EFFECT OF STOCKING DENSITY ON SUNSHINE BASS (*MORONE CHRYSOPS* X *M. SAXATILIS*) PRODUCTION AND WATER QUALITY AT MINOR CLARK FISH HATCHERY, KENTUCKY.

A Thesis

Presented to

the Faculty of the College of Science and Technology

Morehead State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

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December 15, 2004

Accepted by the faculty of the College of Science and Technology, Morehead State University, in partial fulfillment of the requirements for the Master of Science degree.

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15 DEC 2004

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The sunshine bass (*Morone chrysops* X *M. saxatilis*) has recently become a popular game fish, increasing demand for hatchery production. These hybrids however, have a low percent survival when reared in hatcheries. In similar instances with other fish species facing the same dilemma hatcheries have tried varying stocking densities to increase production. At Minor Clark Fish Hatchery, Morehead, Kentucky, six randomly selected 0.41 ha ponds were stocked with varying densities of five-day old fry. On May 28, 2003, we stocked three ponds at a low density (250,000 fry/pond) and three at a high density (450,000 fry/pond). Biweekly monitoring of nutrient concentrations and zooplankton took place over the seven-week study period. Along with the biweekly sampling, DO, pH, specific conductivity, and temperature were monitored every 15 minutes. Survival in the low-density ponds was higher than that of the high-density ponds (22.1% vs 14.8%), but this was not significantly different due to high variability. Survival rates from both stocking densities were comparable to those of most published results of this hybrid. There were no significant improvements or degradations of water quality due to the

differing stocking densities. Production was probably mostly affected by zooplankton production.

Accepted by: ______________________, Chair

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Acknowledgments

First and foremost I would like to thank Dr. Brian Reeder for his unending patience and guidance throughout this process. Many thanks are also extended to the other members of my committee, Dr. David Eisenhour and Dr. David Smith, for their assistance with the production of this manuscript. I would like to thank Rod Middelton, Minor Clark Fish Hatchery, for his guidance and use of the hatchery's facilities during the project. I would also like to thank Kasey Caldwell and Josh Hunt for their assistance in collecting samples and laboratory analysis. Lastly I would like to thank my family and friends, without them this project could not have been completed.

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1.0 Introduction

Growing popularity in hybrid striped bass fishing has led to an increased demand for production of hybrid striped bass in hatcheries. The sunshine bass (*Morone chrysops* X *M. saxatilis*) has become a popular sport fish, especially in the southeast United States, because they are hardy and grow fast (Hodson 1989, Harrell et al. 1990). Carlberg et al. (2000) estimated that hybrid striped bass accounted for $28 million of the U.S. food fish crop, which would place it as the fourth most valuable food fish in the U.S. Webster et al. (2000) estimated that annual production of sunshine bass in 1990 was around 636,000 kg, increasing to 3.8 million kg in 1997. In 1995 sunshine bass were being cultivated by 83% of fish growers in the U.S. (Denson and Smith 1997). These fish thrive in slow moving streams, large reservoirs, lakes, and ponds (Hodson 1989). Sunshine bass do well in large reservoirs because they are piscivorous and most reservoirs provide sufficient gizzard shad (*Dorosoma cepedianum*) and threadfin shad (*D. petenense*), which are the preferred prey items for these fish (Hodson 1989).

Survival of hybrid striped bass fry in hatchery systems has varied among hybrid species and is lower than that of other species. Initially, the sunshine bass was not considered a good candidate for hatchery production because of the adhesiveness of the white bass eggs, which makes determination of fertilization and observation of embryonic development difficult (Harrell et al. 1990), and small fry size (typically 4-6 mm [Ludwig 1997]). Improvements of culture techniques including: 1) the use of human chorionic gonadotropin which is more effective economical, and readily available (Hodson and Hayes 1989) rather than other common hormones used to stimulate ovulation; 2) availability and more successful induced spawning of white bass females as compared to striped bass females (Harrell et al. 1990, Denson and Smith 1997); 3) improvements in stocking procedure with regards to when ponds are filled prior to stocking (Ludwig 1997); and 4) advances in zooplankton and fertilization management techniques that increase fry survival (Hodson and Hayes 1989, Harrell et al. 1990). With these advances sunshine bass survival rates have risen from <10% to an average of 10-25% (Hodson and Hayes 1989, Hodson and Jarvis 1990, Ludwig 2002). Ludwig (2000) found that fry spawned from June to July in Arkansas, had survival rates above 25%, as compared to fry spawned within the normal spawning period of the two species involved in the cross, of mid- March to May.

Palmetto bass (*Morone saxatilis* X *M. chrysops*) which are a slightly larger hybrid, with fry ranging from 6-9mm, have typical survival percentages of 45%, where sunshine bass (*M. chrysops* X *M. saxatilis*) have a typical survival percentage of 10-25% (Hodson and Hayes 1989, Hodson and Jarvis 1990, Ludwig 2002) in earthen ponds; however in indoor water recirculation systems sunshine bass have had increased survival and growth compared to palmetto bass (Kelly and Kohler 1996). Sunshine bass are more desirable because of the availability of brood fish and ease of spawning compared to palmetto bass (Harrell et al. 1990, Denson and Smith 1997). Determining ways to increase the survival percentages of sunshine bass, up to levels comparable to those of the palmetto bass, would be advantageous for fisheries managers. One aspect examined in other species is altering stocking densities to maximize survival. Increases in fish production due to increased stocking density have been shown in hybrid sunfish (*Lepomis cyanellus* X *L. macrochirus*) (Tidwell et al. 1994), and also in Atlantic cod (*Gadus morhua*) (Baskerville-Bridges and Kling 2000), while no or negative effects of increasing stocking density on fish production have been shown in sea bass (*Dicentrarchus labrax*) (Kestmont et al. 2003), South American trout (*Brycon cephalus*) (Gomes et al. 2000), and largemouth bass (*Micropterus salmoides*) (Kubitza and Loushin 1997).

Understanding pond ecosystem dynamics is critical, especially given variability in hatchery data. Without this information it is hard to determine what are the controlling factors for fry survival in hatchery ponds. Other studies have looked at the effect of water quality on trophic structure of hatchery ponds, but have not looked at this aspect in respect to stocking density.

This study will determine the effect that sunshine bass stocking density has on hatchery ponds ecosystems by examining nutrient concentrations, primary productivity, zooplankton community structure and abundance, as well as periphyton community structure and abundance. An understanding of these variables should provide insight on the effect of fish stocking density on water chemistry and fry survival. The objective of this study is to see if different stocking densities of sunshine bass fry have a significant effect on these variables, as well as overall survival of the fry.

2.0 Literature Review

The sunshine bass is a hybrid striped bass formed by the cross of a female white bass (*M. chrysops*) and a male striped bass (*M. saxatilis*). The genus *Morone* belongs in the family *Moronidae* and the order *Perciformes* (Hodson 1989, Page and Burr 1991). The sunshine bass is silver, with continuous stripes above the lateral line and broken stripes below the lateral line, attains a maximum weight of 10 kg, and has a life span of 5-6 years (McClane 1978, Hodson 1989). The sunshine bass was first produced in South Carolina by Bayless in 1969, but was not successfully cultured until 1973 in Florida (Harrell et al. 1990).

The current accepted method for preparing ponds for the stocking of sunshine bass is to fill ponds five days or fewer prior to stocking (Ludwig 1997), and stocking between 250,000-500,000 fry/hectare (Hodson and Hayes 1989). The normal striped bass pond schedule (filling 2-3 weeks prior to stocking) proved inadequate because rotifers needed for food for the small sunshine bass fry did not bloom at the appropriate time (Ludwig 1993), but predators, such as back swimmers, water boatmen, diving beetles, dragon fly larvae, and water scorpions, did establish on this filling schedule (Ludwig 1997). Culver et al. (1993) and Culver et al. (1988) found that in walleye ponds it was important to stock immediately after filling due to a decline in phytoplankton and zooplankton that occurs in hatchery ponds after 4-5 weeks, Geiger et al. (1985) also noted this trend in striped bass ponds.

Five-day old fry are stocked because the fry have fully formed mouthparts and a complete gut by the fourth day after hatching (Harrell et al. 1990). By this time the fry still have some yolk sac remaining but are also capable of grazing upon rotifers (Ludwig 1997). The stocking density of these fry can vary from 250,000-500,000 fry/hectare (Hodson and Hayes 1989). These fry remain in the ponds for a period of 30-45 days (Hodson and Hayes 1989, Dunning and Daniels 2001). After this time the fingerlings reach a size range of 2.5-6.1 centimeters and are removed from the culture ponds (Harrell et al. 1990).

Many hatcheries experiment with differing stocking rates to try to increase survival of the fish species being raised, as well as size of fish. Tidwell et al. (1994) found that stocking large juvenile hybrid sunfish (*Lepomis cyanellus* X *L. macrochirus*) at 12,350 fish/ha, led to the production of more harvestable fish per hectare (> 110 g/ha). Baskersville-Bridges and Kling (2000) found that between two different stocking densities of Atlantic cod (300 larvae/L and 150 larvae/L) that fish stocked at high densities had significantly higher survival percentages than those stocked at low densities (41.6% vs. 29.4%), but found no significant difference when four stocking densities (50, 100, 200, 300 larvae/L) were used. Kestmont et al. (2003) found that perch (*Perca fluviatilis*) larvae have survival percentages proportional to stocking densities, but post-larval perch had increased survival at low to intermediate stocking. Kestmont et al. (2003) also found that there was no significant difference in survival of sea bass (*Dicentrarchus labrax*) larvae at differing stocking densities, but post-larval sea bass had decreased survival with increased stocking density. Gomes et al. (2000) found that differing stocking densities of 30, 60, and 120 larva/m^2, did not have a significant effect on survival of

South American trout (*Brycon cephalus*) but did have a negative impact on length of fish harvested. Rowland et al. (2004) found that silver perch (*Bidyanus bidyanus*) survival was not significantly affected by stocking density when fish were stocked at 50, 100, and 200 fish/m^2. Myers et al. (1996) found that walleye (*Stizostedion vitreum)* survival decreased with increased stocking density. Kubitza and Lovshin (1997) found that largemouth bass (*Micrpoterus salmoides*) stocked at 2470 and 7410 fish/ha showed no significant difference in percent survival (81% vs. 76%).

Improved understanding of water quality and nutrients that are needed for successful sunshine bass culture also has led to improved survival from fry to fingerling life stage (Table 1). Organic and inorganic fertilizers are used to enhance water quality and nutrients in hatchery ponds (Culver et al. 1993, Harrell et al. 1990, Ludwig 1997, Ludwig 2000, and Ludwig 2002). By increasing available nutrients in hatchery ponds, primary production can increase, which can lead to an increase in production of zooplankton utilized as a food source for fry, and increasing fingerling production (Ludwig 2002).

Pond fertilization is a key component of sunshine bass fry survival. Combinations of inorganic and organic fertilizers are used to increase zooplankton concentrations in hatcheries (Ludwig 2002). Fertilizers that are used have low nitrogen to phosphorus ratios (Culver et al. 1993) as well as a low carbon to nitrogen ratios (Hodson and Hayes 1989). The low N:P ratio is important because high phosphate levels favor filamentous algae, which are beneficial to fry production (Culver et al. 1993). However a sufficient N:P ratio must be maintained in order to

Table 1. Recommended hybrid striped bass water quality levels.

Variable	Bonn et al. (1976)	Daily and Economon (1983)	Hodson (1989)	Ludwig (1997)	Ludwig (2000)
Temperature (°C)	14 - 24		4-33	18-24	20
PH	6.5 - 8.5		2.5-8.5		
Dissolved Oxygen (mg/L DO)	Saturation	75 % sat.	1-12	> 5	
Alkalinty	0 - 5		20-100		
Salinity (ppt)			0-25		
NO_3 (mg/L)	< 2.0				
NO_2 (mg/L)	< 0.2				
NH_4 Un-ionized (mg/L)	< 0.02				

provide enough nitrogen and phosphorus in useable forms to allow for fast colonization of bacteria and algae that are food sources for zooplankton (Hodson and Hayes 1989).

Organic fertilizers must provide carbon, nitrogen, and phosphorus, which are needed for primary and secondary production of zooplankton (Hodson and Hayes 1989). Organic fertilizers increase primary production by supplying nutrients for phytoplankton uptake (Kurten et al. 1999). Organic fertilizers are commonly used because they decay slowly which leads to more sustained production of zooplankton (Hodson and Hayes 1989). Common organic fertilizers used for hybrid striped bass cultivation include cottonseed meal, Bermuda hay, and alfalfa pellets (Hodson and Hayes 1989, Ludwig 2002). Kurten et al (1999) found that ponds used for largemouth bass cultivation in Florida, treated with cottonseed meal had higher production in terms of fish size, and a higher density of zooplankton than ponds that were not treated with cottonseed meal. Common inorganic fertilizers utilized by hatcheries are mixtures consisting of 10-30-0, nitrogen-phosphate-potash (Ludwig 2002). Inorganic fertilizers should contain ammonium nitrate at a concentration of 52% N, and phosphoric acid at a concentration of 32% P_2O_2 (Hodson and Hayes 1989).

