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

G.W. Glenney*[1], G.S. Libey[2]

[2]Department of Fisheries and Wildlife Sciences
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061 USA

Corresponding author, present address:
[1]*2071 Old Highway 12
Starkville, MS 39759 USA

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.

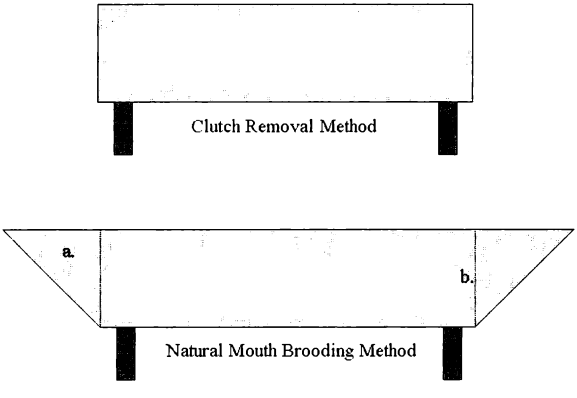

Figure 1. Side view of broodfish tanks- breeding area (2.4 x *1.2* x *0.6 m). a.- Sloped area for fry to congregate. b.- 38 mm mesh net barrier.*

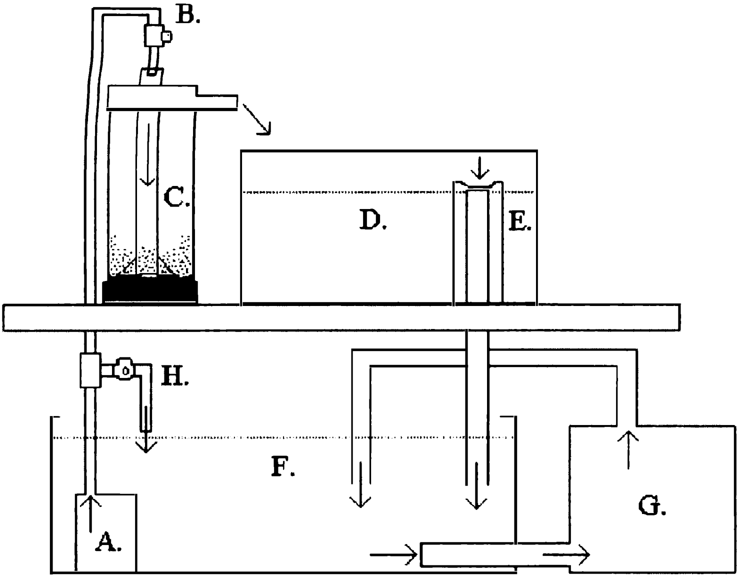

Figure 2. Side view of hatchery system used to incubate tilapia eggs. Arrows indicate the direction of water flow. A.- Pump. B.- 1.27 cm ball valve. C.- Seven liter round-bottom hatching jar. D.- Swim-up fry catch basin. E.- Stand pipe and fry mesh screen. F.- Water reservoir. G.- Activated carbon filter, 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 ($p<0.02$).

