COMPARISON OF THREE COTTONSEED MEAL APPLICATION REGIMENS FOR PALMETTO BASS (*Morone saxatilis* x *M. chrysops*) FINGERLING PRODUCTION IN PLASTIC-LINED PONDS

by

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ABSTRACT

Three cottonseed meal fertilization regimens were compared in plastic-lined ponds to determine the best regimen for production of fingerling palmetto bass (*Morone saxatilis* x *M. chrysops*). Treatments consisted of initial cottonseed meal applications of 70 kg/ha each to ponds on each of days 4 and 7 pre-stocking while ponds were filling with water, follow-up applications on days 0 (fry stocking day), 5, 10, and 15 of 56 kg/ha for treatment 1, 112 kg/ha for treatment 2 and 168 kg/ha for treatment 3. Water quality, zooplankton and fish production were monitored in all ponds and compared. No significant differences were found among treatment means for water quality and fish production variables. Mean density values of rotifers, cladocerans and nauplii also did not differ significantly among treatments. Treatments 2 and 3 produced significantly more copepods than treatment 1. Temporally, treatments 2 and 3 had significantly greater densities of copepods and rotifers on day 11, and cladocerans on day 18, than treatment 1. The cost of producing fish (cost of fertilizer/kg of fingerlings) was significantly lower for treatment 1 compared to treatment 2 or 3, while the difference between treatments 2 and 3 was not significant. With production variables not statistically different among treatments, it appears treatment 1 offered a cost advantage. However, the relatively greater production and harvest lengths in treatments 2 and 3 are biologically and economically meaningful and do outweigh the associated costs of additional fertilizer. Additionally, zooplankton quantity and quality were better in treatment 2 or 3. Thus, either treatment 2 or 3 is recommended for palmetto bass fingerling production ponds.

INTRODUCTION

Successful pond production of phase-I (25-38 mm) striped bass *Morone saxatilis* and their hybrids is dependent upon the availability of adequate food resources. Strategies for producing and maintaining adequate supplies of zooplankton are based upon the use of organic and inorganic fertilizers (Geiger and Turner 1990; Geiger 1983a, b). Suitable water quality is also critical, particularly since striped bass and palmetto bass (*Morone saxatilis* x *M. chrysops*) fry are susceptible to elevated pH levels (Bergerhouse 1993; Anderson 1993a; Barkoh 1996).

Organic fertilizers have been successfully used in the production of striped and hybrid striped bass (Geiger and Turner 1990). They stimulate development of phytoplankton, bacteria and protozoa that serve as food for zooplankton which are necessary for successful fingerling production (Geiger 1983a, b). Cottonseed meal and alfalfa meal are organic fertilizers used in moronid production ponds (Piper et al. 1982; Geiger 1983a, b; Geiger and Turner 1990; Anderson 1993b, c). Enhanced fingerling production has been linked to the use of cottonseed meal (Smith and Swingle 1939; Geiger et al. 1985). However, recommendations on the selection of organic fertilizers for fingerling production have varied (Barkoh and Rabeni 1990; Ludwig and Tackett 1991; Mims et al. 1993; Anderson 1993b, c) and final selections depend upon individual site characteristics, fertilizer availability and cost (Anderson 1993a, b; Barkoh 1996). The selection of cottonseed meal over alfalfa meal has led to better water quality, increased production of desired zooplankton and increased production of palmetto bass at the Possum Kingdom State Fish Hatchery, Texas (Buurma et al. 1996). The next challenge is to develop a cottonseed meal application strategy to maximize fingerling production. The objective of this study was to compare the effects of three cottonseed meal application regimens in plastic-lined ponds on palmetto bass fingerling production, water quality and zooplankton densities to determine the best regimen.

MATERIALS AND METHODS

Pond Management

This study was conducted at the Possum Kingdom State Fish Hatchery, near Graford Texas. Six ponds averaging 0.25 ha or 2,715 m^3 were used. Ponds were filled with water by gravity flow from Possum Kingdom Lake from a depth of 13.6 m. Characteristics of the source water were: alkalinity = 78 mg/l as $CaCO_3$, hardness = 418 mg/l as $CaCO_3$, and chlorides = 800 mg/l. Pond filling began eight days prior to palmetto bass fry stocking, were half full on the day of fry stocking and full at 10 days post-stocking.

Ponds were randomly assigned to three treatments of two replicates each. The three treatments (Table 1) received an initial cottonseed meal application of 70 kg/ha on each of days 4 and 7 prior to fry stocking. On days 0 (day of fry stocking), 5, 10, and 15 treatment 1 received 56 kg/ha, treatment 2 received 112 kg/ha, and treatment 3 received 168 kg/ha. Cottonseed meal (40% protein) was broadcast by hand onto the pond water surface.

