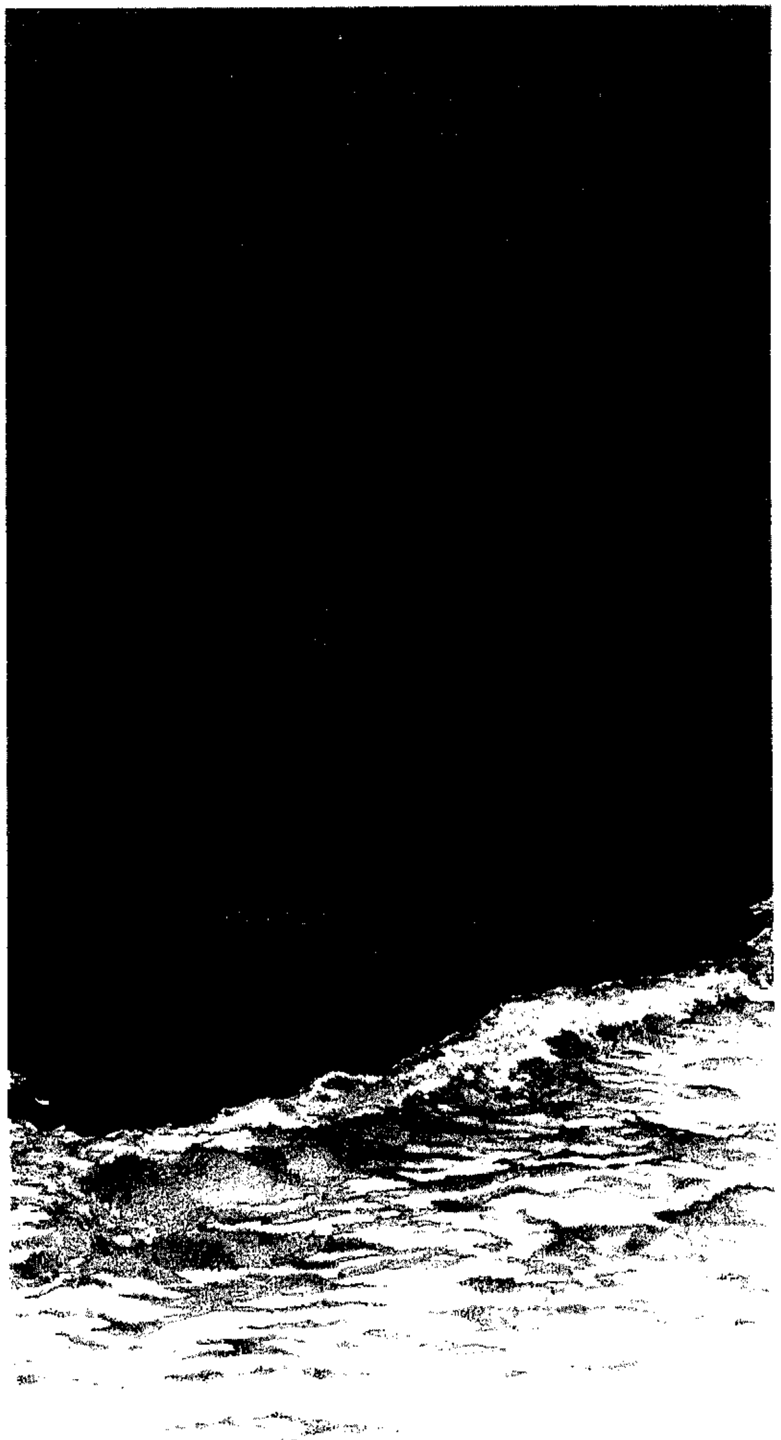

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EFFECTS OF RICE BRAN, COTTONSEED MEAL AND ALFALFA MEAL ON pH AND ZOOPLANKTON

by

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ABSTRACT

Rice bran (RB), cottonseed meal (CSM) and alfalfa meal (ALF) were compared as organic fertilizers in limnocorrals without fish for their effects on pH and zooplankton abundance. The treatments (CSM, RB-N, RB-W, ALF-N and ALF-W) were based on weight (W) and nitrogen (N) content of cottonseed meal, which was the control. Temperature, dissolved oxygen, chlorophyll *a*, ammonia nitrogen, and zooplankton densities were monitored for eight weeks to simulate the average period for the management of ponds for phase 1 (38-50 mm) striped bass *Morone saxatilis* production. Rice bran promoted better water quality than alfalfa meal or cottonseed meal when application rates were based on nitrogen content. The pH was lowest for RB-N, intermediate for ALF-N and highest for CSM ($P < 0.05$). Chlorophyll *a* was a function of nitrogen input and the high-nitrogen treatments (CSM, ALF-N and RB-N) supported similar ($P > 0.05$) phytoplankton standing crops that were 38-62% greater than their corresponding low-nitrogen treatments (ALF-W and RB-W). Water temperature and dissolved oxygen concentrations were statistically similar among treatments, and total ammonia nitrogen was below detectable levels in all treatments. Overall mean densities of rotifers, nauplii, adult copepods, adult crustaceans and total zooplankton did not differ significantly among treatments ($P > 0.05$). However, mean density of cladocerans was significantly higher for RB-N and ALF-N than for ALF-W. The density of cladocerans, adult copepods, adult crustaceans or total zooplankton correlated strongly with chlorophyll *a* but not with the quantities of fertilizers applied as treatments, indicating that phytoplankton was the primary food for zooplankton. Based on pH, RB was a better fertilizer than ALF or CSM when application rates are based on nitrogen. The effects of these organic fertilizers on zooplankton abundance were essentially similar.

