

## Food Selection by Larval Paddlefish *Polyodon spathula* Supplied with Rice Bran to Promote Production of Live Foods, with Prepared Diets, or with their Combination in Earthen Ponds

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### Abstract

Food selection of larval paddlefish *Polyodon spathula* was evaluated by gut analysis in nine 0.02-ha ponds either fertilized with rice bran (RB) to promote zooplankton production, supplied with prepared diets (PD), or with a combination of rice bran and prepared diet (CB). After 40 d, mean fish yields in RB and CB ponds were significantly higher ( $P < 0.05$ ) than fish yield in PD ponds, but there was no significant difference ( $P > 0.05$ ) in survival among treatments. Cladocerans were the main live food items selected by paddlefish in RB and CB ponds. Chironomid larvae were the main food items selected by paddlefish in PD ponds. Less than 10% of the food found in paddlefish guts was prepared diets in PD and CB ponds. Prepared diets apparently were not as available to the fish or were not as preferred by the fish as live foods. Levels of un-ionized ammonia were significantly greater in CB and PD ponds than that in RB ponds. Decomposition of uneaten high-protein diets in PD and CB ponds probably led to production of ammonia. Elevated un-ionized ammonia levels during week 5 caused abnormal swimming behavior and some paddlefish mortalities in PD and CB ponds. Based upon these results, use of RB or other organic fertilizers to promote zooplankton production is recommended over direct feeding or a combination of fertilization and feeding for larval paddlefish in earthen ponds.

Paddlefish *Polyodon spathula* larvae, less than 120 mm in total length (TL), are traditionally raised in ponds organically fertilized which enhance production of live foods (Michaletz et al. 1982; Semmens 1982; Mims et al. 1991; Mims et al. 1993). Larvae prefer large, slow-moving prey such as cladocerans (Rosen and Hales 1981; Michaletz et al. 1982; Mims and Schmittou 1989) until gill rakers of paddlefish ( $\geq 120$  mm in TL) are sufficiently developed to allow them to filter feed on a more diverse diet of zooplankton (Michaletz et al. 1982).

Recently, there has been a trend by fish culturists to feed commercially-available ictalurid or salmonid prepared diets to larval paddlefish stocked into ponds rather than relying on traditional organic fertilizers to promote zooplankton. However, Webster et al. (1991) reported that commercially-available prepared diets, though accepted by paddlefish larvae, yielded poor growth and survival while a zooplankton (*Daphnia* spp.) diet resulted in better growth and survival in intensive culture. They found that the prepared diet particles consumed by paddlefish were not completely digested and suggested that a functional digestive enzyme system for the larvae was incomplete

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TABLE 1. Quantities and application schedules of rice bran (RB) and prepared diets (PD) for 0.02-ha ponds stocked with 61,775 larval paddlefish per hectare.

Week	Treatment			
	Rice bran (kg/ha)	Prepared diet <sup>1,2</sup> (kg/ha)	Combination rice bran and prepared diet (kg/ha)	Inorganic <sup>3</sup> fertilizer (L/ha)
0 <sup>4</sup>	1,410	—	1,410 (RB)	37.0
1	310	350	350 (PD)	4.6
2 to 5	157	350	350 (PD)	9.3
6	—	250	250 (PD)	—

<sup>1</sup> Types of Purina Trout Chow diets by manufacturer's codes and fed to fish were: week 1 #5100; week 2 #5101; week 3 #5102; week 4 #5103; week 5 #5104; and week 6 #5105.

<sup>2</sup> Diets were distributed hourly by an automatic feeder at a rate of 1 kg/24 h.

<sup>3</sup> All ponds received the same amount of liquid 10-34-0 fertilizer.

<sup>4</sup> RB was applied to the filled ponds 3 times per week during a 2-wk period prior to stocking.

(Dabrowski 1979; Webster et al. 1991). Mims (1992) reported that a prepared diet applied as an organic fertilizer resulted in higher survival; and the prepared diet could have served as a supplemental feed to paddlefish when live food items were not available.

No systematic investigation has compared organic fertilization for production of zooplankton with feeding prepared diets exclusively or in combination with organic fertilization and prepared diet for production of juvenile paddlefish in earthen ponds. The objective of this study was to determine by food and gut analyses if paddlefish larvae stocked in earthen ponds were consuming live foods, prepared diets, or a combination of live foods and prepared diets.

