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Influences of feeding habitat and age composition on the growth patterns, length-frequency and gut contents in maternal paddlefish *Polyodon spathula* (Walbaum, 1792).

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ABSTRACT

The study aims to study the impact of the diet shift and feeding behavior of the paddlefish different age groups on the growth during the spawning season. Habitat affected paddlefish prey selection or foraging success is a striking force that promotes either dominance or submissiveness of brood fish to reproductive or growth performance under Russian aquaculture conditions. Four age groups were studied depending on years of commissioning (age composition: 23, 18, 17, 16; for years 1997, 2002, 2003 and 2004, respectively) and registered divergence in their growth rates throughout spawning season 2020. Growth patterns of 59 mature paddlefish were studied based on length-weight relationship, length frequency, and the growth coefficient (K).

The results revealed that moderate growth pattern was observed in the 2nd group Isometric (b = 3), whereas the rest groups recorded negative allometric growth (b < 3). The data obtained from gut contents were consistent with the previous growth performance results. Major five prey categories (benthic macro-invertebrates, macro-invertebrate larvae, detritus, zooplankton and phytoplankton) were observed within fish stomach, which exposed through natural feeding in the rearing ponds. The values of chi-square test (χ^2) results showed that frequencies in one or more prey items of a four age were strong significance (p-value = 8.93E-17 & χ^2 = 1.219, 1.051, 1.066 & 1.032; for 2nd, 3rd, 4th and 1st, respectively). Overall results propose maternal paddlefish prey election and growth patterns can be highly variable, and merely heavily reliant on feeding habitat and swimming behavior across spawning season.

INTRODUCTION

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Paddlefish (*Polyodon spathula*) facilities are centered in the regions of southern Russia, especially those located in the Delta-Volga River, where the activities of aquaculture are observed intensively (**Vasilyeva and Elnakeeb, 2019**). Estuary of the Volga River and the North Caspian basin are unique territories due to its distinctive food

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base structure (e.g., Macro-invertebrate larvae, benthic macroinvertebrates, zoo and phytoplankton, detritus and sand) in lentic ecosystems (Rosen and Hale 1981; Ruban *et al.*, 2019).

The American paddlefish is a pelagic filter feeder throughout most of its post-juvenile life stage. It is, therefore, ecologically efficient via promotes water purification from rapidly developing microalgae, zooplankton, and higher vegetation (Hintz *et al.*, 2017). It can switch prey size selection across their lifespan depending on aquatic environmental conditions, variation in water flow, abundant in prey items, etc. (Makrakis *et al.*, 2005; Nunn *et al.*, 2007). The previous studies revealed that, there is a correlation between prey-size and age of paddlefish, particulate-feeding for young fish mainly select smallest and speeding prey (i.e., chironomid larvae, copepods, and cladocerans). While, maternal and mature-females have the capability to the various elective size of prey (i.e., *Daphnia spp.*, *Diaptomus spp.*, and *Cyclops spp.*) by ram-filter (Ruelle and Hudson 1977; Kozfkay and Scarnecchia 2002). Fishpond area and water quality management have been recognized as prime conditions, particularly in the breeding season when zooplanktonic prey ample in the ecosystem (Hoxmeier and DeVries 1997).

Mims and Shelton (2015) reported, P. *spathula* has a relatively short gut in keeping with prey-size, and characteristic of the digestive system of carnivorous fish (gut length represent 70% of cavity body length). Adults paddlefish filter-feeding models are a more comprehensive array through detected the prey by electroreception (Rosen and Hales 1981; Grossman *et al.*, 2002; Wilkens *et al.*, 2003). Diet shifts in mature females concerning of growth performance during spawning season are related to age composition, Length frequency distribution, body- length relationships, condition factor, gut content measurements (Carlander 1969; Rosen and Hale, 1981; Michaletz *et al.*, 1982; Graham *et al.*, 1986; Brown and Murphy, 1993; Mims and Knaub, 1993; Stell *et al.*, 2018; O'Keefe, 2020; Yang *et al.*, 2020).

Numerous studies indicate that age groups have two concurrent models to selection and foraging prey that is subjected to the free space for feeding, hierarchic structure forms, competition for food, stocking density, and the required energy, whether for growth or reproduction (Gershanovich, 1983; Miner and Stein 1993; Onders *et al.*, 2005; Dasgupta *et al.*, 2008; Mims *et al.*, 2009; Nelson *et al.*, 2013; Hupfeld *et al.*, 2016). Thus, during rearing season, the growth disparity with differential growth between brood fish is magnified, and the direction of necessary energy perhaps it may have deviated to growth rather the formation of gonadotropin (Onders *et al.*, 2011).