Fertilization regimes need to be closely monitored. Heavy fertilization can lead to increases in primary productivity, which can greatly increase respiration and decomposition rates. These increased respiration and decomposition rates can lead to decrease in dissolved oxygen (Ludwig 2002, Middelton and Reeder 2003). If dissolved oxygen concentrations fall below 5 mg/l or 75 % saturation, aeration must occur in order to prevent a fish kill (Daily and Economon 1983, Harrell et al. 1990, Ludwig 1997). A second concern with fertilizer application is that increased concentrations of nitrogen and phosphorus lead to increased ammonia formation and photosynthesis, which can lead to increased pH levels and increased concentrations of un-ionized ammonia, which is harmful to sunshine bass fry at concentrations above 0.02 mg/l (Bonn et al. 1976).

Along with fertilization, zooplankton management is key to increasing the survival of sunshine bass fry in hatchery ponds. As fry grow to fingerling size their feeding habits follow the succession of the zooplankton community (Ludwig 1997). Having the appropriate sized zooplankton at the appropriate time during the rearing period is crucial. Typical hatchery pond zooplankton succession follows the pattern of initial rotifer community, followed by a crustacean community, and completed with the final succession stage (Harrell et al 1990). Average sizes for zooplankton found in each stage of succession are: rotifers <0.1 mm-1.0mm, adult copepods <0.5 mm-2.0 mm, and adult cladocerans 0.2-18 mm (Ludwig 2002).

The initial rotifer community occurs 7-14 days after the pond has been filled (Harrell et al. 1990 and Ludwig 1997). This stage is crucial because small sunshine bass fry rely on rotifers as their main food source in early stages of growth (Hodson and Hayes 1989, Ludwig 1997). The most common rotifer genera found in this stage are *Brachionous, Keratella,* and *Polyarthra*. Fourteen to 28 days following pond filling rotifer densities start to decline and concentration of copepods and cladocerans increases (Harrell et al. 1990, Ludwig 2000). This shift from rotifers to crustacean zooplankton marks the crustacean community. Cladocerans along with cyclopoid and calanoid copepods are common during this stage of succession (Harrell et al. 1990). The final stage of zooplankton succession occurs between 28-45 days after pond filling. While in the final stage both cladocerans and copepod densities quickly decline, and rotifers begin to dominate again. This is mainly a response to increased grazing by the fingerlings. Cannibalism may occur in the later stages of zooplankton succession if there is a great size difference among the fingerlings, and will subsequently reduce fry survival (Harrell et al. 1990).

Proper zooplankton management can help delay and even prevent the onset of the final stages of zooplankton succession. By appropriately fertilizing ponds to increase zooplankton food sources, and performing selective inoculation of zooplankton to ensure there is a proper initial stock of zooplankton in your hatchery ponds, four major goals of zooplankton management can be obtained: 1) minimized duration and impact of initial rotifer community, 2) rapid development of crustacean zooplankton community, 3) maintenance of largest possible crustacean biomass 10-20 days after pond filling and stocking, and finally 4) delayed onset of the final stages of zooplankton succession (Harrell et al. 1990).

At Minor Clark Hatchery, Morehead, Kentucky sunshine bass fry were stocked at two different densities (250,000 and 450,0000 fish/acre) to determine if stocking density had an affect on overall percent survival of the fry. Along with looking at percent survival as a result of stocking density, the effect that stocking density had on fourteen different water quality variables was also monitored. Water quality was monitored in order to determine whether or not stocking density would have an adverse effect on the variables analyzed that possibly could effect fry survival.

3.0 Materials and Methods

3.1 Study Site

Minor Clark Fish Hatchery (MCFH), Morehead, Kentucky, is situated on 122 ha of the Licking River alluvial flood plain. Cave Run Lake Reservoir is the water source for MCFH. Water can be drawn from three different levels of the reservoir to control dissolved oxygen (DO) and temperature (Kentucky Department of Fish and Wildlife Resources 1976, Middelton and Reeder 2003). Culture ponds used in the study had a surface area of 0.41 ha, and depths that ranged from 1.5 m in the "kettle" end to 0.6 m in the shallow end.

3.2 Pond Preparation and Stocking Regime

Six randomly selected ponds were filled from 25 May 2003 through 28 May 2003. On 26 May 2003, each of six ponds were fertilized with 150 lbs of soy. An additional 250 lbs of alfalfa were added per pond on 27 May 2003. After pond filling was completed on 28 May 2003, five-day-old sunshine bass fry were stocked. Ponds 1, 39, and 47 were stocked with 250,000 fry. Ponds 9, 40, and 62 were stocked with 450,000 fry.

3.3 Water Sampling Method

Duplicate integrated water column samples were taken from each pond biweekly from 28 May 2003, through 12 July 2003, and brought back to the laboratory within 24 hours of collection. Field measurements included Secchi depth (using a 20 cm diameter black and white disk), temperature, DO, pH, and specific conductivity profiles were taken with a recently calibrated YSI 600 XLM Multi

Parameter Water Quality Monitor datasonde. Recording YSI 600 XLM Multi Parameter Water Quality Monitor datasondes were suspended at a depth of 0.75 m in all six ponds to record temperature, DO, pH, and specific conductivity every 15 minutes throughout the duration of the experiment. Insolation (photosynthetic active radiation) was collected on site at Pond 39 by the use of a LI-COR LI-1000 datalogger light meter. Submersible photometers were placed at the top of the "kettle" of Pond 39 and suspended in the pond at a depth of 0.75 m. The light meter collected incident light data for the entire hatchery.

3.4 Water Chemistry

Water samples were analyzed for chlorophyll *a* flourometrically using a Turner model 10-AU flourometer after alkalized acetone extraction (Arar and Collins 1997). Alkalinity was determined by titration of with 0.02 N H_2SO_4 to an endpoint of 5.2 (Larson and Henely 1955, Wetzel and Likens 1991). Soluble reactive phosphorus (SRP) was determined using the ascorbic acid method, while total phosphorus was anlalyzed as SRP after perchloric acid digestion (Sommers and Nelson 1972). Nitrate was analyzed as nitrite after cadmium reduction (Henrikson and Selmer-Olsen 1970, Nydahl 1976). Nitrite was analyzed by the use of the sulfanimide method (Henrikson and Selmer-Olsen 1970, Nydahl 1976). Ammonium was analyzed using Nesselerization (Jenkins 1967). Total organic nitrogen (TON) and total organic carbon (TOC) data was collected with the use of a Perkin Elmer Series II CHNS/O 2400 Analyzer (Culmo et al. no date).

3.5 Zooplankton Analysis

Zooplankton were collected with a 12-L Schindler Patalis plankton trap, with a 64-micrometer mesh net. Samples from each site were preserved using 2% diluted formalin (Wetzel and Likens 1991) and stained with a drop of Rose Bengal. Zooplankton samples were counted in two increments: 10 mL and 1 mL samples to ensure both small and large species were enumerated. Ten mL samples were counted with the use of a 10 mL counting wheel with dissecting microscope at 30x magnification. One mL samples were counted with the use of a 1-mL Sedgwick-Rafter cell and compound light microscope at 150x magnification (Wetzel and Likens 1991). Identification of zooplankton to the genus level was accomplished using Pennak (1989). Zooplankton sample counts were calculated by use of equations from Wetzel and Likens (1991).

3.6 Periphyton Analysis

Periphyton were collected and analyzed from the six sample ponds. Periphyton samplers, consisting of eight suspended 2.5x7.6 cm glass slides, were placed in the kettle ends of each pond for two-week intervals. Glass slides are used because they are inexpensive, have a uniform surface, and periphyton are easily removed (Aloi 1990, Cronk and Fennessy 2001). Two sample periods were recorded for the study. Four slides were removed from each sampler after each two-week period and preserved in 2% formalin for counting. Four more slides also were removed at the same time and placed in 90% acetone and used for chlorophyll *a* analysis.

Periphyton counts were performed by scraping the contents of each glass slide preserved in formalin into a 1-mL Sedgwick-Rafter cell. Identification to the genus level of the contents of the preserved slides was completed using Jahn et al. (1949), Prescott (1978), and Pennak (1989). Chlorophyll *a* analysis of the periphyton was performed by scraping a 2.54 cm^2 area of each slide preserved in 90% acetone. The material scraped from the slide was placed in a centrifuge tube with 10 mL of 90% acetone and centrifuged for 20 minutes. The supernate was poured into a 1 cm cuvette and analyzed flourometrically.

3.7 Fish Density Determination

Initial stocking densities were determined by hatchery estimates and sample counting (Middelton, personal communication). Sample counting involves taking a sample of fry and placing them in a calibrated beaker filled with a known amount of water until 25 mL of water is displaced, the number of fry needed to displace 25 mL is then counted. With this estimate, hatchery personnel are able to ensure that the number of fry that are to be placed in a certain pond is correct. Final density was determined using a sample count, similar to the method used for fry.

3.8 Statistical Analysis

Statistical analysis was performed using a Kruskal-Wallace test. This procedure was chosen because the data were non-parametric as determined by a normality test. The software used for statistical analysis was Minitab Student 12.

4.0 Results

There were no significant differences between high- and low-density stocked ponds for the fourteen water quality parameters measured, nor in fish survival. Individual parameters did show some seasonal trends and differences between ponds; however, ecologically all ponds functioned similarly as indicated by the trends depicted in Figures 1-19b. Zooplankton trends were also similar between high- and low-density stocked ponds, with rotifers dominating the zooplankton communities of both. Each pond varied somewhat in zooplankton density and dominance, but no differences could be attributed to fish density. Similarly, no significant differences were observed in water quality or fish survival between high- and low-density ponds; however there was still variability in fish yields between individual ponds.

4.1 Conductivity and Temperature

Seasonal specific conductivity for both high- and low-density stocked ponds followed a similar pattern of gradual increase over the study period, with low-density ponds starting with a mean of 161 μS/cm and ending with 209 μS/cm; high-density ponds starting at a mean of 158.6 μS/cm and ending with 231.2 μS/cm. High-density ponds started to have higher mean averages on 7 June 2003 and continued until the end of the sampling period (Figure 1). High-density stocked ponds had a higher mean conductivity than the low-density stocked ponds (Table 2). Specific conductivity readings that were measured every 15 minutes throughout the sampling period show a similar pattern of gradual increase over time (Figure 2), similar to the pattern seen in the seasonal measurements. High-density ponds had a higher daily

Table 2. Average nutrient data for MCFH 2003 sunshine bass ponds. P-values represent results of Kruskal-Wallace tests.

Variable	High-density mean +/- SEM	Low-density mean +/- SEM	p-values
Conductivity (µS/cm)	196.42 +/- 8.72	183.47 +/- 6.37	0.233
Conductivity(µS/cm*)	210.70 +/- 0.31	168.40 +/- 0.30	0.317
Temperature (°C)	23.21 +/- 0.76	23.29 +/- 0.81	0.691
Temperature (°C *)	24.00 +/- 0.03	24.20 +/- 0.03	0.317
NH_4^+ (µg/L)	223.48 +/- 66.13	276.74 +/- 85.89	0.895
NO_3 (mg/L)	430.65 +/- 108.48	437.79 +/- 88.89	1.00
NO_2^- (mg/L)	0.04 +/- 0.02	0.04 +/- 0.02	0.930
TON (mg/L)	15.03 +/- 2.12	12.38 +/- 1.75	0.453
SRP (mg/L)	6.56 +/- 0.74	9.32 +/- 1.65	0.200
TP (mg/L)	77.83 +/- 9.68	72.54 +/- 9.18	0.691
Alkalinity (mg/L)	62.12 +/- 5.83	58.13 +/- 4.54	0.480
TOC (mg/L)	143.32 +/- 21.36	125.43 +/- 18.11	0.171
DO at 0.1 m	7.46 +/- 0.32	7.00 +/- 0.24	0.331
DO at 1.0 m	7.15 +/- 0.34	6.78 +/- 0.21	0.627
DO at 1.5 m	5.37 +/- 0.76	5.61 +/- 0.41	0.757
DO at 1.0 m *	8.50 +/- 0.03	8.30 +/- 0.03	0.317
pH	7.45 +/- 0.05	7.33 +/- 0.04	0.093
Daily change pH	0.31 +/- 0.08	0.22 +/- 0.08	0.270
pH *	7.00 +/- 0.004	7.60 +/- 0.004	0.317
Chlorophyll *a* (µg/L)	119.28 +/- 22.22	82.88 +/- 13.74	0.064
Secchi depth (cm)	83.75 +/- 7.29	96.55 +/- 8.82	0.233

* Denotes data that was collected every 15 minutes by the recording datasondes.