INTRODUCTION

Zooplankton and pH are important factors for successful culture of planktivorous fry, such as striped bass *Morone saxatilis* (Culver et al. 1993; Anderson 1993a). Culturists have used various combinations of organic and inorganic fertilizers to manage ponds to increase zooplankton populations, via primarily increased phytoplankton biomass, to promote fingerling production. Unfortunately, the increased phytoplankton biomass also can cause elevated pH in fishponds, through excessive photosynthesis, that can be detrimental to fish production. Anderson (1993a) found no striped bass in ponds with pH >9 in the week following fry stocking and reported that successful production of fingerling striped bass requires pH $\leq$ 8.5 until the fish are 14 d old. Similarly, Barkoh (1996) found survival of fingerling striped bass to be <50% when mean pH was >8.5 during the production period. Generally, susceptibility of fish to pH varies with species and age (Bergerhouse 1993), and both low pH (e.g., pH <5) and high pH (e.g., pH >10) can be harmful to fish (Kwain et al. 1984; Brewer and Rees 1990).

To maintain suitable pH in fingerling production ponds, some researchers (e.g., Anderson 1993a; Barkoh 1996) have recommended use of organic fertilization alone. This recommendation was based on the observation that inorganic fertilizers better stimulate phytoplankton blooms to promote the onset of high pH earlier in the culture period, when fish are most vulnerable, than organic fertilizers. Concurrent with using only organic fertilizers, some culturists have drastically reduced fertilizer application rates (e.g., initial fertilization from 225 kg/ha to 112 kg/ha) and frequencies (e.g., follow-up fertilization from weekly applications to 2-3 times per production cycle) to attempt to maintain moderate pH (D. Smith, Texas Parks and Wildlife Department, Electra, Texas, personal communication). The potential disadvantage of these strategies is inadequate zooplankton forage base to support maximum fish production, which is the objective of fingerling culturists. This is because zooplankton production depends on phytoplankton production (Wetzel 1975), which in turn depends on supply of nutrients (Geiger and Turner 1990).

Adequate populations of zooplankton and suitable pH for fish in hatchery ponds may be achieved by using organic fertilizers with high carbon and low nitrogen (N) and phosphorus (P) contents. Cottonseed meal with low carbon and high N and P (Geiger 1983; Anderson 1993b) has promoted high pH (pH > 10) in striped bass fingerlings production ponds resulting in low fish survival (Anderson 1993a). Conversely, rice bran with high carbon and low N and P (Anderson 1993b) has achieved moderate pH (range = 7.4-8.6) and better water quality and zooplankton production than cottonseed meal or distiller's grain in paddlefish *Polyodon spathula* production ponds (Mims et al. 1993). Ludwig and Tackett (1991), however, found no significant difference in pH in ponds fertilized with rice bran verses those fertilized with cottonseed meal.

The results of fertilization studies appear mixed partly because of differences in study conditions and the fact that organic fertilizers differ in their physical properties, decomposability, available nutrients, and nutritional value to zooplankton (Barkoh and

Rabeni 1990; Anderson 1993a). Despite the mixed results, there is evidence that certain organic fertilizers may be better fertilizers than others in promoting zooplankton production and moderate pH. Barkoh and Rabeni (1990) found alfalfa meal superior to wheat shorts or cottonseed meal in promoting survival and reproduction of *Ceriodaphnia dubia* and *Daphnia magna* in laboratory cultures and attributed the results partly, at least, to differences in pH. In their study, the pH for most of the study period was 6.0-6.5 for alfalfa meal, 6.0-5.5 for cottonseed meal and <5.0 for wheat shorts. Also, DePauw et al. (1981) produced and maintained high densities (e.g., 10,000/L) of *D. magna* on rice bran in laboratory cultures. The pH of these cultures varied from 8.2 to 7.6, which overlapped the optimal pH range (6.8-7.8) for the culture of daphnids (Ivleva 1973). It appears that organic fertilizers that directly serve as source of food for zooplankton could better enhance secondary productivity and ultimately fish production when they also promote moderate pH (Mims et al. 1993).