## Materials and Methods

### Pond Management

Nine 0.02-ha earthen ponds located at Kentucky State University Aquaculture Research Center in Frankfort, Kentucky were randomly assigned to one of three treatments. All ponds received a pre-flooding

TABLE 2. Purina Trout Chow diet types and sizes<sup>1</sup> fed to larval paddlefish stocked at 61,775/ha and cultured 40 d in prepared diet (PD) and combination (CB) ponds.

Product number	Type	Diameter (mm)
5100	sink	powder
5101	sink	0.8
5102	sink	1.5
5103	sink	2.5
5104	sink	3.2
5105	float	4.0

<sup>1</sup> Pellet size was increased each week over the culture period. Pellet sizes were mixed for 3 d before switching totally to next larger pellet size.

application of liquid Hydrothol at a rate of 2 kg/ha to reduce filamentous algae. Each pond was equipped with a continuously operated air-lift pump (Parker 1979).

Three treatments were evaluated: 1) fertilization with rice bran (RB) for production of zooplankton (control); 2) feeding a prepared diet (PD) as a non-living food source; and 3) a combination (CB) of fertilization with RB and direct feeding with PD. Automatic feeders (Sweeney, Inc., Texas, USA) were installed and regulated to release prepared diets hourly in PD and CB ponds over a 24-h period. Quantities and application schedules for RB, PD, and CB treatments are provided in Table 1. Rice bran was chosen as the recommended organic fertilizer (Mims et al. 1991). Purina Trout Chow diets (manufacturer's codes #5100 through #5105, Louisville, Kentucky, USA; Table 2) were selected based on research by Webster et al. (1991) and current feeding practices of paddlefish larvae stocked in earthen ponds (J. Hamilton, personal communication, Blind Pony Hatchery, Springfield, Missouri, USA 1990).

All ponds were filled with water from a surface water reservoir and filtered through 385- $\mu$ m saran filter cloth socks. Zooplankton, predominately *Daphnia pulex*, were collected and enumerated (Lind 1985), and inoculated at a rate of 125,000 crustaceans/pond in RB and CB ponds. Zooplankton

TABLE 3. Mean yield, individual total length and weight and survival of paddlefish larvae (SE) stocked at 61,775/ha and cultured 40 d in 0.02-ha ponds fertilized with rice bran, fed prepared diets, or treated with a combination rice bran and prepared diets. Values followed by the same letter in each column are not significantly different at  $P > 0.05$ .

Treatment	Yield (kg/ha)	Individual		Survival (%)
		Total length (mm)	Weight (g)	
Rice bran	174 ± 18a	150 ± 12a	9.1 ± 1.0a	31 ± 4a
Prepared diet	81 ± 10b	113 ± 8b	4.7 ± 0.5b	28 <sup>1</sup> ± 4a
Combination	145 ± 22a	148 ± 10a	9.0 ± 0.8a	26 ± 5a

<sup>1</sup> After 6 d of feed training in hapas, mean survival of fish was 42 ± 3% in PD treatment.

were not added to PD ponds, but instead, dylox (80% soluble powder) was applied at 0.10 mg/L immediately after filling and at 0.03 mg/L on successive weeks in PD ponds. Dylox was added at this rate to eliminate cladocerans and other crustacean zooplankton, but not to adversely affect the health of the paddlefish larvae (Johnson and Finley 1980).

Paddlefish larvae, produced from broodfish collected in Kentucky, were held in wooden boxes (60 × 60 cm) with window-screen bottoms and were supplied with flow-through water sprayed onto the water surface. Boxes were floated within a rectangular fiberglass tank (5 × 1 m). After 8 d when mouth parts were well-developed, peristalsis had begun, and larvae were actively seeking feed (Graham et al. 1986), fish were stocked loose into RB and CB ponds, whereas in PD ponds fish were stocked into circular hapas (diameter = 1.0 m; height = 1.2 m). Each treatment was stocked at a rate of 61,775/ha. Larvae for the PD ponds were trained to feed on a prepared diet for 6 d. Automatic feeders were mounted directly over the hapas and feed was dispensed every hour. Surviving fish (Table 3) were released into the PD ponds. Fish from all treatments were harvested after 40 d.