Table 3. Averages for individual ponds MCFH 2003 sunshine bass ponds.

	High pond			Low pond		
Variable	9	40	62	1	39	47
Conductivity (µS/cm)	202.8	198.3	188.2	173.1	198.4	184.4
Conductivity(µS/cm*)	218.7	189.2	197.1	151.5	196.1	157.4
Temperature (°C)	23.1	23.1	23.5	23.6	23.1	23.5
Temperature (°C *)	23.8	24.0	24.1	24.4	24.0	24.2
NH_4^+ (µg/L)	182.3	280.8	207.3	33.1	257.4	239.8
NO_3 (mg/L)	409.8	363.8	539.8	427.8	446.0	418.3
NO_2^- (mg/L)	0.052	0.045	0.033	0.049	0.039	0.042
TON (mg/L)	13.7	19.5	11.9	8.9	15.9	12.3
SRP (mg/L)	5.5	8.1	6.1	13.9	6.5	7.6
TP (mg/L)	65.9	88.0	79.7	70.1	81.7	65.8
Alkalinity (mg/L)	67.4	60.1	58.8	50.9	63.6	60.0
TOC (mg/L)	170.6	133.2	126.2	93.1	151.3	131.9
DO 0.1 m	7.0	7.5	7.9	6.7	8.0	6.3
DO 1.0 m	6.7	7.3	7.5	6.4	7.9	6.0
DO 1.5 m	5.2	5.5	5.5	5.8	5.9	5.2
DO 1.0 m *	8.2	9.0	8.2	7.9	10.1	6.8
pH	7.5	7.4	7.5	7.2	7.5	7.2
Daily change pH	0.14	0.40	0.41	0.21	0.29	0.14
pH *	7.9	7.8	7.9	7.5	8.0	7.4
Chlorophyll *a* (µg/L)	78.4	149.8	129.7	46.0	146.2	56.4
Secchi depth (cm)	82.2	91.0	78.1	107.3	82.6	99.8

* Denotes data that was collected every 15 minutes by the recording datasondes.

change in specific conductivity (0.029 μS/cm vs. 0.007 μS/cm), as well as a higher mean conductivity (Table 2).

Seasonal temperature for both high- and low-density stocked ponds was similar throughout the study, showing a gradual increase ranging from 20°C up to 27°C (Figure 3) as a result of increased air temperature. Mean temperature was highest in the low-density stocked ponds, but this was by less than 1°C (Table 2). Temperature that was recorded every 15 minutes shows that both high- and low-density stocked ponds had starting temperatures on 28 May 2003 of roughly 20°C and ending temperatures of nearly 30°C on 25 June 2003 (Figure 4). Low-density ponds had a higher mean temperature, which was only slightly higher than the high-density ponds (Table 2), as well as, a slightly higher daily change.

4.2 Nitrogen

Seasonal pattern for ammonium in both high- and low-density stocked ponds shows that ammonium follows a similar pattern for both treatments with low-density ponds starting at 24.5 μg/L and finishing with 216 μg/L, and high-density ponds starting at 26.7 μg/L and ending with 389 μg/L (Figure 5a). Low-density ponds do have a higher mean NH_4^+ concentration than the high-density ponds (Table 2), caused by spikes on 4 June 2003 (436.2 μg/L) and 11 June 2003(745 μg/L). Nitrate seasonal patterns are similar between both high- and low-density ponds, with both showing two spikes followed by great decreases in NO_3 concentrations. High-density ponds started with 423.5 μg/L and ended with 61.7 μg/L, while low-density ponds started with 518.3 μg/L and ended with 95.7 μg/L (Figure 5b). The low-density ponds had

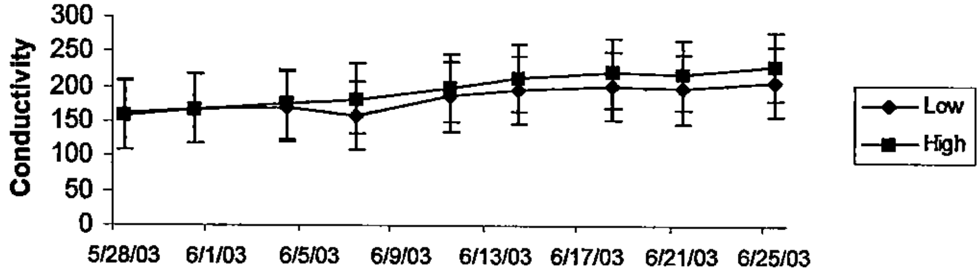


Figure 1. Comparison of seasonal conductivity between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003, measured in μS/cm.

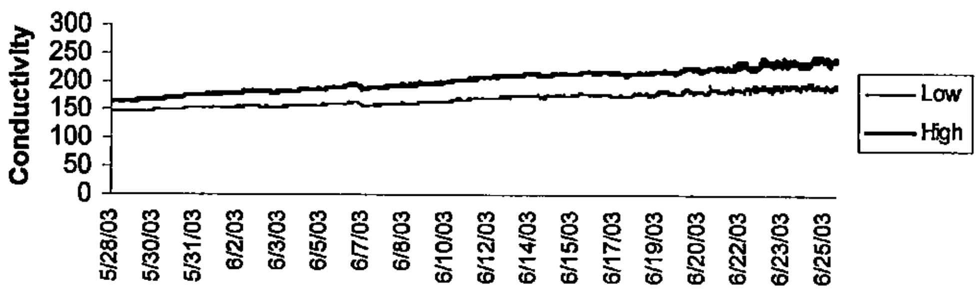


Figure 2. Comparison of conductivity measured every 15 minutes between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003, measured in μS/cm.

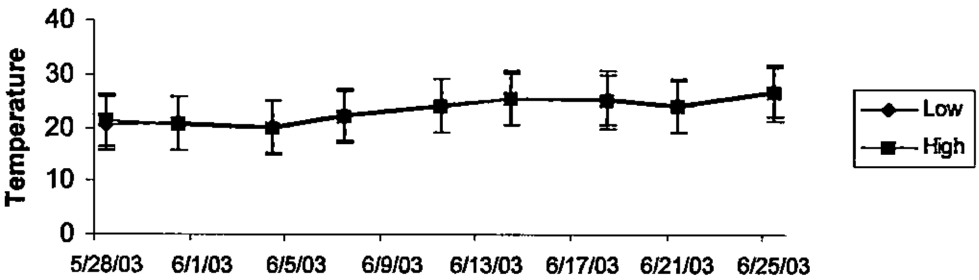


Figure 3. Comparison of seasonal temperature between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003, measured in °C.

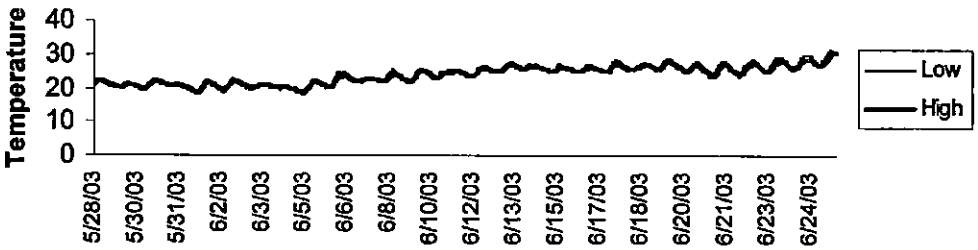
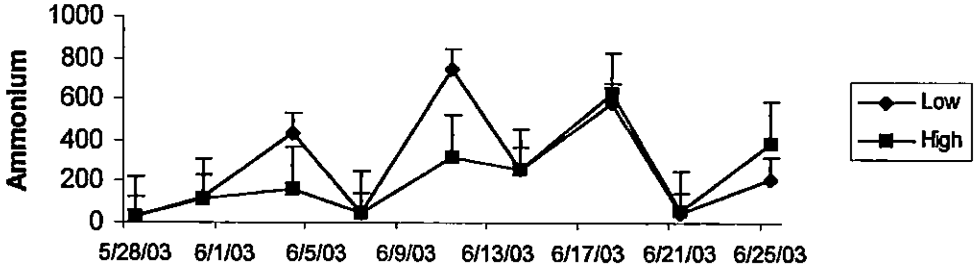


Figure 4. Comparison of temperature measured every 15 minutes between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003, measured in °C.

the highest mean NO_3 concentration (Table 2), despite a large spike in the high-density ponds on 7 June 2003 (Figure 5b). The seasonal patterns for nitrite between the two treatments are similar with both treatments having peaks on 11 June 2003 (high-density 0.18 mg/L, low-density 0.15 mg/L). High-density ponds started with 0.016 mg/L and ended with 0.041 mg/L, while low-density ponds started with 0.009 mg/L and ended with 0.039 mg/L (Figure 5c). The mean NO_2^- concentration of both high and low-density stocked ponds is 0.043 mg/L (Table 2). Total organic nitrogen (TON) seasonal patterns are similar between high- and low-density ponds, with both ponds showing a gradual increase in TON throughout the sampling period. High-density ponds started with 8.4 mg/L and ended with 27.1 mg/L, while low-density ponds started with 10 mg/L and finished with 18.6 mg/L (Figure 5d). High-density stocked ponds had a slightly higher mean TON concentration than the low-density ponds (Table 2).

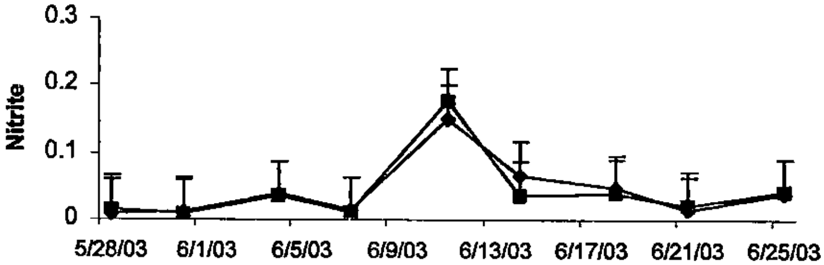
4.3 Phosphorus

The seasonal pattern for soluble reactive phosphorus (SRP) between high- and low-density ponds is similar up to 14 June 2003, and then low-density ponds show a trend for higher values. High-density ponds started with 10.2 mg/L and ended with 6.2 mg/L, while low-density ponds started with 9.8 mg/L and ended with 12.3 mg/L (Figure 6a). Low-density ponds had a slightly higher mean SRP (Table 2). The total phosphorus for the high and low-density treatments show similar seasonal patterns, with both treatments showing a gradual increase over time. High-density ponds started out with 77.3 mg/L and ended with 115.2 mg/L, where low-density ponds

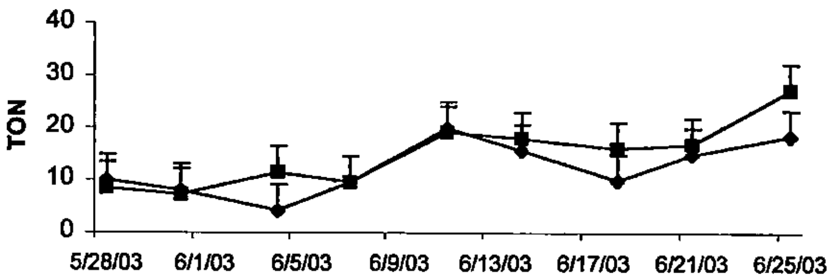


(a)

(b)



(c)



(d)

Figure 5. Comparison of ammonium µg/L (a), nitrate µg/L (b), nitrite mg/l (c), and total organic nitrogen mg/L (d) concentrations between high- and low-density ponds, Minor Clark Fish Hatchery 2003.

