

Research Article

Nutrient distribution in different tissues of subadult Dabry's sturgeon, *Acipenser dabryanus* (Duméril, 1869)

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Abstract

Low fertilization rate and hatchability of cultured Dabry's sturgeon, *Acipenser dabryanus*, are bottlenecks to the fish species protection, while adequate nutrients in body of subadult fish are crucial to successful reproduction. The present study was conducted to preliminarily understand the nutritional status of male and female subadults by characterizing the proximate composition, fatty acid and amino acid profiles in muscle, liver and gonad of farmed Dabry's sturgeon with gonads at stage II through chemical analysis, gas chromatograph and high pressure liquid chromatography. The results showed that lipid content (47.24%) in gonad of females was significantly higher than those in liver (29.26%) and muscle (7.72%), while lipid content (39.43%) in liver of males was significantly higher than those in gonad (18.40%) and muscle (6.68%) ($p < 0.05$). The protein content (5.81%) in gonad of females was significantly lower than those in liver (10.77%) and muscle (22.82), while protein contents in gonad (9.23%) and liver (8.72%) of males were significantly lower than that in muscle (20.83%) ($p < 0.05$). The contents of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in gonad and muscle were the most abundant n-3 polyunsaturated fatty acids (n-3 PUFAs). The content of arachidonic acid (ARA, 0.65%) in gonad of females was significantly higher (0.33%) than that in gonad of males ($p < 0.05$). The results suggest that lipid and highly unsaturated fatty acids, such as ARA, EPA and DHA are accumulated in the gonads at stage II, which may play pivotal roles in gonad development.

Key words: Dabry's sturgeon, Nutrients, Gonad, Liver, Muscle

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Introduction

Dabry's sturgeon (*Acipenser dabryanus*, Duméril, 1869), a potamodromous fish, is distributed mainly in the main stream and some tributaries of the upper reaches of Yangtze River system (Zhang *et al.*, 2011). In recent decades, the natural population of this sturgeon sharply declined, and it was listed as a critically endangered species (Zhang *et al.*, 2011; Wu *et al.*, 2014). Various activities for species protection and population recovery of Dabry's sturgeon have been widely implemented since 2000, such as fishing bans, construction of nature reserve and release program of cultured fish (Zhang *et al.*, 2011). Dabry's sturgeons achieve sexual maturity at the age of 4-6 years for males and of 6-8 years for females in the wild, and have a body weight of 2.5-5 kg when their gonads are at stage II. However, cultured Dabry's sturgeon can grow rapidly with a weight gain of up to 3.5 kg each year, and get sexual maturity earlier than the wild (Zhuang *et al.*, 1997). Artificial reproduction of Dabry's sturgeon was firstly investigated in 1976, and was successful to obtain juvenile fish from the broodstock captured in the wild. Although researches on controlled reproduction for Dabry's sturgeon were steadily carried out, the fertilization rate and hatchability in the 21st century are still lower (Gong *et al.*, 2013).

Broodstock nutritional reserves have been proven to affect the reproductive performance, including fertilization, hatching and survival rates of larvae

(Cerdá *et al.*, 1994; Fernández-Palacios *et al.*, 1997; Izquierdo *et al.*, 2001; Watanabe and Vassallo-Agius, 2003). The importance of lipid and protein in broodstock nutrition had been addressed previously (Izquierdo *et al.*, 2001). Nutrients for reproductive activity might be obtained from fish body, such as liver and muscle tissues. The lipid in muscle was used as energy source in sockeye salmon *Oncorhynchus nerka* (Walbaum, 1792) and turbot *Scophthalmus maximus* (Linnaeus, 1758) during ovarian maturation (Lie *et al.*, 1993). In Atlantic cod *Gadus morhua* (Linnaeus, 1758), a large quantity of lipid stored in the liver was mobilized during gonad development and spawning (Jangaard *et al.*, 1967). Furthermore, polyunsaturated fatty acids (PUFAs) were selectively transferred from the muscle to the ovary for storage in silver pomfret *Pampus argenteus* (Euphrasen, 1788) broodstock during the reproductive season (Huang *et al.*, 2010). In addition, it was reported that protein derived from the body was deposited into the gonad of sockeye salmon during the gonad development (Idler and Bitners, 1960) and that the changes of amino acid profiles among tissues are associated with the gonad development at different stages (Huang *et al.*, 2009). Therefore, understanding the nutritional status in different tissues of fish during gonad development is necessary for improving the reproductive performance of broodstock.

