Comparison of live food organisms and prepared diets as first food for paddlefish, *Polyodon spathula* (Walbaum), fry

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Abstract. A feeding experiment was conducted in aquaria to evaluate growth, survival and food consumption by paddlefish. Polyodon spathula (Walbaum), fry fed live Daphnia pulex, brine shrimp nauplii, Artemia sp. (L.), from two different geographical sources, or one of three commercial dry diets. Fry were fed from first feeding (day 8 post-hatch) to day 17 post-hatch. All diets had similar percentages of crude protein and lipid. Fatty acid composition was similar in all diets except for Great Salt Lake brine shrimp nauplii which had a higher percentage of linolenic acid, 18:3 (n-3), and a lower percentage of eicosapentaenoic acid, 20:5 (n-3). Paddlefish fed live food organisms were significantly (P < 0.05) larger than those fed non-living diets. Survival was significantly higher (P < 0.05) for fish consuming live Daphnia (95.3%) than all other treatments. Percentage of fish with food in the digestive tract did not significantly differ (P > 0.05) among treatments. Poorer growth and survival by fish fed prepared diets may have been due to limited digestibility of the dry diets by paddlefish.

Introduction

Paddlefish, Polyodon spathula (Walbaum), is valued as a commercial fish for its roe and as a sport fish (Carlson & Bonislawsky 1981). Approximately 570 metric tons per year are harvested by commercial and sport fisheries in North America. With the advent of successful spawning techniques, paddlefish are now reproduced by state and federal hatcheries for mitigation programmes. In ponds, large cladocerans (i.e. Daphnia sp.) are generally the first food for newly hatched paddlefish (Mims & Schmittou 1989). However, in hatcheries, the production or procurement of live food is time consuming and is subject to inconsistent supply (Buddington & Doroshov 1984). Live brine shrimp nauplii, Artemia sp., are the primary food for initial feeding of many fish larvae and comprise 85% or more of the live foods fed to larval fishes worldwide (Sorgeloos 1980). However, growth responses in larval fish fed brine shrimp from different sources have been inconsistent, especially when brine shrimp originated from different geographical areas. Variability appears to be caused by differences in fatty acid composition (Watanabe, Oowa, Kitajima & Fujita 1980).

Variation in nutritional quality among different sources of brine shrimp creates an increased need for a high quality prepared diet. Prepared diets, without supplementation with live foods, have been used successfully for feeding fry of bighead carp, Aristichthys nobilis (Richardson) (Carlos 1988), milkfish, Chanos chanos (Forskal) (Santiago, Banes-Aldaba & Songalia 1983), and gamitana, Colossoma macropomum (Cuvier) (Eckman 1987).

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The objective of this study was to evaluate growth, food consumption, and survival of newly hatched paddlefish fry fed live *Daphnia*, two sources of brine shrimp nauplii, and three commercially prepared diets as the only food for paddlefish fry.

Materials and methods

Experimental design

The study was conducted in aquaria at the Aquaculture Research Center, Kentucky State University, Kentucky, USA. Adult male and female paddlefish were collected from Cumberland Lake, KY, injected with luteinizing hormone releasing hormone (LHRHa), and spawned as described by Graham, Hamilton, Russell & Hicks (1986). When the hatched fry were 8 days old and had begun to search actively for food, 80 fry (individual mean weight 19.8 ± 0.3 mg) were hand-counted into each of 28 7.5-l circular glass aquaria which had a continuous flow of dechlorinated tap water at a rate of 0.51/min. Continuous, incidental lighting was provided by fluorescent ceiling lights on the opposite side of the laboratory so as to minimize direct light intensity (Mims & Schmittou 1989). Water quality in each aquarium was measured daily. Water temperature and dissolved oxygen were measured using a YSI Model 54 (YSI Instruments Co., Yellow Springs, OH), and pH was measured using an electronic pH meter (Omega Engineering, Inc., Stamford, CT). Total ammonia nitrogen was measured using a DREL/5 spectrophotometer (Hach Company, Loveland, CO). There were no significant differences (P < 0.05) among treatments in water quality parameters at any sampling period. Data were subsequently pooled. Overall means (± SE) were: water temperature, 2.06° C \pm 0.16; dissolved oxygen, 6.14 mg/l \pm 0.17; pH, 7.47 \pm 0.07; total ammonia, $0.89 \,\text{mg/l} \pm 0.05$.