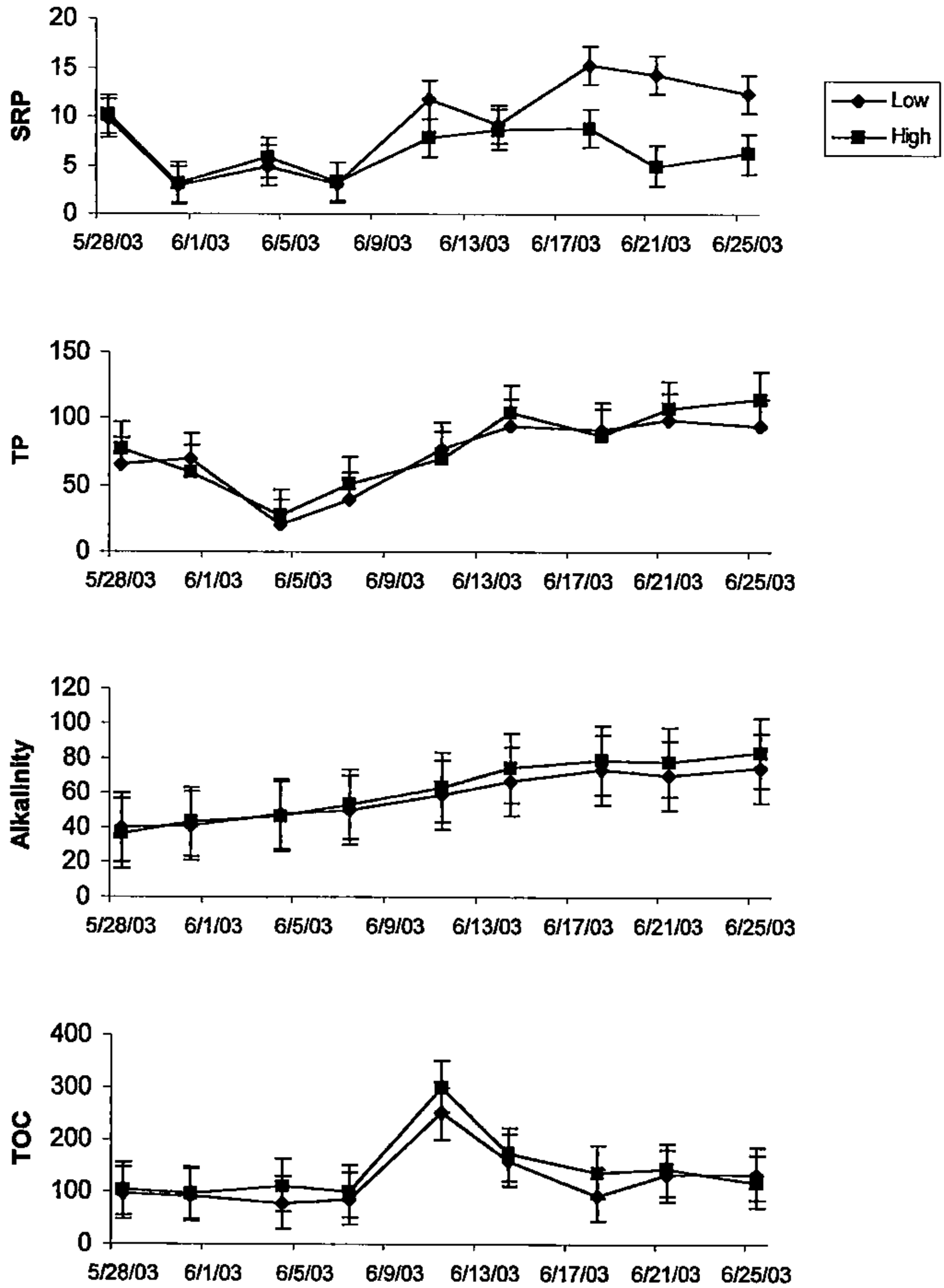
started with 65.2 mg/L and ended with94.8 mg/L (Figure 6b). The high-density ponds had a slightly higher mean TP (Table 2).

4.4 Inorganic and Organic Carbon

Seasonal patterns for alkalinity (inorganic carbon) were similar for both high- and low-density ponds with a gradual increase in alkalinity through out the sampling period, and low-density ponds starting to show a higher seasonal pattern on 11 June 2003, continuing until the end of the sampling period. High-density ponds started with 36.5 mg/L and ended with 83.6 mg/L, where low-density ponds started with 39.8 mg/L and ended with 74.3 mg/L (Figure 6c). Mean alkalinity was highest in high-density ponds (Table 2). The seasonal trends in total organic carbon are similar between both high- and low-density ponds, with both treatments peaking on 11 June 2003 (high-density 301.3 mg/L, low-density 251.5mg/L). High-density ponds started with 103.9 mg/L and ended with 119.2 mg/L, where low-density ponds started with 97.2 mg/L and ended with 134.5 mg/L (Figure 6d). High-density ponds had a higher mean TOC than the low-density stocked ponds (Table 2).

4.5 Oxygen and pH

Seasonal patterns for surface DO (0.10m) for both high- and low-density ponds were similar with both showing a slight gradual decrease over time, with high-density ponds starting at a mean DO of 8.65 mg/L and ending with a mean of 7.65 mg/L, while low-density ponds started with a mean DO of 7.51 mg/L and ended with a mean of 6.59 mg/L (Figure 7a). High-density ponds had a slightly higher mean DO concentration (Table 2). Mean saturation at the 0.10m depth was similar for both



(a)

(b)

(c)

(d)

Figure 6. Comparison of seasonal soluble reactive phosphorus mg/L (a), total phosphorus mg/L (b), alkalinity mg $CaCO_3$/L (c), and total organic carbon mg/L (d) between high-and low-density ponds, Minor Clark Fish Hatchery 2003.

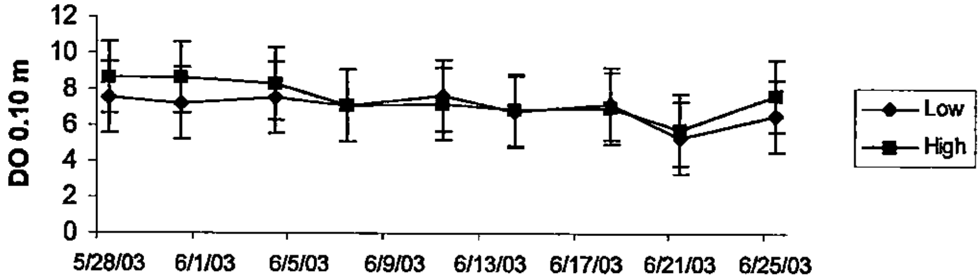
high- and low-density ponds, with high-density ponds having a slightly higher DO saturation (86.9% vs. 82.1%). The only measurements below 75% DO saturation were on 21 June 2003 for both ponds (high-density 68.1%, low-density 63.5% [Figure 9a]). Seasonal patterns for mid-depth DO (1.0m) were similar for both high- and low-density ponds also following a pattern of gradual decrease, with high-density ponds starting with 8.45 mg/L and ending with 6.38 mg/L, while low-density ponds started with 8.45 mg/L and ended with 7.32 mg/L (Figure 7b). Mean mid-depth DO was slightly higher in the high-density ponds (Table 2). Mean DO saturation for both high- and low-density ponds at 1.0 m was similar with the high-density ponds having a slightly higher saturation and with the low-density ponds averaging slightly below the required saturation of 75% (83.3% vs. 71.5%). On 21 June 2003 the saturation for both high- and low-density ponds fell below 75% DO saturation (high-density 65.2%, low-density 63.7% [Figure 9b]). Bottom DO (1.5m) seasonal patterns of rapid DO decline were similar in both treatments, with high-density ponds starting with 8.31 mg/L and ending with 3.71 mg/L, while low-density ponds started with 7.32 mg/L and ended with 4.8 mg/L (Figure 7c). Mean bottom DO was slightly higher in the high-density ponds (Table 2). Mean percent saturation for both ponds was similar, with low-density ponds being slightly higher, and with both falling below the 75% saturation base line (65.1% vs. 61.9%). Both treatments fell below 75% saturation starting 7 June 2003 through the end of the sampling period (Figure 9c). Only bottom DO went below 5 mg/L (Figure 7a-7c), and fell below 75% saturation more than the 0.10m and 1.0 m depths (Figure 9a-9c).

Daily changes in DO were similar for both high- and low-density ponds (Figure 8), with high-density ponds having a mean daily change of 2.49 mg/L DO and a mean daily change of 2.41 for low-density ponds. High-density ponds fell below 5 mg/L on multiple occasions (Figure 11b and 8): 9 June 2003 (1hr 45 min), 12 June 2003 (2 hr), and 15 June 2003 (4 hrs 15 min). Low-density ponds also fell below 5 mg/L on multiple occasions (Figure 11b and 8): 12 June 2003 (1 hr), 13 June 2003 (30 min), 15 June 2003 (15 min), and 23 June 2003 (15 min). The daily minimum and maximum DO day have an even spacing between both densities, with high-density ponds having greater spacing between min-max DO towards the end of the sampling period (Figure 10a and 10b).

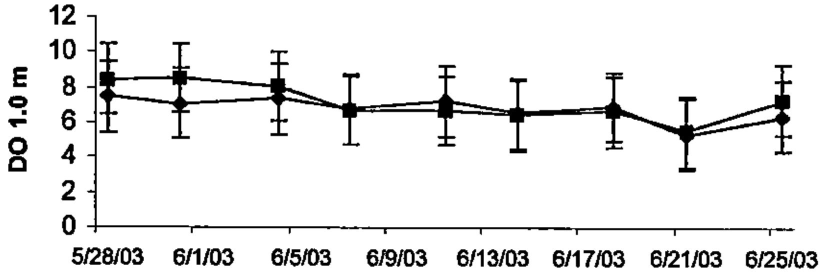
There were similar seasonal pH patterns for both high- and low-density ponds, with both treatments showing gradual increase over time, with high-density ponds starting with a pH of 7.35 and ending at 7.73, where low-density ponds starting at 7.19 and ending at a pH of 7.54 (Figure 12a). High-density ponds had a higher mean pH due to slightly higher pH readings taken between 28 May 2003 and 4 June 2003 (Table 2). Daily change in pH also had similar patterns between high- and low-density ponds, again with both showing increase over time, with high-density ponds following a higher pattern starting 7 June 2003. High-density ponds started with a daily change in pH of 0.13 and ended with a daily change of 0.63, where low-density ponds started with a daily change in pH of 0.034 and ended with a daily change of 0.55 (Figure 12b). Mean daily change in pH was slightly higher in the high-density ponds (Table 2). The pH that was recorded every 15 minutes had similar daily pH changes for the high- and low-density ponds (high-density 0.44 and low-density 0.33). High-density ponds had numerous instances of the pH being measured above 8.5 (Figure 13): 1 June 2003 through 2 June 2003 (8 hrs 45 min), 2 June 2003 (11 hrs 45 min), 21 June 2003 (5 hrs 15 min), 22 June 2003 (3 hrs 45 min), and 24 June 2003 (3 hrs 30 min). Low-density ponds only had one day when pH was greater than 8.5 (Figure 13), 25 June 2003 (3 hrs 15 min). Neither high- nor low-density ponds ever fell below a pH of 6.5.

4.6 Chlorophyll *a* and Secchi Depth

Chlorophyll *a* seasonal patterns were similar throughout the sampling period between high- and low-density stocked ponds, with both ponds starting out with low concentrations of chlorophyll *a* and ending with high concentrations. Both high- and low-density ponds started with chlorophyll *a* concentrations of 9.6 μg/L, but high-density ponds ended with a concentration of 182.3 μg/L and low-density ponds ended with a concentration of 92.2 μg/L. High-density ponds started to separate from, and have a higher seasonal pattern than the low-density ponds on 4 June 2003 till the end of the sampling period (Figure 14a). Mean chlorophyll *a* concentrations were highest in the high-density stocked ponds (Table 2). Secchi depth also shared a similar seasonal pattern between high- and low-density stocked ponds, with both treatments showing a gradual increase in turbidity over time, with high-density ponds having the lowest secchi depths throughout the sampling period. High-density ponds started with a Secchi depth of 105.2 cm and ended at 57.4 cm, where low-density ponds



(a)



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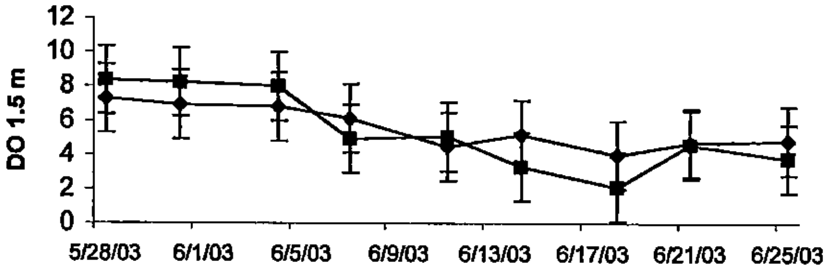


(c)

Figure 7. Comparison of dissolved oxygen concentrations taken at a depth of 0.10 m (a), 1.0 m (b), and 1.5 m (c) between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003, measured in mg/L DO.