Ponds were inoculated with approximately 759,000 zooplankters/ha each that consisted of rotifers (52%), cladocerans (31%), copepod nauplii (13%), and adult copepods (6%). The zooplankton inoculant was collected from a zooplankton production pond and introduced into study ponds daily for five consecutive days beginning five days prior to stocking of palmetto bass fry. The inoculant was collected by pumping water from the pond (95 L/min) and filtering it through a 250-µm-screened vat over a 24-h period (Graves and Morrow 1988a). Each zooplankton inoculant was thoroughly mixed in a bucket before equal volumes were distributed among treatment ponds. The zooplankton production pond (0.3 ha) was filled with water and fertilized with 280 kg/ha of cottonseed meal 19 days prior to fry stocking.

Zooplankton populations were monitored weekly by collecting samples from treatment ponds for identification and enumeration. Samples were taken with a tube sampler (Graves and Morrow 1988b) from the deepest part of each pond at 0700-0730 hours. One liter of each sample was concentrated by filtering the water through an 80-µm Wisconsin plankton net and preserving the concentrate in a 100-ml container. Two 1-ml subsamples of each concentrate were examined separately on a Sedgewick-Rafter counting slide under a dissecting microscope. Zooplankters were identified into four categories: rotifers, copepod nauplii, cladocerans and adult copepods, and densities were expressed as number of organisms/L.

Water quality monitoring began three days prior to stocking palmetto bass fry into ponds and continued for 40 days post-stocking. Dissolved oxygen (mg/L), temperature (^{o}C) and pH measurements were collected. Water quality measures were taken on weekdays at 25-30 cm below the pond surface at 0700-0730 and 1600-1630 hours. Phytoplankton biomass was estimated twice weekly using a 20-cm diameter black and white Secchi disc (Boyd 1984).

Fingerling Production

Palmetto bass fry were produced at the Possum Kingdom State Fish Hatchery using methods described by Warren (1993). Eggs were enumerated volumetrically and incubated at 18^{o}C until they hatched approximately three days later. Two-day-old fry were randomly distributed into research ponds at mean stocking rates of 579,167-644,481 fry/ha. Fry were tempered indoors with pond water, enumerated volumetrically and stocked into ponds.

Supplemental feeding of fish began 14 days post-stocking. Rangen swim-up feed (50% protein) was provided twice daily, totaling 13 kg/ha/day for each pond. As fingerling size increased, adjustments were made to the feed size offered to the fish: starter to crumble #3 to 1.6-mm sinking pellets. Feeding of fish continued until pond draining was started to harvest the fish. Weekly sampling of fingerlings (N = 25 per pond) for fish length (mm) and weight (g) data also began on day 14 post-stocking. All ponds were harvested within an 11-day period. At harvest, fish samples were taken for length (N = 50 per pond) and weight (N = 300 per pond) measurements. Total harvest biomass was determined for each pond. The cost of producing fish was calculated for each pond as cost of fertilizer per kg of fish produced.

Data Analysis

Statistical analyses were performed using the ANOVA general linear models (GLM) procedure and a standard error P difference test for post-hoc testing (SAS Institute 1985; Cody and Smith 1987; Hintze 2001). Differences were considered significant at $P \leq 0.05$. Mean fish production (kg/ha), survival, harvest length (mm) and cost of producing fish were compared among treatments. Water quality variables and zooplankton densities also were compared among treatments.

RESULTS AND DISCUSSION

No significant differences were found among treatments for mean production (kg/ha), survival and harvest length (mm) of palmetto bass fingerlings (Table 2). While differences among treatments were not statistically significant, production (kg/ha) for treatments 2 and 3 were 46.2 and 62.8 kg/ha greater than that of treatment 1, respectively. Also, mean harvest length for treatments 2 and 3 were 4.4 mm and 2.6 mm greater than that of treatment 1, respectively. The small number of replicates and high variability associated with the data most likely prevented the difference from being statistically significant. The cost of producing fish ($/kg fish) for treatment 1 ($0.51) was similar to that of treatment 2 ($0.68) but was significantly lower than treatment 3 ($0.90). Treatments 2 and 3 did not significantly differ in cost of producing fish.

Fish survival rates were high among treatments with averages ranging from 84.1 to 105%. Three of the six replicates had returns ranging from 102.2 to 109.3%, indicating that fish enumeration was not accurate. These errors were probably due to the fact that two different methods were used to estimate numbers of fish that yielded the survival values and the fact that each has inherent sources of error as well as associated human error. The fry that were stocked into ponds were enumerated volumetrically while the fingerlings harvested from the ponds were enumerated gravimetrically. Excess water associated with the fry samples possibly caused underestimation of the fry, and non-representative samples of the fingerlings and excess water associated with weighed fish contributed to enumeration errors that resulted in survival values exceeding 100%. Human error could have come from inconsistency in fish sampling and in following enumeration procedures.

