high, but about equal to values for Atlantic salmon (*Salmo salar*) (Storebakken *et al.* 2000). The LMB in the present study had an average PER value of 2.4% compared with a value of 1.7% reported by Portz *et al.* (2001). They found the best SGR values of 0.66 to 0.70 for a diet with 46.0 to 50.0% crude protein (CP), which compared with a mean SGR value of 0.41 in our study using a feed with 46% CP.

Brown *et al.* (1992) reported a PER value of 1.2% at 50% CP for small (2.7-11.4 g) HSB, similar to the 1.3% for HSB in the present study for fish with average weight of 108 g, fed a feed with 50.2% CP.

Juvenile RBT (98 to 216 g) fed a commercial diet 48.4% CP had nitrogen retention of 37.3% (Vielma *et al.* 2002), substantially greater than the value of 12.0% for the much-larger RBT (643 to 651 g) in the present study. Such large differences are likely related to differences in size and growth rates of the fish between the two studies. Vielma *et al.* (2002) reported 29.7% P retention for a commercial feed with 1.36% total P compared with the 14.8% P retention we calculated for commercial diet

with 1.51% total P. Although the effects of age or size on digestibility of feed ingredients has not been well addressed in fish (Gallagher 1997), Ronsholdt (1995) noted that the phosphorus content of rainbow trout decreased with increasing weight. However, the association between fish size and nutrient retention is dependent on whether the comparison is for fish length or weight. The findings of Ketola (1991) show that the phosphorus requirement for bone mineralization (i.e., growth in length) is higher than that for body weight gain. The N and P retention values of LMB were comparable to values reported for Atlantic salmon, but substantially higher than that reported for RBT, hybrid tilapia or European sea bass (Table 8).

The range in water quality variables was always within a desirable range of values suitable for intensively cultured fish: DO 6.8, pH 7.2, alkalinity 61 mg/L, BOD 9.4 mg/L, TAN 0.65 mg/L, TP 24.5 mg/L, TDS 1,615 mg/L, and TSS 49.5 mg/L.

Although environmental temperature within the species' normal range for growth has little effect on digestibility (National Research Council 1993), Kibria *et al.* (1998) reported that environmental temperature closest to the optimal temperature produced significantly higher retentions of both P and N in silver perch (*Bidyanus bidyanus*). In the present study, water temperatures ranged from 15.8-24.1°C with a mean of 19.9°C. The four species cultured in our study represented cold-, cool- and warmwater categories, each with different optimal temperatures for growth. Largemouth bass, a warmwater species, have optimal growth at temperatures above 25°C (Stickney 1994). Walleye and HSB are considered coolwater species that have optimal growth between 15 and 25°C, which suggests that, all other things being equal, HSB and the smaller size group of WYE would exhibit the highest relative growth and nutrient retention because the water temperature stayed predominantly in the coolwater range. The smaller size group of WYE did show the highest P retention and second highest N retention of the 5 groups of fish studied. Although the temperature for LMB was less than their expected optimum, they nevertheless had faster growth, and displayed the second highest P retention and the highest N retention. The HSB had the lowest P retention and the median N retention of the fish cultured. In our study, growth (SGR) of RBT was significantly lower than that of the other species,

which seems related to both their slow growth rate and the negative effect of relatively high water temperature (24.1°C), although less than the 28°C threshold for mortality of rainbow trout (Hardy *et al.* 2000), it was near the 25°C upper limit for growth (Hardy *et al.* 2000), and far above the optimum for growth, which has been reported to be between 10 and 16°C (Stickney 2000), or between 16.5-17.2°C (Jobling 1994).

There was a strong correlation between food conversion ratio (FCR), which is the inverse of feed efficiency, and nitrogen retention. Thus, when fish are overfed, the feed lacks palatability, or it has poor digestibility for the species, then a higher proportion of N in the feed will be excreted. High FCR values will lead to high ammonia concentrations in the tank effluent. Thus, when designing a new recycle aquaculture system, estimates of TAN production (g/kg feed) should be given consideration in order to avoid ammonia toxicity and excessive levels of ammonia in the effluent. Although it is useful to use a generalized value for ammonia excretion per unit of feed fed (i.e., 0.25-0.35 kg ammonia kg<sup>-1</sup> of feed), the relationship varies with species (Table 8) and protein efficiency ratio (PER).

The equation by Tucker and Boyd (1985), cited by Lawson (1995), to estimate TAN production using values for NPU and protein content of the diet has TAN production rectilinearly related to protein content of the feed. A feed with a protein content of 42 to 49%, as used in the present study, has an estimated nitrogen content of 67 to 78 g/kg. For the NPU values calculated in our study, the estimated TAN production would range from 2.9 to 6.9% of daily feeding rate. Values of 2 to 3% were used by Westers (2001) to estimate TAN from feeding rates. Lawson (1995), Huguenin and Colt (1989), and Tucker and Robinson (1990) suggest TAN can be estimated by 3% of feeding rate.

Nutritional research on protein retention (i.e., N-retention) has demonstrated an interaction between fish age or size, growth rates, temperature, feeding rates, and protein concentration of the feed. Phosphorus retention is strongly affected by phosphorus content of the feed in excess of the fish's requirements, as well as the digestibility of plant products, because the phosphorus in phytin (in plant tissues) substantially reduces P retention and the fiber content is largely

indigestible. Unless a focus is placed on the size and species of fish as well as the characteristics of the feed, it will be difficult to prevent substantial error in estimates of nutrient content of effluents derived from nutrients in the feed.

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