Steady and high quality of fingerling production is important for species protection of Dabry's sturgeon (Huang *et al.*, 2010). The low fertilization rate and hatchability in controlled reproduction of Dabry's sturgeon may be due to the lack of some essential nutrients in broodstock (Gong *et al.*, 2013). In sturgeon aquaculture, difficulties in the transition from previtellogenic oocyte (gonad at stage II) to vitellogenic oocyte during gonadal development were suggested in Chinese sturgeon, *Acipenser sinensis* (Gray, 1835) (Li *et al.*, 2014), white sturgeon, *Acipenser transmontanus* (Richardson, 1836) (Doroshov *et al.*, 1997) and Siberian sturgeon, *Acipenser baeri* (Brandt, 1869) (Williot and Brun, 1998). However, information about the nutritional status of Dabry's sturgeon is limited, except that nutrient composition in muscle of farmed sturgeons has been reported by Gong *et al.* (2012). The objective of this study was to explore the nutritional status of subadult Dabry's sturgeon with gonad at stage II in controlled rearing system and to characterize the key nutrients for gonad development by comparing the nutrient distribution with other sturgeons.

Materials and methods

Ethics statement

All fish handling and experimental procedures were approved by the Animal Care and Use Committee of the Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences.

Experimental fish and sample collection

The subadult Dabry's sturgeons for sampling were 3 years old with gonad at stage II identified by ultrasound images (Fig. 1) and biopsy (Du *et al.*, 2017), which were cultured at the density of 130 fish/pond in an earthen pond with the area of 0.1 hectare and water depth of 2 m in Taihu Experimental Station, Yangtze River Fisheries Research Institute, Chinese Academy of Fisheries Sciences and fed with artificial diets in the past two years before sampling. The culture conditions in the pond were as follows: rural water temperature ranged from 5 to 28°C, pH was 7.0-8.0, and dissolved oxygen was 6.5-7.5 mg/L controlled by continuous aeration (Zhou *et al.*, 2017). Fish were fed with 70% slow-sinking extruded feed plus 30% frozen forage fish at a feeding rate of 0.2-1.5% of body weight according to the water temperature. The extruded feed included 43% crude protein, 4% lipid, 2% lysine and 10% moisture. Frozen capelin (*Mallotus villosus*) from North Atlantic Ocean containing 14% crude protein, 8% lipid, and 75% moisture was used as forage fish. Three males (total length (TL), 98.0±5.3 cm; body weight (BW), 9.8±2.0 kg) and four females (TL, 93.3±3.2 cm; BW, 8.3±0.5 kg) were randomly sampled and euthanized after anaesthetization by tricaine methanesulfonate (MS-222, 75 mg/L) (Sigma, USA), and white muscle, liver and gonad were collected and then were frozen at -80°C until analysis.