No significant differences were identified among treatment means for water temperature, dissolved oxygen, pH and Secchi disc depth (Table 3; Figures 1-3). Mean water temperatures were 16.6-17.1°C and 22.2-22.9°C for mornings and afternoons, respectively (Table 4). Temperatures (Figure 1) were within acceptable ranges for good survival and growth of *Morone* spp. Brewer and Rees (1990) reported that 5-day-old striped bass fry survived temperatures of 12.8-24°C, with an optimum range of 17.8-20°C, and temperatures of 18.3-30°C are considered acceptable for survival and growth of striped bass. Bonn et al. (1976) reported optimum feed conversion and growth for striped bass at 18-19°C. In contrast, Worwode and Adelman (1984) reported optimum growing temperatures for 8-g and 130-g palmetto bass to be 31°C and 35°C, respectively and best food conversion at 19°C and 26.7°C, respectively.

Mean dissolved oxygen values were 8.4-8.6 mg/L and 9.2-9.8 mg/L for mornings and afternoons, respectively. Brewer and Rees (1990) recommended dissolved oxygen levels above 6 mg/L for successful striped bass culture. Except for the final 7-10 days of the study, dissolved oxygen levels remained above 6 mg/L. During the final few days of the study (i.e., between days 34 and 41 post-stocking), morning dissolved oxygen levels dropped to 4-6 mg/L. These low dissolved oxygen levels, however, did not appear to have had a significant adverse affect on fish survival.

Appropriate pH levels are critical to survival of striped bass and palmetto bass fry during the first few weeks of stocking (Anderson 1993b). Bergerhouse (1993) reported 6-hour mortality thresholds for 4-day-old palmetto bass fry at pH 9-9.4, 13-day-old fry at pH 8.8-9.2 and 20-day-old fry at pH 9.2-9.4. Barkoh (1996) and Anderson (1993c) recommended keeping pH levels at or below 8.5 until striped bass fry are at least 14 days old. In this study, all treatments provided acceptable pH conditions for phase-I palmetto bass fingerlings. Mean pH values were 8.4-8.6 and 8.8-8.9 for mornings and afternoons, respectively. The pH trends revealed initial moderate pH values, around 8.5, then rising to 9-10 after the first two weeks following fry stocking. In earlier studies at Possum Kingdom State Fish Hatchery, Buurma et al. (1996) reported that pond water pH reached 9.5 two days before fry stocking without apparent mortalities. In the present study, it was nearly three weeks after fry stocking before pH reached above 9.5, when the fry had achieved considerable tolerance of high pH values (Anderson 1993b; Bergerhouse 1993).

Mean Secchi disc depths (62.3-64.8 cm) were not significantly different among treatments (Table 3). Secchi disc depth is used as a surrogate measure of productivity as it correlates closely with particulate organic matter and chlorophyll *a* levels (Boyd 1984). Temporally, Secchi disc depth was significantly lower for treatment 1 than for treatment 2 or 3 on day 11 post-stocking (Figure 4). These differences in Secchi disc depth appear to relate to differences in zooplankton abundance and phytoplankton biomass among treatments. Densities of rotifers and adult copepods during this period were significantly greater in treatment 2 and 3 ponds, thus were able to reduce phytoplankton biomass through grazing, than in treatment 1 ponds.

Mean densities of rotifers, cladocerans and copepod nauplii were statistically similar (Table 4). Conversely, copepod density was significantly greater for treatment 2 or 3 compared to treatment 1 and similar between treatments 2 and 3 (Table 4). Temporally, rotifer and copepod densities were significantly higher in treatments 2 and 3 ponds than in treatment 1 ponds on day 11, while copepod densities were similar between treatments 2 and 3 (Figure 5). Similarly, densities of cladocerans were higher for treatments 2 and 3 than for treatment 1 on day 18 post-stocking. Treatments 2 and 3 supported better succession of zooplankters throughout the culture period, thus making food available for fish. Conversely, cladocerans and copepods, the preferred food items of moronid fingerings (Parmley et al. 1985), were relatively scarce in treatment 1 ponds. Geiger and Turner (1990) reported that zooplankton succession in ponds consisted of rotifers predominating the population 7-14 days after pond filling, followed by crustaceans 14-28 days after pond filling. The succession in this study followed a similar pattern, except that the rotifer population peaked on day 19 after pond filling and remained in relatively high densities throughout the study.

