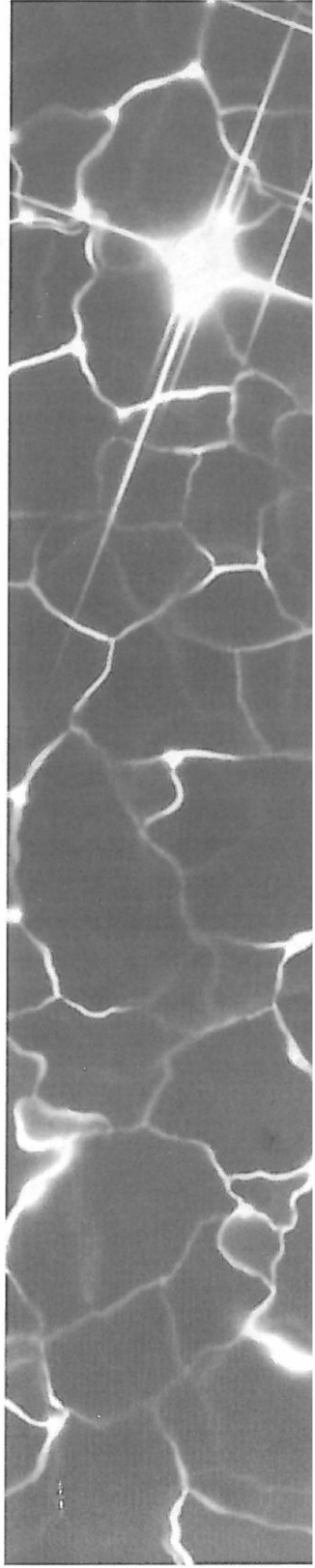
International Journal of Recirculating Aquaculture



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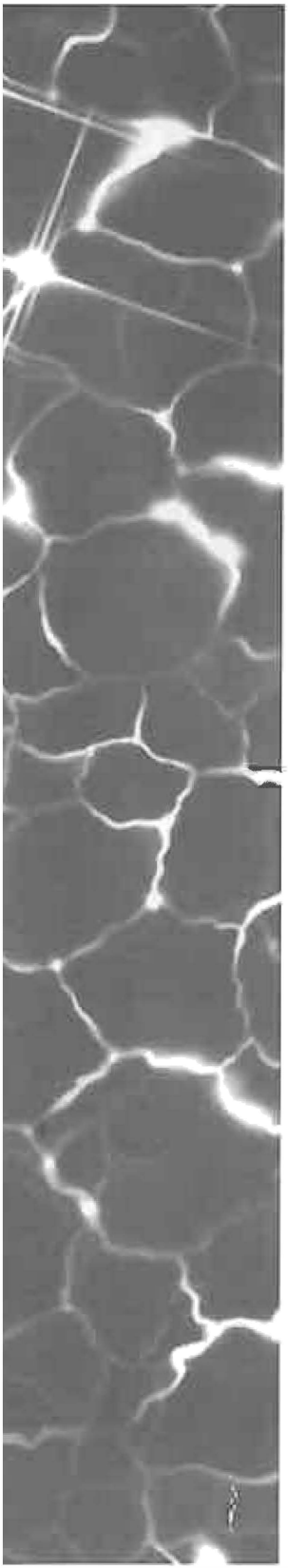

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Dear Readers:

Greetings! We are pleased to present the third volume of the International Journal of Recirculating Aquaculture (IJRA). This peer-reviewed publication continues on its mission to provide a forum for the open exchange of reliable information on all aspects of recirculation aquaculture, including:

- Water Conservation
- Systems Management
- Waste Management
- Species Selection & Evaluation
- Breeding & Genetics
- Larval Rearing
- Growth & Nutrition
- Fish Health/Aquatic Medicine
- Diagnostics & Therapeutics
- Food/Product Quality
- Food Safety
- Marketing & Economics

We continue to encourage the solicitation of manuscripts and reports on all topics concerning recirculation technology. Please keep in mind that we will consider submissions from both academic and non-academic sources (aquaculture farms, hatcheries, aquaria, etc.), understanding that sharing real-world experiences can easily be as valuable as the presentation of scientifically-derived work. Feel free to communicate with our production office concerning any comments or suggestions you may have concerning the journal.

As more and more freshwater and marine species are cultured in recirculating aquaculture systems, new technologies will need to be investigated and developed. This journal will continue to serve as a conduit to deliver ths information to the industry.

Sincerely,

Stephen A. Smith, Executive Editor

A Low Cost Bar Grader for the Harvest of Hybrid Striped Bass (*Morone chrysops* x *M. saxatilis*)

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ABSTRACT

Four bar graders were designed and built to separate a mixed-size population of market-size reciprocal cross hybrid striped bass *Morone chrysops* x *M. saxatilis* in rectangular culture tanks in an indoor, recirculating aquaculture system. Grader frames were constructed of 5.1 cm (2 inch) PVC pipe and fittings. PVC electrical conduit (1.27 cm, 0.5 inch) was used to form a series of parallel, equally spaced vertical bars within the frame. Bar slot spaces were 1.90, 2.54, 3.20, or 3.80 cm (0.75, 1.0, 1.25, 1.5 inches). A strip of flexible vinyl siding was attached to the outer edges of the bar grader to permit a tight fit between the grader and tank walls, and prevent fish from swimming around the grader. The graders were placed sequentially into one end of the tank, largest slot size first, and maneuvered to the opposite end. Each grader

remained in the tank for 60 min. Fish too large to pass through a grader were netted and measured for weight, length and width. Regression analysis was performed for average weight retained at each bar spacing ($Y = 7.13619 + 0.070716\ X$; $r^2 = .9987$). By rearrangement, an equation was derived which allows a culturist to select a bar spacing that retains fish of a predetermined weight :

Grader bar slot size (mm) = Fish weight (g) – 177.9 / 31.7)

Construction of each grader required approximately 2 h, and materials cost $20.

INTRODUCTION

Striped bass *Morone chrysops* and their hybrids are cultured under extensive or semi-intensive conditions in ponds, or at high densities in raceways, recirculating water systems, or cages (Van Olst and Carlberg 1990; Trosclair 1992). Fish grown at high densities, including hybrid striped bass, exhibit non-uniform growth (Nunley 1992; Bromage and Shepherd 1990). Variable growth within a year-class often leads to low feed conversion efficiency and survival (Huner et al. 1984, Kirby et al. 1987). Therefore, grading of small fish during growout is necessary to increase size uniformity and reduce cannibalism (Smith et al. 1985). However, grading can be labor intensive and injurious to fish, making it economically unattractive to culturists. As a result, a mixed-size population often exists at harvest. Commercial hybrid striped bass markets often demand fish of uniform size because of intended product use and customer desires. Therefore, fish culturists must selectively harvest from a mixed population only those individuals meeting size requirements of the buyer.

Panel graders, grader boxes, grading baskets, sorting or grading tables, mechanical pumps with graders, and live cars or socks are common tools designed to separate fish into size categories (Jensen 1990). The preferred sorting method is dictated by the size and species of fish and farm conditions (Huner et al. 1984). Fish may be graded from a variety of culture vessels, including ponds, raceways, tanks, or holding vats. Panel graders are the most common technique separating fish of various sizes in raceways and rectangular tanks. They are constructed as a

vertical or angled assembly of equally-spaced, parallel bars. Aluminum is the most common construction material (Jensen 1990). Fish are separated by girth according to their ability to pass through the spaces between bars (Huner et al. 1984). Bar space-fish size relationships for small, fingerling (83 to 163 mm; 3.3 to 6.4 inch) striped bass have been developed (Ludwig and Tackett 1991), but have not been determined for market-size (>260 mm; >10.2 inch) striped bass. This paper describes an inexpensive, easily constructed, bar grader used to separate live, market-size hybrid striped bass into specified size gradations using two laborers.

METHODS

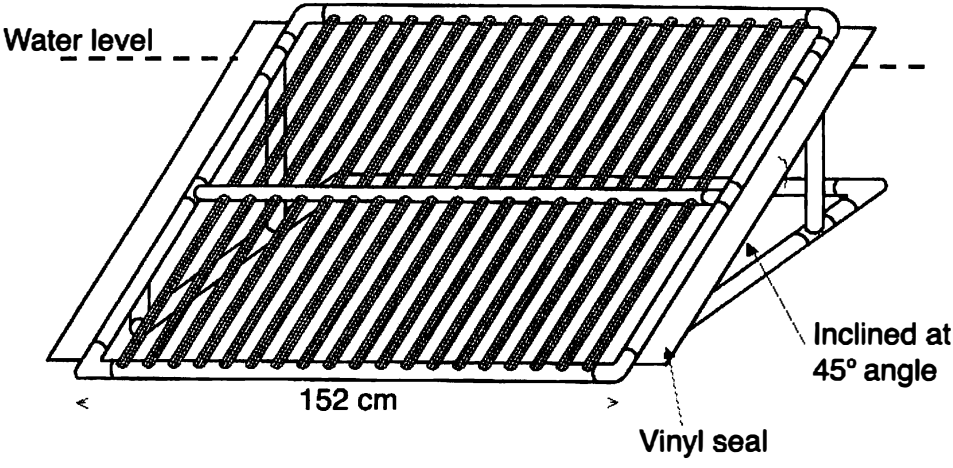
Four bar grader panel frames of 152 x 152 cm (60 x 60 inches) were constructed of 5.1 cm (2 inch) PVC pipe and fittings. Parallel, vertical bars were built of 1.27 cm, PVC conduit and spaced (internal slot distance) at 1.90, 2.54, 3.20, or 3.80 cm (0.75, 1.0, 1.25, 1.5 inch) intervals. Bars were attached to the grader frame by drilling 1.27 cm (0.5 inch) sockets into the top and bottom of the frame and inserting the partially flexible PVC, allowing 360 degree rotation of the bars. The grader frame and bars were inclined at a 45° angle and supported on a PVC base (Figure 1). Inclined bars facilitated fish passage and reduced entrapment by redirecting fish movement downward and through the bars. Holes were drilled in the bar grader base to allow water entry, sinking of the grader, and to prevent fish from swimming under the frame. Flexible 0.64 cm (0.25 inch), vinyl house siding was attached to the sides of the grader frame to provide a close fit against the tank walls. The production tank (1.53 m x 6.11m x 1.22 m; 4.9 x 19.7 x 3.9 feet) was part of a 12,390 L (3,261 gal) recirculating fish culture system at the Virginia Tech Aquaculture Center.

Hybrid striped bass (n=300), length (TL) 271.3 ± 27.7 mm (mean ± SE), weight 288.9 ± 98.7 g, and width 39.3 ± 6.3 mm, were size-sorted by sequentially placing the graders (largest to smallest slot size) into one end of the culture tank and slowly sliding each, one at a time, completely to the opposite end. Each of the 3 grading trials took a total of 240 min , or 60 min for each of the 4 slot sizes tested. Fish not passing through each sequential grade were removed by net, weighed (nearest 0.1 g), and measured for total length and maximum width (nearest mm) (Table 1).

Table 1. Total mean weight (g), +/- SE, length (mm), and width (mm) of hybrid striped bass retained in sequential grading of a mixed-size population (n=300)

Bar Space (cm)	Weight (g)	Width (mm)	Length (mm)
3.80 - 3.20	387.4 +/- 51.9	45.3 +/- 2.4	298.6 +/- 13.8
3.20 - 2.54	301.5 +/- 58.7	39.8 +/- 2.8	277.6 +/- 15.5
2.54 - 1.90	237.2 +/- 62.4	35.5 +/- 3.7	258.9 +/- 20.5

Figure 1. Low-cost bar grader



RESULTS and CONCLUSIONS

Regression analysis was performed (SAS Institute 1985) with mean fish weights against bar width. An equation describing this line ($Y = 177.9 + 31.7\ X$; $r^2 = 0.9968$) was developed and can be used to predict the appropriate slot-size for selection of fish of a certain size:

$$\text{Slot size (mm)} = \text{Fish Weight (g)} - 177.9 / 31.7$$

By substituting the desired fish weight into the equation, the fish culturist can determine the appropriate bar spacing to retain harvest-size reciprocal hybrid striped bass from a population of mixed size. The bar-grader harvesting system was inexpensive ($20 per grader) and constructed in approximately 2 h. The graders were highly mobile and maintenance is expected to be low. Two individuals were needed to push the grader through the rectangular tanks. Harvest-sized fish can be graded in an hour, and handling minimized to netting and removing big fish. This relatively low-cost grader system can be used to harvest and selectively sort (by weight or length) striped bass and other commonly cultured fish species once the initial relationships between grader bar spacing and fish sizes (weight or length) retained are validated.

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Comparisons of Tilapia Seed Production Under Various Broodstock Densities and Fry Stocking Densities.

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ABSTRACT

Three types of seed (eggs, sac-fry, and fry) production for Rocky Mountain White® hybrid tilapia, (*O. niloticus* x *O. aureus*), were compared under green water conditions over a six-month period in an environmentally-controlled greenhouse at the Virginia Tech Aquaculture Center. Rectangular tanks were stocked with broodstock (mean wt. 680 g), at a sex ratio of 3 females to 1 male. Nine tanks were stocked at one of three densities (1, 2, and 4 females/m^2), and seed was collected from the females' mouths weekly. Three additional tanks were stocked at a density of 2 females/m^2, and fry were collected from the edges of the tanks daily.

Average number of viable fry produced by the clutch removal method at 1 female/m^2 was significantly higher than the combined average production of densities at 2 and 4 females/m^2 ($p< 0.02$). Even though there was no significant difference between viable fry production per meter sq. ($p>0.05$), the highest density consistently produced more fry/m^2. No significant difference was observed in viable fry production

between the clutch removal method and the natural mouth-brooding method ($p>0.05$). The mean monthly hatchery seed survival was 65.7 ± 2.3%, which varied largely depending on initial seed developmental stage.

The effects of stocking density on growth and survival were evaluated by stocking 14-16 day old artificially incubated fry (25.5 ± 0.32 mg, 12.1 ± 0.04 mm), into 150-liter troughs at three densities (3, 6, and 12 fry/liter) under green water conditions for 30 days.

Significant differences were observed between mean weight, length, survival, and feed conversion ratios among the various fry stocking densities ($p<0.05$). The greatest growth was at a density of 3 fry/liter, while survival was not affected until a density of 12 fry/liter was reached.

These results suggest that to maximize fry production and reduce labor, a density of 4 females/m^2 or higher be used under the natural mouth-brooding seed collection method. They also suggest a fry stocking density between 6 to 12 fry/liter should be used with periodic grading or sex reversal to reduce cannibalism and increase growth.

INTRODUCTION

In the past thirty years, tilapia (Family *Cichlidae*) have become one of the most important groups of fish to the worldwide aquaculture industry (Lovell 1980). Tilapia often exhibit rapid growth rates, are easily reproduced, have a desirable white, flaky flesh, and express high tolerance to many of the environmental stresses associated with recirculating aquaculture practices. Tilapia are generally considered extremely prolific; however, being mouth brooders, the number of eggs per clutch tends to be relatively low. This complicates the collection of large numbers of "seed" (eggs or sac-fry) of the same developmental stage for production purposes.

Two different seed collecting methods are commonly used. The first method entails collecting the fry after the brooding females have released them. The second involves periodically removing the seed clutches from the brooding females' mouths. The removal of seed from a mouth-

brooding female can reduce the inter-spawning interval by half (Rana 1988). By freeing the female of mouth-brooding and fry protection, she can resume feeding and condition herself more rapidly for the next spawning cycle. Berrios-Hernandez and Snow (1983) felt that the egg removal method was not worth the additional time investment, since there was no significant difference between seed numbers in the two collection methods. However, Watanabe et al. (1992) concluded that the clutch removal method, even though more labor intensive, yielded higher numbers of viable fry. They attributed the lower fry numbers in the natural mouth-brooding to cannibalism.

Tilapia markets are developing outside of traditional equatorial regions. Indoor recirculating aquaculture systems (RAS) have made it possible to expand tilapia production to the temperate zones. Further information is needed for year-round intensive fry production to efficiently supply tilapia producers using RAS.

The objectives of this study were to compare fry production among various broodstock densities under green water conditions, to compare the clutch-removal method and natural mouth-brooding fry collection methods in terms of fry production and to compare the growth and survival of swim-up fry at various stocking densities for thirty days under green water conditions. Stickney (1994) defines green water conditions as "the maintenance of sufficient concentrations of phytoplanktonic algae in the culture tanks of the aquaculture target species to provide a green color".

MATERIALS AND METHODS

The study was conducted in an environmentally-controlled greenhouse at the Virginia Tech Aquaculture Center in Blacksburg, Virginia (USA). The tilapia used for the study were the "Rocky Mt. White" hybrid (*O. niloticus* x *O. aureus*).

