Factors Influencing Zooplankton Production in Organically Fertilized Ponds for Culture of Paddlefish, *Polyodon spathula*

Steven D. Mims
Julia A. Clark
John C. Williams
David R. Bayne

ABSTRACT. Zooplankton production was evaluated using either rice bran, distillers dry solubles, or a prepared diet as organic fertilizers in nine 0.02-ha earthen ponds. Each pond was inoculated with *Daphnia pulex* and stocked with larval paddlefish at 61,775/ha. Mean seasonal abundance and biomass of adult copepods was significantly higher ($P \leq 0.05$) than cladocerans in each treatment. Mean abundance of cladocerans larger than 0.6 mm in total length was significantly lower ($P \leq 0.05$) than cladocerans smaller than 0.6 mm in total length. Mean biomass of large cladocerans in PD ponds was significantly greater ($P \leq 0.05$) than biomass of large cladocerans in DS ponds. Large cladocerans were preferred food items by paddlefish, compared to adult copepods or small cladocerans. Low *Daphnia* abundance after week 4 may have been caused by heavy fish predation, competition within the zooplankton community, potential algal toxicity, or elevated phosphorus. Rice bran or prepared

Steven D. Mims and Julia A. Clark, Aquaculture Research Center, Community Research Service, Kentucky State University, Frankfort, KY 40601 USA.
John C. Williams, Research Data Analysis, Auburn University, AL 36849 USA.
David R. Bayne, Department of Fisheries and Allied Aquacultures, Alabama Agricultural Experimental Station, Auburn University, AL 36849 USA.

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diet were found to provide the best environment for *Daphnia* development during the study; however, paddlefish production in ponds fertilized with PD was about 3 times more expensive than production in ponds fertilized with RB.

INTRODUCTION

Cladocerans larger than 1.0 mm in total length (TL), especially *Daphnia* spp., are preferred food items for paddlefish, *Polyodon spathula*, larvae (Michaletz et al. 1982; Mims et al. 1991). Young paddlefish smaller than 120 mm in TL are particulate feeders (Rosen and Hales 1981) and require *Daphnia* for satisfactory fish survival and yield.

Major declines in the *Daphnia* population are attributed to food deficiency, dissolved oxygen less than 3 mg/L (Ivleva 1973), and predation by invertebrate and vertebrate (Gliwicz 1981). Other factors that affect *Daphnia* longevity and reproduction are water temperature, high phosphorus and pH levels, and phytoplankton biomass (Ivleva 1973; Bhanot and Vass 1976). Michaletz et al. (1983) found low food availability, not fish predation, to be the cause for reduction in the population of *Daphnia* in ponds fertilized with a combination of alfalfa meal (227 kg/ha), brewer's yeast (453 kg/ha), cow manure (453 kg/ha), and 10 bales of clover hay/ha. Recently, Mims et al. (1991) reported that paddlefish rearing ponds stocked at 61,775 larvae/ha, fertilized with rice bran (1,742 kg/ha), and inoculated with *Daphnia pulex* could sustain a *Daphnia* population over a 40-d culture period.

The objectives of this study were: (a) to assess zooplankton responses to two commercially available agro-industrial by-products and one prepared diet applied as organic fertilizers in paddlefish rearing ponds and (b) to determine the selectivity of larval paddlefish on zooplankton produced in these ponds.

MATERIALS AND METHODS

Pond Management

Nine 0.02-ha earthen ponds located at Kentucky State University Aquaculture Research Center in Frankfort were used. Three ponds were randomly assigned to each of three treatments. Ponds received a pre-filling treatment with liquid Hydrothol at a rate of 2 kg/ha to reduce filamentous algae. Ponds were filled to a depth of 1.1 m with water taken from a surface-water reservoir and filtered through saran cloth socks (385-µm mesh size).