Previous studies have shown diet shift in the young paddlefish that subjected consume macro invertebrates and zooplankton, but not in maternal stages identified in this study. The current study aims to investigate: 1) prey diversity consumed by maternal paddlefish, 2) variance of prey selectivity, and its effect on gut content (i.e., stomach fullness) and diet shift during the spawning season, and 3) fluctuations of growth performance within age-groups.

MATERIALS AND METHODS

Study sites and data collection

Specimens were collected for the study from experimental fishponds that operated under supervision the official research institute, Federal state scientific institution "BIOS-CaspNIRKh" located at Delta-Volga River territories and the north Caspian Sea, west of Astrakhan Oblast (**Fig. 1**). Indoor fish farm with planting paddlefish brood-stocks was consisting of three earthen ponds (2 ha/pond). Each fishpond has a different age class of brood fishes. The herd of paddlefish generations in the "BIOS" institute, during 1993-2020, includes 320 individuals with a total biomass of 3174.40 kg, which mature females is 219 with total weight approximately 2422.80 kg. The female's proportion is 68% of producers. In the present spawning season, spring appraisal was carried out for 63 mature females with biomass 960 kg.

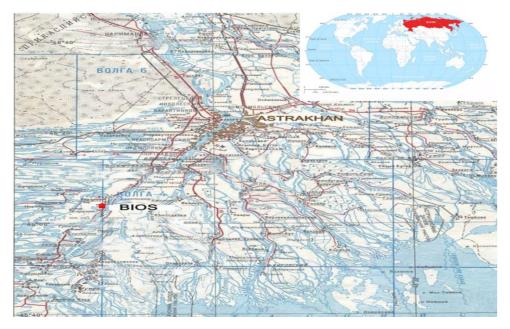


Fig. 1: Map of the official research institute "BIOS- CaspNIRKh", Astrakhan, Southern Russian federation.

The measurements were performed bi-weekly at the present spawning season from April through June 2020. *Polyodon spathula* brood-stocks are fed on zooplankton, benthic macro-invertebrates, and macro-invertebrate larvae in monoculture ponds with a stock density of 850 kg/ha. Samples were transported from the earthen ponds to the circular concrete hatchery ponds in preparation for spawning. After that, the mature-females sorted depending on age classes and various other morphological aspects, and these measurements were calculated as an average (e.g., total length "TL, cm," standard length "SL, cm," and weight "Total weight, Kg"). In total, 63 mature-females were divided into six groups based on age composition and years of commissioning (25; 23; 18; 17; 16; and 15 years) for the age-groups (1995, 1997, 2002, 2003, 2004 and 2005, respectively (**Table 1**). Consequently, two groups (1995 and 2005) were excluded due to

the lack of sufficient repetition permits the expression of statistical significance, therefore the herd became attained four age-groups.

 Table 1: Ranges of paddlefish Biometric values and Mean ± Standard deviation (S.D.) according to the year of commissioning/age composition.

| Age group | Year of age groups | No.* | Age (Years) | Weight (Kg) | | Total length (TL, cm) | | Standard length (SL, cm) | |
|-----------------|--------------------------|------|----------------|-------------|------------|-----------------------|-------------------|--------------------------|-------------|
| | | | | Ranges | Mean ± S.D | Ranges | Mean ± S.D | Ranges | Mean ± S.D |
| 1^{st} | 1997 | 25 | 23 | 8.9-16.6 | 12.15±2.05 | 131-157 | 146.08 ± 7.30 | 121-146 | 134.04±6.48 |
| 2^{nd} | 2002 | 9 | 18 | 7.8-14.6 | 10.87±2.32 | 136-155 | 146.78±7.13 | 127-141 | 133.33±5.07 |
| 3 rd | 2003 | 18 | 17 | 10.4-17.5 | 12.81±1.95 | 130-148 | 141.11±6.95 | 119-137 | 128.56±6.59 |
| 4 th | 2004 | 7 | 16 | 9.4-14.6 | 11.50±2.18 | 135-152 | 143.86±6.78 | 124-137 | 131.43±4.35 |

* Number of paddlefish.