The physical and chemical properties of rice bran and alfalfa meal may make them better fertilizers than cottonseed meal for zooplankton-based production ponds. Most of the cottonseed meal applied to ponds settles as sludge at the pond bottom whereas rice bran or alfalfa meal stays in suspension longer with the greatest probability of being consumed directly by zooplankton (Anderson 1993a). Rice bran and alfalfa meal have relatively high carbon and low N and P compared to cottonseed meal (Anderson 1993b). Several researchers (e.g., Lugwig and Tackett 1991; Mims et al. 1991, 1993; Kurten et al. 1995; Buurma et al. 1996) have compared different organic fertilizers, but we are unaware of any study comparing rice bran, cottonseed meal and alfalfa meal side-by-side in the absence of fish and with emphasis on pH and zooplankton population dynamics. We conducted this study to determine if rice bran promotes lower pH and higher densities of zooplankton than cottonseed meal or alfalfa meal. To prevent the confounding effect of differential fish grazing on zooplankton and ultimately on phytoplankton, we conducted this study in limnocorrals without fish.

MATERIALS AND METHODS

We conducted this study in an outdoor water-storage reservoir (57,418-m^3) at the A. E. Wood Fish Hatchery in San Marcos, Texas. Twenty limnocorrals were constructed from 208-L clear plastic bags, each secured to a 42- X 42- X 21-cm Styrofoam box (floatation device) with a 14-gauge wire. The limnocorrals were linearly linked together and secured at regular intervals to the water flow diversion curtain of the reservoir. The limnocorrals were suspended in 1.5 m of water with the openings about 15 cm above the water surface. The limnocorrals were filled with approximately equal volumes (189 L) of reservoir water. Characteristics of the water were: alkalinity 240 mg/L as $CaCO_3$; total hardness 193.5 mg/L as $CaCO_3$. The limnocorrals were numbered (1 through 20) and randomly assigned to treatments, resulting in four replicates per treatment. We tested the fertilizers on weight and nitrogen-content basis because hatchery managers calculate fertilization rates based on these variables. Because the use of cottonseed meal (CSM) is commonplace, we considered it as the control. Based on weight of CSM, treatment rates of each of CSM, alfalfa meal (ALF-W), and rice bran (RB-W) were 39 mg/L for the initial application and 7.8 mg/L for the follow-up. Based on the nitrogen content of CSM, initial application rates of alfalfa meal (ALF-N) and rice bran (RB-N) were 95 mg/L and 129 mg/L, respectively. The follow-up rate was 19 mg/L for ALF-N and 26 mg/L for RB-N. These fertilization rates assumed the protein contents of CSM, ALF and RB to be 41%, 17% and 12.5%, respectively based on the manufacturer's descriptions. The fertilizers were mixed with water from the limnocorrals to make slurries before application. To prevent high pH, all fertilization was discontinued after week 3 when pH $\geq$9 were measured in some limnocorrals. The control (CSM) rate and the stop-fertilization criterion were similar to the CSM fertilization protocol for striped bass production ponds at Texas hatcheries.

Water quality variables were measured twice weekly in the limnocorrals beginning the day after filling. Water quality variables and water samples were taken at 25-30-cm depths in the afternoon (1400-1500 hours). Water temperature, pH and dissolved oxygen were measured with a Yellow Springs Instruments model 650 MDS meter equipped with a 600 XLM probe. Total ammonia nitrogen was measured with a Denver Instruments model 250 meter equipped with an Accumet Ammonia Ion Selective Electrode.

Zooplankton samples were taken shortly after sunrise with a tube sampler (Schedule 40 PVC pipe, 620 mm long and 15-mm inside diameter) by swirling the content of each limnocorral with the tube, then taking three vertical grab samples into a container. The composite was measured with a graduated cylinder and filtered through a Wisconsin plankton net (80-µm mesh) to concentrate the zooplankton. The filtrate was returned to the appropriate limnocorral. The zooplankters were identified and enumerated into six categories: rotifers, nauplii, cladocerans, adult copepods, adult crustaceans and total zooplankton (all organisms combined) and densities expressed as number of organisms per liter. Water samples for chlorophyll *a* measurements were taken immediately following zooplankton sampling. Chlorophyll *a* was assessed by the acetone extraction and fluorometry (Turner TD–700 Fluorometer) method (APHA et al. 1995) at 0830-0900 hours. Data collection ended eight weeks after initial fertilization to simulate the average period for the management of ponds for the production of phase 1 (38-50 mm) striped bass.

Data Analysis

The temperature, dissolved oxygen, pH, zooplankton density and chlorophyll *a* data were analyzed by a repeated measures version of analysis of variance, analogous to a split-plot design, using the linear models (GLM) procedure of SAS (SAS Institute 1999). When differences among treatment means or treatment-sampling date interactions were significant, the differences between means were tested using the Tukey's procedure. The relationship between zooplankton density and chlorophyll *a* or amount of fertilizer applied to treatment was determined by Pearson's correlation analysis. Significance level was set at $P \leq 0.05$.