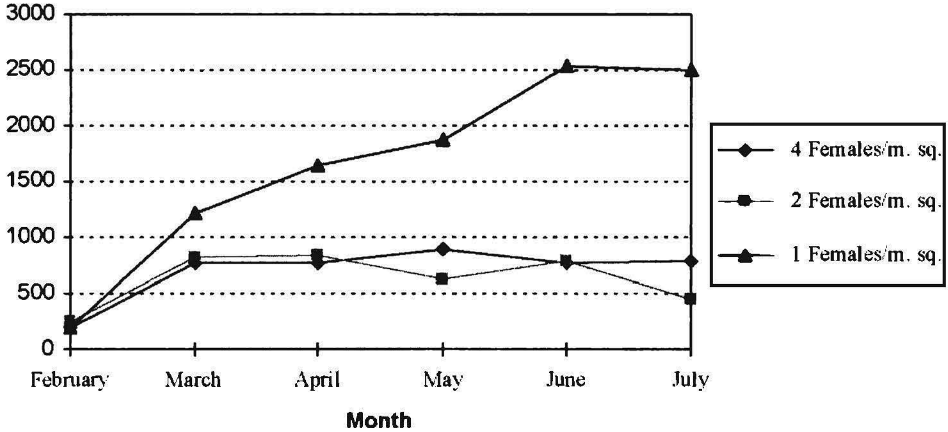

Table 1. Broodstock tank water quality parameter means, ± S.E., and ranges by density treatment. (D.O., Temp. (°C), pH, TAN, NH_3, NO_2, NO_3, Alkalinity, Hardness (mg/l), Absorbance (at 750 nm). No significant difference between treatments was observed ($p>0.05$).

	1 Female/m^2			2 Female/m^2			4 Female/m^2		
	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/m^2				**Two Females/m^2**				**Four Females/m^2**			
	Fry/female		**Fry/m^2**		**Fry/female**		**Fry/m^2**		**Fry/female**		**Fry/m^2**	
	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.

REFERENCES

Abdelghany, A.E. Food and Feeding Habits of Nile Tilapia From the Nile River at Cairo, Egypt. In Proceedings of the First International Conference on Fish Farming Technology, Reinersten, H., Dahle, L. Jorgensen, A., Tvinnereim, K. Eds., **1993**. Trondheim, Norway.

Bautista, A.M., Carlos, M.H., San Antonio, A.I. Hatching Production of *Oreochromis niloticus* L. at Different Sex Ratios and Stocking Densities. *Aquaculture* **1988**. *73*, 85-95.

Berrios-Hernandez, J.M., Snow, J.R. Comparison of Methods for Reducing Fry Losses to Cannibalism in Tilapia Production. *The Progressive Fish Culturist* **1983**. *45*, 116-118.

Buttner, J., Soderberg, R., Terlizzi, D. **1993**. An Introduction to Water Chemistry in Freshwater Aquaculture. Northeastern Regional Aquaculture Center P-4. University of Massachusetts, Dartmouth, MA, USA.

Daud, S. Hasbollah, D., Law, A. Effects of Unionized Ammonia on Red Tilapia (*O. mossambicus* x *O. niloticus* hybrid) Fry. In Second International Symposium In Aquaculture. Pullin R.S.V., Bhukaswan, T., Tonguthai, K., Maclean, J.L., Eds. **1988**. Bangkok, Thailand.

Dambo, W.B., Rana, K.J. Effects of Stocking Density on Growth and Survival of *Oreochromis niloticus* (L.) Fry in the Hatchery. *Aquaculture and Fisheries Management* **1992**. *23*, 71-80.

Don, J., Koiller, M., Yeheskel, O., Avtalion, R.R. Increased Tilapia Viability Using Ultraviolet Irradiation in a Closed Recirculating Zugar-bottle System. *Aquacultural Engineering* **1987**. *6*, 69-74.

Piper, R.G., McElwain, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G., Leonard, J., Eds. **1984**. Fish Hatchery Managment, pp 60-63, 406-467. U.S. Department of the Interior, Fish and Wildlife Service. Washington, D.C., USA.

Guerrero, R.D., Guerrero, L.A. Further Observations on the Fry Production of *Oreochromis niloticus* in Concrete Tanks. *Aquaculture* **1985**. *47*, 257-261.

Head, W., Watanabe, W.O. Economic Analysis of a Commercial Scale, Recirculating, Brackish Water Hatchery for Florida Red Tilapia. *Journal of Applied Aquaculture* **1995**. *5*, 1-23.

Lovell, T. Feeding Tilapia. *Aquaculture Magazine* **1980**. *7*, 42-43.