On economic and biological basis treatment 3 had the best fish production followed by treatment 2, then treatment 1. Although the differences in production were not statistically significant, the higher fertilization rates appear to have promoted relatively greater fish production. Similarly, treatments 2 and 3 had relatively greater densities of zooplankton over the course of the study than treatment 1 (Figure 5) and may partially explain the relatively higher fish production for treatments 2 and 3. Copepod density was significantly different among treatments. Temporally, rotifers, cladocerans and copepods were significantly more abundant for treatments 2 and 3 than treatment 1.

The results reveal that treatments 2 and 3 are better choices that treatment 1. While treatment 1 had a statistically significant lower cost of producing fish compared to treatment 3, the zooplankton quantity and quality and fish growth and production differences provide a stronger reason for choosing either treatment 2 or 3 for palmetto bass ponds. Sufficient quantity and quality of zooplankton at the proper stages of fry development are essential to successful fingerlings production. Zooplankton abundances were better in treatment 2 or 3 than treatment 1. Because the results were statistically inconclusive mainly because of inadequate replication of treatments and small sample sizes, further studies are required to determine which of treatments 2 and 3 would promote better water quality, zooplankton population and fish production. These studies should increase pond replicates within treatments to minimize the variability within treatments to allow differences to be statistically determined

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Table 1. Rates (kg/ha), amounts (kg/treatment) and costs of cottonseed meal applied to palmetto bass fingerling rearing ponds at the Possum Kingdom State Fish Hatchery, spring 1996. Day 0 = fry stocking date.

Day		Treatment 1	Treatment 2	Treatment 3
-7		70	70	70
-4		70	70	70
0		56	112	168
5.		56	112	168
10		56	112	168
15		56	112	168
	Total amount	182	317.5	406
	Cost	$57.66	$100.59	$128.62

Table 2. Stocking and production summary for palmetto bass rearing ponds subjected to three fertilization regimes at the Possum Kingdom State Fish Hatchery, spring 1996. Values are means ± SD with minimum and maximum values in parentheses.

	Treatment 1	Treatment2	Treatment3
Mean pond size (ha)	0.25 ± 0.04 (0.22 - 0.28)	0.27 ± 0.04 (0.24 - 0.30)	0.25 ± 0.04 (0.22 - 0.28)
Mean stocking rate (fry/ha)	644,481 ± 52,803 (607,143 - 681,818)	579,167 ± 64,818 (533,333 - 625,000)	639,160 ± 4,592 (636,364 - 642,857)
Mean days in production	31 ± 4 (28 - 34)	36 ± 5 (32 - 39)	31 ± 3 (29 - 33)
Mean survival (%)	93.2 ± 7.1 (88.2 - 98.3)	84.1 ± 26.1 (65.7 - 102.6)	105 ± 5 (102.2 - 109.3)
Mean production (kg/ha)	224.2 ± 33.5 (200.5 - 247.9)	270.4 ± 56.6 (230.3 - 310.4)	287 ± 3.7 (284.3 - 289.6)
Mean harvest length (mm)	34.2 ± 2.3 (32.5 - 35.8)	38.6 ± 4.9 (35.1 - 42)	36.8 ± 0.9 (36.1 - 37.4)
Cost ($kg/fish)[A]	$0.51	$0.68	$0.90

[A]Cost is based only on fertilizer cost and harvested fish biomass.

Table 3. Mean ± SD water quality variables in palmetto bass rearing ponds at the Possum Kingdom State Fish Hatchery, spring 1996. All values were similar among treatments ($P > 0.05$).

Water Quality	Treatment 1	Treatment 2	Treatment 3
Morning			
Temperature (°C)	17.0 ± 8.1	17.1 ± 7.7	16.6 ± 8.5
pH	8.4 ± 4.9	8.6 ± 4.9	8.6 ± 4.9
Dissolved oxygen (mg/L)	8.5 ± 0.7	8.6 ± 0.9	8.4 ± 0.8
Afternoon			
Temperature (°C)	22.9 ± 3.6	22.8 ± 4.1	22.2 ± 4.2
pH	8.8 ± 0.6	8.9 ± 0.6	8.8 ± 0.5
Dissolved oxygen (mg/L)	9.2 ± 0.9	9.3 ± 1.3	9.8 ± 3.8
Secchi disc (cm)	62.3 ± 9.9	64.4 ± 6.8	64.8 ± 10.7

Table 4. Mean ± SD zooplankton densities in palmetto bass rearing ponds at Possum Kingdom State Fish Hatchery, Spring 1996. Values bearing the same letter are not significantly different ($P > 0.05$)

Zooplankton	Treatment 1	Treatment 2	Treatment 3
Rotifers/L	879.1 ± 681.3a	1500.9 ± 1659a	918 ± 1014a
Cladocerans/L	7.6 ± 12.3a	65.1 + 145.7a	73.2 ± 150a
Copepods/L	7.7 ± 14.8a	28.4 ± 32.7b	19.1 ± 23.7b
Copepod nauplii/L	110.1 ± 118.9a	185.3 ± 174.1a	177.5 ± 209.1a