RESULTS

The total amounts of fertilizers applied as treatments were 34.18 g for RB-N, 20.74 g for ALF-N, and 10.92 g each for CSM, ALF-W and RB-W. Water quality differed significantly among treatments, with the pH being highest for CSM, followed by ALF-N, and then RB-N. The pH did not significantly differ among ALF-N, ALF-W, and RB-W (Table 1). Trends of pH were similar for all treatments, except for the CSM treatment that promoted a steady increase of pH over a 3-week period (Figure 1). The overall mean chlorophyll *a* values varied from 2.27-3.45 µg/L for ALF-W and RB-W to 5.08-5.97 µg/L for CSM, ALF-N and RB-N, but the differences were not significant (P = 0.06). However, temporal differences were significant on 6 of 12 sampling days (Figure 1). The daily chlorophyll *a* values for CSM, ALF-N and RB-N were similar, but each was significantly higher than those of AF-W and RB-W on several occasions. Water temperature and dissolved oxygen were statistically similar among treatments (Table 1). Total ammonia nitrogen concentrations were below the minimum detectible limit (0.05 mg/L) throughout the study.

Overall mean densities of rotifers, nauplii, adult copepods, adult crustaceans and total zooplankton did not differ significantly among treatments ($P > 0.05$). Conversely, mean densities of cladocerans were significantly higher for ALF-N and RB-N than for ALF-W, whereas the densities for CSM and RN-W did not significantly differ from that of ALF-N, RB-N or ALF-W (Table 2). The density trends for nauplii, rotifers, adult copepods or total zooplankton were similar for all treatments (Figures 2 and 3), although adult copepods peaked highest in ALF-N on one occasion. The density trends of cladocerans differed among treatments, with densities peaking for extended periods for the RB-N and ALF-N treatments (Figure 2). Density of cladocerans was least variable in ALF-W and more variable in the other treatments. The density of adult copepods, cladocerans, adult crustaceans or total zooplankton correlated strongly with chlorophyll *a* (r^2 = 0.79-0.96; $P < 0.05$) but not with the amounts of fertilizers applied to treatments.

DISCUSSION

Rice bran promoted better water quality than alfalfa meal or cottonseed meal when application rates were based on nitrogen content. Possibly, the larger quantity of rice bran provided more carbon to generate enough carbon dioxide to prevent the pH from rising as much as it did in the ALF-N and CSM treatments. The ALF-N, second in quantity and carbon content to RB-N, also promoted a lower pH than the CSM treatment but a higher pH than RB-N. These results concur with those of previous studies that demonstrated that RB produced lower pH in laboratory culture media (DePauw et al. 1981) or in fish production ponds (Mims et al. 1993).

Phytoplankton abundance was a function of nitrogen input: treatments with similar nitrogen contents supported similar chlorophyll *a* concentrations. The high-nitrogen treatments supported phytoplankton standing crops that were 38-62% greater than their corresponding low-nitrogen treatments. However, the low chlorophyll *a* concentrations and the high dissolved oxygen levels suggest that the fertilizer application rates could be increased. Our chlorophyll *a* concentrations were $\leq$50% than those recommended for fertilized ponds for the production of fingerling striped bass (Geiger and Turner 1990).

Zooplankton succession roughly followed the typical pattern. Rotifers dominated the population first, followed by cladocerans, and then copepods. The lack of significant differences in densities of zooplankters among treatments, except for cladocerans, suggests that the suit of environmental conditions that support population growth was similar in their combined effects on most, if not all, of the zooplankton populations. Temperature and quantity and quality of food are the important factors that influence zooplankton population growth (Allan 1976, Geiger and Turner 1990), and these factors were statistically similar among treatments. The density of cladocerans correlated strongest with chlorophyll *a,* indicating a better utilization of this food resource by cladocerans compared to the other zooplankton groups. Cladocerans are reported to be more efficient feeders than rotifers and copepods (Allan 1976; Geiger and Turner 1990). The strong positive correlation of zooplankton abundance with chlorophyll *a*, and not with the quantities of fertilizers applied to treatments, suggests that phytoplankton was a major food item of the zooplankton diet, whereas fertilizer was probably not. The fertilizer amounts applied to treatments were probably inadequate to support efficient feeding by most zooplankters. The highest concentration of fertilizer (RB-N) used in this study was 9.5 mg/L/d during the first three weeks when fertilization was administered. DePauw et al. (1981) reported that a minimum of 60 mg/L/d of RB was required to assure the minimum particle concentration for efficient filter feeding by daphnids.

The biological objective of fertilization programs for fingerling fish production ponds is to stimulate the development of all aspects of zooplankton food resources (Geiger and Turner 1990). Most of these fertilization programs recommend the use of organic fertilizers with high nitrogen and low carbon contents, such as CSM, to achieve faster decomposition and increased productivity (e.g., Geiger 1983, Geiger and Turner 1990). However, where management practices have the dual objective to achieve increased pond productivity and moderate pH, organic fertilizers with high carbon contents, such as RB, should be considered. Apparently, the high carbon:nitrogen ratios of these fertilizers would probably be less of a factor to their decomposition rates if particle size is as small as possible (Barkoh and Rabeni 1990). Our results revealed RB as the best of the organic fertilizers compared in this study as well as the need to increase the fertilization rates. Future studies should maximize RB fertilization rate while maintaining oxygen levels that are conducive for fish production.