Environmental Maintenance

Eighteen 200-Watt incandescent bulbs were used to establish a 12:12 light-dark photoperiod (Rothbard and Pruginin 1975). The lamps were controlled with dimmer switches to produce a fifteen-minute artificial

sunrise and sunset. Lamp use was discontinued when the natural photoperiod exceeded a 12:12 photoperiod. Three 300-Watt heaters were used in each tank to maintain spawning temperatures between 25 to 30° C (Rothbard and Pruginin 1975). Water quality parameters were measured daily (Temperature, DO, pH), every other day (TAN, Algae-Absorbance), or weekly (Nitrite, Nitrate, Alkalinity, Hardness) by standardized Hach procedures. Algae concentrations were measured with a Hach 1800 spectrophotometer (Hach Company, Loveland, CO, USA) set at 750nm (absorbance), to establish relative means for comparison (Standard Methods, 1992). Water quality was managed to maintain parameters according to the following criteria: dissolved oxygen concentration- > 5.0 ppm, ammonia (NH_3)- < 0.2 mg/l (TLC) (Daud et al. 1988), nitrite- <0.5 ppm, nitrate- < 500 ppm, alkalinity- between 20 to 300 ppm , hardness- > 50 ppm (Buttner et al. 1993).

A 1.0 Hp. regenerative blower supplied aeration using airstones. The broodstock tanks were managed as a green water algae system. Each tank was siphoned every two weeks with a one-third water exchange. Phytoplankton blooms were managed by water flushes to flatten out population cycling. All broodfish were fed a 40% protein floating pellet (Zeigler Bros. Inc., U.S.) to satiation three to four times daily.

Broodfish Density

Four treatments were established by arbitrarily stocking a total of 108 broodfish, (male- mean wt. 853.9 ± 40.3 g , mean total length 33.9 ± .70 cm; female- mean wt. 508.8 ± 18.2 g , mean total length 29.3 ± .29 cm), into twelve polyvinyl-lined broodtanks (2.4 X 1.2 x 0.6 m). The fish were individually passive integrated transponder (PIT) tagged, weighed, and stocked at a sex ratio of one male per three females (Berrios-Hernandez and Snow 1983; Snow et al. 1983; Santiago et al. 1985; Subasinghe and Summerville 1992; Watanabe et al. 1992). Spawning activity was observed within ten days of stocking. On February 1, all females' mouths were emptied of seed to initiate the study. Seed production was compared by establishing three replicates per density (1.0 female/m^2, 2.0 females/m^2, and 4.0 females/m^2), using nine tanks.

Seed Collection Methods

Nine tanks were managed under the clutch removal method, and sampled every seven days (Verdegem and McGinty 1987). At sampling, a fine meshed bottom net was lowered at one end of the tank. After being crowded over the bottom net, each fish was individually dip netted, identified, and the female's mouths were checked for seed. If no seed was present, the females were checked for internal eggs by gentle abdominal pressure, while the males were checked for milt by manual stripping. A bottom net was used to collect uncontrolled seed expulsion by brooding females. The collected seed was segregated by developmental stages: eggs, or sac-fry. All broodfish in these tanks were weighed monthly when sampling for seed.

The three remaining tanks were stocked with broodfish at a density of 2.0 females/m^2. Fry were collected after the females completed the natural mouth-brooding process. Each tank possessed sloping wings on each end to provide a location for the fry to congregate (Figure 1). A net barrier was placed at the beginning of the slope to establish the same surface area for breeding as the nine clutch removal tanks. A 38 mm mesh size net allowed for the passage of fry but prevented broodfish from entering the wing area. The wing area of these tanks was inspected daily for swim-up fry, which if present, were collected and counted. Broodfish in these tanks were weighed at the beginning and end of the experiment.

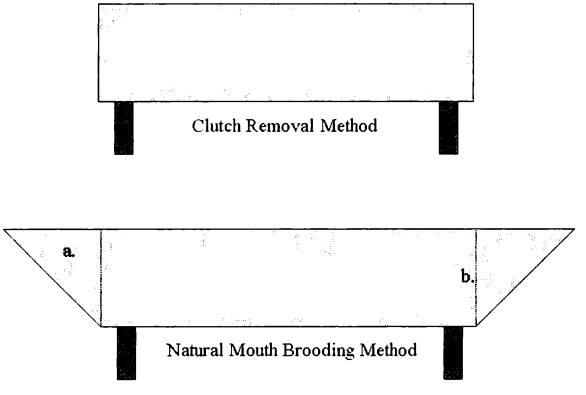

Figure 1. Side view of broodfish tanks- breeding area (2.4 x 1.2 x 0.6 m). a.- Sloped area for fry to congregate. b.- 38 mm mesh net barrier.

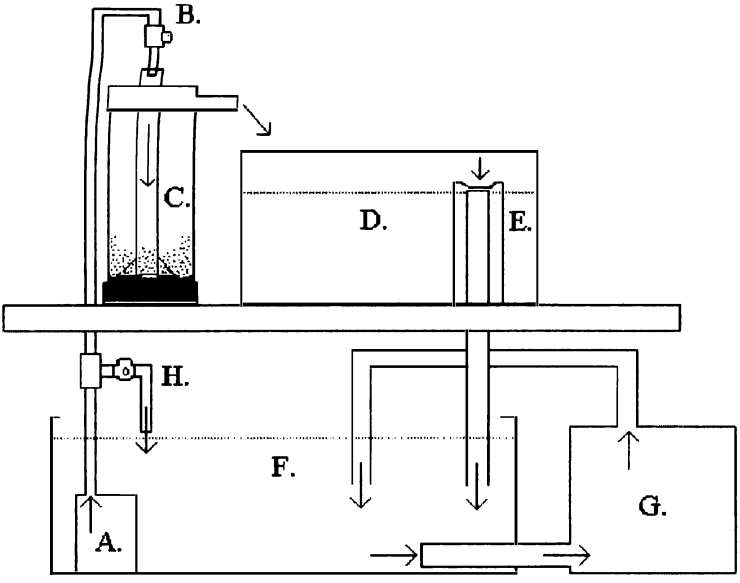

Figure 2. Side view of hatchery system used to incubate tilapia eggs. Arrows indicate the direction of water flow. A.- Pump. B.- 1.27 cm ball valve. C.- Seven liter round-bottom hatching jar. D.- Swim-up fry catch basin. E.- Stand pipe and fry mesh screen. F.- Water reservoir. G.- Activated carbon filter, a corrugated tube filter, and ultraviolet irradiation. H.- Over-flow valve.

Hatchery

The hatchery consisted of nine 7-liter round-bottomed up-welling hatchery jars (Rana 1986) (Figure 2). The water was filtered and sterilized by an aquarium filtration unit at 47 liter/min. The unit contained an activated carbon filter, a corrugated tube filter (16 micron), and a 25-Watt ultraviolet irradiation bulb (Subasinghe and Summerville 1985; Don et al. 1987). The hatchery was cleaned once a week along with a 75% water exchange. The sterilization unit was cleaned every two weeks. Make-up (well) water for the hatchery was stored and maintained between 25-30° C. with immersion heaters (Subasinghe and Summerville 1992). The hatchery unit was managed as a clear water system. The collected eggs from each tank were segregated by developmental stage. The eggs were counted using grids. After counting, the eggs were placed in hatching jars (400-8,000 eggs/jar). An arbitrary composite sample of sixty eggs was taken from all the eggs and measured and weighed. During incubation, dead eggs were removed to deter fungal growth. Upon swim-up, the fry were counted to estimate hatching success.

Fry Rearing

A randomized block design consisting of four trials was used. Each trial consisted of nine 300-liter fry troughs, each divided into two 150-liter sections. Three replicates were conducted within each trial. The fry were stocked at densities of 3, 6, and 12 fry/liter, with each density being arbitrarily stocked twice per replicate (Watanabe et al. 1992; Dambo and Rana 1992). Dechlorinated make-up (city) water was stored and heated to 25-30°C with 200-Watt immersion heaters. Each fry trough was siphoned twice daily with a water exchange of two-thirds the total volume. The fry troughs were managed as a green water algae system. The same water quality parameter regime was used as the breeding trials above. A 1.0 Hp. regenerative blower supplied aeration using airstones.

Swim-up fry (6-8 days post-swim-up) were pooled from the hatchery, and measured for average length (mm) and weight (mg). The fry were fed a 55% protein salmon starter (Zeigler Bros. Inc., Gardners, PA, USA) to satiation four to five times a day, and as size increased, a 44% protein semi-floating diet (Rangen Inc., Buhl, ID, USA) was used. Each trial lasted for thirty days. At the end of the thirty days, an arbitrary sample of sixty fish was taken from each 150-liter density section per replicate and measured and weighed. Total counts were estimated by weight to determine survival by treatment.

Statistics

Analysis of variance (split plot design) was used to statistically analyze for differences between the mean numbers of eggs produced per female/month and per meter sq./month at the various broodstock densities over time. Orthogonal contrasts were conducted to establish which specific broodstock densities differed in terms of egg production per female/month and per meter^2/month.

Analysis of variance was used to analyze the difference between the mean seed numbers produced per female/month and per meter^2/month by the two seed collection methods: clutch removal at seven day intervals, and natural mouth brooding with daily fry collection.

Analysis of variance (randomized block design) was used to statistically analyze for differences between the means of fry length and weight among the three fry stocking densities. Tukey's multiple

comparison analysis was conducted to establish which specific fry stocking densities differed in terms of their effects on growth by testing the significance of variation among mean length and weight.

RESULTS

Density comparisons, clutch removal seed production

Four water quality parameters (D.O., pH, Absorbance, and Hardness) changed significantly through time for the three broodfish densities (1.0 female/m^2, 2.0 females/m^2, 4.0 females/m^2) over the 180-day study period ($p < 0.05$). However, there were no significant differences between treatments over time for all water quality parameters ($p > 0.05$) (Table 1).

No significant difference was observed in cell abundance between the dominant populations of planktonic green algae, *Scenedesmus* and *Eudorina* (identified by Dr. Bruce Parker of Virginia Tech), or between treatments throughout the entire sampling period ($p > 0.05$).

Seed production continued throughout the 180-day study period among all treatments. Monthly fry production per female and per meter sq. was compared among density treatments. There were no significant

Figure 3. Mean fry production per female by treatment for the six-month study period. A significant difference was observed by contrasting a combination of treatments 2 and 4 females/m^2 against treatment 1 female/ m^2 ($p < 0.02$).

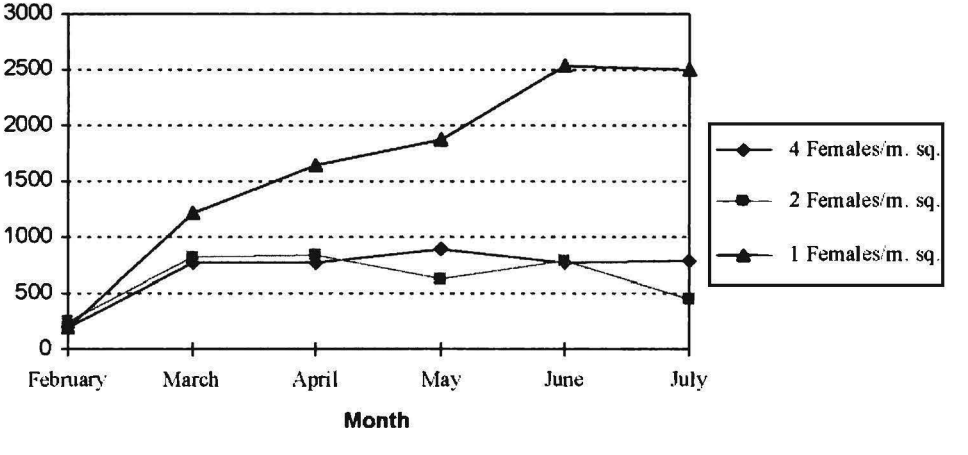

Table 1. Broodstock tank water quality parameter means, ± S.E., and ranges by density treatment. (D.O., Temp. (°C), pH, TAN, NH_3, NO_2, NO_3, Alkalinity, Hardness (mg/l), Absorbance (at 750 nm). No significant difference between treatments was observed (p>0.05).

	1 Female/m²			2 Female/m²			4 Female/m²		
	Mean	**S.E.**	**Range**	**Mean**	**S.E.**	**Range**	**Mean**	**S.E.**	**Range**
Dissolved Oxygen	5.7	0.1	1.9	5.4	0.1	1.4	5	0.1	1.8
Temperature	26.2	0.1	1.8	26.2	0.2	2.5	25.9	0.2	2.0
pH	7.8	0.05	0.6	7.7	0.05	0.6	7.5	0.07	1.0
TAN	0.371	0.032	0.634	0.424	0.04	0.789	0.478	0.021	0.321
Un-ionized (NH_3)	0.0137	0.0026	0.0143	0.0178	0.0049	0.0305	0.0134	0.0049	0.0299
Nitrite (NO_2)	0.044	0.008	0.107	0.051	0.008	0.107	0.082	0.007	0.121
Nitrate (NO_3)	6.07	0.55	11.45	5.69	0.47	6.78	10.86	0.93	13.05
Alkalinity	122.0	8.0	107.0	103.0	6.0	87.0	77.0	7.0	94.0
Hardness	129.0	7.0	90.0	125.0	5.0	69.0	133.0	4.0	55.0
Absorbance (algae)	0.259	0.021	0.289	0.259	0.017	0.261	0.28	0.021	0.309

Table 2. Fry production per female and per meter sq., expressed as monthly means and ± S.E. by density treatment (Expressed as number of fry after swim-up from hatchery). No statistically significant differences were observed over time in fry production per female or per meter sq. for all treatments ($p>0.05$). A statistical significant difference was observed in fry production per female between treatments with a time interaction ($p<0.05$). Fry production per meter sq. was not statistically significant between treatments ($p>0.05$).

	One Female/m²				Two Females/m²				Four Females/m²			
	Fry/female		Fry/m²		Fry/female		Fry/m²		Fry/female		Fry/m²	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
February	309	309	309	309	362	83	723	166	374	56	1497	225
March	1095	285	1095	285	724	83	1448	165	594	193	2376	772
April	1650	631	1650	631	836	79	1670	157	767	73	3072	292
May	1872	740	1872	740	626	301	1250	601	891	176	3560	706
June	2537	399	2537	399	788	223	1575	444	763	108	3050	432
July	2497	355	2497	355	439	167	877	334	781	113	3122	452

differences in fry production per female or per meter sq. for all treatments through time ($p>0.05$) (Table 2). However, there were significant differences in fry production per female between treatments with a time interaction ($p<0.05$). Orthogonal contrasts were conducted between treatment densities. There was a significant difference when treatments 2 and 4 females/m^2 were combined and contrasted against treatment 1 female/m^2. ($p<0.02$) (Figure 3). Differences in fry production per meter sq. were not significant between treatments ($p>0.05$).

Mean condition factors were calculated monthly by tank and compared (condition factor = weight/length^3) (Piper et al., 1982). A significant difference was observed through time for all treatments ($p<0.05$). However, no significant differences were observed between treatments or between treatments with a time interaction ($p>0.05$).

Weekly seed production status was determined by treatment. Observations were categorized by the presence of seed, and if present, developmental stage. The females within the density of 1 female/m^2 collectively produced seed 35% of the time. At the two higher densities of 2 females/m^2 and 4 females/m^2, seed was produced 22 and 25% of the time, respectively. Females of all treatments without seed possessed

Table 3. Fry production per female and per meter sq., expressed as monthly means and ± S.E. by egg collection method (Expressed as numbers of fry after swim-up from hatchery for clutch removal method). No statistically significant difference was observed in fry production per female or per meter sq. between the two seed collection methods ($p>0.05$).

	Clutch Removal Method				**Natural Mouth-Brooding Method**			
	Two Females/m^2				**Two Females/m^2**			
	Fry/female		**Fry/m^2**		**Fry/female**		**Fry/m^2**	
	Mean	**S.E.**	**Mean**	**S.E.**	**Mean**	**S.E.**	**Mean**	**S.E.**
February	362	83	723	166	221	133	443	267
March	724	83	1448	165	433	297	865	594
April	836	79	1670	157	732	282	1463	563
May	626	301	1250	601	654	250	1306	499
June	788	223	1575	444	579	345	1156	690
July	439	167	877	334	502	156	1004	313

internal eggs about 60% of the time. While milt was rarely visible, it was seen 5% of the time in the two higher densities. The males in the lowest density never expressed visible milt.

Clutch Removal vs. Natural Mouth Brooding

No significant differences were observed in water quality parameters between the two seed collection treatments ($p>0.05$) except for the parameter alkalinity (clutch removal method- mean 102, ± 3.2 mg/l; and natural mouth brooding method- mean 115, ± 3.6 mg/l) ($p<0.05$). Significant differences were not observed in fry production per female or per meter sq. between the two collection methods (clutch removal method- monthly mean of 629 fry/female, 1257 fry/m^2; and natural mouth brooding method- monthly mean of 520 fry/female, 1039 fry/m^2) ($p>0.05$) (Table 3).

Hatchery

The hatchery was maintained and operated throughout the six-month sampling period. Mean water quality parameters were: temperature (26.8 ± 1.89°C), dissolved oxygen (5.9 ± 0.83 mg/l), pH (8.5 ± 0.23), TAN (0.342 ± 0.312 mg/l), alkalinity (311 ± 52.6 mg/l), and hardness (488 ± 89.7 mg/l).