Two commercially available agro-industrial by-products (rice bran, [RB] and distillers dried solubles, [DS]) and a prepared diet [PD] (Purina trout chow starter; manufacturer's code #5100, Richmond, Indiana[1]) were evaluated as organic fertilizers. Fertilizer quantities for each treatment were based on the nitrogen content of RB (control) as described by Mims et al. (1991) (Table 1). Total amount of nitrogen applied to each pond as organic fertilizer was 43.1 kg/ha. Organic fertilizers were analyzed according to Horwitz (1980); carbon-to-nitrogen ratios were determined with an elemental analyzer for macrosamples (Leco CHN, model 600, St Joseph, Michigan) (Table 2). Each pond received an additional 11 kg/ha of nitrogen as liquid 10-34-0 ammonium polyphosphate applied at varied amounts and time intervals over the experimental period (Table 1).

Ponds were inoculated with zooplankton, predominately *Daphnia pulex*, at a rate of about 125,000/pond by 15 April 1990. Each pond was equipped with one continually-operated, 5-cm polyvinyl chloride, air-lift pump to provide thorough mixing of nutrients and zooplankton during the study (Parker 1979). On 27 April, each pond was stocked with 61,775 larval paddlefish/ha. After 40 days fish were harvested to determine survival and yield for each treatment.

Water Quality Analysis and Management

Each day, at 0700 and 1500 hours, dissolved oxygen and water temperature were measured with a polarographic dissolved oxygen meter and thermistor (YSI Model 54A, Yellow Springs, Ohio), and pH was measured using an electronic pH meter (Omega pHH-43 meter, Stanford, Connecticut). Water samples were collected twice per week from each pond and analyzed for total filterable orthophosphates (PO_4-P) using a Hach DREL/5 (Loveland, Colorado). Chlorophyll-*a* was extracted weekly from composite phytoplankton samples (Boyd 1979) with acetone and measured spectrophotometrically (APHA et al. 1980). Emergency aeration was provided with 0.33-hp surface aerators whenever dissolved oxygen concentrations were predicted by graph to decline below 40% of saturation (Andrews et al. 1973).

1. Use of trade or manufacturer's name does not imply endorsement.

TABLE 1. Organic and inorganic fertilizer application rates in 0.02-ha ponds stocked with 61,775 larval paddlefish per hectare.

Week	No. of applications	Organic fertilizers: Rice bran (kg/ha/week)	Organic fertilizers: Distillers dried solubles (kg/ha/week)	Organic fertilizers: Prepared diet (kg/ha/week)	Inorganic[1] fertilizer (L/ha/week)
0[2]	6	1410	600	345	37.0
1	3	310	132	76	4.6
2-5	2	157	67	39	9.3
Total	17	2348	1000	577	78.8

[1] All treatments received the same amount of liquid 10-34-0 fertilizer.

[2] Three weekly applications of fertilizers were applied to the filled ponds during a two-week period prior to stocking.

Zooplankton Sampling

Zooplankton samples were collected with a tube sampler as described by Graves and Morrow (1988) twice each week from randomly selected locations at 0.3- and 1.1-m depths in each pond. Sampling continued until a 10-L water sample was obtained. The water sample was filtered through an 80-μm mesh size, Wisconsin-style plankton net, and the concentrated organisms preserved in cold 5% buffered formalin with sucrose (Haney and Hall 1973). Zooplankton were counted in a Sedgewick-Rafter counting chamber with a compound microscope. Cladocerans were identified to species, and adult copepods and copepodites to suborder (Ward and Whipple 1959; Pennak 1978). Randomly selected organisms were measured for total length (TL; mm). Estimated zooplankton abundance, as organisms/L, (McCauley 1984) and biomass, μg/L, (Dumont et al. 1975) were calculated. Species of cladocera were grouped according to their average size, either as small (<0.6 mm in TL) or large (≥ 0.6 mm in TL) organisms (Mims et al. 1991). Rotifers and copepod nauplii were not included because they are not preferred food organisms for larval paddlefish (Michaletz et al. 1982; Mims et al. 1991).