LITERATURE CITED

Allan, J. D. 1976. Life history patterns in zooplankton. American Naturalist 110:165-180.

APHA et al. (American Public Health Association, American Water Works Association, and Water Pollution Control Federation). 1995. Standard methods for the examination of water and wastewater. 19th edition. APHA, Washington, D. C.

Anderson, R. O. 1993a. Apparent problems and potential solutions for production of fingerling striped bass *Morone saxatilis*. Journal of Applied Aquaculture 2(3/4):101-118.

Anderson, R. O. 1993b. New approaches for management of fertilized hatchery ponds. Journal of Applied Aquaculture 2(3/4):1-8.

Barkoh, A. 1996. Effects of three fertilization treatments on water quality, zooplankton, and striped bass fingerling production in plastic-lined ponds. Progressive Fish-Culturist 58:237-247.

Barkoh, A., and C. F. Rabeni. 1990. Biodegradability and nutritional value to zooplankton of selected organic fertilizers. Progressive Fish-Culturist 52:19-25.

Bergerhouse, D. L. 1993. Lethal effects of elevated pH and ammonia on early life stages of hybrid striped bass. Journal of Applied Aquaculture 2(3/4):81-100.

Brewer, D. L., and R. A. Rees. 1990. Pond culture of phase 1 striped bass fingerlings. Pages 99-120 *in* R. M. Harrell, J. H. Kerby, and R. V. Minton, editors. Culture and propagation of striped bass and its hybrids. Striped Bass Committee, Southern Division, American Fisheries Society, Bethesda, Maryland.

Buurma, B. J., A. Barkoh, and G. A. Alexander. 1996. Effects of cottonseed and alfalfa meals as fertilizers on production of palmetto bass Morone saxatilis x M. chrysops fingerlings in plastic-lined ponds. Journal of Applied Aquaculture 6(3):59-70.

Culver, D. A., S. P. Madon, and J. Qin. 1993. Percid production techniques: timing, enrichment, and stocking density manipulation. Journal of Applied Aquaculture 2(3/4):9-31

DePauw, N, P. Laureys, and J. Morales. 1981. Mass cultivation of *Daphnia magna* on rice bran. Aquaculture 25:141-152.

Geiger, J. G. 1983. A review of pond zooplankton production and fertilization for the culture of larval and fingerling striped bass. Aquaculture 35:353-369.

Geiger, J. G., and C. J. Turner. 1990. Pond fertilization and zooplankton management techniques for production of fingerling striped bass and hybrid striped bass. Pages 79-98 *in* R. M. Harrell, J. H. Kerby, and R. V. Minton, editors. Culture and propagation of striped bass and its hybrids. Striped Bass Committee, Southern Division, American Fisheries Society, Bethesda, Maryland.

Ivleva, I. V. 1973. Mass cultivation of invertebrates: biology and methods. English translation: Israel Program for Scientific Translations, Jerusalem.

Kurten, G., L. Hall, and N. Thompson. 1995. Comparison of cottonseed meal and alfalfa meal for fertilizing earthen Florida largemouth bass spawning and rearing ponds. Management Data Series Number 113. Texas Parks and Wildlife Department, Austin.

Kwain, W., R. W. McCauley, and J. A. MacLean. 1984. Susceptibility of starved, juvenile smallmouth bass, *Micropterus dolomieui,* to low pH. Journal Fish Biology 25:501-504.

Ludwig, G. M., and D. L. Tackett. 1991. Effects of using rice bran and cottonseed meal as organic fertilizers on water quality, plankton, and growth and yield of striped bass *Morone saxatilis* fingerlings in ponds. Journal of Applied Aquaculture 1(1):79-94.

Mims, S. D., J. A. Clark, J. C. Williams, and D. B. Rouse. 1993. Comparisons of two by-products and a prepared diet as organic fertilizers on growth and survival of larval paddlefish, *Polyodon spathula*, in earthen ponds. Journal of Applied Aquaculture 2(3/4):171-187.

Mims, S. D., J. A. Clark, and J. H. Tidwell. 1991. Evaluation of three organic fertilizers for paddlefish, *Polyodon spathula*, production in nursery ponds. Aquaculture 99:69-82.

SAS Institute. 1999. SAS/STAT User's Guide, version 8. SAS Institute, Inc., Cary North Carolina.

Wetzel, R. G. 1975. Limnology. W. B. Saunders Company, Philadelphia

Table 1. Mean water quality values ($\pm$ SD) for five organic fertilization treatments compared for 8 weeks (May 20 - July 12, 2002) in limnocorrals suspended in an outdoor water-storage reservoir at the A. E. Wood Fish Hatchery, Texas. Where applicable values in a row bearing the same letter are not significantly different (P <0.05).