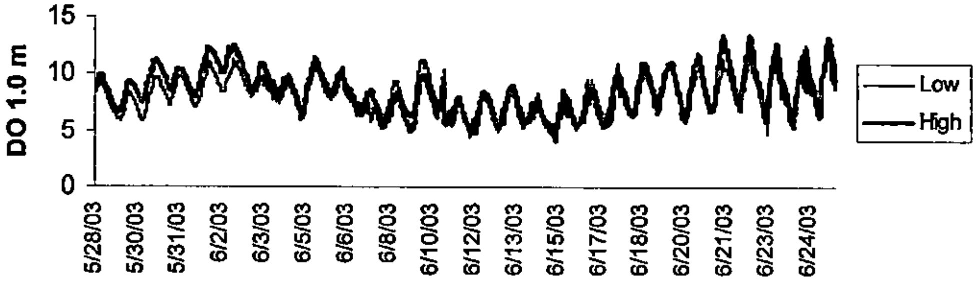
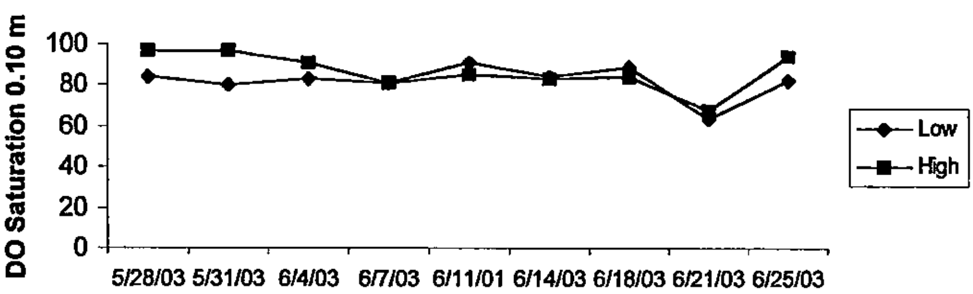
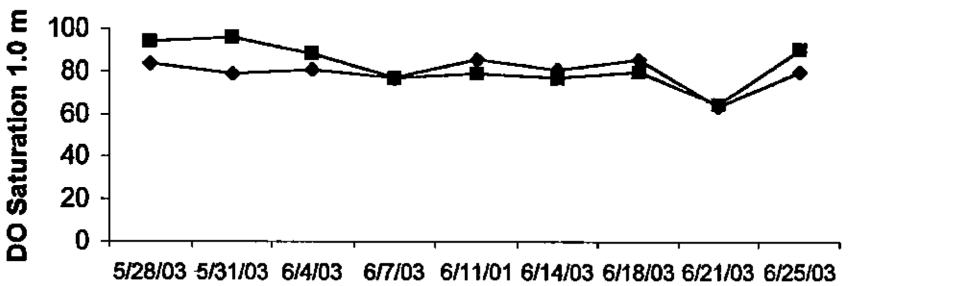


Figure 8. Comparison of dissolved oxygen concentrations measured every 15 minutes at a depth of 1.0 m between high- and low-density ponds, Minor Clark Fish Hatchery 2003, measured in mg/L DO.



(a)



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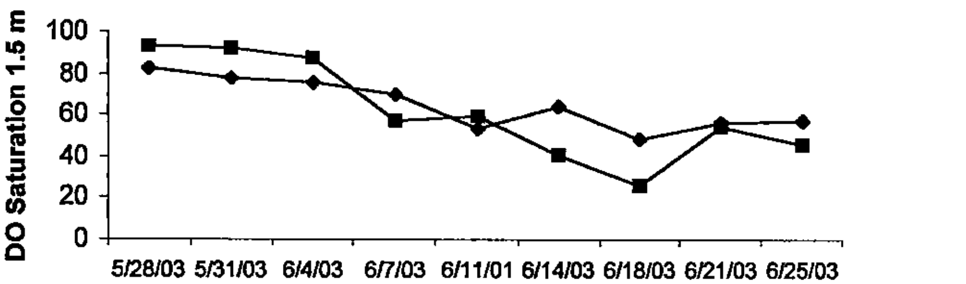
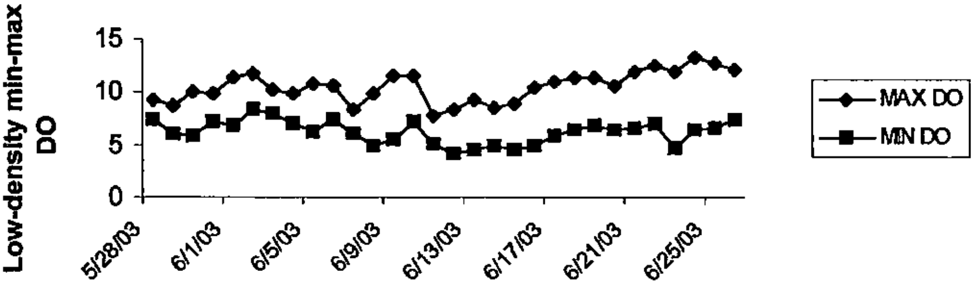
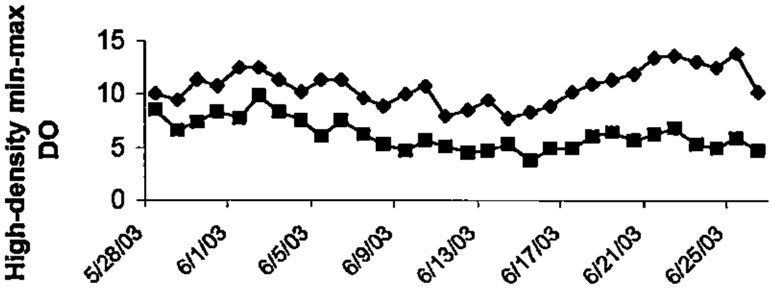


(c)

Figure 9. Comparison of dissolved oxygen saturation at a depth of 0.10 m (a), 1.0 m (b), and 1.5 m (c) between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003.

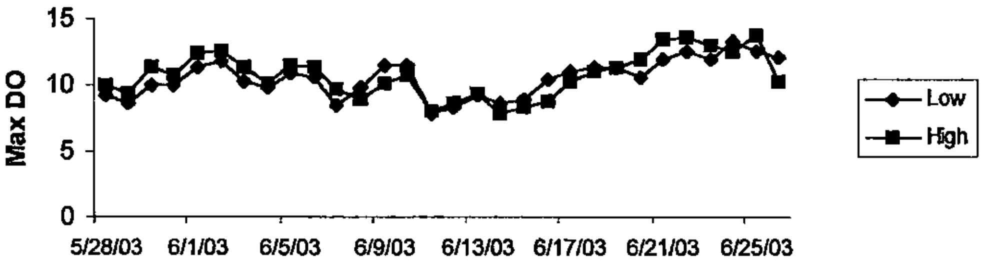


(a)

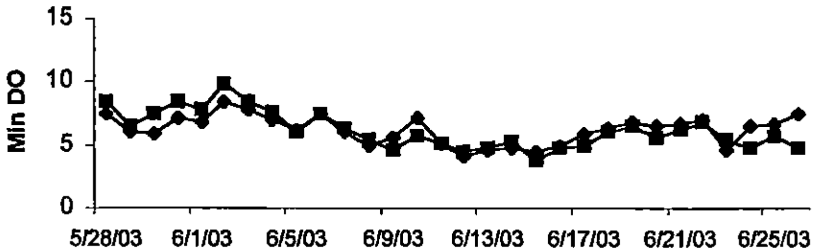


(b)

Figure 10. Seasonal minimum and maximum dissolved oxygen concentrations for low-density ponds (a) and high-density ponds (b), Minor Clark Fish Hatchery 2003, measured in mg/L DO.

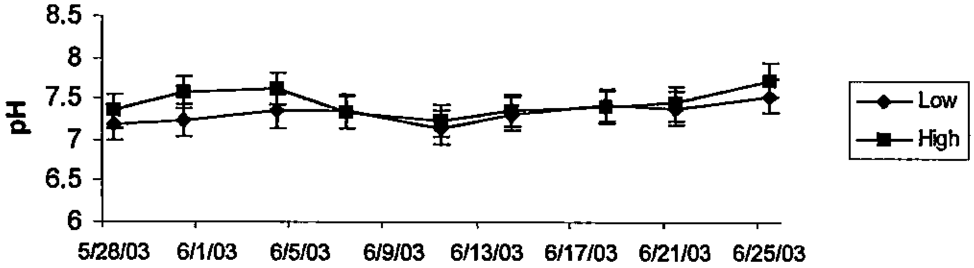


(a)

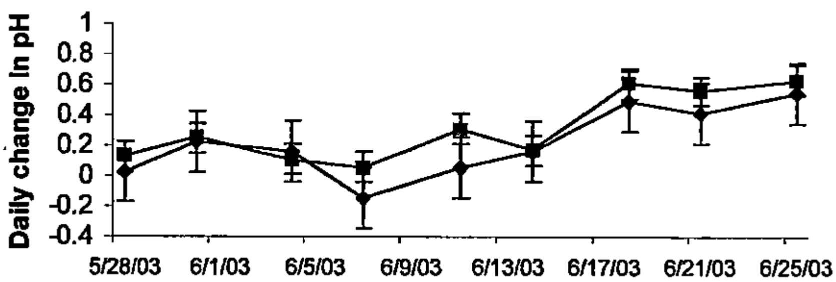


(b)

Figure 11. Comparison of seasonal maximum dissolved oxygen concentrations (a) and minimum dissolved oxygen concentrations (b) between high- and low-density ponds, Minor Clark Fish Hatchery 2003, measured in mg/L DO.



(a)



(b)

Figure 12. Comparison of seasonal pH (a) and daily change in pH (b) between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003.

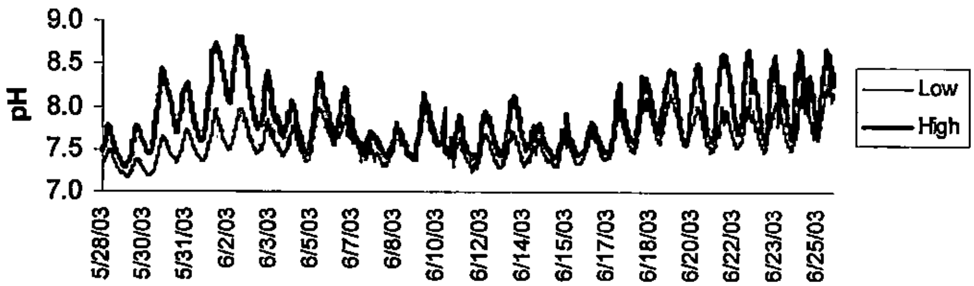


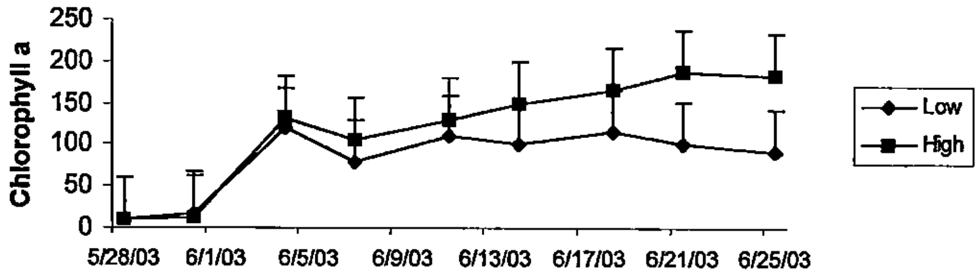
Figure 13. Comparison of pH measured every 15 minutes between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003.

started at 91.8 cm and ended at 87.4 cm (Figure 14b). Mean Secchi depth was greatest in the low-density ponds (Table 2).

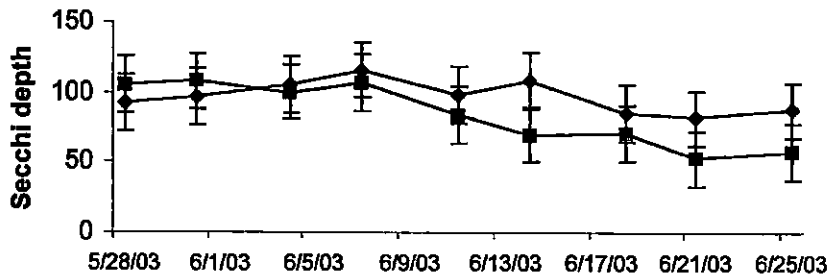
4.7 Zooplankton

Fine scale (1 mL) zooplankton counts for both high- and low-density ponds were dominated by rotifers, copepods, cladocerans and nauplii, and pyrrophyta during the first days of sampling, with rotifers dominating the zooplankton community during the latter sampling. The rotifer communities for both pond treatments were dominated by *Asplannchna, Brachionus, Kerratella, Kellicotia,* and *Polyarthra* species. Calanoid and cyclopoid species were found in the copepod counts, and *Daphnia* was the only cladoceran species found for cladoceran counts. The pyrrophyta counts were dominated by *Ceratium*. High-density ponds had more rotifers (Table 4) (Figure 16b), with rotifers comprising 77.9% of the community (Figure 16b). High-density pond rotifers peaked mid-June (1088.8/L and 1120.6/L [Figure 16b]). Similarly, rotifers comprised 79.1% of the community of the low-density ponds (Figure 15a). Rotifers peaked earlier in low-density ponds (11 June 2003 at 1901.7/L [Figure 16a]). More nauplii and copepods were found in low-density ponds; whereas cladocerans and pyrrophyta were more abundant in high-density ponds (Table 4).