Length-weight relationship and condition factor

The body weight W (kg)-total length TL (cm) relationship of mature females' specimens was computed by the following equation:

$\mathbf{W} = \mathbf{a} \, \mathbf{L}^{\mathbf{b}} \tag{Froese, 2006}$

The constants value "a" and "b" were estimated by the least-squares method whose rely on logarithms as a function to describe growth patterns:

Log W = Log a + b Log L (Froese *et al.*, 2014)

Where: W is the total body weight (kg), TL is the total length (cm), "a" the intercept, and "b" the exponent.

To assess paddlefish condition in the fish farms at the southern parts of the Russian Federation was calculated Fulton's factor (K) from the relationship between total length (L) in cm and total weight (w) in kg by the function:

K=100W/L³ (Froese, 2006)

Gut content analysis

A number of samples were selected for gut content measurements; the tri-Cainemethane sulfonate bath (MS-222) was used to euthanize. The whole stomach preserved in 70% ethanol. Paddlefish mature-females stomachs were weighed of the three age groups, and gut contents were extracted and determined the weight of contents. Stomach fullness was expressed through the stomach mass proportion (g) attributed to stomach contents (g), accounted as:

Stomach fullness (%) = <u>Weight (stomach contents)</u> <u>Weight (stomach contents)</u>

This computing is based on the premise that the size and feeding activities of paddlefish are strictly related to the larger stomachs, also in the hatchery stage, where it shows the directing of energy to either growth or gonadotropin synthesis. The identification and counting of prey species were carried out by using dissecting stereo-microscope (M5A). For each individual and taxonomic group, the mean number of prey

per stomach, proportion by number (P_j) , and occurrence frequency (Oi) were calculated. It determined proportion by number as:

$$\boldsymbol{P}_j = \frac{N_j}{\sum_{i=1}^{Q} N_i}$$

(Chipps and Garvey 2007)

Where: Ni= the number of organisms is for item I; Q= number of prey items. Occurrence frequency (Oi) also estimated as:

$$Oi = \frac{Ji}{P}$$

Where: J is the number of fish in their stomachs containing prey i and P is number of fish with a stomach filled by contents.

Statistical analyses

The occurrence frequency of different prey categories taxa (e.g., Domain, subphylum, class, order, and family) was compared using analysis of chi-square across diverse age groups of paddlefish stomachs (**Chipps and Garvey 2007**). The null hypothesis of chi-square was that all items of prey have opportunities to expose with equal probability to occur in paddlefish stomachs of age groups as a result of unknowing the prey availability in ponds.

The comparison between TL of age-groups females and the length of prey consumed was conducted (**Michaletz** *et al.*, **1982**). One-way analysis of variance (ANOVA) was performed to determine the differences among the levels of stomach fullness to identify diet shift direction before spawning times. Statistical analyses were conducted by using XLSTAT[®] version 2019.2.1 (**XLSTAT**, **2019**). The significance level was set at P < 0.05 (confidence interval and tolerance are %95 and 0.0001, respectively).

RESULTS

Length frequency distribution

According to (**Gulland**, 1971), analysis of length-frequency may be used to forecast growth and estimation yield models, such as production per recruit group of species. Length-frequency for the four age-groups revealed that there is significant variability in their distributional patterns, illustrated in **Fig.** (2).

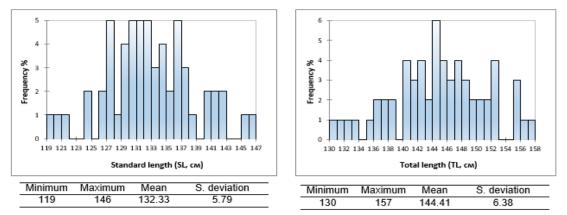


Fig. 2: Total and Standard Length frequency distribution of the mature paddlefish.

For the 1st group, SL located in a range between 121 to 146 cm and TL from 131 to 157 cm, Min-Max values. Additionally, Min-Max length-frequency data also occur infrequently for the other remaining 2^{nd} , 3^{rd} , and 4^{th} groups between (127-141; 119-137; and 124-137 cm SL & 136-155; 130-148; and 135-152 cm TL, respectively). In total, standard-total length of 59 mature brood fish ranged from 130 & 119 to 157 & 146 cm, and mean 144.41 & 132.33 cm (**Fig. 2**). A positive relationship was identified between the total length of age-group paddlefish and average prey length consumed.