	Treatments					
Water quality variables*	Cottonseed meal	Alfalfa meal (nitrogen equivalent)	Rice bran (nitrogen equivalent)	Alfalfa meal (weight)	Rice bran (weight)	*P*-value
P.M. pH	9.39 $\pm$ 0.59a	9.25 $\pm$ 0.57b	9.08 $\pm$ 0.62c	9.17 $\pm$ 0.58bc	9.13 $\pm$ 0.64bc	0.005
Chlorophyll-a (*ug*/L)	5.08 $\pm$ 3.20	5.97 $\pm$ 5.58	5.56 $\pm$ 6.69	2.27 $\pm$ 2.2	3.45 $\pm$ 4.56	0.061
Dissolved oxygen (mg/L)	15.15 $\pm$ 2.47	15.00 $\pm$ 2.71	14.76 $\pm$ 2.94	14.87 $\pm$ 2.87	14.67 $\pm$ 2.87	0.762
P.M. temperature (oC)	30.44 $\pm$ 1.88	30.48 $\pm$ 1.89	30.47 $\pm$ 1.93	30.44 $\pm$ 1.92	30.40 $\pm$ 1.88	0.427

*Total ammonia nitrogen was below detectable levels (i.e., < 0.05 mg/L) in all treatments.

Table 2. Mean zooplankton densities (± SD) for five organic fertilization treatments compared for 8 weeks (May 20 - July 12, 2002) in limnocorrals suspended in an outdoor water-storage reservoir at the A. E. Wood Fish Hatchery, Texas. Where applicable values in a row bearing the same letter are not significantly different ($P < 0.05$).

	Treatments					
Zooplankton (number/L)	Cottonseed meal	Alfalfa meal (nitrogen equivalent)	Rice bran (nitrogen equivalent)	Alfalfa meal (weight)	Rice bran (weight)	*P*-value
Rotifer	151 ± 291	101 ± 191	127 ± 181	110 ± 170	118 ± 155	0.76
Nauplii	228 ± 279	205 ± 238	144 ± 193	209 ± 229	179 ± 225	0.076
Cladocerans	203 ± 275ab	241 ± 275a	249 ± 325a	116 ± 170b	166 ± 250ab	0.025
Adult copepods	71 ± 108	68 ± 120	81 ± 154	48 ± 66	59 ± 74	0.59
Adult crustaceans	425 ± 430	411 ± 381	458 ± 485	274 ± 257	343 ± 350	0.28
Total zooplankton	653 ± 553	616 ± 502	602 ± 563	483 ± 360	522 ± 499	0.38