Mean monthly survival for unpigmented eggs (< two days old) was 37.2 ± 3.4%, 70.2 ± 2.6% for pigmented eggs (>two days old), and 89.7 ± 1.2% for sac-fry (non-swimming) to yolk-sac absorption. The monthly mean hatchery survival for all three seed stages combined to yolk-sac absorption was 65.7 ± 2.3%. The weekly mean egg weight was 6.22 ±

Table 4. Fry weights and lengths, expressed as means, and ± S.E. by stocking density (30 day trial). Means within the same column followed by different letters are significantly different ($p<0.05$).

Weight (grams)	Mean	S.E.	Length (mm)	Mean	S.E.
3 fry/liter	1.85	0.048 a	**3 fry/liter**	42.90	0.35 a
6 fry/liter	1.62	0.043 b	**6 fry/liter**	41.15	0.329 b
12 fry/liter	1.42	0.039 c	**12 fry/liter**	39.26	0.309 c

0.030 mg, while the mean major axis was 2.59 ± 0.006 mm.

Fingerling density, growth, and survival

No significant differences were observed in all water quality parameters between stocking densities 3 fry/liter, 6 fry/liter, and 12 fry/liter ($p>0.05$). Significant differences were observed between mean length and weight among treatments, with a density of 3 fry/liter showing the highest growth. ($p<0.05$) (Table 4).

A significant difference was observed in survival rates between fry densities, but was not seen until a density of 12 fry/liter was reached ($p<0.05$) (Table 5). A significant difference was also observed in feed conversion between fry densities. However, feed conversion was not significantly reduced until a density of 12 fry/liter was reached ($p<0.05$) (Table 5).

DISCUSSION

Density comparisons, clutch removal seed production

The numbers of viable fry obtained per female and per meter sq. in this study are among the highest reported for intensive tilapia production (Berrios-Hernandez and Snow 1983; Guerrero and Guerrero 1985; Lovshin and Ibrim 1987; Bautista et al. 1988; Smith et al. 1991; and Watanabe et al. 1992). One reason for the higher production could be the weekly collection of seed. The 7 day interval was used in the present study based on prior observations by Verdegem and McGinty

Table 5. Fry survival and feed conversion ratio means and ± S.E. by density treatment (30 day trial). Means within the same row followed by different letters are significantly different ($p<0.05$). (Feed conversion ratio = total feed eaten for thirty days / total final weight).

	Survival			Feed Conversion Ratio		
	Mean	**S.E.**		**Mean**	**S.E.**	
3 fry/liter	54.4%	5.0%	a	1.15	0.079	a
6 fry/liter	43.7%	4.9%	ab	1.11	0.097	a
12 fry/liter	37.5%	4.0%	b	0.9	0.036	b

(1987), who observed that this period produced more abundant seed of a more uniform and suitable age for artificial incubation when compared with 2, 4, and 10 day intervals.

Average broodfish size used in the present study was larger than other tilapia breeding studies reviewed (Berrios-Hernandez and Snow 1983; Guerrero and Guerrero 1985; Santiago et al. 1985; Lovshin and Ibrim 1987; Smith et al. 1991; and Watanabe et al. 1992). According to Siraj et al. (1983), seed-hatching rates increased as the size of *T. nilotica* broodstock increased from a mean weight of 49 to 294 grams. The large broodfish used in the present study may have produced more viable seed, which increased survival through the artificial hatching process.

The present study's algae populations may also have aided seed production by supplying the broodstock with essential proteins and vitamins. Abdelghany et al. (1993), observed plant material to compose 63% of wild *O. niloticus*'s diet, with phytoplankton composing up to 36%. The presence of algae in culture tanks has been noted to enhance growth and survival of a number of species (Stickney 1994).

The numbers of viable fry observed per female for the density of 1 female per meter sq. were two to three times higher than seen in comparable stocking rates in previous studies (Berrios-Hernandez and Snow 1983; Snow et al. 1983; Santiago et al. 1985; Subasinghe and Summerville 1992; Watanabe et al. 1992). This may be due to the use of small units, with only one male per tank. The high production rate may have resulted from the male consistently pressuring the females to breed, without having to contend with competing males. Rana (1988), noted that in some circumstances, when using the clutch removal method under crowded conditions, the inter-spawning interval was not reduced, and was similar in length to a normal female's breeding cycle. In the present study, the clutch removal method may have been effective in increasing production at the lowest density while being negated at the higher densities. The low density may also have enabled each female to establish a "safe territory" in which to reproduce consistently. To maximize seed production per female, a density of 1 females/m^2 should be used. This low density may be appropriate for small producers, selective breeding programs, or research. Regarding fry production per female, the upper limiting density did not appear to be reached in the present study.

Although fry production per meter sq. did not differ statistically between treatments, there was consistently higher production at the density of 4 females/m^2 (Table 2). Hence, to maximize seed production per meter sq., a density of at least 4 females/m^2 should be used. However, since production per female did not decline at 4 females/m^2, higher densities may be possible without sacrificing production per female.

There was a significant difference in broodstock condition factor over the 6-month breeding period, but not between treatments. Broodstock in the highest fry producing treatment of 1 females/m^2 had the lowest condition factors. This would indicate that a resting period for breeding stock may be warranted after a period of consistent production. Lovshin and Ibrim (1987) found a 16% increase in egg and fry production over a 105-day period by exchanging *O. niloticus* males and females every 21 days. Guerrero and Guerrero (1985) suggested that the breeding process be terminated once a peak production period of 17 to 20 days after stocking had been reached, and that these breeders be replaced. However, it appears from the present study that "Rocky Mt. White" hybrid (*O. niloticus* x *O. aureus*) broodstock can produce consistently for up to six months if properly fed and kept in suitable breeding conditions.

The fish in the present study produced consistently from February to July, with only a slight decline in July. Head and Watanabe (1995) noted that Florida red hybrid tilapia, produced greater numbers of seed from February to July as opposed to August to January. However, utilizing a 6-month broodstock rotation cycle, photoperiod manipulation, and consistent environmental conditions associated with indoor facilities, seasonal fluctuations in seed production may be reduced for consistent year round production.

When seed was present, the lowest density did show higher percentages of sac-fry. This indicates that females in the lowest density were completing the spawning process more quickly than females in the other two densities. Again, this may have been due to the single male being able to consistently pressure each female to breed, or that the advantage of decreasing the inter-spawning interval with the clutch removal method was more prevalent at the lowest density. Smaller females have been noted to complete the spawning and fertilization process more quickly than larger females (Siraj et al. 1983). It is

doubtful that this occurred in the present study, as no significant difference was found between average female weight between treatments ($p > 0.05$).

Clutch Removal-vs-Natural Mouth Brooding Method

Although there was no statistically significant difference between the two egg collecting methods, the clutch removal method did produce, on average, 109 more fry per female/month than the natural mouth-brooding method. In terms of labor associated with the two methods, it appears that the additional fry production associated with the clutch removal method would not be worth the additional labor necessary. These results agree with those of Berrios-Hernandez and Snow (1983), who asserted that the clutch removal method was not worth the additional effort when breeding *T. aurea* in fertilized swimming pools. Study results conflict with those of Watanabe et al. (1992), who found that Florida red tilapia produced significantly higher numbers of seed with the clutch removal method as opposed to the natural mouth-brooding method. Their use of an extended 8-16 day fry collection period may have facilitated cannibalism, opposed to the daily collection practiced in the present study.

Another explanation for the equal production of seed between the two methods could involve the sloping end design of the natural mouth-brooding method tank. The net barrier intended to establish equal breeding areas between the two treatments also established a shallow protected area that broodstock could not access, thus reducing the possibility of cannibalism. Finally, algae populations were encouraged to grow to supply food and reduce visibility, both of which would support fry survival.

By decreasing the inter-spawning interval using the clutch removal method, production can be increased from an average of one spawn per month to two spawns per month (Rana 1988). However, by increasing the number of spawns per female, the number of seed per clutch or condition of the seed may be reduced. This was not examined in the present study, but may be another reason for the similar production rates between the two seed collection methods.

The clutch removal method did synchronize the broodstock on a breeding cycle, thus providing seed of a more uniform developmental

stage. The natural mouth-brooding method produced seed sporadically and of varying developmental stages, which in turn require grading. Production between females was quite variable within all the clutch removal treatments. The clutch removal method does enable the producer to distinguish which females are breeding, and which females should be replaced. Also, by tagging broodfish, the culturist can confidently select for desired traits. The practicality and value of tagging broodstock for selective breeding on a commercial scale would have to be determined by each producer.

Breeding Observations

Breeding activity was noted to increase during the final hours of light. Activity was only observed at the surface of the water, since the green water obstructed total observation. Females were noted to occupy the corners and sides of the tank's surface water. Males were seen to aggressively nudge and push females down in the water column. Aggressive activity between males was observed by the splashing of water, and periodic thumping on the sides of the brood tanks.

Several of the males were noted to have sores upon their lower jaws. These sores may have been due to fighting between males, or caused by the males attempting to build nests upon the polyvinyl liner.

Hatchery

Seed survival to the yolk-sac absorption stage within the hatchery was related to the developmental stage of the seed when first collected. The lowest survival rate (37.2%) was seen with unpigmented eggs (< two days old). Similar results were stated by Rothbard and Hulata (1980), who also observed reduced survivorship and some times total loss of unpigmented egg batches. The present study's results contradict the findings of Rana (1986), who did not report reduced survivorship with unpigmented eggs. He attributed this to the use of ultraviolet irradiation, which was also used in the present study. In the present study, seed densities ranged from 57/liter to 1143/liter, and no differences were noted between density and survival.

Factors determining seed survivorship are mechanical stress and bacteria /fungal infection. Rana (1988), observed a 17% increase in hatchability by using round bottomed hatching jars instead of conical

jars. This increase was attributed to a reduction in mechanical stress. Egg blebs and ruptures in the chorionic layer due to mechanical stress were observed on occasion in the present study even though round bottom hatching jars were used. It was also noted that survivorship in the present study increased when bacterial or fungal infected eggs were removed as soon as possible. Thus, to increase hatchery survival, a combination of U.V. sterilization and prudent dead egg removal would be good management practices.

Another method of increasing survival would require reducing the number of unpigmented eggs used. This could be done by using smaller broodfish that complete the spawning process more quickly (Siraj et al. 1983), or by extending the time between seed collection. However, extending the time between seed collection would reduce the total number of spawns over a given amount of time.

Fry density, growth, and survival

The fry density growth trials were conducted using only artificially incubated seed. Results from the present study conditions show (Tables 4, 5) that to maximize growth, a density of 3 fry/liter should be used. Survival was not significantly affected until a density of 12 fry/liter was reached. However, the feed conversion ratio (F.C.R.) was inversely related to stocking density. This may have been attributed to the increase in fry abundance and cannibalism, leading to a decrease in F.C.R.

It was likely that the low survival in the present study was predominantly due to cannibalism, and that higher survival could be reached by grading the fry after two weeks from initial hatchery swim-up, and thereafter as needed. Two weeks after initial swim-up seemed to be the crucial time when the fry started to differentiate in size. Furthermore, sex reversal of swim-up fry would be a productive method to decrease size variability, and to reduce cannibalism and increase growth (Varadaraj et al. 1994).

Water quality parameters in the present study did include daily pulses in total ammonia nitrogen levels (max. 10.9, min. 0.14 mg/l), unionized ammonia (max. 0.659, min. 0.00059 mg/l), and pH (low 7.7 to high 9.5). Water exchange was limited to only two-thirds of total volume per day. Fluctuating water quality conditions may have also played a role in the reduced survival seen with the fry density trials.

SUMMARY

The present study indicates that indoor commercial scale tilapia seed production can be successfully conducted in the temperate zones. This study showed that "Rocky Mt. White" hybrid, *Oreochromis* broodstock will consistently produce seed for six months when provided consistent optimal spawning conditions. To maximize fry production and reduce labor, a density of 4 females/m^2 or higher should be used with the natural mouth-brooding seed collection method. A fry stocking density of 6 fry/liter should be used along with regular grading or sex reversal to reduce cannibalism and stimulate growth. The present study has also provided information that can be used as a foundation for designing intensive commercial tilapia hatcheries.

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Water Quality Impacts of Three Biofilter Designs in Recirculating Aquaculture Systems

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ABSTRACT

Nine recirculating aquaculture systems utilizing three biofilter types were placed on line and stocked with yellow perch, *Perca flavescens*, fingerlings. Biofilter type differed among systems, and included upflow pulsed bed bead filter, packed tower trickling filter, and rotating biological contactor. Following filter acclimation, a comparative analysis of biofilter performance was conducted, involving measurement of temperature, pH, dissolved oxygen, total ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, alkalinity, total hardness, carbonaceous biochemical oxygen demand, dissolved organic carbon, and total suspended solids. Filter bed emergence promoted effective carbon dioxide stripping, pH maintenance, and consistent nitrification performance in trickling filters and rotating biological contactors. Higher total ammonia nitrogen mass removal rates were observed in trickling and rotating biological contactor filters than in bead filters. Low total ammonia nitrogen mass removal rates and nitrification efficiencies for all filters resulted from relatively high carbonaceous biological oxygen demand loadings. Analysis of areas under mass removal curves showed that RBC filters were surface area limited. Foam formation in trickling filters effectively removed total suspended solids from the culture water. Filter type did not have a significant effect on median

organic water quality parameter values in the production tanks. Although differences in nitrification performance and certain water quality parameters were observed between filter types, the data set did not indicate that one filter type should be considered generally most effective at treating wastewater produced in a recirculating aquaculture system.

INTRODUCTION

Effective biofiltration is a key part of recirculating aquaculture systems (Libey and Miller 1985; Wheaton et al. 1991). Biofilters maintain chemoautrophic bacteria, including nitrifiers which biochemically oxidize total ammonia (NH_4^+-N and NH_3-N) to nitrate, thereby allowing recirculation of culture water. Although nitrification occurs throughout the culture system (Rogers and Klemetson 1985; Losordo 1991), high levels of sustained nitrification cannot be attained without use of a biofilter. Organic degradation within the culture environment can significantly deteriorate system water quality and increase biofilter clogging (Lucchetti and Gray 1988). The majority of organic wastes stem from uneaten feed, sloughed biofilm, and fecal matter (Libey 1993; Piedrahita et al. 1996).

Biofilters used in production aquaculture include submerged bead reactors, fluidized sand reactors, trickling filters, rotating biological contactors, and rotating drums (Miller and Libey 1985; Rogers and Klemetson 1985; Malone et al. 1993; Honeyfield and Watten 1996; Summerfelt 1996; Westerman et al. 1996). This raises the question of which configuration expresses the greatest number of positive attributes regarding treatment effectiveness, filter operational characteristics and filter management needs under waste loading conditions characteristic of production aquaculture. This study evaluated three types of biofilters used for production of yellow perch (*Perca flavescens*) in recirculating aquaculture systems. The biofilter designs evaluated were upflow pulsed bed bead filter, packed tower trickling filter, and rotating biological contactor (RBC).

Specific objectives of this study were:

1. To evaluate acclimation times of the respective filter types,

2. To evaluate system water quality as a function of filter type,

3. To relate treatment efficiencies for each filter type (as a function of filter waste loading rates in g/m^2/d), and

4. To evaluate filter performance as a function of filter design and operational characteristics.

MATERIALS AND METHODS

Culture Methods

Stocking and System Characterization — Nine recirculating systems at the Virginia Tech Aquaculture Center were placed on line and stocked with yellow perch at a density of approximately 455 fish m^{-3} (Schmitz, 1999). Fingerlings measured approximately 9 cm total length, with a mean weight 5.0 g.

Each system consisted of an 8,330 L rectangular culture tank (6.1m x 1.5m x 1.2m), micro-screen drum filter (Aqua-Manna, Ladoga, IN, USA), biofilter, U-tube with pure oxygen injection, and three 0.75 kW pumps (Figure 1). The drum filter employed a 120-micron mesh screen and a vacuum device for solid waste removal, and was the site for new water additions to the system. Biofilter type (Figure 1 a,b,c) differed among systems. Degassing chambers were employed before bead and trickling filters. Three replicates were used for each filter type. Biofilters were randomly assigned to culture systems to avoid any bias of position effects within the culture facility. System flow rates were adjusted to obtain approximately two system turnovers per hour.

The systems were located in an aluminum frame building (33.5m x 15.2m x 4.8m). Lighting was low to minimize algal growth and stress responses of fish to activity around the tanks. An automatic timer produced a 16-hour light: 8-hour dark photoperiod. An exhaust fan and four propane gas heaters were used to regulate ambient air temperature.

Biofilter Characterization — Media characteristics for the upflow pulsed bed bead filter, packed tower trickling filter, and rotating biological contactor are given in Table 1.

The upflow pulsed bed bead filters (Figure 1a) included three stages, each column (0.74 m diameter x 2.11 m height) comprising one stage. Each stage employed a bed of 2 x 3 mm ABS (acrylonitrile, butadiene and styrene) plastic beads with a specific gravity of 1.04 (International Polymer Corp., Allentown, PA, USA). Water was pumped upward through the stages to expand the beds. Expansion promoted bed turnover and agitation of the biofilm on the beads. Each bed was expanded for approximately 1 minute, and allowed to settle for 2 minutes (Honeyfield and Watten 1996). Water flow was controlled with a timed electric ball valve assembly.