Larval Fish Sampling and Gut Analysis

Ten fish were collected from each pond weekly, preserved in 10% buffered formalin, and the gut contents analyzed at a later date. Each gut

TABLE 2. Analysis of organic fertilizers applied to 0.02-ha ponds stocked with 61,775 larval paddlefish per hectare.

Composition	Rice bran (%)	Distillers dried solubles (%)	Prepared diet (%)
Crude Protein	11.1	27.2	47.1
Fat	14.1	6.2	24.7
Crude Fiber	12.8	3.5	1.1
Moisture	9.5	4.4	7.5
Phosphorus	1.5	1.3	1.6
Potassium	1.5	1.6	0.6
Magnesium	0.8	0.5	0.2
Calcium	1.1	0.1	2.5
C:N ratio	22:1	10:1	6:1

was removed, placed in a Sedgewick-Rafter counting chamber, and carefully opened. Contents were rinsed into the counting chamber with distilled water and examined microscopically at 40 × magnification. Zooplankton were counted and identified to the same taxonomic levels as described previously.

Prey Selection

Chesson's (1978) alpha (α) electivity index was used to compare the prey consumed by predators with availability of the prey in the environment: $\alpha_i = r_i / p_i / (\Sigma_i r_i / p_i)$, where r_i is the proportion of prey taxon eaten and p_i is the proportion of the same prey taxon in the environment. The expected value for random feeding with this index is a function of number of food items: 1/n where n is the number of types of food in the sample. The index varies between 0 and 1 with values above 1/n indicating preference and below 1/n indicating avoidance. The α index has the advantage of not being affected by relative abundance of food types and is useful for making meaningful comparisons among samples where abundances may differ (Lechowicz 1982).

Data Analysis

Natural logarithmic transformation on zooplankton abundance and biomass was conducted to reduce heterogeneity of variances. Zooplankton abundance and biomass, α index, and water quality data observed weekly in the ponds were analyzed by a repeated measures version of ANOVA (split plot design) for a completely randomized design (SAS 1990). Mean differences between selected treatment means (*a priori* comparisons) were tested with contrasts. Weekly trends in measured variables were tested using orthogonal polynomial contrasts. The probability level for tests was 0.05.

RESULTS

Fish

At harvest, mean paddlefish yield in ponds fertilized with RB (219 ± 22 kg/ha) was significantly greater ($P \leq 0.05$) than in ponds fertilized with DS (129 ± 27 kg/ha). Mean fish survival in ponds fertilized with RB (55 ± 5%) was not significantly different ($P > 0.05$) than that from ponds fertilized with DS (50 ± 6%). Mean yield in ponds fertilized with RB (219 ± 22 kg/ha) was not significantly different ($P > 0.05$) than that from ponds fertilized with PD (258 ± 22 kg/ha). However, mean survival in ponds fertilized with PD (79 ± 5%) was significantly higher ($P \leq 0.05$) than that from ponds fertilized with RB (55 ± 5%). Fish sampled from ponds fertilized with RB and PD averaged 130 and 120 mm TL, respectively, whereas DS fish averaged only 105 mm.

Zooplankton

Mean abundance and biomass of total zooplankters during the experimental period were similar among treatments (Table 3). Mean abundance and biomass of adult copepods and copepodites were significantly higher and greater ($P \leq 0.05$), respectively, than that of either large or small cladocerans. Mean abundance of large cladocerans was significantly lower ($P \leq 0.05$) than abundance of small cladocerans; however, there was no significant difference ($P > 0.05$) in averaged biomass between large and small cladocerans.

Mean biomass of large cladocerans in PD ponds was significantly greater ($P \leq 0.05$) than biomass of large cladocerans in DS ponds; how-

TABLE 3. Mean seasonal abundance (AB) and biomass (BM) ± standard error (S.E.) of adult microcrustacean zooplankton collected from 0.02-ha ponds fertilized with rice bran (RB), prepared diet (PD), or distillers dried solubles (DS) and stocked with 61,775 larval paddlefish per hectare. Different letters in columns indicate significant differences ($P \leq 0.05$).