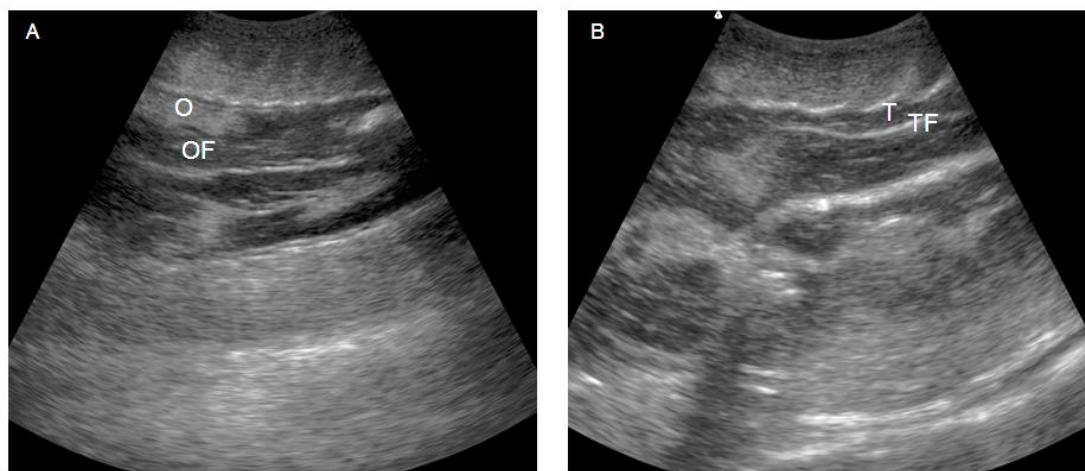


Figure 1: The ultrasound images of ovary and testis at stage II of Dabry's sturgeon. (A. Ovary: O – ovary; OF-ovary fat. Ovary was located mostly on the surface of the ovarian fat with irregular margin. The parenchymal echogenicities of the ovary were more hyperechoic (brighter) than surrounding fat and the body wall. B. Testis: T-testis; TF- testis fat. The testis was mostly embedded within the testicular fat and with smooth and echogenic (bright) margins. The parenchymal tissue of the testis was more hypoechoic (darker) than surrounding fat and the body wall.)

Proximate analysis

Fresh samples were dried by a vacuum freeze-drier (Labconco Freezone 2.5, USA) for various assays. Moisture content was determined by oven-drying at 105°C. Crude protein content was determined by Kjeldahl method (Association of official analytical chemists (AOAC, 1995), and a conversion factor of 6.25 was used to convert total nitrogen to crude protein. Total lipid was determined according to the method described by Bligh and Dyer (1959).

Amino acids determination

Amino acids analysis was performed according to Lindroth and Mopper (1979). Briefly, lyophilized samples were hydrolyzed by 6 M hydrochloric acid (HCl) at 110°C for 24 h under nitrogen. The hydrolyzed samples (10 µL) were separated and quantified by a

reversed-phase high performance liquid chromatography (HPLC, Agilent 1260, USA) equipped with a C18 chromatographic column (Agilent Eclipse-AAA, 150×4.6 mm, 3.5 µm, USA). Precolumn derivatization was performed using ortho-phthalaldehyde (OPA) for the amino acids detection. The data of amino acids were expressed as ratios of each amino acid to lysine content.

Fatty acids analyses

Fatty acids composition of muscle, liver and gonad samples were analyzed according to Querijero *et al.* (1997). Briefly, total lipid was extracted according to Bligh and Dyer (1959), fatty acid methyl esters (FAMES) were then produced by methylation with boron trifluoride (BF₃) in methanol. FAMES sample (1.0 µL) were analyzed using gas chromatography (Agilent

6890, Santa Clara, CA, USA) with a capillary column (HP-FFAP, part number: 19091F-413, 30 m×0.32 mm×0.25 μm, Agilent, USA). The temperature of injector and flame ionization detector (FID) was set at 250°C and 260°C, respectively. High-purity nitrogen was used as the carrier gas at a flow rate of 1 ml/min. The oven temperature program used was 100 °C with a temperature hold of 2 min, 100 to 210°C at a rate of 8°C min⁻¹ with a final temperature hold of 5 min, 210 to 220°C at a rate of 2°C min⁻¹ with a final temperature hold of 7 min, and then 220 to 240°C at a rate of 8°C min⁻¹ with a final temperature hold of 10 min. FAMES standard containing 40 FAMES (Nu-Chek Prep. Inc, Elysian, MN, USA) was used for qualitative analysis. Identified fatty acids were presented as area percentage of total fatty acid.

Statistics

Data were analyzed using one-way ANOVA to detect the significance of tissue treatment and means were compared using Duncan's multiple range tests with SPSS (Version 19.0, SPSS Inc). Independent sample *t*-test was performed to compare the difference between males and females. Data were expressed as mean±SD and the statistical significance was $p<0.05$ (Beheshtipour *et al.*, 2018).