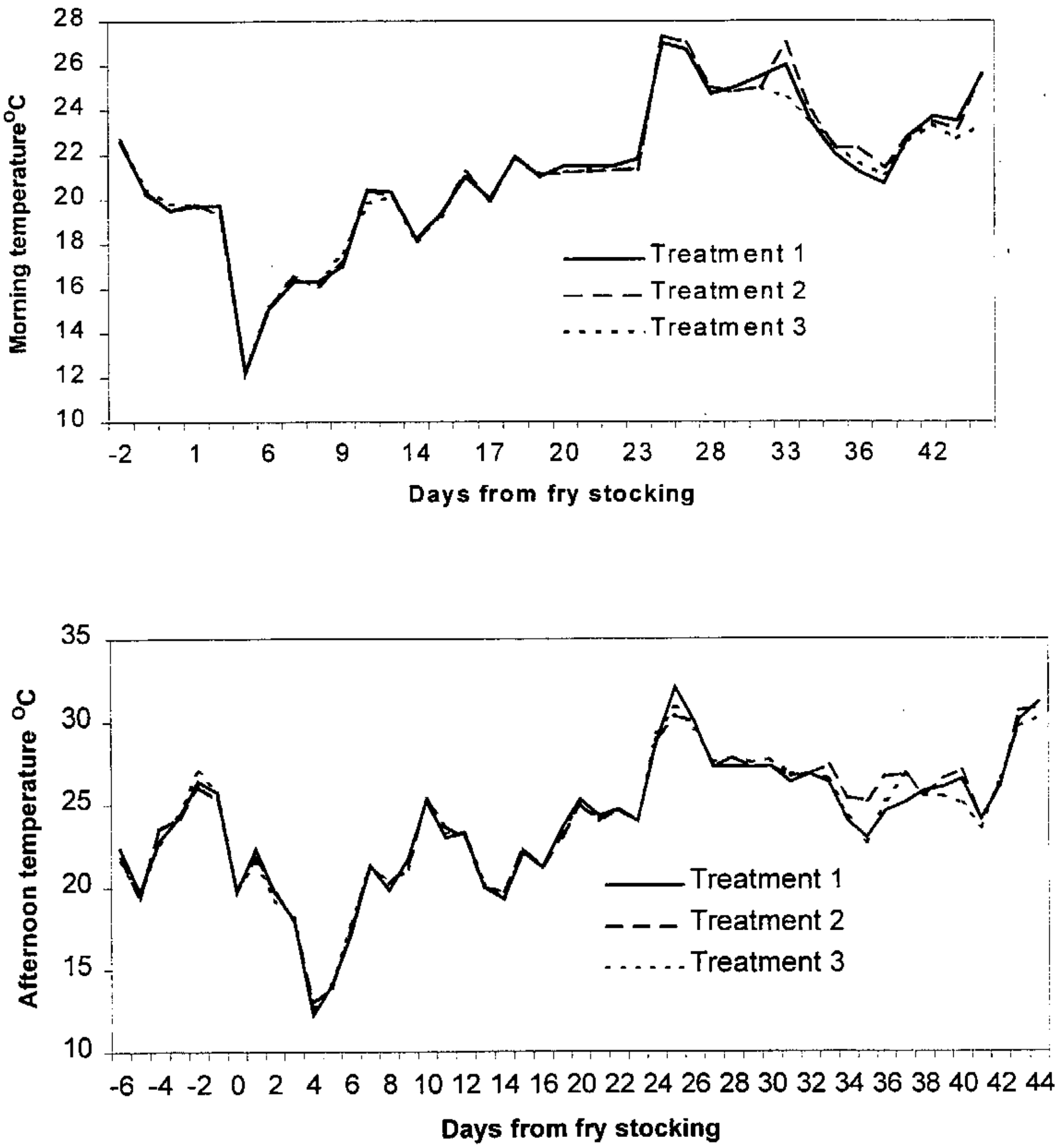
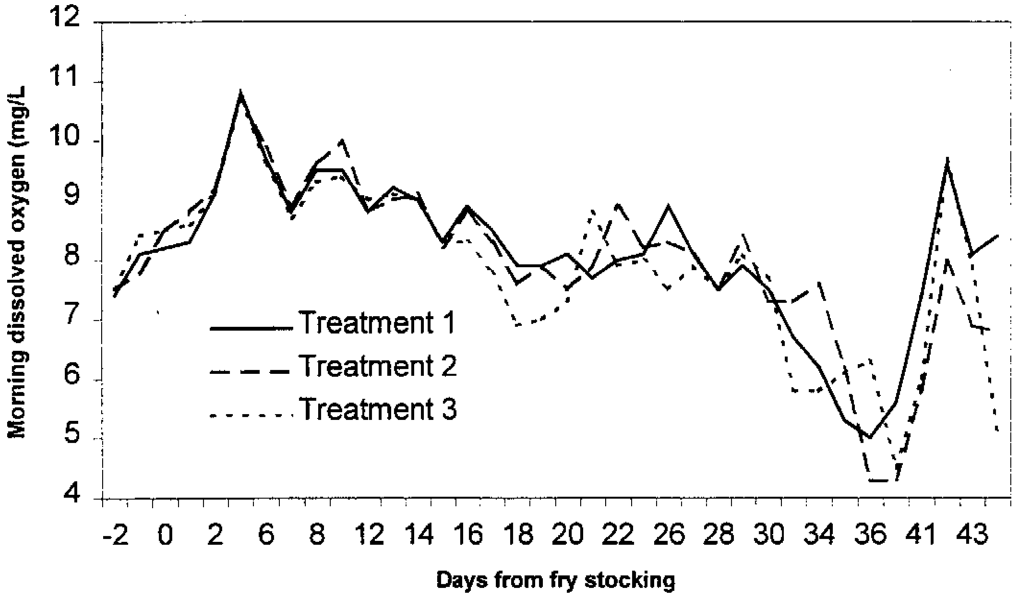