Packed tower trickling filters (Aqua-Manna, Inc., Ladoga, IN, USA) consisted of a cylindrical vessel packed with a single-face corrugated plastic medium (0.76 m diameter x 0.76 m height) positioned parallel to water flow (Figure 1b). Water was pumped approximately 2.4 m through a center pipe to the top of the medium and was distributed by a rotating spray bar. As water trickled downward throughout the medium, it was aerated and CO_2 was stripped.

Table 1. Media characteristics and median system flow rates (95% CI) for each biofilter type.

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

Rotating biological contactor filters (Fresh-Culture Systems, Inc., Breinigsville, PA, USA) consisted of a cylindrical drum (1.22 m diameter x 1.52 m length) rotated at approximately 1 rpm by air injected below a series of louvers located around the center of the drum (Figure 1c). Rotation of the filter resulted in emergence of the biofilm from the water column, meeting the biofilm's oxygen requirements and stripping CO_2.

Biofilter Acclimation — After stocking, concentrations of total ammonia-nitrogen (TAN) and nitrite-nitrogen (NO_2^--N) were monitored daily to assess nitrification activity. Feeding rates through this period rose from 500 g initially to 1000 g/system/day. Water exchanges were used as necessary to prevent prolonged exposure of fish to elevated TAN and NO_2^--N concentrations. Biofilters were considered fully acclimated when TAN and NO_2^--N levels consistently remained below 0.5 mg/L. Following acclimation, studies on biofilter performance began.

Daily Operations and Water Quality Parameters — All systems were initially filled with well water. Municipal water was utilized for daily water replacements. New water was introduced into the systems each morning following water sampling. Well water also was used for emergency water exchanges. Targeted ranges for basic water quality parameters were chosen to optimize environmental conditions for both fish and nitrifiers: NH_3-N <0.05 mg/L (Colt and Armstrong 1981) NO_2^--N < 1.0 mg/L (Losordo 1991), NO_3^--N < 100 mg/L (Losordo 1991), dissolved oxygen > 5 mg/L (Kaiser and Wheaton 1983; Losordo 1991), pH 6.5-8.0 (Meade 1989), temperature 22-23°C (Schmitz 1999), alkalinity > 100 mg/L (Meade 1989; Losordo 1991), and hardness > 100mg/L (Meade 1989). $NaHCO_3$ additions were made to a system when pH and alkalinity levels dropped below 7.0 and 100 mg/l (as $CaCO_3$), respectively. Surface agitators were added as needed to bead filter systems to maintain targeted pH levels to maintain fish.

Feed Administration — Fish were fed a 42% crude protein, 12% fat, 3% crude fiber and 13% moisture floating pellet diet (Rangen, Inc., Buhl, ID, USA) two to three times daily. Rations were recorded to track system feed input (Figure 2). Schmitz (1999) reported data on fish production.

Table 2. Median values (95% CI) for basic water quality parameters. Parameters in each column with same superscript are not M significantly different at the p < 0.05 level. M

Filter Type	TAN[1] (mg/L)	NH_3-N_2 (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	DO (mg/L)	pH	Temp. (°C)	Alkalinity (mg/L)	Hardness (mg/L)	Feed (kg/d)
Bead	1.06 [a] (1.00-1.09)	0.006 [a] (0.0051-0.0063)	0.390 [a] (0.340-0.435)	66 [a] (62-71)	9.5 [a] (9.3-9.7)	7.12 [a] (7.09-7.14)	23.2 [a] (23.2-23.4)	136 [a] (124-148)	217 [a] (192-253)	1.95 [a] (1.92-2.53)
Trickling	0.90 [b] (0.85-0.94)	0.008 [b] (0.0077-0.0084)	0.400 [a] (0.345-0.472)	77 [b] (69-83)	9.2 [a] (9.0-9.4)	7.28 [b] (7.26-7.33)	23.2 [a] (23.1-23.3)	112 [b] (104-123)	202 [a] (178-237)	1.99 [a] (1.78-2.37)
RBC	0.85 [b] (0.82-0.90)	0.006 [a] (0.0055-0.0061)	0.381 [a] (0.355-0.405)	77 [ab] (67-85)	9.5 [a] (9.2-9.8)	7.19 [c] (7.16-7.23)	22.3 [b] (22.1-22.4)	128 [a] (118-137)	223 [a] (192-253)	2.22 [a] (1.92-2.53)

[1] Maximum TAN values for systems with each filter type: bead, 1.89; trickling, 1.82; RBC, 1.74 mg/L.
[2] Maximum NH_3-N values for systems with each filter type: bead, 0.015; trickling, 0.018; RBC, 0.071 mg/L. as $CaCO_3$.

Nitrogenous Wastes and Physical Characteristics — Daily water sampling commenced at 8 AM, prior to the first fish feeding. Samples were taken from the production tank prior to mechanical and biofilter treatment (sample point 1) (Figure 1). Grab samples were taken periodically from biofilter influents and effluents (sample points 2 and 3) to monitor filter performance. Filter performance also was monitored at 4-hour intervals during analysis of diurnal system dynamics.

Temperature (°C), pH, dissolved oxygen (DO) and TAN were measured daily. Nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N) and alkalinity (as $CaCO_3$) were measured weekly. Total hardness (as $CaCO_3$) was tested periodically. All tests followed protocols presented in the Standard Methods handbook (APHA et al. 1995). A YSI Model 58 dissolved oxygen meter (YSI Co., Yellow Springs, OH, USA) was used for temperature and DO measurements, and a Hanna Instruments Model HI 1270 pH probe (Hanna Instruments, Woonsocket, RI, USA) was used to monitor pH. TAN, NO_2^--N and NO_3^--N were analyzed using a Hach DR/2000 spectrophotometer (Hach Co., Loveland, CO, USA). Total alkalinity and total hardness both were analyzed via Hach titrations. Calculations of NH_3-N were made using equations presented by Emmerson et al. (1975).

Organic Wastes – Monitoring of carbonaceous biochemical oxygen demand ($cBOD_5$), dissolved organic carbon (DOC), and total suspended solids (TSS) analysis began on days 126, 259, and 108 of the study, respectively, and continued for the remainder of the production cycle. $cBOD_5$ samples were drawn from sample points 1 and 3 for each system. Samples were drawn in triplicate and immediately analyzed for initial DO concentrations. Final DO concentrations were measured following a 5-day incubation period (APHA et al. 1995). A YSI model 5905 BOD probe (YSI Co., Yellow Springs, OH, USA) was used to obtain both initial and final DO concentrations. DOC samples were drawn from sample points 1 and 3 for each system. Samples were immediately filtered through 0.45 micron membrane filters (Gelman Sciences Inc., Ann Arbor, MI, USA) and stored at 4°C until analysis (APHA et al. 1995). A Dohrmann Model DC-80 TOC Analyzer (Rosemount Analytical Inc., Lansdowne, PA, USA) and Horiba Model PIR-2000 Infrared Gas Analyzer (Horiba Instruments Inc., Irvine, CA, USA) were used for analysis. TSS were estimated using the filtration method (APHA et al.

1995). Grab samples were collected from all system sample points and stored at 4°C until analysis within 7 days of sampling (APHA et al. 1995).

Statistical Analysis

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

Data for all water quality test parameters were analyzed by filter type for systems that proved viable throughout the entire 292-day study. Data from systems 3 (RBC), 7 (trickling), and 8 (bead) were not included in the analysis. In system 3, all fish died following a break in the aquaculture facility's main water distribution pipe, when cold, chlorinated water entered the culture tank. Data from system 7 (trickling) was excluded from final analysis due to low fish numbers, resulting either from initial understocking or high rates of perch cannibalism (Schmitz 1999). In system 8, mortalities resulted from an unknown cause, resulting in a > 60 % population reduction within the system (Schmitz 1999). Hence, statistical analysis included data from two replicates for each filter type.

Table 3. Median values (95% CI) for organic water quality parameters. None of the values in a given column are significantly different at the $p < 0.05$ level.

Filter Type	$cBOD_5$ (mg/L)	DOC (mg/L)	TSS (mg/L)
Bead	48 (32-64)	14 (12-20)	13 (10-19)
Trickling	45 (31-58)	13 (10-14)	11 (8-16)
RBC	44 (33-55)	15 (14-19)	8 (5-13)

Table 4 Median influent and mass loading values for TAN and organic water quality parameters (95% CI). Values in same row with different superscripts are significantly different at $p < 0.05$.

Test Parameter	BEAD		TRICKLING		RBC	
	Influent (mg/L)	Mass Loading (g/m -d$^-$)	Influent (mg/L)	MassLoading (g/m -d$^-$)	Influent (mg/L)	Mass Loading (g/m -d$^-$)
TAN	1.10 [a] (0.10-1.23)	0.14 [a] (0.13-0.15)	0.91 [b] (0.83-0.99)	0.96 [b] (0.87-1.04)	0.88 [b] (0.79-1.02)	8.17 [c] (7.41-9.48)
$cBOD_5$	48 [a] (32-64)	6 [a] (4-8)	45 [a] (31-58)	48 [b] (35-58)	44 [a] (33-55)	409 [c] (298-560)
DOC	14 [a] (12-20)	1.8 [a] (1.5-2.5)	13 [a] (10-14)	13.6 [b] (10.4-15.0)	15 [a] (14-19)	136.5 [c] (126.8-174.7)
TSS	17 [a] (10-21)	2.1 [a] (1.3-2.7)	13 [a] (9-25)	13.3 [b] (9.0-26.5)	10 [a] (6-15)	96.9 [c] (54.4-138.2)

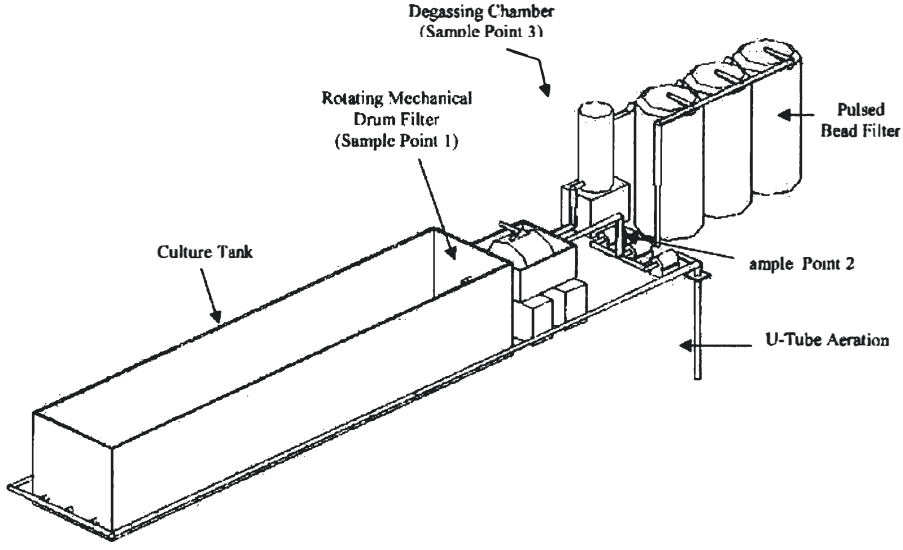

Figure 1a.

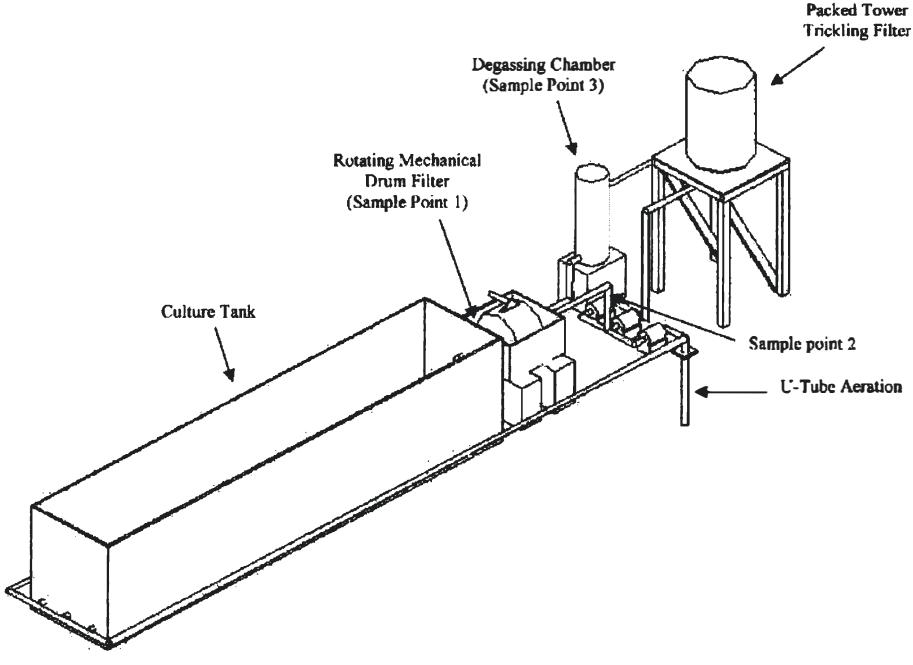

Figure 1b.

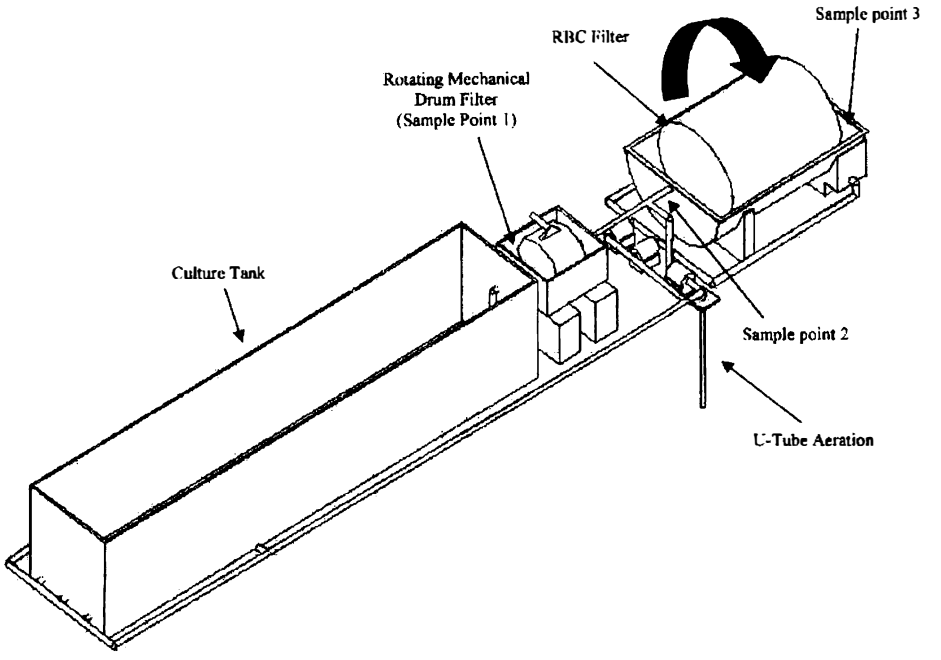

Figure 1c.

RESULTS

Biofilter Acclimation

For all biofilter types, TAN and NO_2-N levels increased to a peak, and then decreased to steady state conditions (Figures 3 a,b). The troughs in curves before the peaks result from episodes of water flushing used to reduce TAN concentrations before filters were fully acclimated. TAN concentrations for all filter types peaked between days 22 and 25. Bead and RBC filters peaked at TAN concentrations of 3.68 and 2.92 mg/L, respectively, with trickling filters peaking at a lower concentration of 1.60 mg/L. For NO_2-N concentrations, peaks were observed for all filter types between days 40 and 43. The RBC filters peaked at 4.06 mg/L, while the bead and trickling filters peaked at 2.41 and 2.03 mg/L, respectively. The rate of decline to steady state conditions in nitrogenous waste levels was similar among all filter types. All filters reached steady state conditions for TAN around day 42, and for NO_2-N around day 52. Times to TAN and NO_2-N acclimation were somewhat longer than the 20-35 days reported by Wheaton et al. (1991).

Water Quality Analysis

Basic Water Quality Analysis — TAN, NH_3-N, NO_2^-N (Figure 4a), and NO_3^--N (Figure 4b) concentrations increased through the study in all systems. TAN and NO_3^--N steadily increased until roughly days 98 and 182, respectively; water flushing then was practiced to manage their concentrations. NO_3^--N concentrations were directly reduced via water exchanges. TAN fluctuations were probably a function of NO_2^--N control, and also were directly affected via water exchanges when microbial oxidation was not sufficient for nitrite reduction. Median TAN and NH_3-N values (Table 2) were greater for systems with trickling filters than for those with RBC or bead filters ($p < 0.001$). All median values were well below the upper limit of the target range set for this study. Maximum TAN and NH_3-N values experienced by the filters (Table 2, footnotes 1 and 2) rarely reached levels considered harmful to fish health.