Results

Proximate composition

As shown in Table 1, significant differences in proximate composition were observed among liver, muscle and

gonad ($p<0.05$). In females, gonad showed the lowest contents of moisture and protein, and the highest content of lipid; whereas liver showed the medium values of moisture, protein and lipid; and muscle showed the highest contents of moisture and protein, and the lowest lipid content. However, in males, liver showed lower moisture content and the highest lipid content; whereas muscle showed higher protein content and the lowest lipid content; and gonad showed medium lipid content and lower protein content.

When comparing the differences between sexes, proximate composition in muscle and liver was similar, except that liver lipid content of males was significantly ($p<0.05$) higher than that of females. However, females showed significantly higher lipid content, lower moisture and protein contents in gonad than the males ($p<0.05$).

Amino acid profile

The amino acid profiles in different tissues of Dabry's sturgeon are presented in Table 2. In females, the ratios of alanine (Ala), tyrosine (Tyr), cysteine (Cys), threonine (Thr), phenylalanine (Phe), and leucine (Leu) in liver were significantly higher than those in muscle and gonad; and the ratio of total essential amino acid in liver was higher than that in muscle and gonad ($p<0.05$).

Table 1: Proximate composition in different tissues of Dabry's sturgeon.

Proximate composition (%)	Dabry's sturgeon		
	Males	Females	P-value
Muscle			
Moisture	73.17±0.73 ^A	71.25±4.70 ^a	ns
Protein	20.83±2.17 ^A	22.82±2.73 ^a	ns
Lipid	6.68±0.50 ^B	7.72±1.54 ^c	ns
Liver			
Moisture	50.45±7.99 ^B	57.89±7.22 ^b	ns
Protein	8.72±1.08 ^B	10.77±0.38 ^b	ns
Lipid	39.43±0.34 ^A	29.26±3.82 ^b	*
Gonad			
Moisture	71.47±5.52 ^A	45.84±5.65 ^c	*
Protein	9.23±0.24 ^B	5.81±1.55 ^c	*
Lipid	18.40±7.49 ^B	47.24±5.22 ^a	*

* Data are presented as mean±SD. Values of the same nutrient in each column with different superscripts showed significant difference ($p<0.05$): Lower cases were in females and upper cases were in males. The list of p -values shows the independent sample t -test result of the nutrients in the same tissue between males and females: ns indicated no significant difference, whereas * indicated $p<0.05$

Table 2 Amino acid compositions in different tissues of Dabry's sturgeon.