Brine shrimp cysts from different geographical sources were obtained from a commercial supplier (Biomarine, Inc., Hawthorne, CA). Great Salt Lake (GSL) and China (CH) nauplii were hatched from cysts (GSL can nos. 614438, 614453, 007291; CH can nos. 500107-109, 500127) in 3-l transparent plastic hatching cones containing water with salt (NaCl) added to give a salinity of 30 ppt. Water was continuously aerated and constant illumination was provided by fluorescent lights mounted adjacent to the hatching cones (1000 lux). After 30h of incubation for GSL cysts and 42 h incubation for CH cysts at 28°C, aeration was turned off. Nauplii were collected after settling to the bottom of the hatching cone and placed into a plastic tub containing 31 dechlorinated tap water. After 10 min, nauplii were siphoned from the water column leaving the unhatched cysts. Different incubation times were used because of the slower hatching rate of the CH cysts (Webster 1989). Brine shrimp nauplii were fed immediately after collection. Daphnia were collected every 8h from outdoor ponds. Prepared diets were Biokoyowa larval diet (BioKyowa, Inc., Cape Girardeau, MO), Moore-Clark trout diet (Moore-Clark Co., Inc., Vancouver, BC, Canada), and Purina Trout Chow (Purina Mills, Inc., St Louis, MO). All prepared diets were separated, using sieves, into particles between 0.25 and 0.40 mm.

Fish were fed every 3h for 9 days. *Daphnia* were fed at a rate of five organisms/ml of aquarium water (Mims & Schmittou 1989). *Daphnia* concentrations were determined at each feeding by counting three 1-ml samples using a Hensen-Stempel pipette and a Sedgwick-Rafter counting cell. Great Salt Lake brine shrimp nauplii were fed at a rate of 40-50 nauplii/ml of aquarium water while CH brine shrimp were fed at a rate of 20-30

nauplii/ml of aquarium water. Difference in nauplii density was due to GSL having a greater number of cysts/ml (Webster 1989). Concentration of nauplii in both brine shrimp treatments was much higher than the recommended level of 0.1-5.0 organisms/ml (Elridge, Whipple, Eng, Bowers & Jarvis 1981). Nauplii density was determined by counting nine 1-ml samples taken 15 min after adding nauplii to the aquarium. Dry diets were fed at a rate of 4 g/feeding, which was in excess of consumption. Uneaten food from the previous feeding was siphoned from the aquaria 1h prior to the next feeding.

Every 3 days, 10 fish were randomly sampled from each aquarium. Fish were examined under a dissecting microscope for the presence of food in the transparent digestive tract. Fish were placed in 10% formalin and preserved for length and weight measurements. Total length was measured to the nearest 0.10m using a dissecting microscope and dial calipers. Each fish was blotted dry on paper towels and weighted to the nearest 1.0 mg. Survival was calculated by adding the number of larvae removed at each sampling period to the number of fish surviving at the conclusion of the experiment. A decision to conclude the study when survival of any treatment decreased to 50% was made prior to the start. Specific growth rate (SGR) was computed based on the formula of Weatherly & Rogers (1978), as follows:

SGR (% body weight/day) = $(\log_e \text{ weight}_l - \log_e \text{ weight}_0) \times 100$

where.

 $weight_t = weight of fish at time t$ weight θ = weight of fish at time θ = culture period in days T

Nutrient and lipid analysis on diets

Crude protein (N × 6.25) in all diets was measured using a LECO nitrogen determinator Model FP-228 (LECO Corp., St Joseph, MI) (Sweeney & Rexroad 1987). Percentage moisture was determined by drying to constant weight at 95°C in a convection oven (Association of Official Analytical Chemists (AOAC) 1984). Percentage fat was determined by ether extraction (AOAC 1984) (Table 1).