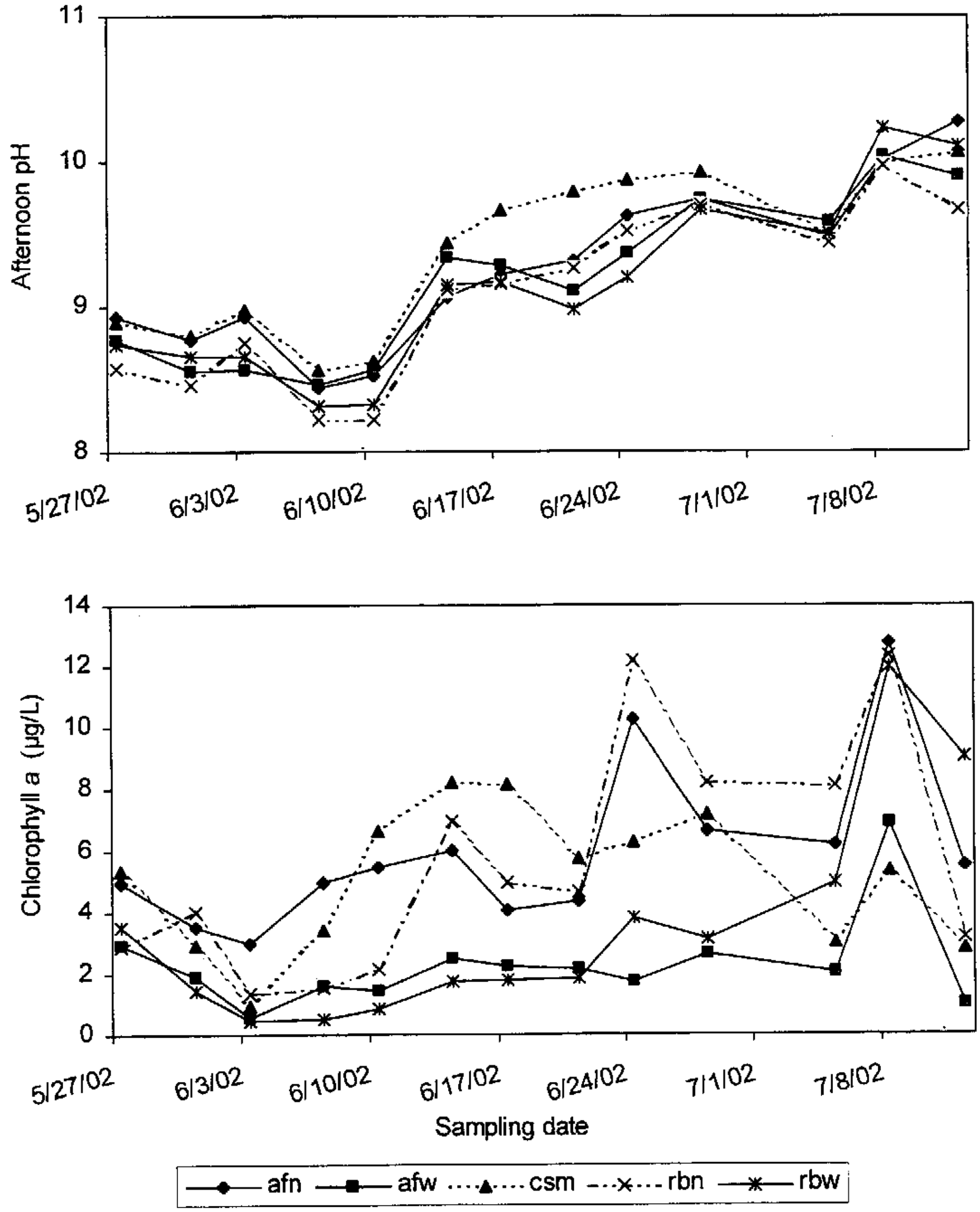

Figure 1. Mean pH and chlorophyll *a* levels for five organic fertilization treatments compared for 8 weeks (May 20 - July 12, 2002) in limnocorrals suspended in an outdoor water-storage reservoir at the A. E. Wood Fish Hatchery, Texas.