Treatment	Total Zooplankton		Large Cladocera		Small Cladocera		Adult Copepoda	
	AB no./L	BM mg/L	AB no./L	BM mg/L	AB no./L	BM mg/L	AB no./L	BM mg/L
RB	89	328	3	69	45	39	218	781
DS	88	281	2	22a	24	22	226	891
PD	90	343	4	83b	37	53	243	891
S.E.	38	186	1	29	22	24	89	336

ever, there were no differences ($P > 0.05$) in mean abundance of large cladocerans among treatments (Table 3). Between weeks 1 and 4 abundance of large cladocerans in RB and PD ponds was significantly higher ($P \leq 0.05$) than in DS ponds (Figure 1), and large cladoceran abundance in PD ponds was significantly higher ($P \leq 0.05$) than in RB ponds. Peak populations of large cladocerans were found on week 3 in PD ponds, on week 4 in RB ponds, and on week 6 in DS ponds. Dominant large cladocerans were *Daphnia pulex*, *D. catawba*, and *Scapholeberis mucronata*.

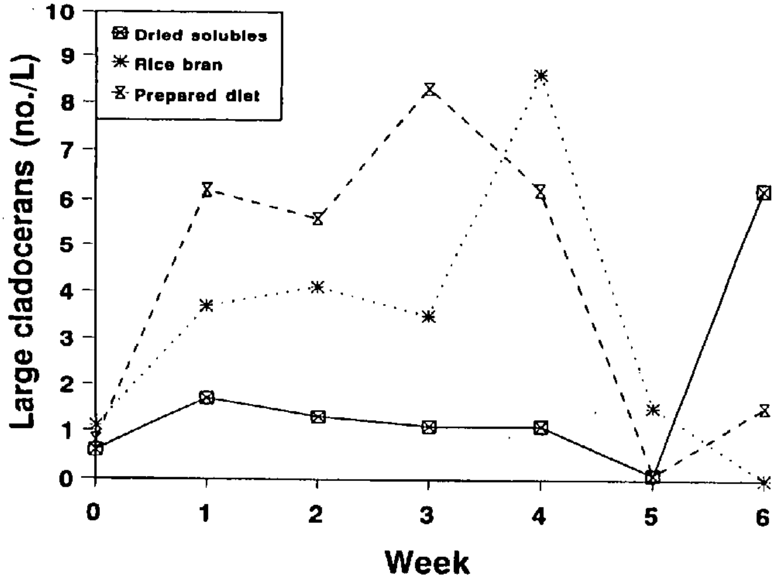
Mean abundance and biomass of small cladocerans were similar among treatments (Table 3). Peak densities of small cladocreans were found on week 4 in RB ponds and week 6 in DS and PD ponds (Figure 2). Dominant small cladocerans were *Bosmina longirostris* and *Chydorus sphaericus*.

Mean abundance and biomass of adult copepods, primarily of suborder Cyclopoida, were similar among treatments (Table 3). Peak population of adult copepods was found during week 1 in RB and PD ponds and week 4 in DS ponds (Figure 3).

Feeding Selectivities

Mean α indices for large cladocerans by larval paddlefish in RB (0.99), DS (0.94), and PD (0.93) ponds were similar ($P > 0.05$) and indicated a high preference for these food items. There were no weekly differences ($P > 0.05$) for large cladoceran selectivity among treatments. Mean α indices

FIGURE 1. Mean weekly abundance of large cladocerans in ponds fertilized with rice bran (RB), distillers dried solubles (DS), or prepared diet (PD) and stocked with 61,775 larval paddlefish per hectare.