Table 1.	Percentage	moisture,	crude	protein,	and	crude	fat	in	living	and
prepared	diets fed to	paddlefish	fry¹							

Diet	Moisture	Crude protein ²	Crude fat ²
Moore-Clark	15·6 ± 0·3	52·2 ± 0·9	22·4 ± 0·3
Purina	10.0 ± 0.1	58·2 ± 0·1	22·4 ± 0·2
Biokyowa	5.5 ± 0.1	68.8 ± 1.1	27·9 ± 2·9
Daphnia	91·2 ± 1·6	65·6 ± 3·2	23.0 ± 0.9
GSL ³	84.4 ± 0.1	52.2 ± 1.4	29·5 ± 0·6
CH ⁴	82.3 ± 0.3	50·6 ± 0·4	29·2 ± 1·3

¹ Values represent means of three replications.

²Dry matter basis.

³GSL = Great Salt Lake source brine shrimp nauplii.

⁴CH = China source brine shrimp nauplii.

Total lipids were extracted from approximately 2-g samples of diets and paddlefish fry by a modification of the procedure of Kates (1986). Samples were frozen with liquid nitrogen (-196°C) and stored (-40°C) until lipid extraction. Initial extraction was in 10ml of methanol/chloroform (2:1, by vol.) for 1·5h. Partially extracted samples were collected by centrifugation $(300 \times g \text{ for } 10 \text{ min})$ and the extraction procedure was repeated two more times with methanol/chloroform (1:1, by vol.) for 1h. Extracts were washed twice with 3 ml of 5% NaCl solution and taken to dryness with nitrogen in a water bath (27°C). Three ml of chloroform was added to the extracted lipid and stored (-20°C) under nitrogen.

Fatty acid methyl esters were obtained by refluxing the extracted lipid for 5min with 1.5ml of 0.5 N methanolic sodium hydroxide. After cooling to room temperature, 2ml of BF₂/methanol were added and refluxed for an additional 20 min (Metcalfe, Schmitz & Pelka 1966).

Fatty acid methyl esters were analysed using a Hewlett-Packard 5890 gas chromatograph equipped with a 30-m fused-silica capillary column DB225 (J & W Scientific, Folsom, CA) and a flame-ionization detector. The carrier gas was helium. Oven temperature was programmed from 170 to 225°C at 1°C/min. Injections were in the split mode with a split ratio of 1:50. Detector response was recorded and quantitated with an electronic integrator-recorder. An internal standard was added and fatty acid methyl esters were identified by comparison of their retention times with those of authentic standards (Nu-Chek Prep, Inc., Elysian, MN).

Statistical methods

Analysis of variance (ANOVA) was computed for fatty acid percentages of all diets and for length, weight, SGR, survival, and food consumption of the paddlefish fry using the Statistical Analysis Systems ((SAS) GLM procedure 1988). Percentage data were transformed to arc sin values for analysis (Zar 1984). Duncan's Multiple Range test was used to compare individual means. Untransformed data are reported to facilitate comparison with results from other related studies.

Results

Fatty acid composition of the diets differed (Table 2). Most notable were the proportions of 16:1 (n-7) (palmitoleic acid) and the n-3 fatty acids, 18:3 (n-3) (linolenic acid), 20:5 (n-3) (eicosapentaenoic acid or EPA], and 22:6 (n-3) (docosahexaenoic acid or DHA). The CH brine shrimp had a significantly higher percentage of palmitoleic acid than the other diets (P < 0.05). The GSL brine shrimp contained a significantly higher percentage of linolenic acid than other diets (P < 0.05). Percentage of EPA in all diets was greater than 1.81% of the diet except the GSL brine shrimp which had a significantly lower percentage (0.98%) of the diet as EPA (P < 0.05). Prepared diets had high levels of DHA while living foods had significantly lower percentages, if any, of DHA present (P < 0.05).

Fish fed live foods had significantly greater lengths and weights than fish fed non-living diets after 17 days post-hatch (P < 0.05) (Table 3; Figs 1 & 2). The specific growth rate (SGR) of paddlefish fry over the 9-day period was significantly less (P < 0.05) in fish fed prepared diets (3.72 to 4.28% per day) than fish fed living food organisms (9.36 to 10.11% per day) (Table 3).