Gross scale (10 mL) zooplankton counts for both high- and low-density ponds for the four dominating groups were comprised of the same genera and species found in the fine scale counts, although more of the smaller rotifer species went uncounted



(a)



(b)

Figure 14. Comparison of seasonal chlorophyll *a* µg/L (a) and Secchi depth cm (b) between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003.

at this magnification. Low-density ponds had more rotifers (Table 5), which comprised 75.2% of the community (Figure 17a). Low-density rotifers peaked in mid-June, reaching maximum density (473.4/L and 362.4/L [Figure 18a]). Rotifers comprised 76.3% of the community in high-density ponds (Figure 17b). Rotifer populations in high-density ponds peaked from mid-June through the end of the sampling period (343.5/L – 409/L [Figure 18b]). More nauplii, cladocerans, and pyrrophyta were found in low-density ponds, whereas cladocerans were highest in high-density ponds.

4.8 Periphyton

The diversity of periphyton for both sampling periods was similar between high- and low-density ponds (Figure 19a and 19b). Low-density ponds had a higher abundance of periphyton for both sampling periods (18.9/cm^3 and 30.41/cm^3 vs. 18.3/cm^3 and 21.7/ cm^3 [Figure 19a and 19b]). Chlorophyll *a* analysis for both sample periods shows that high-density ponds had higher chlorophyll *a* concentrations (1.31 μg/L and 2.15 μg/L vs. 0.89 μg/L and 0.29 μg/L).

4.9 Fish Survival

There was no significant difference between percent survival of fry stocked at 250,000 fry/ha and 450,000 fry/ha (p-value=0.5711). Fish stocked at the low-density had a survival of 22.1%, and fish stocked at the high-density had a survival of 14.8% (Figure 20). High-density ponds had more fish harvested than the low-density ponds (66,396 vs. 55,225). High-density ponds produced a higher weight of fish (39.4 kg), but fewer fish per kg (1684 fish/kg). Low-density ponds produced 25.2 kg of fish, of fish, with 2191 fish/kg. Average length of fish was similar between the two treatments. High-density ponds produced fish with an average length of 2.92 cm, and low-density ponds produced fish that were 2.82 cm in length (Table 6).

Table 4. Zooplankton 1 mL averages (counts/mL).

Pond	Rotifers	Nauplii	Copepods	Cladocerans	Pyrrophyta
1	59.6	23.6	3.2	0.3	28.3
39	1300.5	149.9	35.4	1.3	55.8
47	60.7	29.6	5.2	0.6	41.4
Low mean	473.6	67.7	14.6	0.7	41.8
9	57.9	46.4	10.6	0.5	30.9
40	1288.6	99.7	20.0	1.8	131.8
62	245.6	34.9	7.9	2.8	67.0
High mean	533.7	60.3	12.8	1.7	76.6

Table 5. Zooplankton 10 mL averages (counts/mL).

Pond	Rotifers	Nauplii	Copepods	Cladocerans	Pyrrophyta
1	12.9	11.3	4.6	0.7	10.8
39	514.7	76.0	15.3	0.2	514.7
47	4.5	12.4	4.5	0.3	4.5
Low Mean	177.3	33.2	8.1	0.4	177.3
9	33.0	25.7	8.2	0.4	33.0
40	358.4	43.0	12.1	0.1	358.4
62	116.7	18.9	6.0	0.1	116.7
High Mean	170.4	29.2	8.8	0.2	170.4

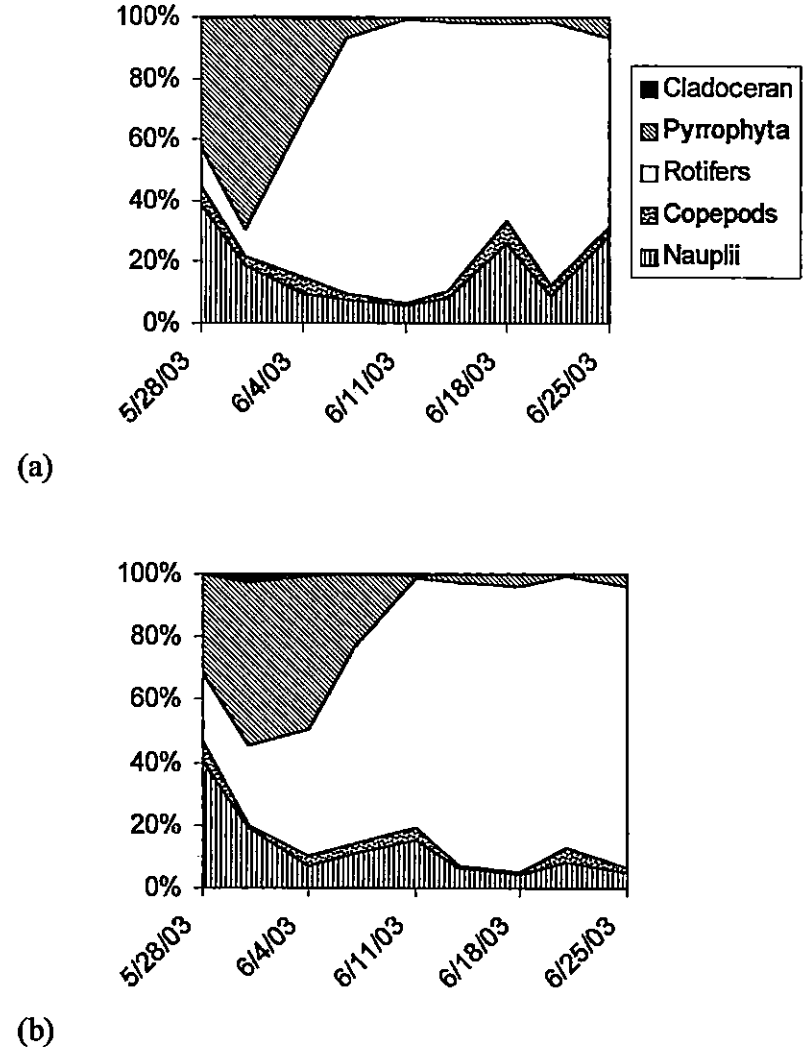


Figure 15. Zooplankton percent composition for 1 mL samples taken from low-density (a) and high-density stocked ponds, Minor Clark Fish Hatchery 2003.

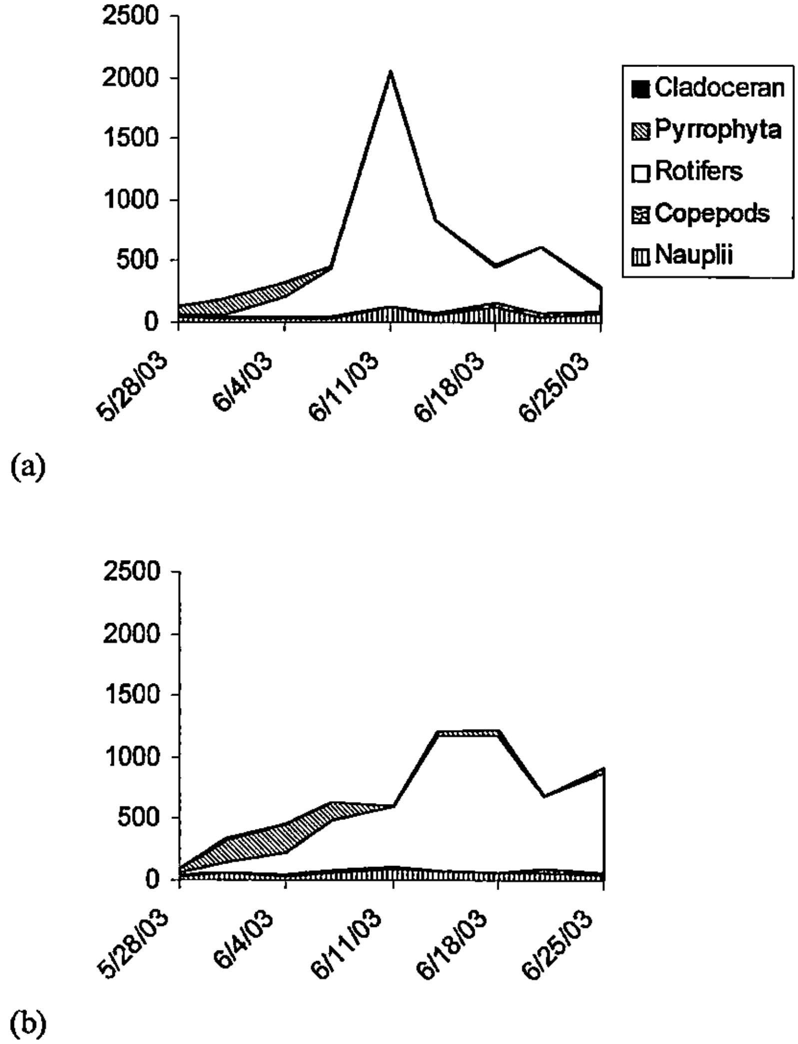
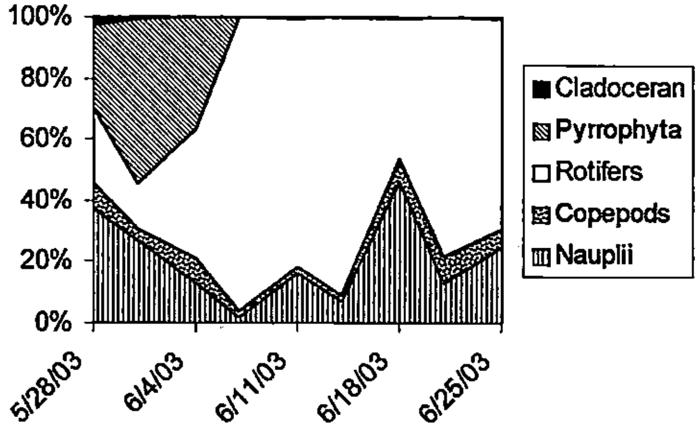
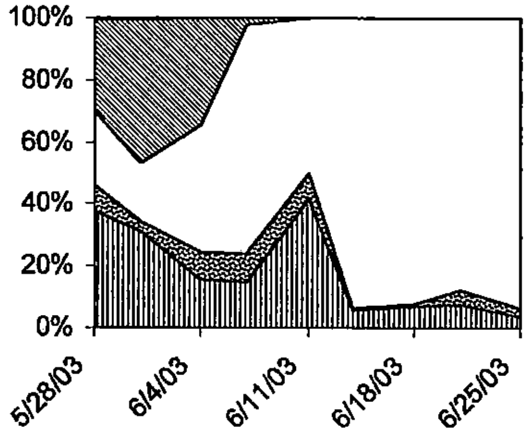


Figure 16. Zooplankton 1 mL sample counts for low-density ponds (a) and high-density ponds (b), Minor Clark Fish Hatchery 2003.

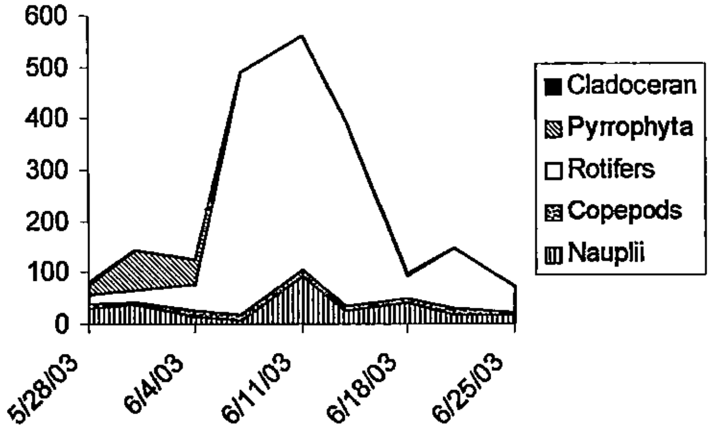


(a)



(b)

Figure 17. Zooplankton percent composition for 10 mL samples taken from low-density ponds (a) and high-density ponds (b), Minor Clark Fish Hatchery 2003.