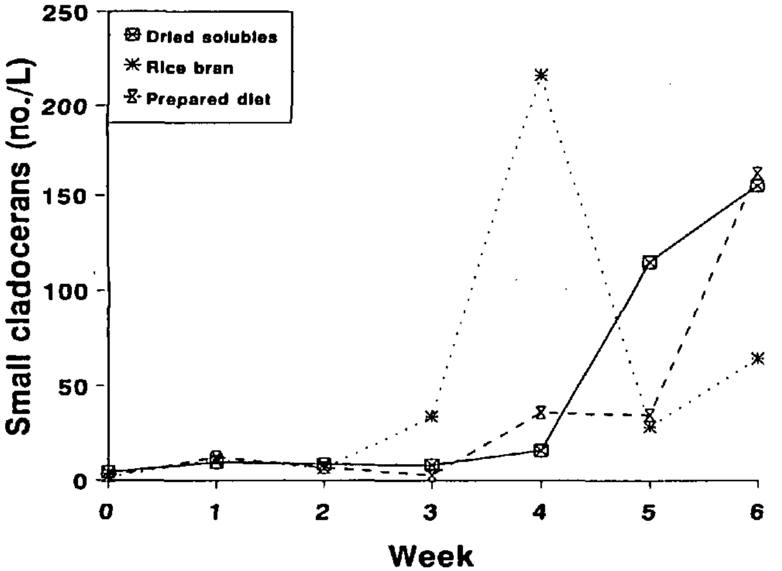


for small cladocerans were zero for each treatment, indicating an avoidance of these food items. There were no significant weekly differences ($P > 0.05$) among treatments for small cladoceran α indices. Mean α index for adult copepods for fish in PD ponds (0.18) was significantly greater ($P \leq 0.05$) than the α index for fish in RB (0.06) or DS (0.00) ponds. The indices indicated an avoidance of adult copepods in all treatments.

Water Quality

Mean early morning dissolved oxygen concentrations for ponds fertilized with RB (7.1 mg/L) was significantly lower ($P \leq 0.05$) than for DS

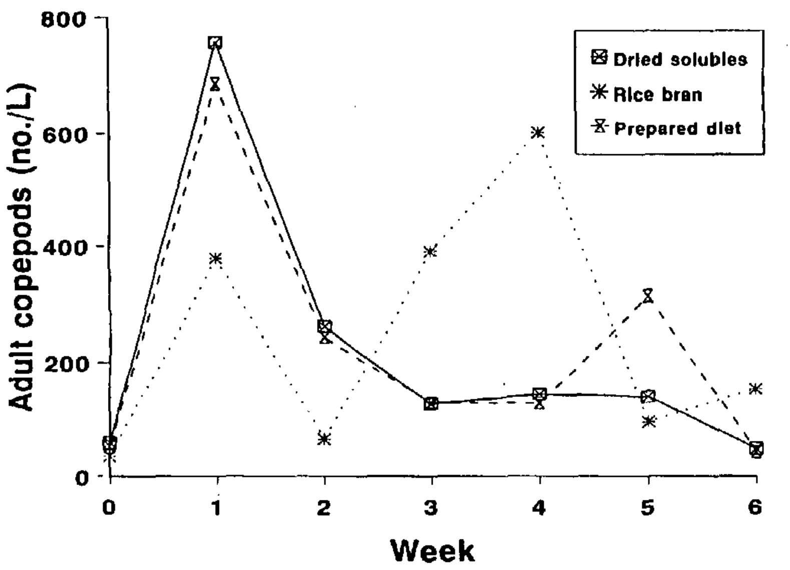
FIGURE 2. Mean weekly abundance of small cladocerans in ponds fertilized with rice bran (RB), distillers dried solubles (DS), or prepared diet and stocked with 61,775 larval paddlefish per hectare.



(7.7 mg/L) or PD (8.6 mg/L) ponds. Mean water temperatures were not significantly different ($P > 0.05$) for RB, DS, and PD ponds and averaged 20.7 °C.