Table 2. Percentages (wt. %) of selected fatty acids in the total lipids extracted from newly-hatched paddlefish fry and diets fed to fry1

	Diet ²							
Fatty acid ³	MC	ВК	Purina	GSL	СН	Daphnia		
14:0	4·3 ± 0·0 ^b	3.8 ± 0.0°	4.5 ± 0.0°	1·1 ± 0·1°	1.8 ± 0.1d	1·7 ± 0·1d		
16:0	17.9 ± 0.1b	18.0 ± 0.1^{b}	18.6 ± 0.1^{a}	13.3 ± 0.3^{d}	12·2 ± 0·1°	$14.7 \pm 0.3^{\circ}$		
16:1 (n-7)	4.8 ± 0.0^{d}	3.4 ± 0.1°	10.7 ± 0.0^{6}	3.0 ± 0.1°	$16.7 \pm 0.2^{\text{a}}$	$6.7 \pm 0.2^{\circ}$		
18:0	4.2 ± 0.0^{a}	3.0 ± 0.0^{b}	3.9 ± 0.1^{a}	4.0 ± 0.3^{a}	4.0 ± 0.3^{a}	3.5 ± 0.2^{b}		
18:1 (n-9)	18.5 ± 0.2^{b}	14·6 ± 0·1d	12.2 ± 0.1°	22.4 ± 0.4^{a}	16·5 ± 0·6°	12·1 ± 0·7°		
18:1 (n-7)	2.8 ± 0.0°	3.6 ± 0.0d	3·0 ± 0·1°	6.5 ± 0.2^{b}	10.8 ± 0.4^{a}	5.4 ± 0.2°		
18:2 (n-6)	10.5 ± 0.0^{b}	11.0 ± 0.0^{a}	5.9 ± 0.1°	6.2 ± 0.1°	5·2 ± 0·1d	5.1 ± 0.2^{d}		
18:3 (n-3)	0.9 ± 0.0^{d}	2.0 ± 0.0^{d}	1·1 ± 0·0d	26.3 ± 0.4^{a}	$4.5 \pm 0.1^{\circ}$	12.2 ± 1.0^{b}		
20:5 (n-3)	8·1 ± 0·1°	11.6 ± 0.1a	11.6 ± 0.0^{a}	3.3 ± 0.1^{d}	12.4 ± 0.2^{n}	10.3 ± 0.7^{b}		
22:6 (n-3)	7.2 ± 0.0^{b}	11.6 ± 0.0^{a}	6.4 ± 0.1^{c}	$0.0 \pm 0.0^{\circ}$	$0.0 \pm 0.0^{\circ}$	1.5 ± 0.5^{d}		
Total fatty acids	99.00	99-20	99-60	97-50	96.50	91-20		
Unknown	1.00	0.80	0.40	2.50	3.50	8.80		
% sat.	27·5 ± 0·0 ^b	25·6 ± 0·1°	32·3 ± 0·2°	19·7 ± 0·3°	19·4 ± 0·2°	23·8 ± 0·1 ^d		
% mono	36.8 ± 0.0^{b}	29.9 ± 0.1d	28.6 ± 0.1°	$33.8 \pm 0.3^{\circ}$	45.6 ± 0.6^{a}	24.4 ± 0.0^{t}		
% diene	11.5 ± 0.1^{a}	11.7 ± 0.0^{a}	8.2 ± 0.0^{b}	6.8 ± 0.1°	6.8 ± 0.1^{c}	$5.9 \pm 0.3^{\circ}$		
% poly	23.3 ± 0.1^{d}	$32.1 \pm 0.1^{\circ}$	30.6 ± 0.1°	37·3 ± 0·1ª	24.8 ± 0.0^{d}	35.1 ± 1.3^{t}		
% w3	20.2 ± 0.1^{d}	28.9 ± 0.0^{b}	24.2 ± 0.1°	34.7 ± 0.1^{a}	17.9 ± 0.1°	27.5 ± 1.4^{t}		
% w6	11·7 ± 0·0 ^b	12.4 ± 0.1^{a}	7.4 ± 0.1^{d}	$7.0 \pm 0.1^{\circ}$	7.4 ± 0.1^{d}	8.6 ± 0.3		
w3/w6	1.7 ± 0.0^{d}	$2.3 \pm 0.0^{\circ}$	3.3 ± 0.1^{b}	5.1 ± 0.1^{b}	2.4 ± 0.0^{c}	3.2 ± 0.1^{t}		

¹Values are means ± SE of two replications. Values in each row with the same superscript are not significantly different (P > 0.05).