NO_2^--N concentrations rose through the study for all filter types (Figure 4a). This lag between loading and *Nitrobacter* community response is typical of biofilters in recirculating aquaculture systems (Lucchetti and Gray 1988). The greatest NO_2^--N fluctuations were observed in trickling filter systems, where a maximum value of 2.24 mg/L was observed on day 201 of the study. These large fluctuations may have resulted from the high rate of feed administration to the trickling filter systems (Figure 2). The median daily rate of feed administrated to trickling filter systems was significantly higher than those to bead ($p < 0.001$) or RBC ($p = 0.01$) systems, which were not significantly different ($p = 0.10$). NO_3^--N concentrations also were greatest in the trickling filter systems, with a maximum value of 143 mg/L, while bead and RBC filter systems both had maximum values of 118 mg/L. Median nitrate levels in bead filter systems were lower than those in trickling filter and RBC systems, although significantly lower only for those in trickling filter systems ($p = 0.04$). NO_3^--N levels were considered non-problematic until levels surpassed 100 mg/L at approximately day 182 of the study (Figure 4b). Thereafter, water exchanges were used for reduction of nitrate concentration.

The pH values for all systems ranged from 7.0-7.4. The pH levels in trickling and RBC systems were typically higher than those in bead systems. Higher pH values most likely resulted from the carbon dioxide

Table 5. Median mass removal rate and percent removed values (95% CI) for TAN and organic parameters over the course f study. Values in same row with different superscripts are significantly different at $p < 0.05$.

Parameter RB	BEAD RB		TRICKLING		C RB	
	Mass RB Removal RB (g/m -d^{-1}) RB	Percent Removed (%	Mass RB Removal RB (g/m -d^{-1}) RB	Percent RB Removed RB (%	Mass RB Removal RB (g/m -d^{-}) RB	Percent RB Removed RB (%
TAN RB	0.004 ^{a} (0.003-0.009)	4.3 ^{a} (2.4-10.2)	0.037 ^{b} (0.006-0.062)	5.2 ^{a} (0.6-9.4)	0.14 ^{b} (-0.18-0.33)	1.9 ^{a} (-1.7-5.5)
cBOD$_5$ RB	0.5 ^{a} (0.2-1.0)	9.7 ^{a} (4.4-12.8)	1.4 ^{ab} (-2.9-7.7)	5.6 ^{a} (-6.5-15.2)	8.3 ^{b} (-2.9-127.7)	3.3 ^{a} (-0.9-30.1)
DOC RB	0.02^{a} (-1.02-0.41)	0.9 ^{a} (-69.0-16.4)	0.33^{a} (-0.40-1.11)	2.7 ^{a} (-3.8-8.6)	2.68 ^{a} (-53.28-22.27)	1.7 ^{a} (-43.7-11.8)
TSS RB	0.4 ^{a} (0.1-1.1)	22.5 ^{a} (4.4-41.5)	8.3 ^{a} (2.1-15.4)	54.6 ^{a} (19.6-66.9)	12.5 ^{ab} (-15.3-54.0)	9.6 ^{a} (-14.8-51.9)

stripping capabilities of the trickling and RBC filters. Elevated dissolved CO_2 also can suppress pH below optimal levels for nitrifiers (Grace and Piedrahita 1994), potentially decreasing nitrification rates. Higher mean alkalinity values in the bead systems (Table 2) were the consequence of more frequent sodium bicarbonate additions. In response to continued pH suppression, at day 156 of the study, surface agitators were placed in all bead filter systems after passage of the water through the filter beds to effect carbon dioxide stripping and maintenance of pH above 7. Higher NH_3-N observed in trickling filter systems than in RBC and bead filter systems may be attributed to elevated pH levels due to the effective carbon dioxide stripping abilities of the trickling filters.

DO typically ranged from 8.5-10.5 mg/L, with 5.2 mg/L being the lowest concentration observed among all systems. DO was not a limiting factor for fish or biofilter performance at these concentrations (Kaiser and Wheaton 1983; Losordo 1991).

Hardness values typically ranged from 150-280 mg/L in all systems. Hardness remained above levels considered critical for fish growth.

Organic Water Quality Analysis — Median organic water quality parameter values did not differ significantly among systems with different filter types (Table 3). Trend lines were fitted to the data to track organic parameter levels during the course of the study.

The greatest $cBOD_5$ increase was observed in bead filter systems, where levels increased by approximately 34 mg/L. $cBOD_5$ values in trickling and RBC systems increased approximately 28 and 29 mg/L, respectively. Since function of nitrifiers was inhibited chemically during $cBOD_5$ analysis, heterotrophic bacteria exerted all of the measured oxygen demand. These high values indicated that heterotrophs were consuming considerable dissolved oxygen, and likely were impacting the activity of nitrifying bacteria.

Bead and RBC systems displayed increases in TSS levels of approximately 7 mg/L, while trickling systems showed almost no increase (1 mg/L). This suggests that trickling filters were effective in suspended solids removal. This was unexpected, since trickling filters are not designed specifically for solids removal. The high specific surface area of the trickling filter media corresponds to a low void ratio, which

may explain why these filters removed suspended solids and became clogged halfway through the study. This particular media may have been a poor choice for use in a trickling filter since long-term performance would be expected to degrade as a result of solids accumulation in the filter. Bead filters were expected to be most efficient for suspended solids control. In a filter comparison study (Westerman et al. 1996), floating-bead biofilters were the only filter type that significantly reduced suspended solids levels (5-6 kg SS/m^3 day^{-1}). Characterizing performance of a floating-bead filter and an RBC operating in series, Delos Reyes and Lawson (1996) also found that the bead filter captured a large portion of the solids in the filter influent.

DOC levels decreased through the study period for all filter types. Bead filter systems showed a DOC reduction of approximately 5 mg/L. Levels in trickling and RBC systems decreased approximately 1 and 2 mg/L, respectively.

Mass Removal Analysis

TAN Mass Removal — Influent TAN in bead filter systems was significantly higher than in trickling ($p = 0.004$) and RBC ($p = 0.03$) filter units (Table 4), and did not differ among RBC and trickling filter systems ($p = 0.48$). Higher influent TAN to bead filters was most likely a function of lower nitrification rates in bead than in trickling or RBC filters. Median mass loading values (g/m^2/d) were greatest in RBC systems (Table 4). The highest TAN mass removal rate (g/m^2/d) was observed in RBC systems, followed by trickling filter systems (Table 5). Bead filters exhibited the lowest removal rate, significantly less than RBC ($p = 0.05$) and trickling ($p = 0.01$) filters. TAN removal rates in trickling and RBC filters were not significantly different ($p = 0.13$). TAN removal efficiencies did not differ among filter types ($p = 0.82$).

Organic Mass Removal — Influent organic levels were not significantly different among filter types (Table 4). Organic mass loading was highest in RBC filters, lowest in bead filters, and significantly different among filter types.

The RBC filters were found to have the highest organic mass removal rates among all filters for all parameters tested (Table 5). $cBOD_5$ removal rate in RBC systems was approximately 17 times greater than in bead

systems (p = 0.03), with total grams removed approximately 5 times B greater. B OD_5 removal rate in trickling filters Bid not Biffer from those B in bead (p = 0.09) or RBC (p = 0.09) filters. B

The TSS removal rate in the trickling filters was approximately 21 B times that in bead filters (p = 0.003). Total grams of TSS removed in B trickling filters were approximately 9 times greater than that in beaB B filters. TSS removal rate in RBC filters Bid not Biffer from those in bead B (p = 0.13) or trickling (p = 0.33) filters. Although trickling filters are not B intended for solids removal, the Bata showed effective solids removal B from the Bulture effluent. BIn one particular system, solids removal was B so great that the trickling filter Blogged two months before the end of the B study. After being taken offline and pressure washed, removing exBess B solids and biofloc, the filter was placed back online and operateB B normally through the remainder of the study. B

A net increase in DOC was observed across bead filter beds, although B DOC levels Becreased in all systems for all filter types over the Bourse of B the study. BOrganic matter was observed to accumulate in the bead beds B throughout the study. Degradation of this matter was most likely B responsible for the net increase in DOC Boncentrations across the filter B beds. Dissolution and Begradation processes may not have occurred B

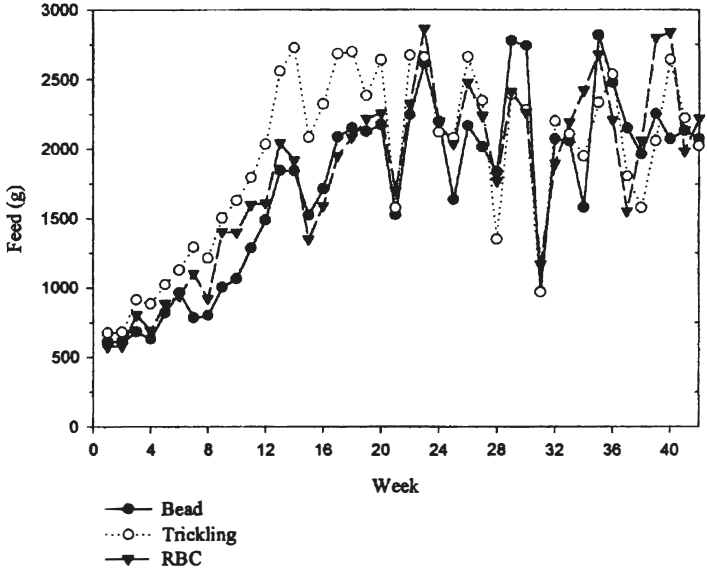

Figure 2. Average weekly feed additions

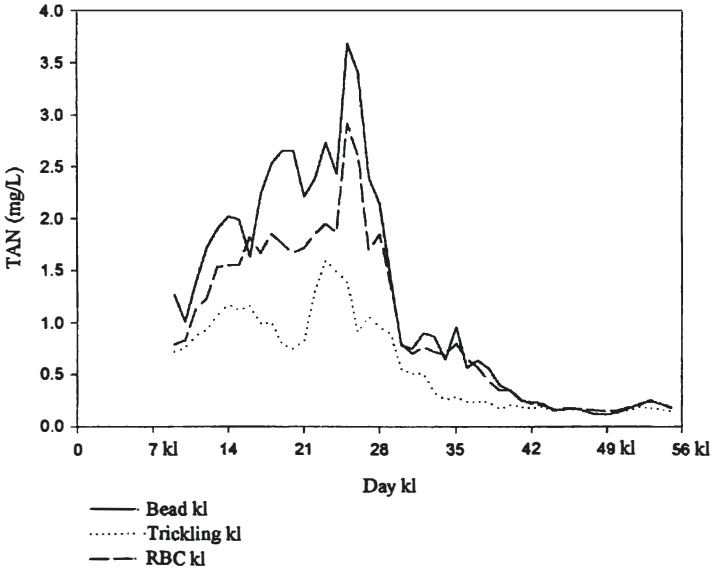

Figure 3a.

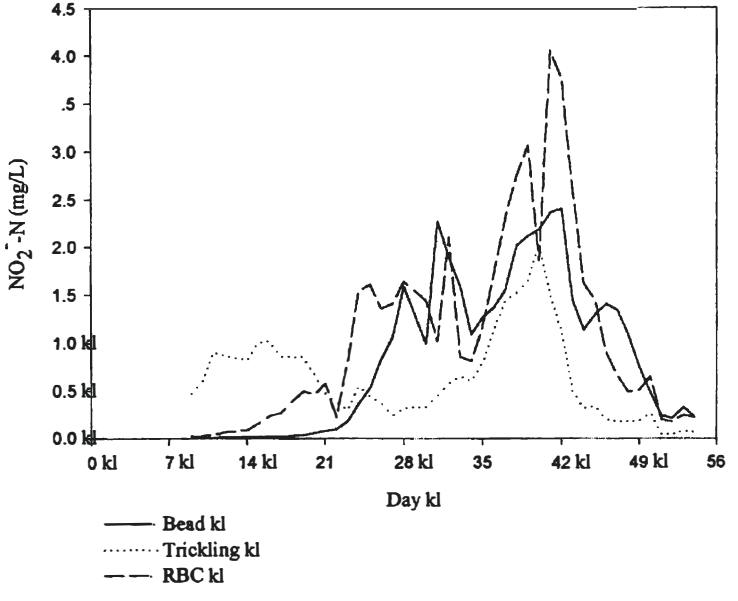

Figure 3b. Biofilter microbial acclimation, showing concentrations of: a) total ammonia nitrogen, and b) nitrite nitrogen (NO_2-N) as indicators of nitrifier population function.

quickly enough to increase system concentrations over the course of the study.

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

Diurnal TAN Analysis

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

TAN mass removal rates increased for all filter types between hours 0 and 4 (Figure 5a). TAN mass removal rates were not significantly different among filter types for hr 0 ($p = 0.51$) or hr 4 ($p = 0.51$). At hr 8, bead systems exhibited lower TAN mass removal rate than trickling ($p = 0.03$) and RBC ($p = 0.03$) filters, among which removal rates did not differ ($p = 0.94$). These relationships also were observed at hr 12. After peaking between hr 12 and 16, mass removal rates declined for all filter types, but did not decline to the levels observed at hr 0. Other diurnal analyses of various biofilters (Twaroska et al. 1997, Westerman et al.

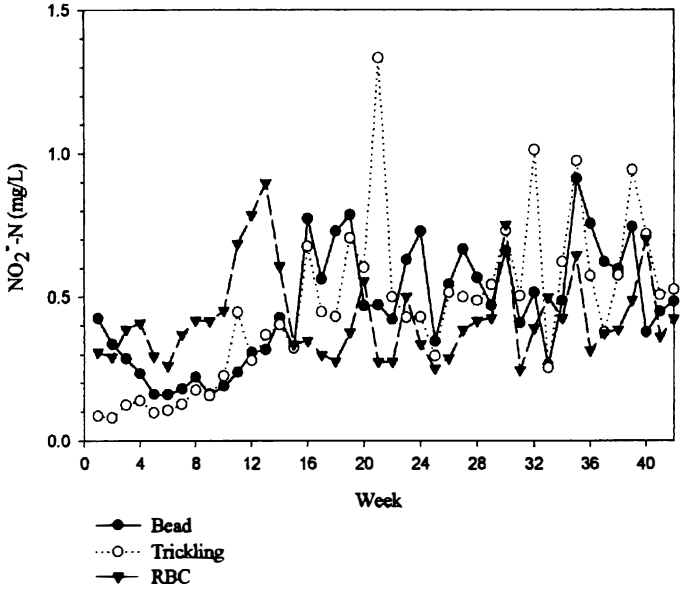

Figure 4a.

1996) showed that TAN removal increased with increasing TAN concentrations before peaking and declining, and also that TAN mass removal rates at hr 24 did not declined to levels observed at hr 0. Nitrification efficiencies increased once adequate feeding-induced ammonia was present (Figure 5b).

Analysis of the area under the concentration curves showed that TAN mass removal per unit surface area of 0.04, 0.11, and 0.10 g/m^2/day for bead, trickling and RBC filters, respectively. Total mass removal for the 24-hr sampling periods was 40, 50, and 33 g for bead, trickling and RBC filters, respectively. TAN mass removed was higher for bead filters than for RBC filters, but mass removed per unit surface area was higher for RBC filters, suggesting that surface area of RBCs was limiting. We estimated the additional RBC surface area (SA) needed to compensate for this removal difference as:

$$\mathbf{SA\ (m^2) = (Bead_{TMR} - RBC_{TMR})/RBC_{MRSA}}$$

where: TMR = total TAN mass removed (g), and MRSA = mass removed per unit surface area (g/m^2/day). We estimated that the RBC filters would have needed an additional 70 m^2 of surface area to remove as much TAN as the bead filters.

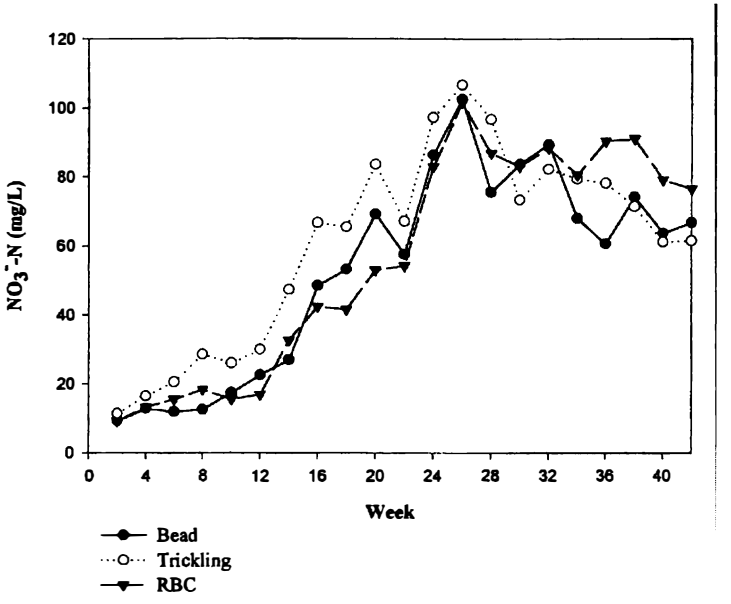

Figure 4b. Median weekly a) nitrite (NO_2-N), and b) nitrate (NO_3-N) concentrations during the course of the study.