(a)

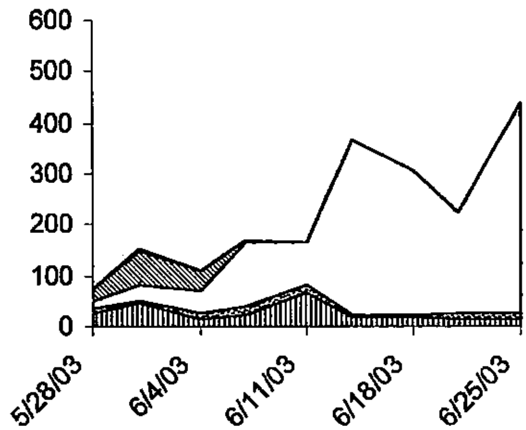


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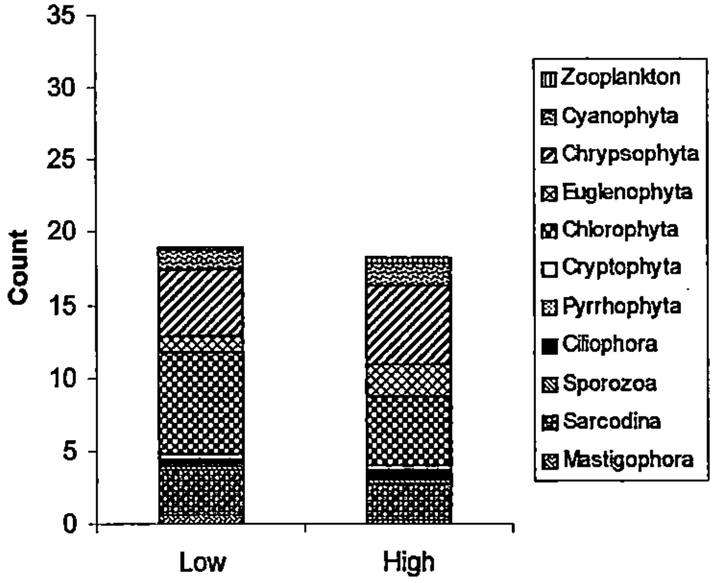
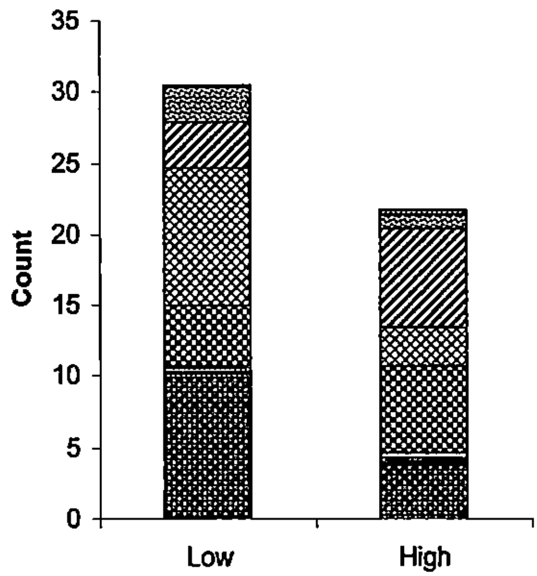
Figure 18. Zooplankton 10 mL sample counts for low-density ponds (a) and high-density ponds (b), Minor Clark Fish Hatchery 2003.



(a)



(b)

Figure 19. Comparison of periphyton counts/cm^2 for collection period 1 (a) and collection period 2 (b) between high- and low-density stocked ponds, Minor Clark Fish Hatchery 2003.

Table 6. Sunshine bass harvest at MCFH, 2003.

Pond	Stocking Density	Fish Harvested	% Survival	kg	Fish/kg	Length (cm)
1	250,000	21,486	8.6	13.0	1646	2.77
39	250,000	113,582	45.4	52.0	2185	2.77
47	250,000	30,607	12.2	10.6	2894	2.92
Low mean		55,225	22.1	25.2	2191	2.82
9	450,000	81,349	18.1	52.0	1565	2.74
40	450,000	54,091	12	37.4	1448	2.95
62	450,000	63,746	14.2	29.0	2200	3.07
High mean		66,395	14.8	39.4	1684	2.92

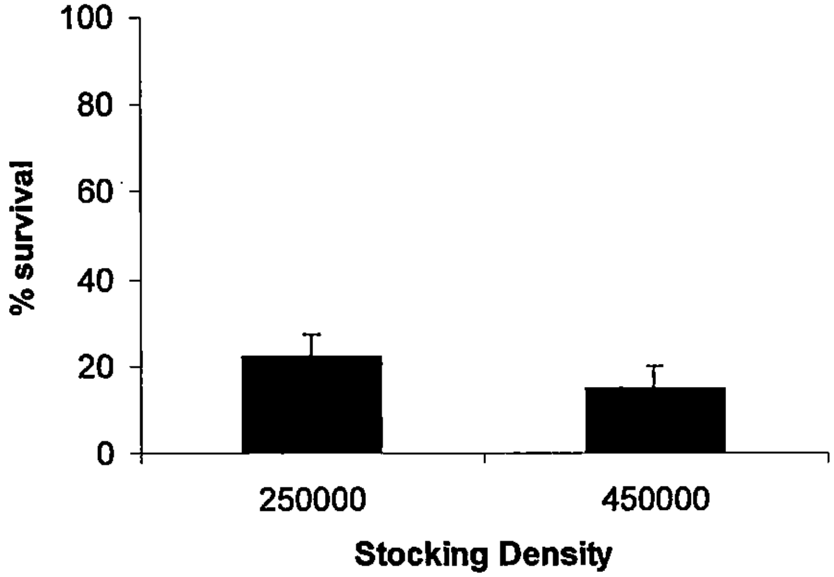


Figure 20. Comparison of percent survival between high and low-density stocked ponds, Minor Clark Fish Hatchery 2003.

5.0 Discussion

No significant differences were found in the water quality parameters measured between high and low-density ponds. Also there was no difference in overall percent survival of fish between the pond treatments. There was however a difference in zooplankton counts between pond treatments. Seasonal changes in the fourteen water quality parameters analyzed could explain the differences in zooplankton due to increased primary production increasing available food, as well as fish production and density variations between individual ponds.

5.1 Conductivity and Temperature

Conductivity measurements gradually increased in both high- and low-density ponds throughout the study period. This was probably due to liming, addition of inorganic and organic fertilizer, and evaporation exceeding precipitation. The gradual increase in conductivity coincided with an increase in temperature. Since Cave Run Lake has a mostly forested watershed, and water is taken from the dam, the water quality of the source water is good with a relatively low conductivity (208.4 µS/cm, Davis and Reeder 2001). The advantage of Minor Clark Hatchery using Cave Run Lake as a water source is that hatchery managers manipulate water that is relatively clean. Both high- and low-density had low conductivities the first sample day (high-density 156.8 µS/cm and low-density 160.7 µS/cm), which were below Cave Run Lake averages (Davis and Reeder 2001). High-density ponds reached 231.2 µS/cm and low-density reached 209 µS/cm at the end of the study period, which is still relatively low, suggesting good water quality.

All ponds had similar temperatures and ponds were filled from Cave Run outflow with an initial temperature of 21°C, which gradually increased to 27°C by the end of the study. Temperature varied diurnally in these shallow systems, with daily changes being as high as 3.8°C, and averaging 1.7°C. Temperatures found for both high- and low-density fall within the optimal ranges for sunshine bass production found in other studies (Bonn et al. 1976, Hodson 1989, and Ludwig 1997).

5.2 Nitrogen

Nitrate concentrations for the first day of sampling for both high- and low-density ponds (423.5 µg/L and 518.5 µg/L) were greater than the Cave Run average of 231 µg/L (Davis and Reeder 2001). This is due to an oxidizing environment in the ponds during the start of the study, and the addition of organic fertilizer before the fry were stocked. Peaks in high-density ponds on 7 June 2003 and 18 June 2003, and in low-density ponds on 11 June 2003 and 18 June 2003 are indicative of bacterial-mediated oxidation of nitrite (Wedemeyer 2001). Nitrate concentrations for both high- and low-density ponds were well below the Cave Run average at the end of the sampling period (61.7 µg/L and 95.7 µg/L), which would indicate a reducing environment converting NO_3 to NH_4^+. High and low-density ponds fell well within the required range for fry rearing given by Bonn et al. (1976).

Nitrite concentrations for both high- and low-density ponds started characteristically low (0.016 mg/L and 0.009 mg/L). High nitrite measurements on 11 June 2003 coincide with high ammonium readings in low-density ponds, which indicates significant microbial or physiochemical nitrate reduction. Ponds averaged

0.043 mg/L nitrite, which is much greater than the Cave Run Lake average (0.003, Davis and Reeder 2001), suggesting mildly reducing conditions are common or microbial activity. High- and low-density mean (0.043 mg/L) nitrite was within the recommended levels for fry rearing (Bonn et al. 1976).

The highly oxygenated Cave Run outflow had low ammonium at the beginning of the study. Starting NH_4^+ measurements for both high- and low-density ponds (26.7 μg/L and 24.5 μg/L) were even lower than the Cave Run average of 104 μg/L (Davis and Reeder 2001). As the season progressed, fertilization and the aforementioned reducing conditions caused the NH_4^+ concentration to increase faster than algal uptake. By the end of the study, NH_4^+ means for both ponds treatments were two times higher than the Cave Run Lake (high-density 223.2 μg/L, low-density 276.7 μg/L). Strong reducing conditions found on 4 June 2003, 11 June 2003, and 18 June 2003 coincide with peak NH_4^+.

5.3 Inorganic Carbon

Alkalinity measurements had a gradual increase throughout the sampling period. This gradual increase coincided with an increase in conductivity, which appears to be driven mostly by liming (r^2=0.9371 p-value=0.387 [Figure 21d]). Davis and Reeder (2001) found the average alkalinity of Cave Run Lake source water to be 30.95 mg $CaCO_3$/L. Both high- and low-density ponds (36.5 mg/L and 39.8 mg/L) started slightly higher than the average for Cave Run Lake. Inorganic carbon concentrations for both high- and low-density ponds (62.12 mg/L and 58.13 mg/L) fall within the optimal range for fry production found in Hodson (1989). However the high nitrogen and total phosphorus may suggest the inorganic carbon may be a limiter to primary productivity. For example, Boyd (1997) suggests alkalinity up to 100 mg/L could be necessary to maintain highly productive hatchery ponds.

5.4 Phosphorus

Soluble reactive phosphorus concentrations in both high- and low-density ponds (10.2 mg/L and 9.8 mg/L) were higher than Cave Run Lake averages (0.022 mg/L, Davis and Reeder 2001). Decreases in SRP at the beginning of the study suggest rapid plankton uptake (Figure 6a). Nevertheless, pond concentrations remained higher than 9 mg/L, which is considered the minimum requirement for plankton in hatchery ponds (Wedemeyer 2001). Total phosphorus is arguably a better correlate of pond productivity (Carlson 1977). Total phosphorus for both high- and low-density ponds (77.3 mg/L and 65.2 mg/L) was higher than the Cave Run Lake average (0.040 mg/L, Davis and Reeder 2001) and was due to fertilization. Decreases in TP at the start of the study coincide with the decreases seen in SRP, which indicate uptake and subsequent sediment deposition of phosphorus by plankton (Figure 6b). Increases in both SRP and TP are indicators of increased productivity (Vollenweider 1968) and coincide with the increases seen in chlorophyll *a* concentrations (Carlson 1977, Wetzel 2001). Increase in algal productivity, as a result of increased phosphorus concentrations, led to increased daily changes in dissolved oxygen.

5.5 Oxygen and pH

Low dissolved oxygen is a limiting factor that affects fish carrying capacity in hatchery ponds (Wedemeyer 2001). Minimum DO needed for fish rearing is 5mg/L or 75 % saturation, and both high- and low-density ponds started out with acceptable DO concentrations and saturations. After two weeks of sampling, the bottom third of the ponds fell below the minimum DO requirement, leaving only two-thirds of each pond capable of supporting fish. DO measured every 15 minutes found the mid-depth level fell below the minimum DO requirement several times, and in some cases lasting for longer than one hour. These instances where DO was below the minimum requirement could have resulted in loss of fry. Low DO levels also affect the different forms of nitrogen found in the water. The steady decline of DO concentrations in the ponds explains the high concentrations of nitrite and peaks in ammonium that were found in both pond treatments.