Table 3. Length, weight, specific growth rate (SGR), percentage survival and percentage of the fish consuming diets for paddlefish fry fed different living and nonliving diets1

Diet	Final individual length (mm)	Final individual weight (mg)	SGR	Survival (%)	Fish consuming diets (%)
Daphnia CH³ GSL² Biokyowa Purina Moore-Clark	27.5 ± 0.3^{b} 29.1 ± 0.8^{a} 27.8 ± 0.1^{ab} 20.1 ± 0.3^{c} 20.0 ± 0.1^{c} 19.5 ± 0.8^{c}	153.4 ± 5.4^{ab} 163.6 ± 9.9^{a} 138.6 ± 3.6^{b} 43.5 ± 2.1^{c} 48.5 ± 1.7^{c} 43.4 ± 4.6^{c}	9·83 ± 0·16 ^a 10·11 ± 0·30 ^a 9·36 ± 0·14 ^a 3·75 ± 0·23 ^b 4·28 ± 0·17 ^b 3·72 ± 0·50 ^b	95·31 ± 1·39 ^a 68·13 ± 7·85 ^{bc} 80·00 ± 6·39 ^b 63·13 ± 4·46 ^{cd} 48·44 ± 2·07 ^{dc} 40·63 ± 1·30 ^c	95.00 ± 2.89^{a} 94.17 ± 3.36^{a} 93.33 ± 4.97^{a} 86.67 ± 6.32^{a} 86.09 ± 6.02^{a} 79.00 ± 7.14^{a}

¹Values are means ± SE for 4 replications. Means within a column with the same superscript are not significantly different (P > 0.05).

²Diets are MC = Moore-Clark trout diet; BK = Biokyowa larval diet; Purina = Purina trout chow; GSL = Great Salt Lake source brine shrimp nauplii; CH = China source brine shrimp nauplii; Daphnia = Daphnia.

³The number preceding the colon indicates the number of carbon atoms; the number following the colon indicates the number of double bonds; the position of the double bond nearest the terminal end is given by the designation 'n (total number of carbon atoms) minus the number of carbon atoms from the end of the chain'.

²GSL = Great Salt Lake source brine shrimp nauplii.

³CH = China source brine shrimp nauplii.

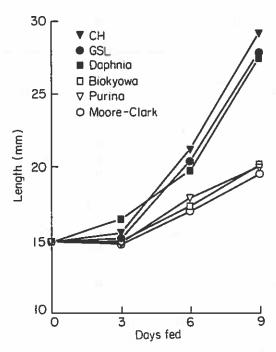


Figure 1. Total lengths (mm) of paddlefish fry fed several living and non-living diets. Data are means of four replications.

Survival was significantly higher (P < 0.05) among fry fed Daphnia (95.3%) than fish fed all other diets. No successful cannibalism (fry in the digestive tract of another fish) was observed during this study. However, larger fry killing smaller fry in an attempt to consume the smaller-size fish was observed, but did not appear to be confined to one particular treatment. Food consumption, based on the percentage of fish with food in their digestive tracts 15 min after feeding, indicated that paddlefish fry consumed all diets equally well (P > 0.05) (Table 3). No difference (P > 0.05) in percentage food consumption was found in any of the three sampling periods among treatments, thus food consumption data were combined. Gross examination of fry with a dissecting microscope revealed no differences among the groups in appearance externally or of internal organs.

Discussion

Growth of paddlefish fry fed prepared diets was less than that of fish fed live food organisms after 17 days post-hatch, indicating that prepared diets are not currently suitable for the initial feeding of paddlefish fry. Prepared diets and the living food organisms had similar percentages of crude protein and fat, and had relatively high percentages of the n-3 fatty acids 20:5 (n-3), EPA, or 22:6 (n-3), DHA, except the Great Salt Lake source of brine shrimp nauplii. All dry diets were assumed to be nutritionally adequate. No difference was found in the number of fish consuming diets. Each aquarium had upwelling currents created by inflowing water which kept the non-living diets suspended. However, they remained in suspension for less time than did the living foods.

Growth and survival of white sturgeon, Acipenser transmontanus (Richardson), larvae fed dry diets were lower than those of fish fed live Tubifex (Buddington & Doroshov 1984).