Figure 1. Mean morning and afternoon pond water temperature in palmetto bass fingerling rearing ponds at Possum Kingdom State Fish Hatchery, spring 1996.



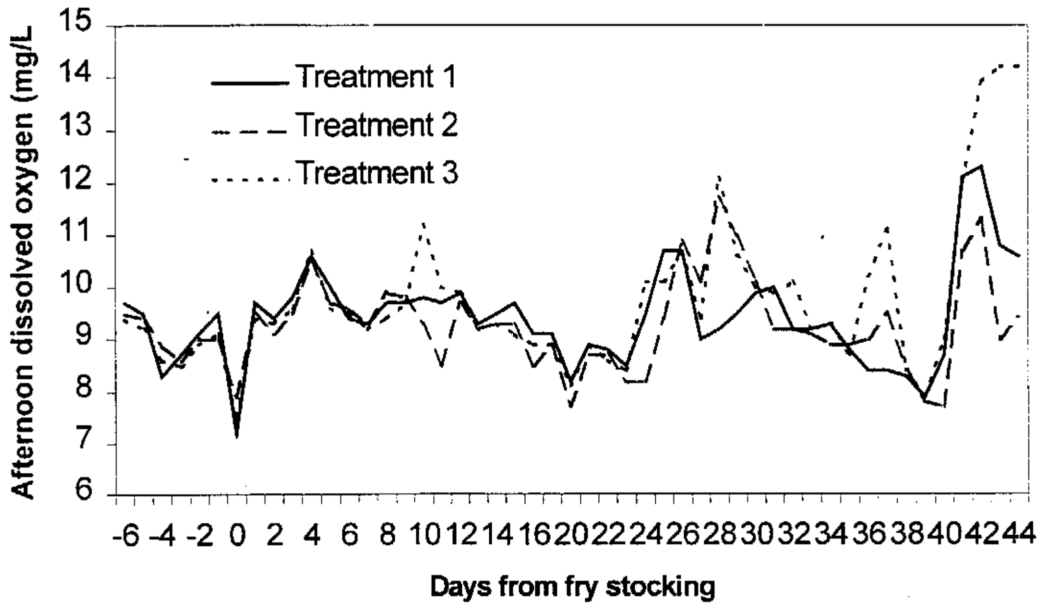


Figure 2. Mean morning and afternoon dissolved oxygen levels in palmetto bass fingerling rearing ponds at Possum Kingdom State Fish Hatchery, spring 1996.