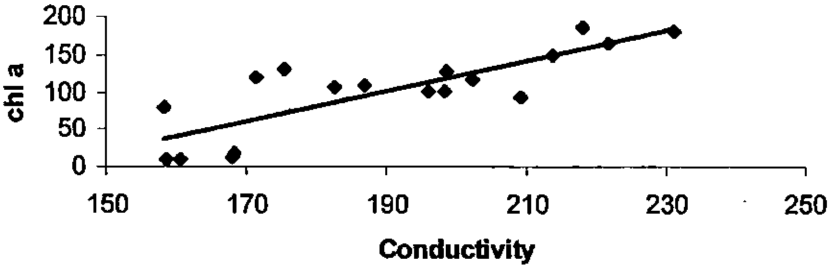
There is a greater difference in daily minimum and maximum DO midway through the sampling period (Figure 10a and 10b). This DO change can be used as an indicator of productivity (Odum and Hoskins 1958). High-density ponds have a greater change indicating a higher level of productivity.

Davis and Reeder found the mean pH of Cave Run Lake to be 7.39. Both high- and low-density ponds started below this average (7.35 and 7.19) but finished with mean pH readings above the Cave Run Lake mean pH (7.54 and 7.73). There were numerous instances where pH that was measured every 15 minutes was above the accepted range of 8.5, which could have resulted in the formation of ammonium hydroxide and a possible fish kill (Bonn et al. 1976 and Hodson 1989). These changes in pH are a result of increased primary production.

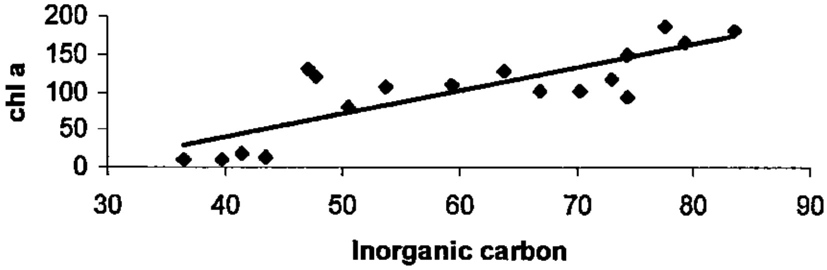
5.6 Chlorophyll *a* and Secchi Depth

Davis and Reeder (2001) found that Cave Run Lake had a mean chlorophyll *a* measurement of 2.57 µg/L. Both high- and low-density ponds stayed above this mean (high-density 119.3 µg/L and low-density 82.9 µg/L). Increases in chlorophyll *a* concentrations started to occur after the rapid depletion of SRP in both ponds indicating that the decrease in SRP was the result of increased productivity. Increased chlorophyll *a* concentrations as a result of pond fertilization resulted in increased photosynthesis rates. Therefore daily DO minimum and maximum changes became dramatic, with a mean daily change of 2.5 mg/L DO in the more productive high-density ponds. The carbon uptake from the activity resulted in high pH levels, with average daily pH fluctuations as a great as 1.06.

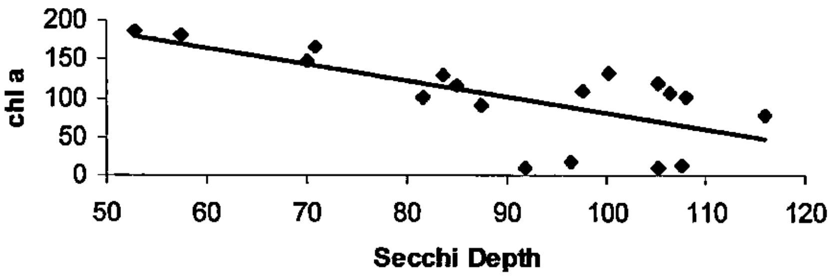
Algal turbidity reduced Secchi depth. Secchi depth was highest in low-density ponds, which had the lowest chlorophyll *a* measurements, as well as a smaller daily change in minimum and maximum DO. Secchi depth was not the strongest predictor for algal biomass (r^2=0.449 p-value=0.454 [Figure 21c]). Conductivity (r^2= 0.669 p-value=0.454 [Figure 21a]) and alkalinity (r^2= 0.666 p-value=0.483 [Figure 21b]) were stronger predictors for algal biomass. However, some Secchi depth readings could have been altered due to non-algal turbidity from stirring of the bottom caused by the boat or the equipment used for the collection of the water samples.



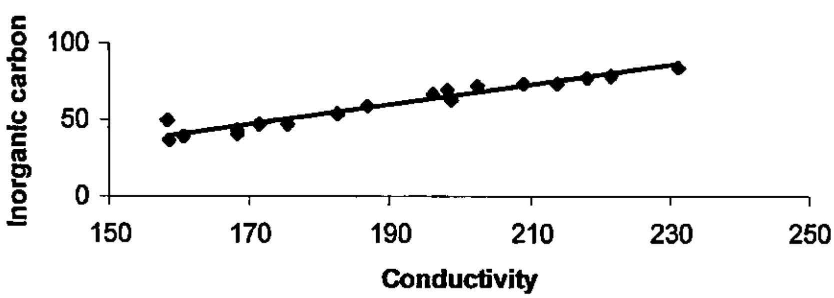
(a)



(b)



(c)



(d)

Figure 21. Regression analysis for the effect of conductivity (a), inorganic carbon (b), and Secchi depth (c) on chlorophyll *a*, and the effect of inorganic carbon on conductivity (d), Minor Clark Fish Hatchery 2003.

5.7 Zooplankton and Periphyton

Both high- and low-density ponds were dominated by rotifers throughout the sampling period. This was expected because the source water is rotifer dominated and rotifers tend to reach maximum densities early in the summer (Wetzel 2001). Rotifers are an essential food item for sunshine bass fry during their first weeks in the ponds (Hodson and Hayes 1989, Ludwig 1997). Peaks in rotifer populations in high- and low-densities during mid-June can be attributed to the fry grazing on larger copepods, cladocerans, as well as artificial fish food. None of the other three plankton groups counted ever dominated the zooplankton as biomass, percentage, or numbers. Peaks in the zooplankton community coincided with increases in chlorophyll *a* concentrations and daily DO changes that indicated increased productivity. These findings are similar to those of other studies that found zooplankton abundance to follow maximum abundance in phytoplankton (Beaver and Crisman 1989, Müller et al. 1991, Weiss 1991, and Laybourn-Perry et al. 1992, 1994). Therefore plankton abundance was controlled from bottom-up fertilization, which is where the addition of fertilizer increases algae production, leading to an increase in zooplankton, but population distribution may have been altered by top-down selective predation, where the addition of piscivores decreases the numbers of planktivores, therefore increasing the numbers of zooplankton, but in this case the addition of artificial feed resulted in a lowered selection for zooplankton as a food source. High counts for both nauplii and rotifers throughout the study can also be attributed to high concentrations of bacteria that also are utilized as a food source for these two groups (Roff et al. 1995, Merrel and Stoecker 1998). Periphyton diversity was similar in both high- and low-density ponds between the two sample periods, however total counts were greater for the second period. The difference in overall counts between period one and period two can be attributed to increased productivity in the ponds over time.

5.8 Fish Survival

Both high- and low-density ponds had fish percent fish survivals that fell within the acceptable range of 10-25% (Hodson and Hayes 1989, Hodson and Jarvis 1990, Ludwig 2003). Within each stocking density grouping there were individual ponds that brought down the overall percent survival rate for their group. For the low-density stocking, Pond 1 had a survival of 8.6%, where Pond 39 had a survival of 45.4% (Table 6). For the high-density ponds Pond 40 had a survival of 12% where Pond 9 had a survival of 18.1%.

The difference in Pond 39 and the two other low-density ponds survival rates could be attributed to the difference in zooplankton counts between the ponds. Pond 39 had a much greater count for all groups of zooplankton counted during analysis. Similar patterns are present in the high-density ponds. Pond 40 had a greater count for each of the individual zooplankton groups. Both Pond 9 and Pond 40 also had extremely high rotifer counts when compared to the ponds in their respective groupings (Tables 4 and 5). Even with these differences in percent survival between ponds within groupings, there are no significant differences that can be attributed to stocking density and percent survival of sunshine bass fry.

High-density ponds for MCFH in 2003 were stocked with 450,000 fry, with a mean harvest of 66,395 fish/acre, 39.4 kg, and a survival of 14.8%. Low-density ponds for MCFH in 2003 were stocked with 250,000 fry, and had a mean harvest of 55,225 fish/acre, 25.2 kg, and a survival of 22.1%. From 1999 through 2002 sunshine bass hatchery ponds at MCFH were stocked with an average of 462,460 fish/acre, with a mean harvest of 53517 fish, 29.6 kg, and a survival of 11.9 % annually (Table 7 and 8) (Middelton, personal communication). Reeder and Caldwell (personal communication) found MCFH to have a mean stocking densities of 430,500 fish/acre, with a mean harvest of 89,628 fish, 45.3 kg, and a survival of 18.2 %. In comparison Reeder and Caldwell (personal communication) found sunshine bass fry raised in Frankfort, Kentucky, to have an average stocking density of 430,500 fish/acre, with a mean harvest of 3517 fish, 4.6 kg, and a survival of only 0.81%. These differences in overall fish production and percent survival between MCFH and Frankfort can be attributed to the source water used to fill ponds. MCFH utilizes Cave Run Lake, which as mentioned before is relatively clean, where Frankfort utilizes the Kentucky River for their source water, which is not as clean due to pollution, as the water provided by Cave Run Lake. The difference in the production of sunshine bass from these two hatcheries lends strength to the argument of the need for monitoring of water quality, and use of source water that has parameters that closely resemble those needed for the production of sunshine bass.

Table 7. Sunshine bass harvest at MCFH 1999-2002.

Year	Stocking density	Total stocked	Fish harvested	Mean percent survival	kg	Fish/kg
1999	350,000-700,000	6,525,000	900,153	13.8	535.5	1681
2000	350,000-500,000	5,725,000	477,076	8.3	235.4	2027
2001	250,000-500,000	4,900,000	942,501	19.2	518	1820
2002	350,000-500,000	5,550,000	318,341	5.7	173.9	1831

Table 8. Comparison of sunshine bass production MCFH 1999-2003.

Year	Average stocking density	Average harvest	Average fish harvested (kg)	Percent survival
1999	501,923	69,243	41.2	13.8
2000	477,083	39,756	19.6	8.3
2001	408,333	78,542	43.2	19.2
2002	462,460	26,528	14.5	5.74
2003	250,000	55,225	25.2	22.1
2003	450,000	66,395	39.4	14.8

6.0 Conclusion

Varying stocking densities of fish, in order to increase production is a commonly practiced technique (Tidwell et al. 1994, Baskerville-Bridges 2000). There was no significant difference as a result of stocking density on percent survival of sunshine bass fingerlings, nor the fourteen water quality parameters that were analyzed. Low-density ponds did however have a slightly higher percent survival and fish/kg count, where high-density ponds had higher averages of fish harvested, kg of fish, and fish length.

The overall survival percentages of 22.1% for low-density ponds and 14.8% for high-density ponds fell within the acceptable survival percentages of sunshine bass fry from other studies (Hodson and Hayes 1989, Hodson and Jarvis, Ludwig 2002), and higher than the results from previous years at MCFH (Table 7-8). Water quality variables also fell within the acceptable ranges, with the exception of DO and pH. DO was recorded numerous times below the required 5 mg/L DO and 75% saturation, where pH was recorded several times above the acceptable range of 8.5. Low DO and high pH can both lead to fish kills, and may explain why some of the individual ponds for both stocking density treatments had survival percentages that fell below acceptable percentages for sunshine bass. Ludwig (1993) states that fry survival is related to a combination of chance, physical, biological, and chemical factors found in the hatchery ponds.

The zooplankton counts revealed that there was a sufficient rotifer bloom available for the fry early in the study however, there were never any high counts made for cladocerans or copepods. Low counts of cladocerans and copepods may be attributed to grazing of the sunshine bass fry prior to the introduction of artificial fish feed. The periphyton counts revealed that there were numerous protozoans that could have also been utilized as a food source for the fry.

Varying stocking density may not have a significant effect on percent survival, but there is the advantage of lower cost for stocking low-density ponds. By stocking ponds at the lower 250,000 fish/0.47ha ($1250) instead of the higher density of 450,000 fish/0.47 ha ($2250), hatchery managers could save $1000 just from the cost of initially purchasing the fry. One disadvantage to stocking at low-densities would be that more ponds would be needed to equal the production of the high-density stocked ponds, which would increase the cost of fish production. A second disadvantage to stocking at low-densities is that the fish have a lower weight than fish stocked at high-densities as shown in the difference between the fish/kg averages between the two stocking densities (2191 fish/kg low-density vs. 1684 fish/kg high-density). Fingerlings that weigh more are healthier and have a higher chance of survival when stocked when compared to smaller fingerlings.

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