Length-weight relationship

The total length-weight relationship was computed to undergo age-group patterns during the current spawning season for paddlefish brood-stocks in southern Russia. **Mahé** *et al.* (2017) discussed vital applications of the power function in the relationship between weight and length to be assigned the variation of the factors related to both spawning season/age criteria.

The relationship between length-weight for the four paddlefish age groups in the present spawning season demonstrated in **Table** (2).

Table 2: Power regression results for the length-weight relationship for the mature- females of paddlefish in the southern Russian region.

| Age-group | Α | В | Growth pattern | \mathbf{R}^2 |
|-----------------------|--------|--------|----------------|----------------|
| 1 st group | 0.0078 | 2.878 | NA | 0.7932 |
| 2 nd group | 0.0039 | 3.0014 | IS | 0.6013 |
| 3 rd group | 0.2416 | 2.1564 | NA | 0.5856 |
| 4 th group | 1.7251 | 1.7948 | NA | 0.2564 |

a= intercept, b=slope, R²= regression coefficients, and the growth pattern NA= Negative Allometric (b<3), IS= Isometric (b=3).

Condition factor

Generally, Biological and physical circumstances can be expressed by condition factor to assess feeding efficiency, the divergence of morphometric features related to growth performance, and other farming factors (Le Cren 1951). Furthermore, in the pond-culture system, the chief role of the body condition factor is to evaluate the season's success (Araneda *et al.*, 2008). In the present study, the values of condition factor of the mature paddlefish *Polyodon spathula* based on year of commissioning per age groups with an average of 0.43, 0.39, 0.37 and 0.44 in 1st; 2nd; 3rd; and 4th group, respectively. The values of condition factor less than one showed the influence of food compositions with different prey items given spawning season stress of paddlefish.

In situ studies of the natural paddlefish feeding in rearing pond

The concentration of zooplankton biomass in ponds varied during the breeding season in the range of $2-25 \text{ g/m}^3$ with an averaging 7.6 g/m³. The structural dynamics of the zooplankton biomass development in the feeding ponds were relatively stable. The species composition of the zooplankton in the experimental ponds was diverse (e.g., *Daphnia longispina*, *D. magna*, *Moina rectirostris*, *Moina macrocopa*, *Bosmina*

longirostris, *Polyphemus pediculus*, *Ceriadaphnia reticulata*, *Chydorus sphaericus*, *Alona guttata*, *Cyclops sp.*, *Diaptomus sp.*, and *Leptodora richardi*). The zoobenthos larvae biomass were dominated by chironomid. In addition, water beetles, water bugs, larvae of the caddisfly, dragonflies, mayflies, and stoneflies were observed.

Gut content analysis

The presence of identifiable preys in gut content, also empty stomachs samples' were investigated. It was difficult to determine the count of fragmented and partial-digested individual prey of the stomach contents.

| Prey category and taxa | N | P_{j} (%) | P _f | O_i (%) |
|--|-----|-------------|-----------------------|-----------|
| Subphylum Crustacea | | | | |
| Class Branchiopoda | | | | |
| Order Cladocera | | | | |
| Family Bosminidae | 20 | 4 | 7 | 53.8 |
| Family Chydoridae | 10 | 2 | 3 | 23.1 |
| Family Daphniidae | 30 | 6 | 8 | 61.5 |
| Family Leptodoridae | 3 | 0.6 | 2 | 15.4 |
| Family Moinidae | 15 | 3 | 4 | 30.8 |
| Class Hexanauplia | | | | |
| Order Calanoida | | | | |
| Family Diaptomidae | 25 | 5 | 7 | 53.8 |
| Order Cyclopoida | | | | |
| Family Cyclopidae | 137 | 27.4 | 12 | 92.3 |
| Other Classes (i.e., Ostracoda, Maxillopoda, and Malocostraca) | 90 | 18 | 10 | 76.9 |
| | | | | |
| Domain Bacteria and Phylum Chlorophyta | 50 | 10 | 9 | 69.2 |
| | | | | |
| Subphylum Hexapoda (Class Insecta) | 73 | 14.6 | 8 | 61.5 |
| Unidentifiable | 47 | 9.4 | 6 | 46.2 |

Table 3: Statistical summary of the consumed prey categories during the spawning season 2020.

The number of organisms (N); frequency of occurrence (P_j); the number of full stomach fish samples with identifiable prey (P_f); percentages of proportion by number (O_i).