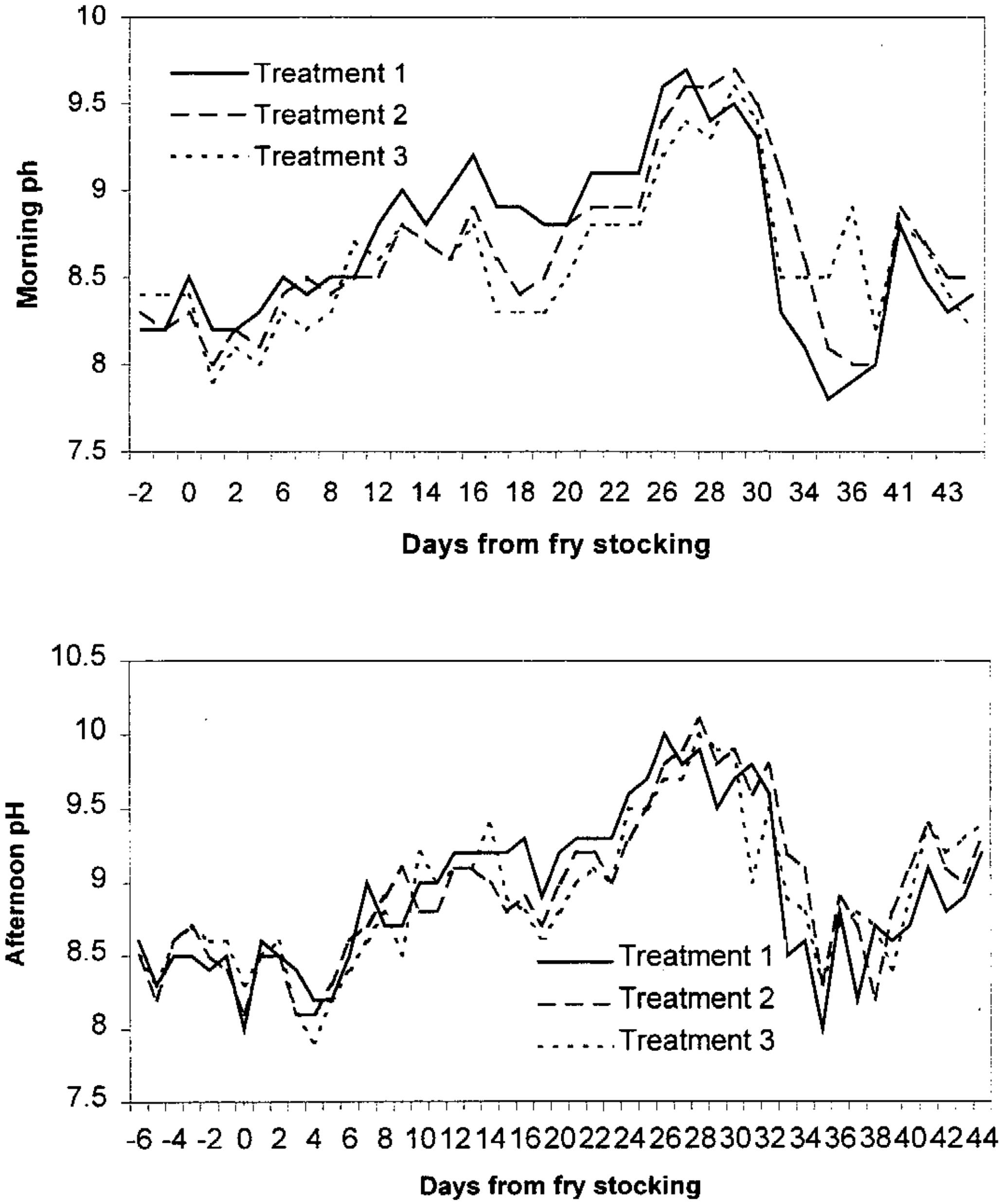


Figure 3. Mean morning and afternoon pH in palmetto bass fingerling rearing ponds at Possum Kingdom State Fish Hatchery, spring 1996.