Mean phytoplankton biomass was estimated by measuring chlorophyll-*a* concentrations. Ponds fertilized with DS (40 μg/L) had a significantly higher ($P \leq 0.05$) mean chlorophyll-*a* concentration than RB (16 μg/L) or PD (20 μg/L) ponds. Chlorophyll-*a* concentrations in DS ponds were significantly higher ($P \leq 0.05$) on weeks 0 and 4 and in PD ponds on week 4 than in RB ponds (Figure 4). Mean afternoon pH values for ponds fertilized with PD (8.4) were significantly higher ($P \leq 0.05$) than for RB (7.8) or DS (8.0) ponds. Weekly afternoon pH in RB ponds was significantly lower ($P \leq 0.05$) on weeks 1, 3, and 4 than in PD ponds ; and pH in DS ponds was significantly lower ($P \leq 0.05$) on week 4 than in PD ponds (Figure 5). Highest afternoon pH was 9.6 in PD ponds. Mean seasonal or

FIGURE 3. Mean weekly abundance of adult copepods in ponds fertilized with rice bran (RB), distillers dried solubles (DS), or prepared diet and stocked with 61,775 larval paddlefish per hectare.



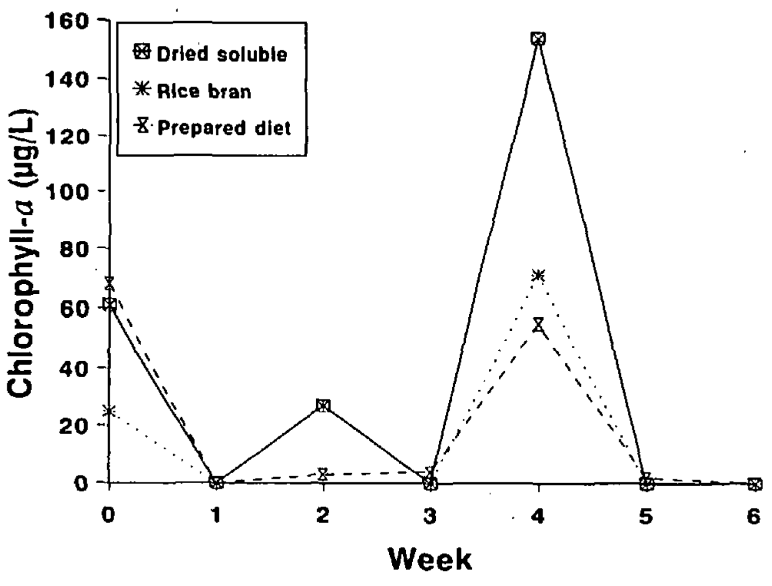
weekly phosphorus levels were not significantly different ($P > 0.05$) among treatments; however, levels of phosphorus above 2.0 mg/L were noted on week 5 for all treatments.

DISCUSSION

Rice bran (RB) and prepared diet (PD) performed better than distiller's soluble (DS) as organic fertilizers to promote large cladoceran production for supporting high paddlefish yields. However, adult copepods were most abundant, compared to large cladocerans or small cladocerans in each treatment.

The greater abundance of adult cyclopoid copepods may be a result of their ability to escape predation by larval paddlefish. Similar results have been reported by Michaletz et al. (1982) and Mims et al. (1991). Random