#### Water Quality Analysis and Management

Dissolved oxygen (DO) and water temperature, (polarographic dissolved oxygen meter and thermistor, model 54A, Yellow Springs, Ohio, USA) and pH (Omega PHH-43 meter, Stanford, Connecticut, USA) were measured at 0700 and 1500 h daily in each

pond. Secchi disk visibility (cm) was measured daily at 0700 h. Water samples were collected weekly from each pond and analyzed for alkalinity (as CaCO<sub>3</sub>), total ammonia-nitrogen (TAN), nitrite-nitrogen (NO<sub>2</sub>-N), nitrate-nitrogen (NO<sub>3</sub>-N), and soluble reactive phosphorus (PO<sub>4</sub>-P) (Hach DREL/5, Loveland, Colorado, USA). Afternoon water temperatures, pH and TAN were used to determine the percentage of un-ionized ammonia in the water of ponds (Boyd 1979). Chlorophyll *a* was extracted from phytoplankton samples (Boyd 1979) with acetone and measured spectrophotometrically (APHA 1985). Emergency aeration was provided by 0.25-kw surface aerators whenever DO concentrations were predicted by graph (Boyd 1979) to decline below 40% of saturation (Andrews et al. 1973). All ponds were treated with 0.1 mg/L liquid Hydrothol once after stocking to control filamentous algae. Ponds in each treatment received 11 kg of sodium chloride to prevent possible nitrite-induced anemia (Tucker et al. 1989).

#### 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 to 1.1 m depths in each pond. Sampling continued until a 10-L water sample was obtained. Water samples were then filtered through an 80-μm mesh, Wisconsin-style plankton net and the collected organisms were preserved in cold 5% buffered formalin with sucrose (Haney and Hall 1973). Cladocer-

ans were identified to species, adult copepods and copepodites to suborder and ostracods to order (Ward and Whipple 1959; Pennak 1978). Species of cladocera were grouped either as small (<0.6 mm TL) or large ( $\geq 0.6$  mm TL) organisms (Mims et al. 1991). Rotifers and copepod nauplii were not included because they are not preferred food organisms for paddlefish larvae (Michaletz et al. 1982; Mims et al. 1991). Zooplankton were counted in a Sedgewick-Rafter counting chamber with a compound microscope. Total lengths of each of four randomly selected large and small cladocerans, adult copepods and ostracods per sample were measured. Abundance (number of organisms/L) and estimated biomass ( $\mu\text{g}$  of organisms/L) of zooplankton were calculated (Dumont et al. 1975).

#### *Larval Fish Sampling and Gut Analysis*

Ten fish were sampled from each pond weekly with either a dip net or a 7.6-m long 5-mm mesh seine. Individual fish were measured (mm TL) and weighed (g) and were preserved in 10% buffered formalin for analysis of gut content. Each gut 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  $40\times$  magnification for the presence of prepared diet (CB and PD treatments) and zooplankton or other live food items. Zooplankton were counted and identified to the same taxonomic levels as described previously. At harvest, an additional 40 fish from each pond were sampled and preserved to obtain final individual TL (mm) and weight (g).

#### *Prey Selection*

Chesson's (1978)  $\alpha$  electivity index,  $\alpha_i = r_i/p_i / \sum r_i/p_i$ , where  $r_i$  is the proportion of prey taxon eaten and  $p_i$  is the proportion of the same prey taxon in the environment, was used to compare prey consumed by predators to the availability of the prey in the environment. The expected value for

random feeding,  $1/N$ , is a function of the number of food items ( $N$ ). The index varies between 0 and 1 with values above  $1/N$  indicating preference and below  $1/N$  indicating avoidance. The  $\alpha$  index is affected by relative abundance of food types and is useful for making meaningful comparisons among samples where abundances may differ (Lechowicz 1982).

#### *Data Analyses*

Overall and weekly determinations of zooplankton abundance and biomass, electivity indices, and water quality parameters were analyzed by a repeated measure version of ANOVA analogous to a split plot design (SAS 1990). Mean differences between selected treatment means (*a priori* comparisons) were tested with contrasts (SAS 1990). Weekly trends in zooplankton abundance and biomass,  $\alpha$  index, and water quality variables were tested using orthogonal polynomial contrasts. Electivity indices ( $\alpha$ ) were tested for deviation from  $1/N$  by *t*-tests. The probability level for tests was 0.05.