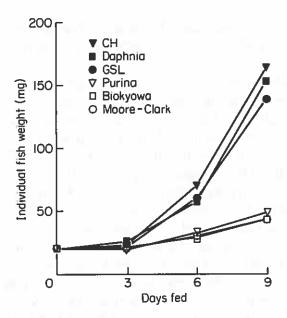


Figure 2. Individual fish weight (mg) of paddlefish fry fed several living and non-living diets. Data are means of four replications.

The reduced performance associated with weaning the white sturgeon from living foods onto dry diets was attributed to imprinting of larvae to live foods after initiation of exogenous feeding. Paddlefish fry used in this study were fed from initiation of exogenous feeding so no imprinting onto live food was possible for fish fed prepared diets. Further, non-living diets were readily consumed by paddlefish fry. However, examination of the digestive tract revealed that many of the diet particles consumed did not appear to be completely digested prior to excretion. This may explain why paddlefish fed prepared diets had lower growth rates and survival than fish fed brine shrimp nauplii and *Daphnia*. Brandt (1978) reported that paddlefish fed a dry diet for 40 days had 16% survival and had a total length of 30 mm. In this study, paddlefish fed for 9 days on dry diets had 50.7% survival and total length of 19.9 mm.

The significance of live food as the first diets of young fish is not well understood. Dabrowski (1979) proposed that initial digestion in larval fish is carried out by the enzymes present in the live food organisms. This implies that larval fish do not have the enzymes to digest non-living diets. However, the major enzymes needed for digestion of protein and starch have been shown to be present at the time of first feeding in the digestive tracts of striped bass, Morone saxatilis (Walbaum) (Baragi & Lovell 1986) and dolphin fish, Coryphaena hippurus (L.) (Divakaran & Ostrowski 1990). Flutcher (1980) suggested that a fat soluble essential growth factor in live brine shrimp nauplii was responsible for the successful rearing of whitefish, Coregonus lavaretus (L.), larvae. Watanabe et al. (1980) reported that the highly unsaturated fatty acids EPA or DHA were necessary in a diet for red sea bream, Pagrus major (Temminck & Schlegl), larvae.

Fatty acid requirement of paddlefish has not been previously investigated. Webster & Lovell (1990) have reported that striped bass, *Morone saxatilis* (Walbaum), larvae require a dietary source of EPA. It has been shown that red sea bream, *Pagrus major* (Fujii & Yone 1976), and turbot, *Scophthalmus maximus* (L.) (Owen, Adron, Middleton & Cowey 1975) require high dietary percentages of EPA and DHA for optimal growth and survival. Other

species, such as channel catfish, *Ictalurus punctatus* (Rafinesque) (Satoh, Poe & Wilson 1989) and grass carp, *Ctenopharyngodon idella* (Cai & Curtis 1990) do not require long-chain n-3 fatty acids.

This study indicates that paddlefish fry may not require the highly unsaturated fatty acids EPA and DHA for at least 15 days post-hatch. The similar growth rate of fish fed GSL brine shrimp (with a high percentage of linolenic acid and a low percentage of EPA) to fish fed CH brine shrimp (with a low percentage of linolenic acid and a high percentage of EPA) would indicate this. However, since paddlefish fry were fed for 9 days, adverse effects on growth rate may not yet have occurred. If fed for a longer time period, differences in growth might have developed since paddlefish fed CH brine shrimp nauplii had a higher individual fish weight than fish fed GSL brine shrimp nauplii after 9 days.

Webster & Lovell (1990) showed that striped bass larvae fed for 12 days on a source of brine shrimp with less than 1.5% EPA (dry matter basis) had reduced growth and survival compared to fish fed brine shrimp with greater than 1.5% EPA. Some species of fish, such as rainbow trout, Oncorhynchus mykiss (Walbaum) (Owen et al. 1975) and kelp bass, Paralabrax clathratus (Girard) (Kayama, Tsuchiya, Nevenzel, Fulco & Mead 1983) can convert linolenic acid to the longer-chain n-3 fatty acids EPA and DHA. It is not clear if paddlefish have a dietary requirement for EPA and DHA or can elongate-desaturate linolenic acids into these long-chain n-3 fatty acids.

In conclusion, it appears that paddlefish fry can utilize sources of brine shrimp with or without a high percentage of EPA for at least 15 days post-hatch. Prepared diets are consumed, but do not appear to be completely digested. Further research on fatty acid requirement of paddlefish fed for longer time periods needs to be conducted.

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