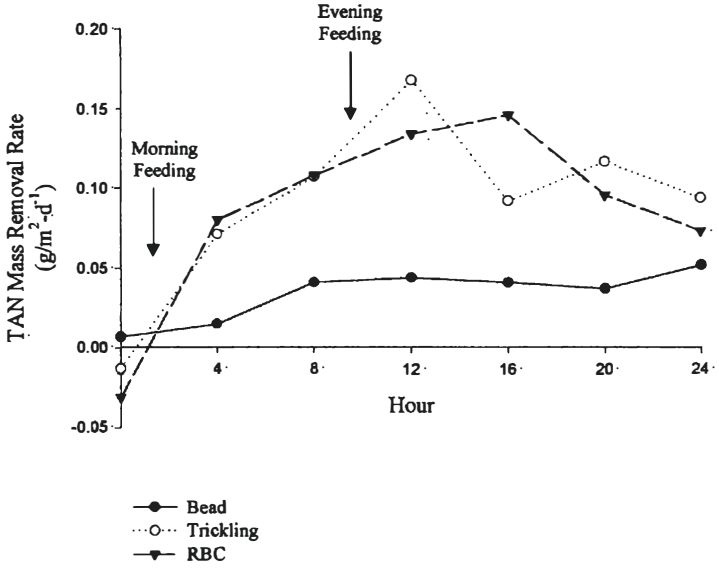

Figure 5a.

DISCUSSION

TAN Mass Removal as a Function of Filter Design

The biofilters employed in this study were commercially available (trickling filter, RBC) or an experimental prototype (bead filter). Flow rates were equalized at two system turnovers per hour, and filters were not sized to provide similar amounts of surface area. To a certain extent, TAN mass removal rates are a function of TAN mass loading rates. Hence, differences in surface area of the filters may have affected filter performance. We found that RBC filters yielded the highest TAN mass removal rate, followed by trickling filters, with the lowest removal rate in bead filters. Other filtration studies also found that RBC filters provided better or more consistent nitrification performance than other filter types. Miller and Libey (1985) found that RBCs yielded the greater TAN mass removal rates than packed tower trickling filters or fluidized bed reactor filters at three fish stocking densities. Rogers and Klemetson (1985) found TAN removal efficiency of more than 90% for an RBC and 50% for a trickling filter. Westerman et al. (1996) reported higher TAN removal rates for bead filters (120-160 g/m^3/d) than for RBCs (101 g/m^3/

d); however, converting these rates into grams removed per unit filter surface area yielded 0.10-0.13 g/m^2/d and 0.27 g/m^2/d, respectively. Malone et al. (1993) found that TAN mass removal rate of a mechanical washed bead filter (0.291 g/m^2/d) slightly exceeded that of a RBC (0.280 g/m^2/d). However, Delos Reyes and Lawson (1996) found that a RBC yielded much higher nitrification performance than a mechanical washed bead filter when operated in series. TAN mass removal rate for the bead filter was 0.056 g/m^2/d, and for the RBC, 0.257 g/m^2/d. Removal efficiencies were 5 and 52 % for the bead filter and RBC, respectively. Our results regarding trickling filter performance were similar to those of Singh et al. (1999), where trickling and bead filter configurations were compared. Systems employing trickling filters maintained lower TAN and NO_2-N than those with bead filters over the course of their study.

One reason why TAN removal in RBCs and trickling filters exceeded that in bead filters is that RBC and trickling filter beds are exposed to air, where atmospheric oxygen is capable of satisfying some of the oxygen demands of the exposed biofilms. Bacterial oxygen demand in bead filters can be met only by oxygen available within the water column. Lower TAN mass removal rates in bead filters than in trickling and RBC filters also may have been due to unintended retention of solids in bead filters. During each upwelling cycle, aggregated solids were to be released from the filter bed to the water flow, allowing the mechanical filter to intercept the solids and discharge them from the system. However, as organic waste loading to the filters increased through the course of the study, upwelling failed to control build-up of organic material in the bead filter beds. Water channelization occurred in the bead filter columns as a result of filter bed clogging.

TAN mass removal rates in our study were somewhat lower than removal rates in the filtration studies cited. We offer two explanations. First, our data were collected using samples drawn at 8 AM, before the fish were fed; TAN concentrations were relatively low prior to first feeding, leading to low estimates of removal rates and nitrification efficiencies. Second, nitrification efficiency decreases with increasing organic concentrations (Bovendeur et al. 1990; Figueroa and Silverstein 1992; Manem and Rittman 1992; Malone et al. 1993). $cBOD_5$ levels in our study ranged from 12-75 mg/L, with median values around 45 mg/L (Table 5). Nitrification rates decrease at $cBOD_5$ levels > 20 mg/L (Figueroa and Silverstein 1992). Nitrification efficiency drops below

10% once total organic carbon (TOC) levels reaches 12 mg/L (Abeysinge et al. 1996), corresponding to a BOD level of about 20 mg/L. Hence, high BOD_5 levels in this study may have contributed to the relatively low TAN removal rates and nitrification efficiencies exhibited by all filter types.

Solids removal in trickling filters

During diurnal sampling, analysis of areas under removal curves showed that trickling filters removed the greatest amount of TAN mass over the 24 hr sampling periods. TSS removal and intermittent foam fractionation observed in the trickling filters are believed responsible for high nitrification rates. Foam condensate was first observed around day 217 of the study and was observed intermittently thereafter. Condensate would emerge from the water column in the degassing chambers (Figure 1a) following discharge from the trickling filters. Fine solids < 30 mm, which predominate in aquaculture effluent particle size distributions (Chen et al. 1991; Easter 1992), are not effectively removed by conventional solids removal devices (e.g., settling tanks and microscreen filters) and accumulate with time. Chen et al. (1993) showed that foam fractionation provided effective solids removal from aquaculture effluents, including organic solids < 30 mm in diameter. The high percentage of TSS removed by trickling filters (Table 7) confirms that these filters were effectively removing solids from the culture water. The small opening sizes (approx. 16 mm^2) of vertical passages in the corrugated plastic filter medium used in this study most likely contributed to foam fractionation and TSS removal.

Commercial application

This study was performed on a pilot-scale. However, trickling and RBC filters of the designs used in this study have been employed in commercial aquaculture facilities, adding insights regarding filter performance. In one particular facility, three trickling filters were employed for each culture tank, with two operated simultaneously, and the third off-line. Filter operation was rotated every 24 hours; an operating filter would be taken offline for cleaning, and the newly-cleaned filter would be placed back on-line. Filter cleaning was considered the major drawback to operation of these filters (J. Bradley, Aqua-Manna, Inc., Ladoga, IN, personal communication). RBC filters

have proven relatively maintenance free, less prone to mechanical failure, and capable of sustaining TAN at or below 3 mg/L when feeding up to 272 kg feed/system/day (D. Prillaman, Blue Ridge Aquaculture, Martinsville, VA, USA, personal communication). The bead filters evaluated in this study have not been used on a commercial scale (B. Watten, U.S. Fish and Wildlife Service, Kearneysville, WV, USA, personal communication). Other upflow bead filtration designs have been used with success in commercial operations (S. Abernathy, TilTech Aquafarm, Robert, LA, USA, personal communication).

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Evaluation of UV Disinfection Performance in Recirculating Systems

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ABSTRACT

The use of ultraviolet (UV) disinfection devices has become increasingly popular in wastewater and aquaculture industries. Although the effectiveness of UV disinfection has been well documented for flow-through operation regimes in wastewater treatment, research focusing on water recirculating systems is still limited. In this study, the performance of single-lamp UV devices were tested on a recirculating system for fecal coliform (FC) disinfection. Experimental results indicated that UV power input, recirculating flow rate and water UV transmittance were three important factors determining UV disinfection efficiency. An UV disinfection model for a recirculating system was developed based on theoretical analysis and experimental data. A key model parameter, namely the first-order inactivation rate constant (k), was determined to be 0.0062 $m^2 J^{-1}$ for FC disinfection. Simulation using the model provided useful information for design and operation of recirculating UV

disinfection systems. The model prediction of disinfection process for other microorganisms is also capable of using reported values of the inactivation rate constant.

INTRODUCTION

Ultraviolet (UV) disinfection is an increasingly popular alternative in wastewater treatment (Hanzon and Vigilia 1999) and aquaculture industries. Absorption of UV radiation causes damage to the genetic material of bacteria, which prevents cell replication (U.S. EPA 1986). The advantages of UV disinfection include being non-toxic, ecologically-friendly, effective with a wide range of organisms, requiring a short contact time, and being easy to control (Moreland et al. 1998; Hanzon and Vigilia 1999). The effectiveness of UV radiation to inactivate pathogenic microorganisms in wastewater has been well documented for wastewater treatment purposes (Johnson and Qualls 1984; U.S. EPA 1986; Darby et al. 1993; Emerick et al. 1999). UV facilities used in the wastewater industry are usually flow-through systems with several banks of lamps in series (Ho et al. 1998). Pathogen inactivation can be described as a first-order reaction with respect to UV dose usually defined as UV light intensity times the exposure time (U.S. EPA 1986). Various models have been developed to describe the response of microorganisms such as fecal coliforms (FC) to UV light to aid in the design of UV disinfection systems (Qualls and Johnson 1985; U.S. EPA 1986; Loge et al. 1996a, 1996b). However, these models were developed for flow-through UV disinfection systems used in the wastewater treatment industry.

UV devices have become an integral part of many recirculating aquaculture operations providing disinfected water to hatchery, rearing, and depuration operations. Recirculation is a major feature of these aquaculture systems, which makes the evaluation of UV disinfection effectiveness different from that in flow-through wastewater treatment systems. Recirculating systems have attracted significant attention in the last two decades for applications in aquaculture. Lack of suitable water supplies and more stringent control of waste and nutrient discharges from pond and raceway facilities drive the demand for recirculating systems. However, little research has been reported on UV disinfection

performance in recirculating aquaculture systems. Fish production generates wastes due to excretion and uneaten food. Without proper treatment, accumulation of these wastes will create unhealthy conditions that may result in reduced fish growth rates, low feed conversion efficiency, disease and elevated mortality. An UV unit for disease disinfection is an important component for a reliable recirculating system. Although significant research efforts have been devoted to recirculating systems in the last two decades (Timmons and Losordo 1994; Losordo 1998a, 1998b), studies focusing specifically on UV disinfection performance are scarce.

The objectives of this study were (1) to evaluate the performance characteristics of UV disinfection devices in recirculating systems under various conditions; (2) to develop an UV disinfection model for recirculating systems, (3) to calibrate model parameter and to validate the model using the experimental data, and (4) to simulate UV disinfection behaviors under various conditions to provide quantitative information for the design and operation of UV disinfection devices used in recirculating systems.

THEORETICAL ANALYSIS

UV radiation absorbed by the nucleic acid of bacteria can damage the genetic material and prevent cell replication (U.S. EPA, 1986). UV disinfection performance in terms of a concentration reduction rate has typically been described as a first-order reaction:

$$\frac{dN_t}{dt} = - kI_{ave}N_t \qquad (1)$$

where N_t = bacterial concentration (CFU per 100 ml) (CFU = colony forming unit); t = time (s); k = first-order inactivation rate constant (m^2 J^{-1}); I_{ave} = average UV intensity (W m^{-2}); For an initial bacterial concentration (N), integrating equation (1) gives a bacterial concentration after exposure to UV (N_t);

$$Nt = N\, e^{-kI_{ave}t} \qquad (2)$$

The average UV intensity inside an UV unit can be calculated using Beer's law (U.S. EPA 1986). For a cylindrical reactor with a single central lamp surrounded by a quartz tube (Fig. 1), the UV intensity at radius r can be expressed as:

$$I_r = \frac{PT_r^{100(r-r_o)}}{2\pi rL} \qquad (3)$$

The average UV intensity is thus obtained using the following equation:

$$I_r = \frac{\int_{r_o}^{R} I_r 2\pi rL\, dr}{V_L} = \frac{P}{100V_L lnT_r} = (T_r^{100(R-r_o)} - 1) \qquad (4)$$

where P = output power of the UV unit (W); I_r = UV intensity at radius r (W m^{-2}); $T_r = UV_{254}$ (254 nm wave length) transmittance through water of one centimeter thickness (cm^{-1}); L = active length of the UV unit (m); V_L = total contact volume of the UV unit (m^3); R = radius of the inner surface of the UV unit cylinder (m); and r_o = radius of the quartz tube of the UV unit (m) (Fig. 1). For the tested 25-W and 40-W UV units in this study, R and r_o were 0.0254 m and 0.011 m, respectively.

Because an UV device behaves hydraulically as a plug-flow reactor (Darby et al. 1993), the average exposure time for a flow-through UV reactor can be determined by dividing the net reactor volume by the flow rate through the system. For flow through an UV unit, bacterial concentration of the treated water can be expressed as:

$$N_\tau = N\, exp\,(-kI_{ave}VL/Q) = N\, exp\,(-kP\,\frac{T_r^{100(R-r_o)} - 1}{100QlnT_r}) \qquad (5)$$

where N_τ = bacterial concentration of the flow through a working UV unit (CFU per 100 ml); Q = flow rate through the UV unit (m^3 /s).

For a recirculating system (Fig. 2), assuming bacterial concentration within a system is homogeneous, the bacterial storage or dissipation rate depends on the balance between the input rate from the source

production and the influent, and the output rate including effluent and reduction by the UV unit. The basic equation can be developed based on mass balance principle.

$$\frac{dN}{dt} = N_s + \frac{Q_e}{V}(N_i - N) - \frac{Q}{V}(N - N_r) \qquad (6)$$

where N_S = bacterial source production rate of the system (CFU per 100 ml), including excretion by fish and growth within the system; Q_e = water exchange rate ($m^3\ s^{-1}$); V = total water volume of the recirculating system (m^3); N_i = bacterial concentration of the influent (CFU per 100 ml).

At steady state, substituting equation (5) into equation (6) results in:

$$N = \frac{N_S V + Q_e N_i}{Q_e + Q\left\{1 - exp\left[-k\,\frac{P}{100Q\ln T_r}(T_r^{100(R-r_o)} - 1)\right]\right\}} \qquad (7)$$

For a closed recirculating system ($Q_e = 0$) of UV disinfection, the following equation can be derived from equation (7).

$$\frac{N_s}{N} = \frac{Q}{V} = \left\{1 - exp\left[-\frac{k}{100Q\ln T_r}\,\frac{P}{V}\,\frac{V}{Q}\,(T_r^{100(R-r_o)} - 1)\right]\right\} \qquad (8)$$

where the term N_S/N is defined as RSRR (relative specific reduction rate). Physically, the RSRR describes the ratio of the bacterial production rate to the equilibrium bacterial concentration in a system. A high value of the reduction rate implies a high disinfection efficiency. The value of Q/V represents the cycle rate of the water through an UV unit, and the ratio P/V gives the UV power input per cubic meter of water. Therefore, equation (8) describes UV disinfection efficiency as a function of water cycle rate, UV power input ratio and water UV_{254}

transmittance, which provides a better understanding of the performance of UV disinfection in a recirculating system.

MATERIALS AND METHODS

The UV disinfection study was conducted using a water recirculating system as shown in Fig. 3. The system consisted of a tank, a recirculating pump, and a single-lamp ultraviolet (UV) unit (Aqua Ultraviolet, CA, USA). Prior to each test, the tank was cleaned and filled with artificial seawater or freshwater (Table 1). The artificial seawater was made using Durex All Purpose Salt (Morton International, Chicago, USA) and de-chlorinated tap water. Wastewater containing a high concentration of microorganisms, collected from the wastewater lagoon of a nearby dairy farm was used as a bacterial source. For each test, one percent of dairy wastewater (v/v) was added into the water bath and mixed with the artificial seawater. An air diffuser was placed in the water bath to maintain dissolved oxygen (DO) concentration at 9.3±0.4 mg l^{-1} (measured using a YSI-50 DO meter, Yellow Springs, Inc., USA). The diffuser also served as a mixer to keep coliform concentration homogeneous within the water bath. The mixed water was pumped from the bath through a one-way valve, and then returned to the bath via two ways: an over flow path and a disinfection path through the UV unit (Fig. 3). One ball valve was used in each path to adjust water flow rates through the UV device according to the experimental protocol. Timing was started once the UV light was turned on. Water samples were collected from the water bath at different disinfection times (Table 1). Before each test, the outside surface of the quartz sleeve of the UV lamp was hand cleaned with commercial cleaning solution so that the effect of sleeve dirt on the disinfection efficiency was virtually eliminated. All of the treatments had a salinity of 15% except treatments 11 (fresh water) and 12 (26% salinity) (Table 1). Among the treatments, UV_{254} transmittance was adjusted by adding a different volume of wastewater. For all the treatments, temperature and pH were maintained at 13.2±2.0 °C and pH 8.15±0.20, respectively.

Sample analyses were performed in the Water Quality and Waste Analysis Laboratory at Washington State University. The bacterial species evaluated for UV disinfection performance was fecal coliform

Table 1 UV disinfection experiments performed in different conditions.