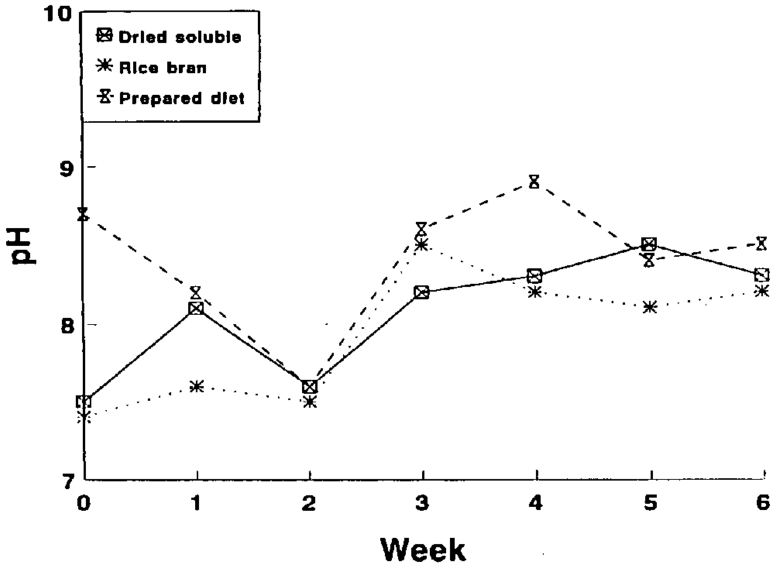
FIGURE 4. Mean weekly chlorophyll-a concentrations (µg/L) in ponds fertilized with rice bran (RB), distillers dried solubles (DS), or prepared diet (PD) and stocked with 61,775 larval paddlefish per hectare.



feeding on adult copepods usually occurs when fish gill raker development permits filter feeding (Michaletz et al. 1982). Before gill raker development is complete, consumption of adult copepods by larval paddlefish is limited because of their inability to stalk and capture fast swimming copepods. Copepods can change direction rapidly at swimming speeds 10 to 50 times greater than cladocerans (Allan 1976).

In contrast, large cladocerans were the least abundant zooplankters sampled. Paddlefish preference for cladocerans and competition between cladocerans and adult copepods for organic matter may explain the low cladoceran abundance. Similar effects of fish predation on zooplankton communities (Allan 1974; Hairston and Pastorok 1975; Jacobs 1978; Gardner 1981) and food competition within the zooplankton community (Allan 1976; Lynch 1980; Porter et al. 1982) have been reported. Additionally, the low abundance of large cladocerans in samples could be due to inade-

FIGURE 5. Mean weekly afternoon pH in ponds fertilized with rice bran (RB), distillers dried solubles (DS), or prepared diet (PD) and stocked with 61,775 larval paddlefish per hectare.



quate sampling methods. Large cladocerans were observed to congregate primarily around the pond perimeters and low in the water column, whereas copepods were more dispersed and pelagic (Mal'tsman 1974).

Cladoceran populations can simultaneously reproduce sexually and parthenogenetically (Ivleva 1973). Sexual reproduction, as evidenced by the formation of ephippia in females, can appear in a young population. However, a large proportion of ephippial females usually occurs when there is food deficiency and overpopulation (Ivleva 1973) indicating the demise of the population. Michaletz et al. (1983) found a high percentage of ephippial females caused by a lack of food when *Daphnia ambigua* and *D. parvula* density reached 50 per liter in paddlefish ponds. Parthenogenetic reproduction of subitaneous eggs is predominant when the population is healthy and growing (Ivleva 1973). In this study, parthenogentic reproduction was evident through week 4 in each treatment, with less than 25% of the reproducing female having ephippia from the zooplankton samples.

Sexual reproduction never became the predominant form of reproduction during the study, which indicated that overpopulation or food deficiency probably did not adversely affect the large cladocerans. However, a sharp decline of *Daphnia* spp. in RB and PD treatments was noted during week 5, despite no change in mode of reproduction. Fish predation was probably not the primary reason for the decline on week 5, because the large cladoceran density in PD treatment increased during week 6. Also, paddlefish detect food by electroreceptors and not by sight (Jorgensen et al. 1972). The possibility of paddlefish removing most large cladoceran from the environment is highly unlikely because the ability of paddlefish to capture *Daphnia* decreases as *Daphnia* densities decline (Mims and Schmittou 1989). Therefore, other environmental factors were examined to determine what may have caused the decline in *Daphnia* spp.