## **Results**

### *Fish*

Mean fish yields in RB and CB ponds were significantly greater than that in PD ponds, but there was no significant difference in survival among treatments (Table 3). At harvest, fish from RB and CB ponds were significantly longer and heavier than those from PD ponds. Fish in PD ponds were observed frequently feeding on or near the surface of the pond and congregating around the feeders throughout the study. The fish appeared to be attracted by the feeder's noise or vibration during operation. Fish in CB ponds did not congregate near the feeders. Fish in CB and RB ponds remained at deeper depths and were evenly dispersed in the water during the study.

### *Water Quality*

Mean Secchi disk visibility in CB ponds (45 cm) was significantly lower than that in

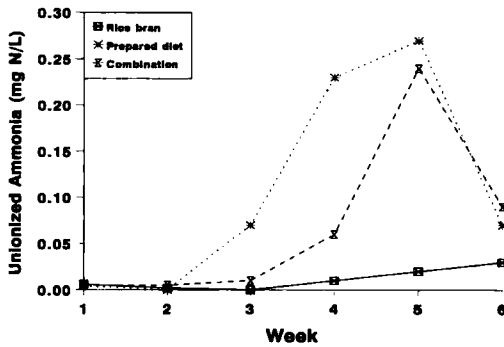


FIGURE 1. Mean weekly un-ionized ammonia (mg N/L) in paddlefish ponds receiving rice bran (RB), prepared diet (PD), or a combination (CB) of RB and PD.

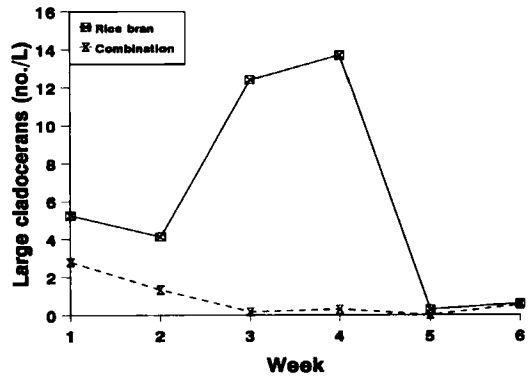


FIGURE 2. Densities of large cladocerans (no./L) in paddlefish ponds receiving rice bran (RB) or a combination (CB) of rice bran and prepared diets.

RB ponds (69 cm), but visibilities in CB and RB ponds were not significantly different from PD ponds (58 cm). Mean phytoplankton biomass, as measured by chlorophyll *a* concentrations, in the CB (139  $\mu\text{g/L}$ ) and PD (159  $\mu\text{g/L}$ ) ponds was significantly higher than that in RB ponds (67  $\mu\text{g/L}$ ). Mean pH in PD (8.8) and CB (8.7) ponds were significantly higher than that in RB (8.1) ponds during the culture period. There were no statistical differences in mean dissolved oxygen, water temperature, pH, soluble reactive phosphorous or alkalinity levels among treatments and were within acceptable levels for larval paddlefish cultured in ponds (Mims 1992). Ponds fertilized with RB had a mean un-ionized ammonia level of 0.007 mg/L which was significantly lower than the un-ionized ammonia level of 0.07 for CB ponds and 0.08 mg/L for PD ponds. After week 3, weekly un-ionized ammonia levels in PD and CB ponds were significantly higher than that in RB ponds (Fig. 1). During week 5 on day 4 most CB and PD ponds had an afternoon pH greater than 10 with un-ionized ammonia levels greater than 1.2 mg/L. Some fish mortalities and abnormal swimming behavior were observed in CB and PD ponds. Flushing with fresh water lowered un-ionized ammonia and stopped abnormal swimming behavior and fish mortalities. Fish in RB ponds were

not exposed to similar ammonia-related problems.

#### Zooplankton in Pond Samples

Water samples from PD ponds contained no large cladocerans and only a few small cladocerans. Adult copepods in PD ponds were similar in number to those found in water samples from RB and CB ponds. Since the intention of the Dylox was to eliminate or reduce the availability of zooplankton (particularly cladocerans) in PD ponds to test direct feeding of fish on prepared diets, the sparse zooplankton data were not included in the analysis.

Overall, there were 6 organisms/L and a biomass of 74  $\mu\text{g/L}$  of large cladocerans in RB ponds and 1 organism/L and a biomass of 8  $\mu\text{g/L}$  in CB ponds. These data were statistically different between the treatments. Ponds fertilized with RB generally had more large cladocerans than CB ponds; however, only during weeks 3 and 4 was abundance of large cladocerans in RB ponds significantly greater than large cladocerans in CB ponds (Fig. 2). Peak populations of large cladocerans were found during weeks 3 and 4 in RB ponds compared with weeks 1 and 2 in CB ponds. Dominant large cladocerans were *Daphnia pulex* and *D. catwba* through week 5 and *Scapholeberis mucronata* during week 6.