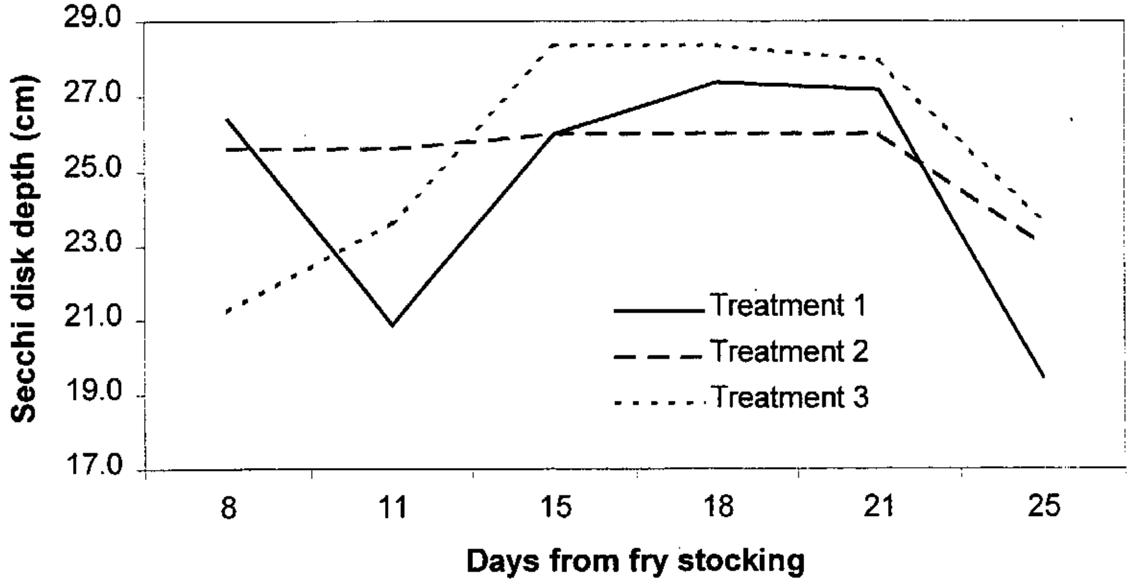


Figure 4. Mean Secchi disk depths in palmetto bass fingerling rearing ponds at Possum Kingdom State Fish Hatchery, spring 1996.

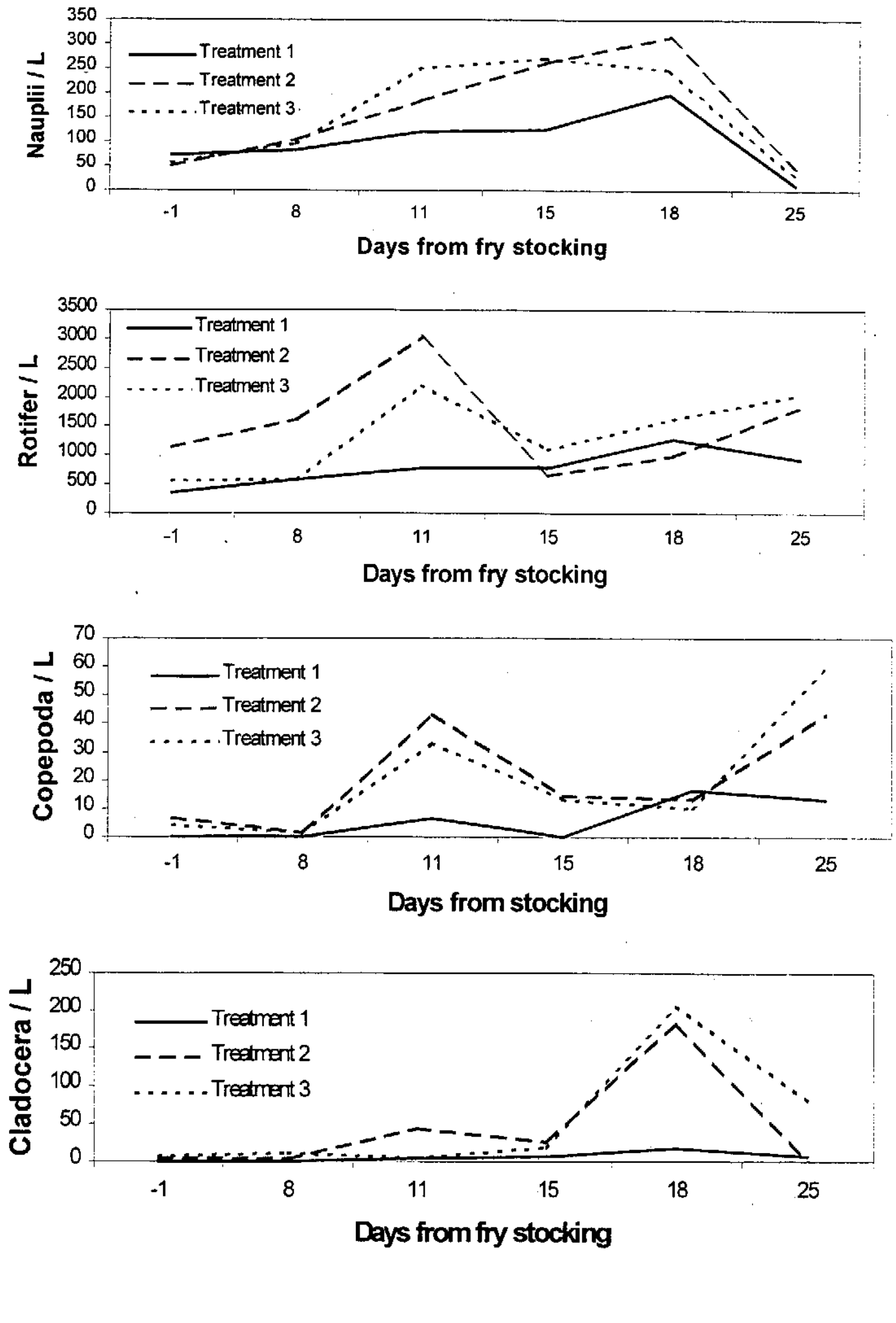


Figure 5. Mean zooplankton estimates in palmetto bass fingerling rearing ponds at Possum Kingdom State Fish Hatchery, spring 1996.