High phytoplankton abundance during week 4 could have been the reason for the decline of *Daphnia* on week 5. Phytoplankton is often assumed to be the chief food for cladocerans; however, organic detritus and bacteria make up the bulk of material ingested (Pennak 1978). Cladocerans are filter feeders and prefer particles of less than 50 µm. Only small, young green algae; diatoms; desmids; and flagellates can be ingested and assimilated by cladocerans (Porter 1977). Algae larger than 50 µm and algae with durable cell walls or with gelatinous sheaths are frequently not ingested or digested, which can cause an overall increase in algal biomass (Porter 1976). Therefore, rapid growth of algae, which is undesirable to cladocerans, could result in large populations of algae able to produce toxins fatal to *Daphnia* (Porter 1977; Lampert 1981; Michaletz et al. 1983; Nizan et al. 1986).

Phosphorus from the inorganic and organic fertilizers could have also impacted the population of *Daphnia* in the ponds. Concentrations of orthophosphate less than 0.5 mg/L are known to stimulate reproduction of *D. pulex* by lowering the age of sexual maturation (Ivleva 1973). However, orthophosphate concentrations between 0.5 and less than 2.0 mg/L can slow parthenogenetic reproduction. Concentrations of orthophosphate greater than 2 mg/L are lethal to young *D. pulex*, though adults may survive, but egg development is impaired. In this study, orthophosphate levels increased to greater than 2.0 mg/L by week 4 and stayed high throughout the remainder of the study. High orthophosphate levels could have limited *Daphnia* reproduction and recruitment and prevented the recovery of *Daphnia* in the ponds.

Organic matter with a low carbon-to-nitrogen (C:N) ratio is known to decompose more rapidly than organic material with a high C:N ratio (Boyd 1979). Prepared diets with a 6-to-1 C:N ratio will decompose more rapidly than DS and RB with C:N ratios of 10-to-1 and 20-to-1, respectively. Rapid decomposition results in increased levels of bacteria available for *Daphnia* consumption. Thus, *Daphnia* populations increase in a shorter period of time in response to fertilization with PD, compared to RB and DS fertilization. Abundance of cladocerans in ponds fertilized with PD and RB was greater during weeks 1 to 4 than in ponds fertilized with DS. However, the rapid bacterial decomposition of high C:N ratio materials that favors cladoceran growth may also increase levels of toxic ammonia and nitrite harmful to fish. Paddlefish ponds fertilized with cottonseed meal with a 7:1 C:N ratio had elevated ammonia and nitrite levels potentially detrimental to larval fish (Mims et al. 1991) but not detrimental to cladocerans (Ivleva 1973). Low ammonia and nitrite levels were measured in waters fertilized with RB which has a C:N ratio of 20:1 (Mims et al. 1993).

Use of RB or PD as pond fertilizers provided higher abundance and biomass of large cladocerans than use of DS during the first 4 weeks after fish stocking. However, greater densities of large cladocerans in PD ponds compared to RB ponds during the early weeks after fish stocking probably increased survival of paddlefish in PD ponds (Mims and Schmittou 1989). Organic fertilizers with a low carbon-to-nitrogen ratio will more rapidly decompose than organic fertilizers with a high carbon-to-nitrogen ratio, and, the release of nitrite into the aquatic environment by heterotrophic bacteria can be detrimental to the larval fish (Mims et al. 1991). Additionally, heavy phytoplankton blooms can increase afternoon pH values in pond waters to levels that cause un-ionized ammonia to reach values harmful to paddlefish (Mims 1992). Phosphorus levels should also be monitored and controlled to prevent levels lethal to *Daphnia* spp., as well as to prevent stimulating large populations of phytoplankton that may release toxins harmful to *Daphnia* growth and reproduction. Another factor that should be considered in selecting an organic fertilizer is cost (Barkoh and Rabeni 1990). Paddlefish production in ponds fertilized with PD was about 3 times more expensive than production in ponds fertilized with RB (Mims et al. 1993).

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