Amino acid (%)	Muscle			Liver			Gonad		
	Males	Females	P-value	Males	Females	P-value	Males	Females	P-value
Aspartic acid	1.21±0.02	1.22±0.03 ^b	ns	1.31±0.08	1.34±0.06 ^a	ns	1.29±0.12	1.27±0.07 ^{ab}	ns
Glutamic acid	1.95±0.03 ^{AB}	1.95±0.11	ns	1.77±0.16 ^B	1.86±0.17	ns	2.19±0.18 ^A	1.90±0.01	*
Serine	0.50±0.01 ^B	0.51±0.03 ^b	ns	0.64±0.06 ^A	0.69±0.02 ^a	*	0.72±0.04 ^A	0.63±0.03 ^a	ns
Glycine	0.54±0.04 ^B	0.59±0.17 ^b	ns	0.72±0.08 ^B	0.89±0.15 ^a	ns	1.05±0.22 ^A	0.79±0.09 ^{ab}	ns
Alanine	0.64±0.02 ^B	0.66±0.08 ^c	ns	1.00±0.04 ^A	0.99±0.06 ^a	ns	0.92±0.16 ^A	0.83±0.02 ^b	ns
Tyrosine	0.37±0.01 ^B	0.37±0.01 ^c	ns	0.47±0.03 ^A	0.47±0.03 ^a	ns	0.43±0.02 ^A	0.43±0.04 ^b	ns
Cysteine	0.07±0.01 ^B	0.08±0.01 ^b	ns	0.13±0.02 ^A	0.13±0.01 ^a	ns	0.11±0.02 ^A	0.08±0.05 ^b	ns
Histidine	0.63±0.05 ^A	0.57±0.10	ns	0.61±0.03 ^{AB}	0.63±0.07	ns	0.54±0.01 ^B	0.55±0.06	ns
Threonine	0.49±0.01 ^B	0.51±0.01 ^b	ns	0.60±0.01 ^A	0.65±0.04 ^a	ns	0.63±0.04 ^A	0.52±0.04 ^b	*
Arginine	0.79±0.04 ^B	0.78±0.03	ns	0.81±0.03 ^{AB}	0.83±0.02	ns	0.92±0.10 ^A	0.82±0.04	ns
Valine	0.51±0.02 ^B	0.48±0.03 ^b	ns	0.68±0.01 ^A	0.64±0.06 ^a	ns	0.56±0.06 ^B	0.60±0.07 ^{ab}	ns
Methionine	0.31±0.01	0.31±0.01 ^a	ns	0.29±0.02	0.28±0.03 ^a	ns	0.28±0.04	0.19±0.05 ^b	ns
Phenylalanine	0.45±0.01 ^B	0.45±0.01 ^c	ns	0.58±0.01 ^A	0.58±0.02 ^a	ns	0.48±0.05 ^B	0.48±0.02 ^b	ns
Isoleucine	0.49±0.01	0.49±0.00	ns	0.51±0.01	0.52±0.04	ns	0.48±0.07	0.50±0.03	ns
Leucine	0.85±0.02 ^B	0.85±0.01 ^c	ns	1.01±0.06 ^A	0.99±0.04 ^a	ns	0.90±0.08 ^{AB}	0.92±0.02 ^b	ns
W _{EAA/L}	4.52±0.14 ^B	4.46±0.08 ^b	ns	5.08±0.06 ^A	5.12±0.24 ^a	ns	4.78±0.33 ^{AB}	4.53±0.10 ^b	ns
W _{NEAAL}	5.28±0.05 ^B	5.38±0.37 ^b	ns	6.03±0.38 ^{AB}	6.36±0.27 ^a	ns	6.72±0.75 ^A	5.91±0.22 ^{ab}	ns

*Data are presented as mean ± SD, and the amino acid profiles were expressed as ratios to lysine content. NEAA/L means the ratio of total non-essential amino acid content to lysine content, and the EAA/L means the ratio of total essential amino acid to lysine. Values of the same amino acid in each row with different superscripts showed significant difference ($p<0.05$): Lower cases were in females and upper cases were in males. The list of p - values shows the independent sample t -test results of the amino acid in the same tissue between males and females: ns indicated no significant difference, whereas * indicated $p<0.05$.

In males, the ratios of serine (Ser), Ala, Tyr, Cys, and Thr in liver and gonad were significantly higher than those in muscle, and the ratios of glycine (Gly) and arginine (Arg) in gonad were significantly higher than those in muscle; and the ratio of total essential amino acid in liver was significantly higher than that in muscle ($p < 0.05$). When comparing the differences between sexes, the ratios of Glu and Thr in gonad of females were significantly lower than those in gonad of males, and the ratio of Ser in liver of females was significantly higher than that in liver of the males ($p < 0.05$).

Fatty acid profile

The fatty acid profile in different tissues from males and females of Dabry's sturgeon are presented in Table 3. In females, the most abundant saturated fatty acid (SFA) was palmitic acid (C16:0) in muscle, liver and gonad, which showed no difference between tissues; followed by C18:0, which was higher in liver than in gonad and muscle ($p < 0.05$). The most abundant mono-unsaturated fatty acids (MUFA) in the three tissues was oleic acid (C18:1n9), followed by C16:1n7, and both of them showed no difference between tissues. The most abundant poly-unsaturated fatty acids (PUFA) in the three tissues was C18:2n6, which showed no difference among tissues; followed by C22:6n3 (DHA), and then C20:5n3 (EPA). Both DHA and EPA contents in gonad and muscle were higher than those in liver ($p < 0.05$).