Treatment number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
UV unit power (W)	25	25	25	25	25	25	25	40	40	40	25	25
Wastewater volume (l)	340	340	340	340	340	340	340	340	340	340	680	680
Salinity (‰)	15	15	15	15	15	15	15	15	15	15	0	26
Flow rate (s^{-1})	1.26	1.26	1.26	2.52	0.63	1.26	2.52	2.52	1.26	1.26	1.26	1.26
TSS (mg l^{-1})	60	74	79	71	51	46	36	36	34	50	36	44
Turbidity (NTU)	13.7	16	32.1	31	14.5	9.4	17.1	15.9	15.7	28.3	29.2	28.8
UV_{254} transmittance (% cm^{-1})	54.2	52.9	30.1	25.3	57.6	69.2	52.1	38.2	39.4	26.8	35.0	31.0
Fecal coliform concentration (CFU per 100ml)												
Disinfection time = 0 (s)	32250	61400	72000	24500	22000	12500	14000	27500	27500	39000	67000	37000
270	19000	39000	48000	12500	13900	7500	10850	16250	15000	19000		
540	8900	27150	27500	8250	8500	3550	3900	4425	5150	8800	34800	29000
810	4800	8900	18800	5950	5500	1180	1550	1300	2300	3150		
1080								350	855	1500		
1350	2000	4050	6600	850	1900	220	400	90	355	895		
1890	375	700	1150	160	1070	60	80					
2160											6620	4460
2700											4940	1950

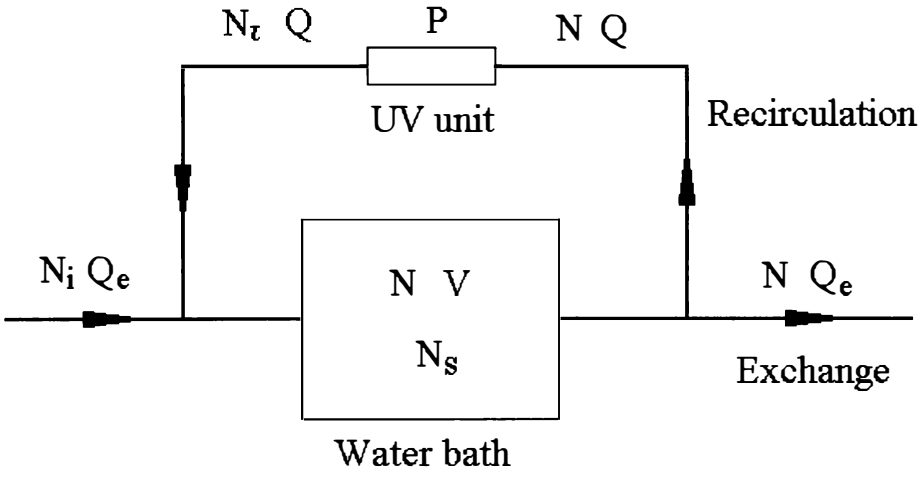


Figure 1. Schematic diagram of a recirculating system for UV disinfection.

(FC). The concentration of FC was determined using the membrane filter procedure specified by the Standard Method of 9222D (APHA 1995). It should be pointed out that fish do not excrete FC. The target for UV disinfection in most aquacultural systems is not FC, but other microorganisms. The reasons for selection of FC as an indicator of UV disinfection were: (a) it is a most common species studied for UV disinfection purposes; (b) a reliable standard method is available (APHA 1995); (c) FC is a target microorganism for depuration systems; (d) the results of this study provide information for reference and comparison with disinfection practices targeting other microorganisms. Initial water samples were collected before each trial (disinfection time = 0 as shown in Table 1). In addition to FC analysis, these samples were also analyzed for UV_{254} (UV light at a wave-length of 254 nm) transmittance using a Spectronic 21-D spectrophotometer (Milton Roy, Brussels, Belgium), turbidity using a 965-A Digital turbidimeter (Orbeco Analytical Systems, Inc., NY, USA), and total suspended solids (TSS) concentration according to the Standard method of 2540D (APHA 1995).

The UV units were highly effective for FC disinfection under all experimental conditions as presented in Table 1. In most cases, a 25-W UV unit disinfected about 99% of FC in the 340-liter wastewater within 31.5 minutes. This indicated that only about 1% FC remained after 7 cycles through a 25-W UV unit. Similarly, the system showed about

Figure 2. Cylindrical geometry of a single lamp UV unit.

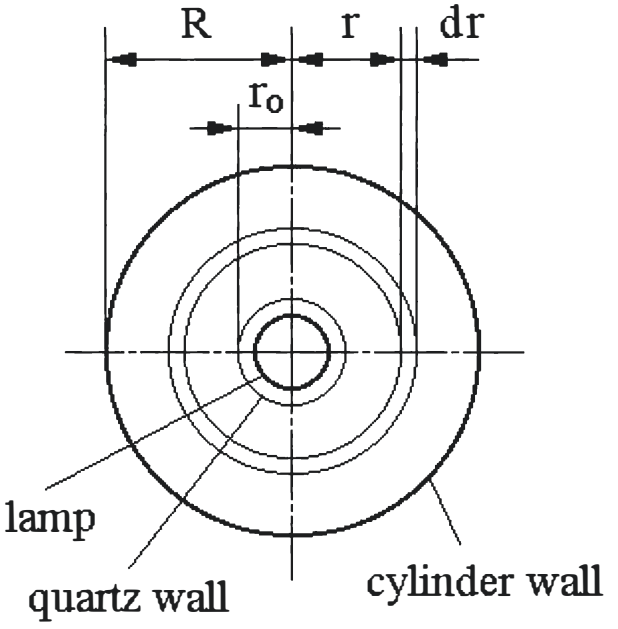


Figure 3. Schematic of the UV disinfection experimental system.

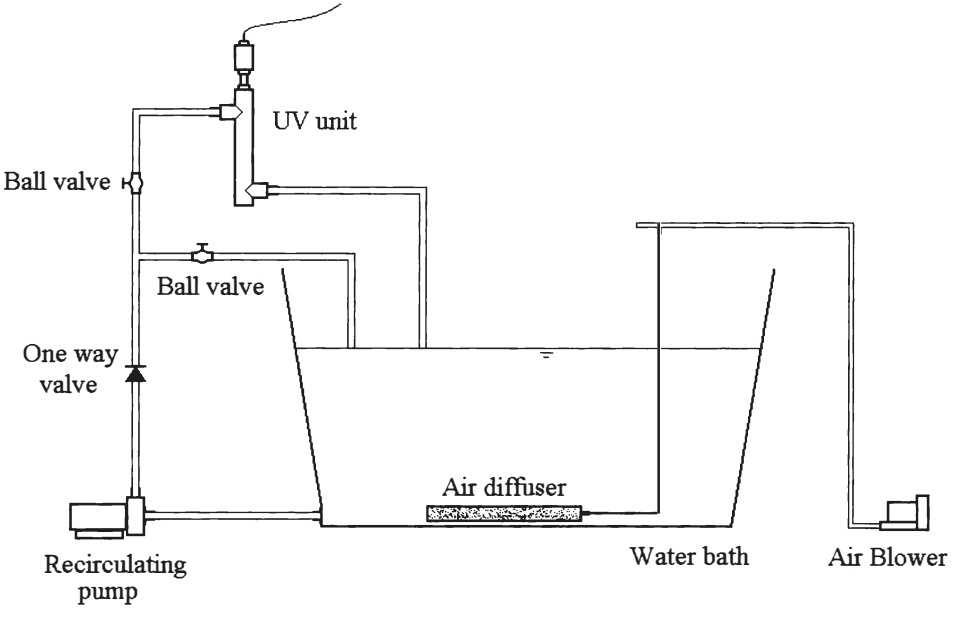


Table 2 Literature values of the inactivation rate constant for some microorganisms.

Microorganisms	Values of k (m^2 J^{-1}), Reference
Total coliform	0.0084-0.0166 (Ho et al. 1998)
Escherichia coli	0.0127 (Nieuwstad et al. 1991)
Fecal streptococci	0.0067 (Nieuwstad et al. 1991)
	0.0084 (Havelaar et al. 1987)
Spores of sulphite-reducing clostridia	0.0014 (Nieuwstad et al. 1991)
Somatic coliphages	0.0159 (Nieuwstad et al. 1991)
	0.0144 (Havelaar et al. 1987)
F-specific bacteriophages	0.0053 (Nieuwstad et al. 1991)
	0.0054 (Havelaar et al. 1987)
MS2 bacteriophages	0.0106 (Havelaar et al. 1990)
Reoviruses	0.0055 (Nieuwstad et al. 1991)

99% of FC removal efficiency after 5 cycles through a 40-W UV unit. The disinfection efficiency of treatment 5 was extremely low compared with those of the others due to the low flow rate. Treatments 11 and 12 were conducted for comparing the impact of salinity on the UV disinfection of fecal coliform. No significant difference ($R^2 = 0.92$, $N = 4$, $P < 0.05$) in the survival ratio was observed due to salination (Table 1). The results in Table 1 generally indicated that UV power, flow rate and UV_{254} transmittance were the three most important factors affecting UV disinfection efficiency.

MODEL PARAMETER CALIBRATION

The first-order inactivation rate constant (k) is a key parameter for the UV disinfection model, which was determined below using experimental data (Table 1). During the tests, there was no bacterial source ($N_S = 0$) and no water exchange ($Q_e = 0$) in the experimental system.

Equation (6) was thus simplified as:

$$\frac{dN}{dt} = \frac{Q}{V}(N - N_\tau) \qquad (9)$$

Integrating equation (9) and subtracting equation (5) gives following expression:

$$\frac{N}{N_o} = exp\{-\frac{Qt}{V}[1-exp(-kP\frac{T_r^{\frac{100}{r}(R-r_o)}-1}{100Q\,lnT_r})]\} \qquad (10)$$

where N_0 = initial fecal oliform concentration (CFU per 100 ml), and the value of N/N_0 represents survival ratio. To determine the rate constant k, equation (10) was changed into following form:

$$ln[1+\frac{V}{Qt}ln(\frac{N}{N_o})] = -k\frac{T_r^{100(R-r_o)}-1}{100Q\,lnT_r}P \qquad (11)$$

Linear regression calculation with equation (11) using the experimental data (Table 1) resulted in k = 0.0062 $m^2\,J^{-1}$ for fecal coliform (R^2 = 0.763, N = 56, P < 0.01). Using this k value, the linear correlation between the calculated and tested survival ratios was statistically significant (R^2 = 0.916, n = 56, P<0.01) (Fig. 4).

MODELING RESULTS AND ISCUSSION

To better understand UV disinfection performance, model simulation was performed for a steady-state recirculating system under variations in aily cycle number, UV power input, and UV_{254} transmittance of the water. Modeling results provide useful information for design and operation of recirculating UV disinfection systems.

The relationship between the relative specific reduction rate (RSRR) and the aily cycle number (Fig. 5) gives a basis for the determination of *daily cycle number for a recirculating UV disinfection system.* The RSRR rises as aily cycle number increases, but the impact of ycle number, when adequately high, is less significant. Therefore, to obtain a

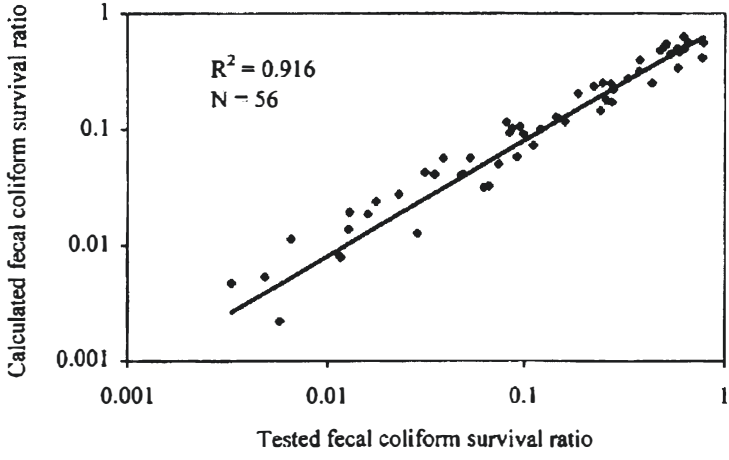
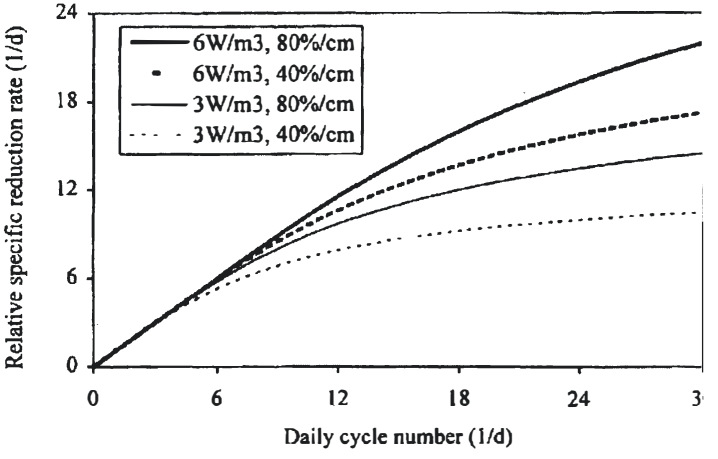


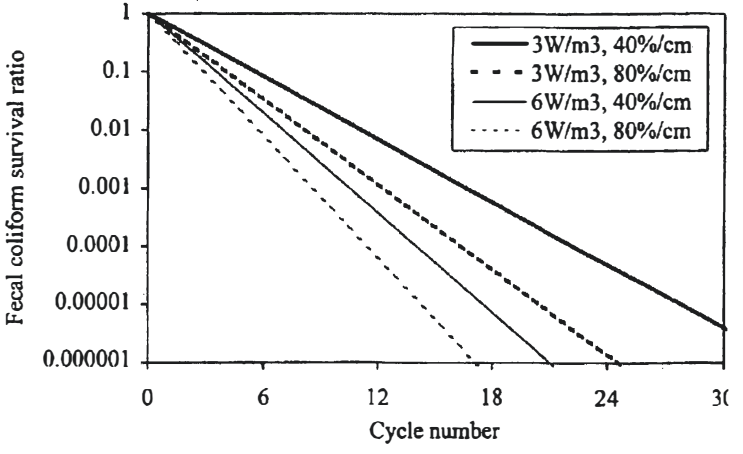
Fig. 4. The linear relationship between calculated and tested fecal coliform survival ratios. R^2 is the determination coefficient of regression, and N is the number of data points.

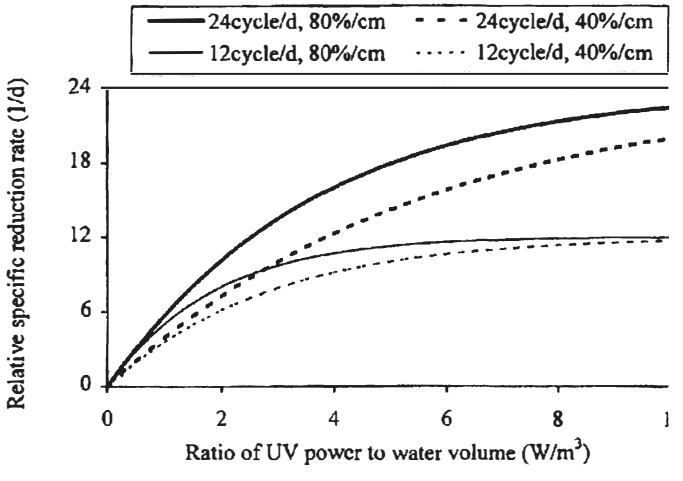
high reduction rate, the number of daily cycles needs to be maintained above a certain level. In recirculating aquaculture systems, the pumping rate is typically determined for a system turnover time of 0.5-1.0 hour (Timmons and Losordo 1994). As indicated in Fig. 5, the daily cycle number required for a high disinfection efficiency is often comparable to that required for nitrification biofiltration purposes.

For a given system without bacterial source production, the FC survival ratio was reduced approximately as an exponential function of the cycle number (Fig. 6). UV power input and water UV transmittance also had significant effects on disinfection efficiency. Given an UV power of 6 W m^{-3}, the cycle numbers required for a survival ratio of 0.00001 were about 17 and 21 for water transmittance values of 80 and 40 % cm^{-1}, respectively. About 24.5 and 33.5 cycles were needed under the same conditions when UV power was 3 W per cubic meter.