There were 72 organisms/L and a biomass of 44  $\mu\text{g/L}$  of small cladocerans in RB ponds and 34 organisms/L and a biomass of 28  $\mu\text{g/L}$  in CB ponds. No statistical differences for abundance and biomass of small cladocerans for the whole study or by individual weeks were found between treatments. Peak populations of small cladocerans were found on weeks 5 and 6 in RB ponds compared to weeks 1, 5, and 6 in CB ponds. Dominant small cladocerans were *Bosmina longirostris* and *Chydorus sphaericus*.

There were 191 organisms/L and a biomass of 882  $\mu\text{g/L}$  of adult copepods and 298 organisms/L and a biomass of 1374  $\mu\text{g/L}$  in CB ponds. Mean differences between treatments were not statistically significant; also, statistical differences were not found for weekly copepod abundance and biomass for RB and CB ponds. A population peak of copepods was found on week 1 in both treatments.

### Food Selection

Mean  $\alpha$  indices of large cladocerans from RB and CB ponds were 0.99 and 0.79, respectively, which indicated a preference for these food items. No statistical difference for large cladoceran  $\alpha$  indices was found between treatments, except during week 5 when the  $\alpha$  index in RB ponds (0.99) was significantly greater than that in CB ponds (0.00) (Table 4). Mean  $\alpha$  indices of small cladocerans and adult copepods from RB ponds were 0.00 and 0.06 and from CB ponds 0.01 and 0.21, respectively, and indicate an avoidance of these foods. During week 5, the indices indicated random selection rather than an avoidance of copepods in both treatments (Table 4). The dominant zooplankton in the guts of fish from RB and CB ponds was large cladocerans. Small cladocerans were not consumed until week 6 in both treatments which indicates fish had begun to filter-feed. Adult copepods were present in the guts of fish from each of the three treatments with heaviest consumption in the PD ponds.

TABLE 4. Chesson's  $\alpha$ <sup>1</sup> food electivity indices calculated for larval paddlefish stocked at 61,775/ha in 0.02-ha ponds and fertilized with rice bran (RB) or fertilized with rice bran and the fish fed a prepared diet (CB).

Week	Treatment	Large Cladocera	Small Cladocera	Adult Copepoda
Week 1	RB	0.99	0.00	0.00
	CB	0.99	0.00	0.00
Week 2	RB	0.99	0.00	0.00
	CB	0.99	0.00	0.00
Week 3	RB	0.99	0.00	0.00
	CB	0.99	0.00	0.00
Week 4	RB	0.99	0.00	0.00
	CB	0.50	0.00	0.00
Week 5	RB	0.99	0.00	0.34 <sup>2</sup>
	CB	0.00	0.00	0.67
Week 6	RB	0.99	0.00	0.00
	CB	0.50	0.04	0.62

<sup>1</sup> Reciprocal of number of prey groups in ponds ( $N = 3$ ;  $1/N = 0.33$ ) represents random feeding; values above the reciprocal value indicate positive selection; values below the reciprocal value indicate avoidance of prey.

<sup>2</sup> Not significantly different from 0.33 at  $P < 0.05$ .

Chironomid larvae and pupae first appeared in the guts of paddlefish from CB ponds during week 1 and from RB and PD ponds during week 2 (Fig. 3). The number of chironomids consumed by fish from CB and PD ponds was significantly greater than the number of chironomids consumed in RB ponds. Ostracods also appeared infrequently in the diets of fish from RB and CB ponds during weeks 5 and 6.