Based on the previous feeding base knowledge, 900 prey items were identified andf categorized into three primary class zooplankton, phytoplankton & insect items in both rearing pond environment and gut content. Four hundred prey species were not determined below genera Insecta and Crustacea. Of the remaining 500 prey items, Chironomidae larvae were the most dominated lake flies – ($P_j = 14.6$ %) of the total biomass (**Table 3**). As well as the biomass of water beetles, water bugs, larvae of the caddisfly, dragonflies, mayflies, and stoneflies accounted for 3.2 to 15.7%. On the other hand, the most abundant of consuming prey within paddlefish gut up to ($P_j = 66\%$) consisted of zooplankton, of which Cyclops prevailed. Furthermore, in the stomachs of some samples, Daphnia was represented ($P_j = 6\%$), Cyclops and Diaptomus about ($P_j =$ 27.4% and 5%) of the total food lump, respectively.

Moreover, there is the presence of Cladocera, Insecta, and Copepoda were reluctant to be eaten by paddlefish approximately ($P_j = 10\%$). Among them, water bugs (Belostomatidae), great silver water beetle (*Hydrophilus piceus*), water mites (Hydrachnidia), mayflies (Ephemeroptera), and dragonflies (Anisoptera), were found singly. In addition to previous organisms, fragments of higher vegetation, and sand were observed. Additionally, Phytoplankton in the fish diet accounted for ($P_j = 2-6\%$), often found from blue-green algae: *Microcystis sp* (cyanobacteria), Aphanizomenon sp. Furthermore, from green algae: *Scenedesmus dimorphus* and *Scenedesmus acuminatus*, *Volvox sp* (chlorophyte green algae), *Pandorina sp.*, *Pediastrum sp.*

The percentages' frequency of occurrence (O_i) for both phytoplankton and detritus in the paddlefish gut contents (**Fig. 3**) did not differ from what would be expected across all age-groups. In contrast, the obtained results of 2^{nd} , 1^{st} , $3^{rd} \& 4^{th}$ groups were recorded significant differences that refer to the most abundant food items (zooplankton; benthic macro-invertebrates; and Macro-invertebrate larvae).

The overall results of chi-square (χ^2 test) showed that frequencies in one or more prey categories of a four age were highly significantly different contingency as the computed (p-value = 8.93E-17) is less than the significance level p < 0.0001; one cannot reject the null hypothesis (H₀). According to the null hypothesis, there is a significant interaction between the rows (Age -group) and the column (Food items,) of the **Table (3)**.

The score of χ^2 results indicated that there is no statistical significance between the 1st age-group and prey types within the detritus, zooplankton, macro-invertebrate larvae, and phytoplankton. On the other hand, a highly significant difference with benthic macro-invertebrates ($\chi^2 = 1.032$, P < 0.0001) was observed. For 2nd, 3rd & 4th age groups P-values of the Chi-square analyses were represented: < 0.0001, 0.004, 0.006 and $\chi^2 =$ 1.219, 1.051 & 1.066, respectively. Four prey-tapes were representatives of five prey categories with an interaction between the age-group from 2nd to 4th and benthic macroinvertebrates, detritus, zooplankton, and phytoplankton (P-values < 0.000, 0.003 and 0.000). Neither, there is no relation of macro-invertebrate larvae to the three previous age groups.

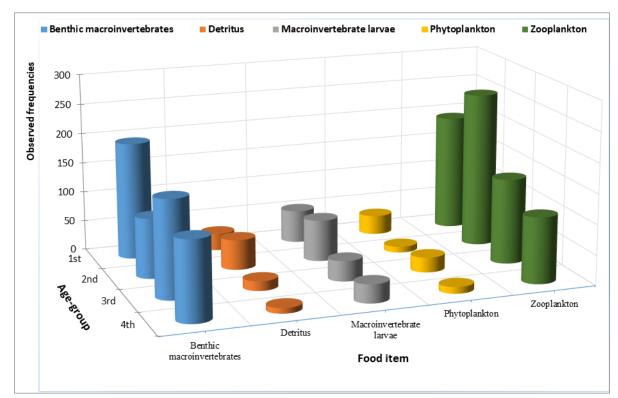


Fig. 3: 3D view of the distributional Contingency (Age-groups / Food items) with chi-square test $(\chi 2)$ frequencies in one or more prey categories of a four age contingency, was set at p-value < 0.001.