Lovshin, L.L., Ibrim, H.H. Effects of Broodstock Exchange on *O. niloticus* Egg and Fry Production in Net Enclosures. In Second International Symposium on Tilapia in Aquaculture, Pullin R.S.V., Bhukaswan, T., Tonguthai, K., Maclean, J.L., Eds. **1987**. Bangkok, Thailand.

Rana, K.J. An Evaluation of Two Types of Containers for the Artificial Incubation of *Oreochromis* Eggs. *Aquaculture and Fisheries Management* **1986**. *17*, 139-145.

Rana, K.J. Reproductive Biology and the Hatchery Rearing of Tilapia Eggs and Fry. In Recent Advances in Aquaculture (Volume 3), Muir, J.F., Roberts, J., Eds. **1988**. Timber Press: Portland, OR, USA.

Rothbard, S., Hulata, G. Closed-system Incubator for Cichlid Eggs. *The Progressive Fish Culturist* **1980**. *42*, 203-204.

Rothbard, S., Pruginin, Y. Induced Spawning and Artificial Incubation of Tilapia. *Aquaculture* **1975**. *5*, 315-321.

Santiago, C.B., Aidaba, M.B., Abuan, E.F., Laron, M.A. The Effects of Artificial Diets of Fry Production and Growth of *Oreochromis niloticus* Breeders. *Aquaculture* **1985**. *47*, 193-203.

Siraj, S., Smitherman, R.O., Castillo-Gallusser, S., Dunham, R. Reproductive Traits for the Three Year Classes of *Tilapia nilotica* and Maternal Effects on their Progeny. In International Symposium on Tilapia in Aquaculture, Fishelson, L., Yaron, Z., Eds. **1983**. Nazareth, Israel.

Smith, S.J., Watanabe, W.O., Chan, J.R., Ernst, D.H., Wickland, R.I., Olla, B.L. Hatchery Production of Florida Red Tilapia Seed in Brackish Water Tanks: The Influence of Brood Stock Age. *Aquaculture and Fisheries Management* **1991**. *22*, 141-147.

Snow, J.R., Berrios-Hernandez, J.M., Ye, H. A Modular System for Producing Tilapia Seed Using Simple Facilities. In International Symposium on Tilapia in Aquaculture, Fishelson, L., Yaron, Z., Eds. **1983**. Nazareth, Israel.

Clesceri, L., Greenburg, A., Trussell, R., Eds. **1992**. Standard Methods for the Examination of Water and Wastewater (17th edition), pp 731-733, 1072-1074. American Public Health Association. Washington, D.C., USA.

Stickney, R.R. **1994**. Principles of Aquaculture pp 260-262. John Wiley & Sons, Inc.: New York, NY, USA.

Subasinghe, R.P., Summerville, C. Disinfection of *Oreochromis mossambicus* (Peters) Eggs Against Commonly Occurring Potentially Pathogenic Bacteria and Fungi Under Artificial Hatchery Conditions. *Aquaculture and Fisheries Management* **1985**. *16*, 121-127.

Subasinghe, R.P., Summerville, C. Effects of Temperature on Hatchability, Development and Growth of Eggs and Yolksac Fry of *O. mossambicus* (Peters) Under Artificial Incubation. *Aquaculture and Fisheries Management* **1992**. *23*, 31-39.

Varadaraj, K., Kumari, S., Pandian, T. Comparison of Conditions for Hormonal Sex Reversal of Mossambique Tilapias. *The Progressive Fish Culturist* **1994**. *56*, 81-90.

Verdegem, M.C., McGinty, A.S. Effects of Frequency of Egg and Fry Removal on Spawning by *Tilapia nilotica* in Hapas. *The Progressive Fish Culturist* **1987**. *49*, 129-131.

Watanabe, W.O., Smith, S., Wickland, R., Olla, B. Hatchery Production of Florida Red Tilapia Seed in Brackish Water Tanks Under Natural Mouth Brooding and Clutch Removal Methods. *Aquaculture* **1992**. *102*, 77-88.