Fig. 7 presents information for the determination of UV power per cubic meter of water in a recirculating UV disinfection system. Clearly, a higher power input resulted in a higher reduction rate, but this means more energy consumption. As indicated in equation (8), the maximum RSRR is Q/V, i.e., daily cycle number if time is measured in days instead of seconds. Fig. 7 shows that, when the power input was 6 W m^{-3} and the daily cycle number was 24, RSRR reached 81% and 66% of its maximum value for water with 80 and 40 % cm^{-1} transmittance,


6W/m3, 80%/cm
6W/m3, 40%/cm
3W/m3, 80%/cm
3W/m3, 40%/cm
Relative specific reduction rate (1/d)
Daily cycle number (1/d)
0
6
12
18
24


3W/m3, 40%/cm
3W/m3, 80%/cm
6W/m3, 40%/cm
6W/m3, 80%/cm
Fecal coliform survival ratio
Cycle number
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
0
6
12
18
24


24cycle/d, 80%/cm
24cycle/d, 40%/cm
12cycle/d, 80%/cm
12cycle/d, 40%/cm
Relative specific reduction rate (1/d)
Ratio of UV power to water volume (W/m3)
0
6
12
18
24
0
2
4
6
8

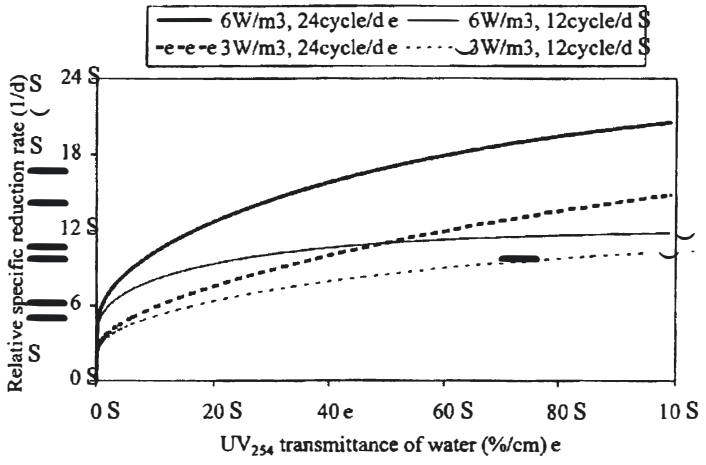
respectively. For a lower daily cycle number, RSRR reached a higher percentage of its maximum. It should be pointed out that the two tested UV units during the experiment were quite new. In reality, the output power of an UV unit depends on its lamp age (U.S. EPA 1986; Darby et al. 1993). For the purpose of UV power design, U.S. EPA (1986) suggested a power efficiency of 0.7 for each UV unit.

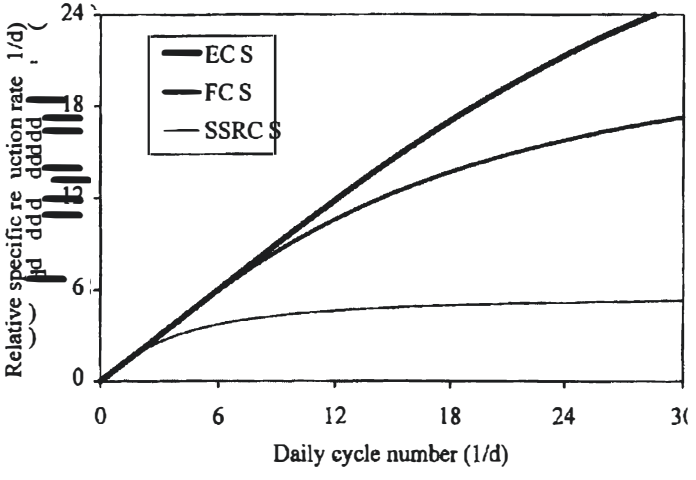
The steady-state relationship between RSRR and water UV_{254} transmittance was plotted in Fig. 8. The reduction rate decreased significantly when the transmittance was less than 20 % cm^{-1}. When the transmittance was above 20 % cm^{-1}, the reduction rate increased slowly with the increase in transmittance, especially for a low daily cycle number. Therefore, it is desirable to maintain the UV_{254} transmittance of the water above 20 % cm^{-1} in order to obtain a satisfactory disinfection efficiency.

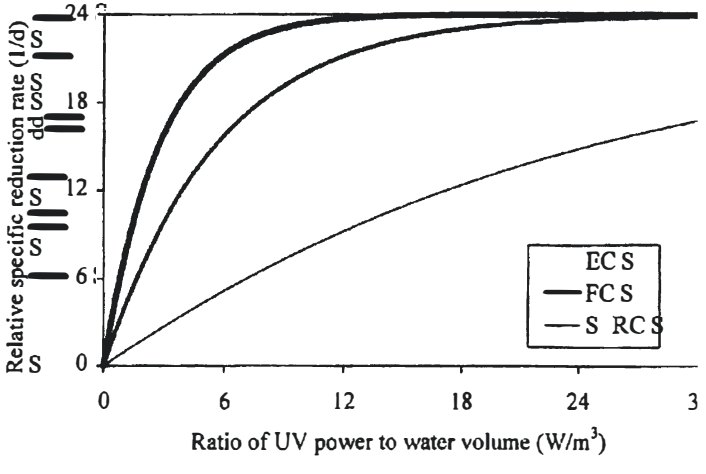
The above modeling results were obtained using the inactivation rate constant of fecal coliform ($k = 0.0062\ m^2\ J^{-1}$). In recirculating aquaculture systems, however, fecal coliform may not be the target for disinfection. Various microorganisms have different survival rates in the UV disinfection process, and thus result in different values of the inactivation rate constant. Table 2 lists some literature values of the parameter for some other microorganism species. Using these k values, respective disinfection effectiveness can be obtained for their species. For the purpose of comparison, Figs. 9 and 10 give the modeling results for two different microorganisms of *Escherichia coli* (EC), spores of sulphite-reducing clostridia (SSRC) by comparison with FC. Clearly, a lower inactivation rate resulted in a lower reduction rate. Fig. 9 indicates that, for the species with a lower inactivation rate, fewer benefits can be obtained through increasing daily cycle number. In this case, UV disinfection efficiency increases more profoundly with increase in UV power input (Fig. 10).

CONCLUSIONS

The experimental results indicated that UV power input, recirculating flow rate and water UV_{254} transmittance were the three most important factors affecting UV disinfection efficiency in a recirculating system.


6W/m3, 24cycle/d e
6W/m3, 12cycle/d
3W/m3, 24cycle/d e
3W/m3, 12cycle/d
Relative specific reduction rate (1/d)
24
18
12
6
0
0
20
40 e
60
80
10
UV254 transmittance of water (%/cm) e


EC
FC
SSRC
Relative specific re uction rate 1/d)
24
18
12
6
0
0
6
12
18
24
Daily cycle number (1/d)


EC
FC
S RC
Relative specific reduction rate (1/d)
24
18
12
6
0
0
6
12
18
24
Ratio of UV power to water volume (W/m3)

The first-order inactivation rate constant (k) is a key parameter in the UV disinfection model. It was determined in this study as $k = 0.0062\ m^2 \cdot J^{-1}$ for fecal coliform.

Simulation results clearly showed the reduction rate of fecal coliform varied with water cycle rate, UV power input and water UV_{254} transmittance. For disinfecting microorganisms with a lower inactivation rate, the model suggests that increasing UV power input is more effective than increasing daily cycle number.

ACKNOWLEDGMENTS

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BOOK REVIEW

Practical Genetics for Aquaculture

C.G. Lutz

Fishing News Books, London. Distributed in the United States by Iowa State University Press, Ames, IA. (2001) xi + 235 pp. ISBN 0-85238-285-5. $US 79.95.

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Genetics is an important and fast-developing discipline within the science and practice of aquaculture. Development and use of a high-performance stock is key to achieving production efficiency and profitability. Although aquaculturists grasp the importance of genetics, most struggle to understand how it might be applied to improve their own production stocks. The struggle is made all the more difficult by the pace and technical complexity of recent developments in aquaculture biotechnology. Hence, many students and practitioners would appreciate a concise and current explanation of genetics as applied to aquaculture. To meet this need, Greg Lutz of Louisiana State University and Fishing News Books have recently published Practical Genetics for Aquaculture.

The intended audience for this book is non-geneticists with an interest in aquaculture, and Lutz strove to achieve a balance between practical application and more technical issues. Strengths of the volume include a straightforward prose style that will prove accessible to a readership with widely varying degrees of familiarity with genetics as a science. The text and case studies offer clear explanations of principles and applications of

aquaculture genetics. The text is supported with black and white pictures and diagrams. Each major chapter includes case studies illustrating application of the principles or techniques described in that chapter to particular species or species groups. Each chapter has its own listing of literature cited, easing the reader's access to further reading. Weaknesses of the volume stem from limited depth on any one topic. Entry into the technical literature will be somewhat more difficult because the literature cited sections for each chapter are not extensive.

A brief description of Practical Genetics for Aquaculture will illustrate its strengths and weaknesses. There are 11 chapters. Chapter 1 provides a brief overview explaining the importance of the field of genetics. After a brief review of basic principles of Mendelian genetics, Chapter 2 addresses qualitative genetics. Coverage of theoretical bases is basic, explaining expression of traits encoded and one and two loci, but does not include explanation of pleiotropy, variable penetrance and expressivity, linkage, and sex linkage. The case studies build on the explanation of theory, and discuss expression of pearl and other colorations in tilapia, coloration patterns in ornamental fishes, and albinism.

The principles and application of quantitative genetics are covered in chapters 3 through 5. Chapter 3 lays out the theory pertaining to partitioning of phenotypic variance in quantitative traits and the estimation of heritability using nested family designs. There is a useful discussion of how the reproductive biology of the species determines which mating designs are most attractive. Case studies focus upon heritability estimation in channel catfish, freshwater prawn, crawfishes, gilthead sea bream, and salmonids. Overall, this is a useful and very accessible chapter. However, absence of key references may frustrate highly interested readers. The classical Introduction to Quantitative Genetics (Falconer and Mackay 1996) is not cited, nor after considerable discussion, are supporting citations for mixed models or software for executing mixed model analyses.

Chapter 4 discusses selection and realized heritability. After further explanation of partitioning phenotypic variance, estimation of heritability using regression or realized heritability designs is presented. More graphics would have helped the reader, for example, in understanding the experimental design for realized heritability. Procedures for multi-trait

and family selection are presented clearly. Some subtleties of experimental designs are not mentioned, e.g., ignoring extreme phenotypes in regression-based analyses, and hence, citations to the more technical quantitative genetics literature would have been useful. A more explicit discussion of founder effects and genetic drift would have supported sections explaining lack of response to selection and conflicting results among selection programs. Useful case studies are presented in the context of brief sections on evaluating available strains, domestication selection, conflicting results, correlated responses, indirect selection, indirect measurement of a trait, environmental tolerances, differences between sexes, and genotype by environmental interactions. The chapter concludes with brief sections on selection for miscellaneous traits on finfishes, mollusks, and crustaceans. Although it would have provided an interesting case study illustrating many of the principles discussed in the chapter, there was no discussion of the prominent Norwegian selection program for Atlantic salmon.

The dominance-based components underlying expression of quantitative phenotypes – inbreeding, crossbreeding and hybridization – are discussed in Chapter 5. After a presentation of theory, there are descriptions of practice, including inbreeding impacts, exploitation of heterosis in production stocks, maternal effects, and breed formation. Case studies include carp, salmonids, monosex hybrids, and invertebrates. Rather cursory presentations are given on calculation of individual inbreeding coefficients and on random genetic drift and effective population size. The latter mentions only the effect of skewed sex ratio, omitting discussion of unequal family size and population bottlenecks, mechanisms of importance that are under the control of aquaculturists. The seminal work of Chevassus (1979) on hybrid salmonids is not mentioned. The seminal work of Kincaid (1976 a,b) demonstrating inbreeding depression in salmonids and estimating the degree of inbreeding in federal and state hatcheries are not mentioned, and his description of rotational line crossing is not referenced for the benefit of interested readers. The discussion of hybrid fish for stocking natural waters does not mention use of splake (brook trout x lake trout), that results are highly location-specific, and that some interspecific hybrids may backcross with parental species in the wild.

Chapters 6 through 10 cover the methods encompassing the rapidly developing field of aquaculture biotechnology. Chapter 6 presents

material on gynogenesis and androgensis, respectively, the production of individuals bearing only maternal or paternal genomes. Subtleties of different methodologies and how they yield different results are well presented. Case studies showing how gynogenesis and androgenesis have been applied to elucidate sex determination, linkage, and partitioning of phenotypic variance are presented. Of the many applications presented, the only omission I noted was the possible application of androgenesis for regeneration of whole fish from cryopreserved sperm. Chapter 7 presents methods and applications for induced polyploidy, the production of individuals with extra sets of chromosomes. Discussion of methods is straightforward, but not simplistic, and well supported with graphics. Case studies are presented on polyploidy in tilapia, cyprinids, salmonids, other finfishes, and bivalves. In what was otherwise a complete chapter, I noted omission of explanation of flow cytometry as a means of evaluating polyploidy, discussion of the pioneering work of Chourrout in inducing polyploidy in salmonids, and mention of commercialization of triploid Pacific oysters and rainbow trout.

Mechanisms of sex determination and control in major aquaculture species are discussed in Chapter 8. Case studies for tilapia, other finfishes, and crustaceans are presented. Flowcharts explaining the steps of sex reversal and progeny testing in indirect methods for sex reversal might have helped the unfamiliar reader. No mention of all-female salmonid stocks was surprising, because all-female production has come to dominate the rainbow trout sector.

Selective breeding and biotechnology both require controlling the reproduction of the species of interest. Chapter 9, covering control and induction of maturation and spawning is the best developed (40 pages) and most fully referenced in the volume. Many practical aquaculturists will appreciate inclusion of this chapter, but purists will question whether the chapter belongs in a genetics book.

The application of gene transfer techniques to aquatic organisms (Chapter 10) starts with a brief presentation on expression vectors and methods for achieving gene transfer. Development and field testing of transgenic lines is mentioned briefly. Case studies cover transgenic Indian catfish, tilapia, Atlantic salmon, and Chinese carp. A number of key works on transgenesis are not mentioned, including the prominent

work of Fletcher and other groups on attempts to improve freeze resistance in Atlantic salmon and other species, and that of the Devlin group on growth enhancement and risk assessment for Pacific salmonids. The development, controversies, and possible commercialization of Atlantic salmon is not mentioned. The author asserts that it is difficult to assess or even speculate on the potential impact of genetically modified organisms on natural systems, although numerous reports have addressed risk assessment and risk management for transgenic aquatic organisms (e.g., ABRAC 1995, Muir and Howard 1999).

Much recent controversy concerns the possible ecological and genetic impacts of stocked or escaped cultured fish upon wild populations. Though it is well that Chapter 11 addresses genetic threats to wild stocks and ecosystems, the treatment is rather shallow and emphasizes the effects of hatchery fish stocked to support sport fisheries, only briefly mentioning the effects of escaped aquaculture stocks on wild populations and ecosystems. The chapter does not discuss the role of aquaculture in the establishment of non-native species, e.g., Atlantic salmon in the west coast of North America, Pacific oyster *C. gigas* in North America and Australia, and tilapia *O. niloticus* on several continents. The chapter lacks references to direct interested readers to landmark publications such as Hindar et al. (1991) or Ryman and Laikre (1991) or symposium volumes such as Schramm and Piper (1995) or Mustafa (1999).

There is a general index listing both topics and species discussed that will ease reader's use of the book. There is, however, no glossary defining key terms. I note the absence of a chapter covering genomic mapping, quantitative trait locus detection, and genetic marker-assisted selection. Although these are not techniques that will be pursued by practicing aquaculturists, it would be well for them to be informed of these developments that will find future application in aquaculture genetics.

Of existing books on aquaculture genetics (Kirpichnikov 1980, Tave 1993, Purdom 1993), this book most resembles Tave's Genetics for Fish Hatchery Managers, now out of print. Lutz's Practical Genetics for Aquaculture will serve many aquaculturists, giving them an entry into principles and applications of genetics in aquaculture, although they will have to read the technical literature if the case studies did not address the species and trait of interest. More serious students or practitioners of

aquaculture genetics will have to supplement this reading with publications from the technical literature and from Falconer and Mackay (1996) or Becker (1992). Teachers of aquaculture genetics will have to supplement this book with assigned readings from the technical literature to convey subtleties of genetic theory, experimental design, and data analysis. Established aquaculture geneticists will be familiar with the theory, but will find the case studies interesting. Overall, I find Practical Genetics for Aquaculture a useful book that I will put to good use in teaching my own aquaculture genetics course.

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Acceptable scientific and common names of fishes are listed in A List of Common and Scientific Names of Fishes from the United States and Canada published by the American Fisheries Society. Scientific names should be italicized (e.g., *Antigonia aurorosea*). Common names may be used throughout each paper, but must be accompanied by a full scientific name when they first appear in a paper. The author must use the full common name, e.g., "yellow perch" not "perch."

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example: Times New Roman 12pt *Italic* **BOLD**
Times New Roman 10pt *Italic* **BOLD**

Subsection headings should be italicized in the manuscript. Spell out one-digit numbers, except when they are used with units of measure – e.g., six ponds, 7 days. Spell out any number that begins a sentence. When two numbers occur sequentially in the text, one of the numbers must be spelled out – e.g., In 2007, fifty fish were stocked. Use commas in numbers of 1,000 or more. Use the 24-hour clock to describe time – e.g., 1300, not 1:00 p.m. Always place a zero to the left of a decimal point if the number is less than one; this includes probability values – e.g., P = 0.05, not P = .05. All units of measurement must be reported via the metric system. Parts per million or milligrams per liter can be reported as either "ppm" or "mg/l," but not as "$mg.1^{-1}$."

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