Stomach fullness concerning to age composition

The stomach fullness index was computed as a mass proportion between the weights of the empty stomach concerning a stomach filled by contents in view variance of age classes. As a result of the diversity in prey classes (Five main categories) and discriminatory distribution patterns, a fullness stomach index was obtained and illustrated in **Fig. (4)**.

Of total specimens, about 81.3% of stomachs samples contained identifiable prey, and the remaining examined set represents 19.7% were empty. The results referred that the frequency of full stomachs by contents of prey items was dominant in the 4th-group (74%). Contrarily, the fullness of the 2nd-group paddlefish stomach appears had slightly lowered among the rest of the other groups (about 62%). However, there is no significant age-groups interaction for the gut fullness when exposed to five prey-categories (F = 1.158 & Pr > F = 0.378 at a significance level, was set P < 0.05).

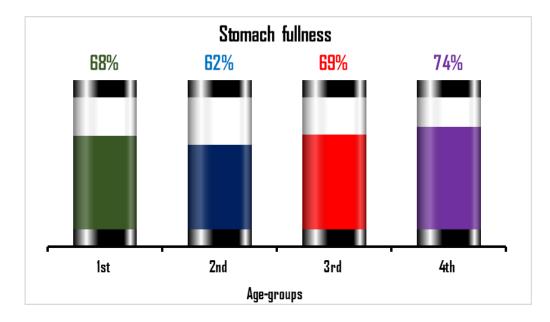


Fig. 4: Percentage of stomach fullness for each (Age group) of *P. spathula* during spawning season.

DISCUSSION

The spawning season of paddlefish occurs from April to June under aquaculture conditions in Russia. In mid-March, maternal paddlefish are usually exposed to physiological stress caused by the gametogenesis and begin entering into spawning season (Larimore, 1950). Temperature and quality of the inlet water source, and what holds of their flora, benthic fauna, and insect larvae in the Volga Delta, which allow fishes to be select from the broad feeding base (Hintz *et al.*, 2017; Vasilyeva and Elnakeeb, 2019). These conditions interfere to effect on the American paddlefish feeding patterns behavior in the rearing ponds, furthermore, the influence of age composition on the growth performance as a function in view measurements of length frequency, length-weight relationship, condition factor, and gut contents analysis.

The results of morphometric measurements demonstrated significant variability across age groups, especially in the 2^{nd} group. There is great variability in total length frequency (S. Deviation = 6.38, P < 0.05) and length-weight relationship (Isometric growth, 2nd group). The variance of distributional growth patterns between the four age groups may depend on the aquatic environmental stressors and genetic pool (Forese, 2006; Mims and Shelton, 2015). In keeping to the previous results, physiological status disorder for well-being conditions and stress that were observed through the breeding season is explained by the low value of the condition factor (Le Cren 1951; Araneda *et al.*, 2008).

The analysis of the paddlefish specimens and water samples in the present study was utilized to investigate whether there is a relationship between morphometric measurements and growth performance with the qualitative and quantitative composition of prey-items, whatever in the fishpond or stomach for the four age groups, and that is the primary purpose of the paper. A drastic decline in maternal vertical migration activity was observed in order to the prey search. *P. spathula* feeding behavior drove based on swimming behaves in ways that vary markedly from pelagic to benthic filter-feeding. Thus, qualitative and quantitative prey items were shifted. Observed five prey categories involved whether, in the water column and gut contents, they were selected naturally depending on numerous factors e.g., fish vitality, swimming speed, growth patterns of the fish, prey-fish length relationship, etc. Therefore, the abundant prey groups were both zooplankton and benthic macro-invertebrates for $2^{nd} \& 1^{st}$ age groups.

Chi-square ($\chi 2$ test) was used to determine which category of prey that was prevalent. The obtained results conclude that the divergence proportion number of prey due to the possibility of consuming prey (capture) and frequency of prey occurrence in the pond environment. There is no statistical significance of stomach fullness analyses. Particularly, the elective prey categories embedded in the gut approximately located in the same range of length (Hintz *et al.*, 2017; Stell *et al.*, 2018; O'Keefe, 2020; Yang *et al.*, 2020). It means competitive interactions stunt among age-groups paddlefish was absent.

CONCLUSION

The current study sheds light on the importance of evaluation of shifting in the feeding and thus growth patterns also, which underlie the disorder of the physiological homeostasis during fish spawning season. Moreover, changing the dynamics of feeding behavior from pelagic to benthic relay on prey diversity in commercial paddlefish farms is considered a view of novel studies.

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