All fish sampled from hapas had prepared diet in their guts after 1 week of feed training. After the fish were released from the hapas in PD ponds, no feed was observed in their guts for weeks 2 and 3. However, 60% of the sampled fish had feed in their guts on week 4. Percentages of sampled fish from PD ponds with feed in their guts increased to 66% on week 5 and 87% on week 6. No feed was found in the guts of fish sampled from CB ponds through week 3. During weeks 4, 5 and 6, respectively, 57, 77 and 10% of the fish sampled from CB ponds had feed in their guts. In both treatments, we observed that the feed was less

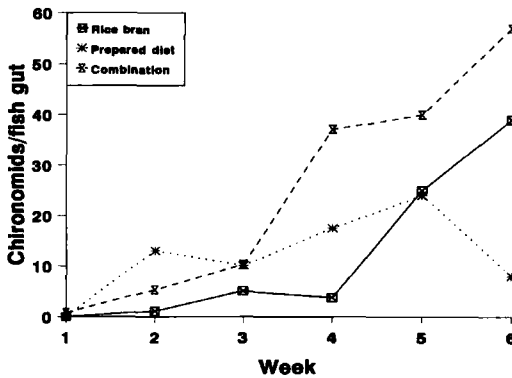


FIGURE 3. Mean number of chironomid larvae and pupae per paddlefish gut in ponds receiving rice bran (RB), prepared diets (PD) or a combination (CB) of RB and PD.

than 10% of the total volume of food consumed by the fish except during week 1 when fish were training on feed in hapas in PD ponds. The majority of the food consumed by the larval fish released in PD ponds was chironomid larvae and pupae.

### Discussion

Hepher (1978) stated that when prepared diets are fed to young fish as a supplement to zooplankton, survival and yields should increase for many fish species. However, some fish prefer zooplankton over prepared diets. Hepher (1979) found silver carp *Hypophthalmichthys molitrix* consumed only zooplankton when offered both prepared diets and zooplankton. Fitzmayer et al. (1986) observed that striped bass *Morone saxatilis* larvae stocked in ponds ingested prepared diets, but growth and survival were poor unless these diets were supplemented with zooplankton. In our study, prepared diets did not improve fish production, but instead contributed as a fertilizer for the production of live foods. Despite the abundance and the frequent availability of prepared diets in CB and PD ponds, paddlefish continued to feed heavily on live foods. This was probably because paddlefish capture food particles almost exclusively in the water column (Kroll et al. 1992) and the sink-

ing PD was only available in the water column for a short time.

Paddlefish, greater than 120 mm in TL, are able to feed on a larger range of zooplankton sizes and species because their gill rakers have grown sufficiently in length to permit filter-feeding (Michaletz et al. 1982). Low survival in this study permitted fish in the RB and CB ponds to achieve average sizes larger than 120 mm in TL because of low fish competition. Fish from PD ponds averaged less than 120 mm in TL at harvest probably because of several factors: 1) lack of *Daphnia* in paddlefish diet reduced growth; 2) sinking prepared diets were not as available for consumption by fish as zooplankton in the water column; and 3) uneaten feed promoted poor water quality such as high ammonia and pH levels which could have adversely affected the fish. Fish that were stocked in the pond and were greater than 60 mm in TL were first observed to be consuming the sinking prepared diets in PD and CB ponds during week 4. The percentage of fish consuming prepared diets increased with increasing age and size of fish. Since paddlefish larvae do not feed by sight, but rather sense food by sensory receptors (Jorgensen et al. 1972), it is apparent that the fish became more efficient in sensing prepared diets in the water column as morphological development of the rostrum (site of sensory receptors) neared completion. Yeager and Wallus (1982) reported rostrum development of paddlefish to be completed when paddlefish were approaching 90 mm in TL.

Fish in CB ponds were able to ingest a 4-mm floating pellet once they were greater than 120 mm TL as reported by Kroll et al. (1992). A smaller floating pellet of 2 mm in diameter might be useful to feed to smaller fish (80 to 120 mm TL); however, such a floating pellet was not available at the time of this study. One disadvantage of feeding a prepared diet, especially floating diets, to larval paddlefish in ponds was their slow, unusual feeding behavior at the water surface. The fish must turn on its side or upside

down to obtain the pellet in its mouth. This behavior during feeding makes the young fish very vulnerable to bird predation. Though losses of fish by birds was not a problem in our study area, culturists should be aware of the potential negative impact to survival if surface feeding is practiced.

Results of this study suggest that live foods promoted by organic fertilization are preferred over prepared diets by larval paddlefish in ponds. Sinking prepared diets in this study were not readily consumed probably because they did not remain in the water column; therefore, they were not available to the fish. If a sinking prepared diet is used in ponds, it should be applied as a fertilizer and not as a direct food source for the fish (Mims 1992). Direct feeding could cause an accumulation of feed in the static system, water quality deterioration, and reduction in fish survival and yields.

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