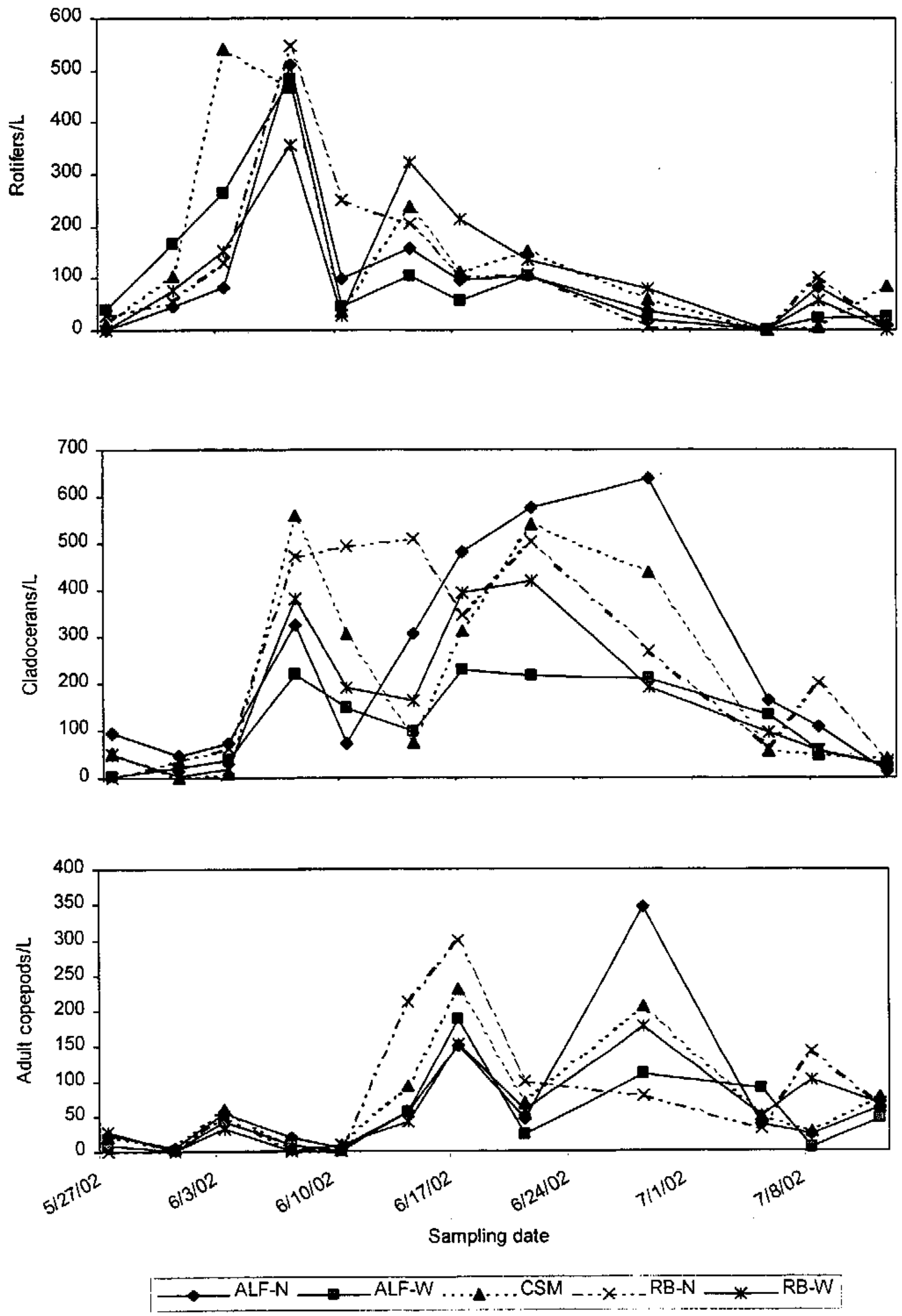

Figure 2. Mean densities of rotifers, adult cladocerans and adult copepods for five organic fertilization treatments compared for 8 weeks (May 20 - July 12, 2002) in limnocorrals suspended in an outdoor water-storage reservoir at the A. E. Wood Fish Hatchery, Texas.

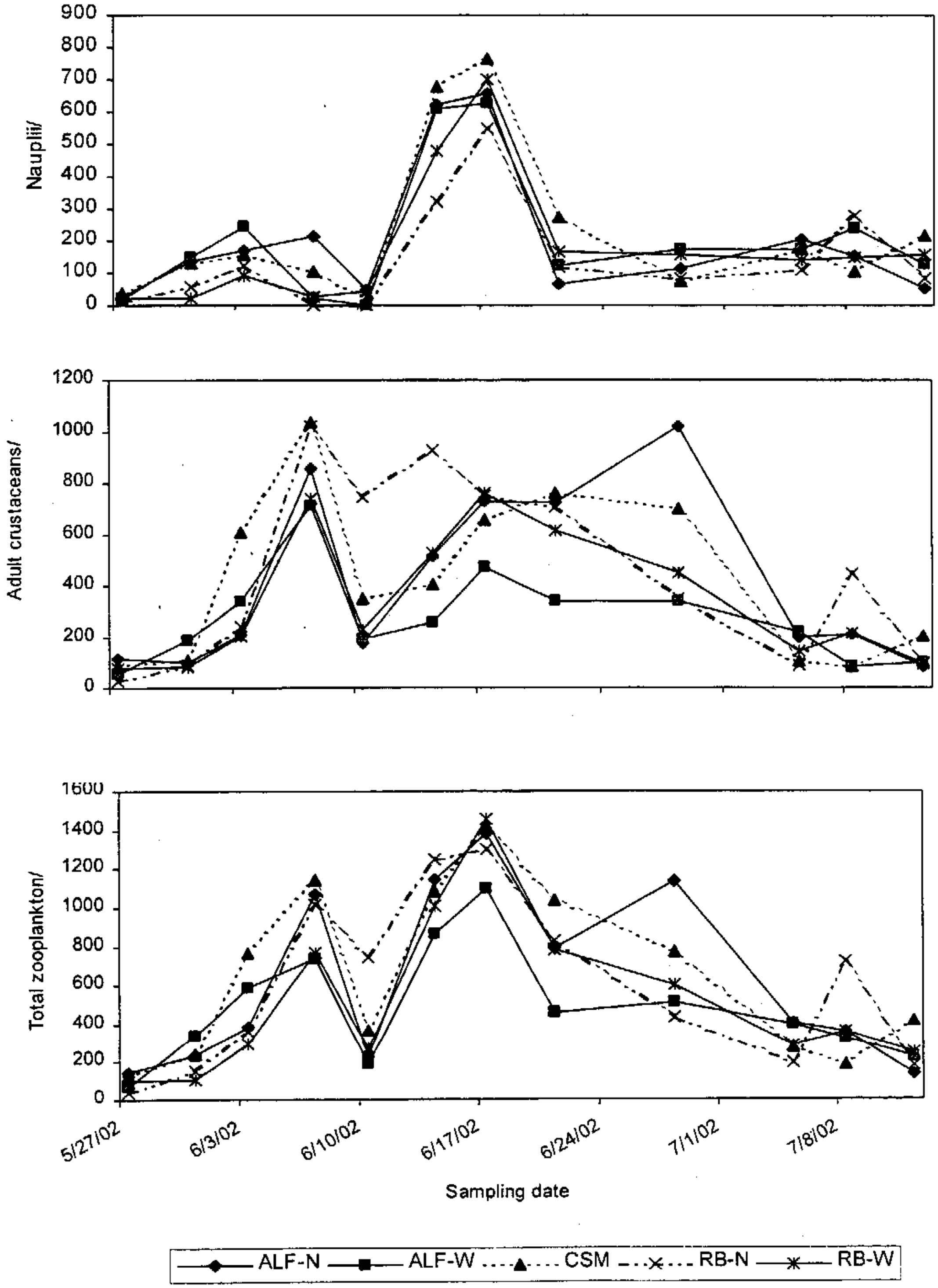

Figure 3. Mean densities of nauplii, adult crustaceans and total zooplankton for five organic fertilization treatments compared for 8 weeks (May 20 - July 12, 2002) in limnocorrals suspended in an outdoor water-storage reservoir at the A. E. Wood Fish Hatchery, Texas.