In males, the most abundant SFA was

C16:0, which was significantly lower in gonad than that in the muscle and liver; followed by C18:0, which was significantly higher in the liver than that in the muscle and gonad ($p < 0.05$). In addition, C14:0 content in gonad was significantly higher than that in muscle and liver ($p < 0.05$). The most abundant MUFA was C18:1n9, which was significantly higher in liver than that in gonad; followed by C16:1n7, which was significantly higher in gonad than that in muscle and liver ($p < 0.05$). The most abundant PUFA was C18:2n6, which was higher in muscle than that in liver; followed by C22:6n3 (DHA), which was lower in liver than that in gonad and muscle ($p < 0.05$). In addition, the contents of C18:3n6 and C20:4n6 (ARA) in liver were significantly higher than those in gonad and muscle ($p < 0.05$), and the contents of C18:3n3 and C20:5n3 (EPA) in muscle and gonad were significantly higher than those in liver ($p < 0.05$).

When comparing both sexes, no difference in the fatty acid profile of muscle and liver was observed, while the contents of C16:0, C18:2n6, C20:3n6 and C20:4n6 (ARA) in gonad of females were significantly higher than those in gonad of males, and the contents of C16:1n7, C22:1n9, C20:3n3 and C22:5n3 in gonad of females were significantly lower than those in gonad of males ($p < 0.05$). The total content of n-6 PUFA in gonad of females was also significantly higher than that in gonad of males ($p < 0.05$).

Table 3: Fatty acid profile in different tissues of Dabry's sturgeon.

Fatty acid %	Muscle			Liver			Gonad		
	Males	Females	<i>P</i> - value	Males	Females	<i>P</i> - value	Males	Females	<i>P</i> - value
C14:0	2.52±0.34 ^B	2.40±0.20	ns	2.31±0.29 ^B	2.33±0.37	ns	5.13±0.62 ^A	2.67±0.39	ns
C15:0	0.41±0.05	0.39±0.03	ns	0.36±0.05	0.38±0.09	ns	0.43±0.07	0.44±0.07	ns
C16:0	21.53±0.87 ^A	20.82±0.49	ns	21.89±0.28 ^A	22.27±2.25	ns	17.78±0.91 ^B	20.67±0.57	*
C17:0	0.38±0.06	0.38±0.06	ns	0.47±0.06	0.48±0.08	ns	0.38±0.09	0.40±0.05	ns
C18:0	3.48±0.38 ^B	3.40±0.63 ^{ab}	ns	5.33±0.05 ^A	4.03±0.65 ^a	ns	3.19±0.59 ^B	2.74±0.29 ^b	ns
C20:0	0.58±0.07	0.50±0.08	ns	-	-	-	-	-	-
C23:0	0.17±0.02	0.17±0.04	ns	0.15±0.05	0.16±0.08	ns	0.21±0.04	0.19±0.03	ns
C24:0	0.27±0.01	0.25±0.08	ns	0.21±0.07	0.18±0.07	ns	0.24±0.06	0.25±0.03	ns
C14:1n5	0.15±0.01 ^B	0.14±0.01	ns	0.14±0.02 ^B	0.15±0.03	ns	0.27±0.03 ^A	0.16±0.03	ns
C16:1n9	0.51±0.01 ^B	0.49±0.03 ^c	ns	0.87±0.06 ^A	0.90±0.07 ^a	ns	0.48±0.12 ^B	0.52±0.03 ^{ab}	ns
C16:1n7	3.80±0.40 ^B	3.82±0.34	ns	3.27±0.01 ^B	3.58±0.31	ns	5.61±0.60 ^A	4.22±0.44	*
C17:1n7	0.45±0.04	0.46±0.01	ns	0.45±0.03	0.50±0.04	ns	0.53±0.07	0.49±0.05	ns
C18:1n9	28.78±3.93 ^{AB}	32.43±4.48	ns	37.74±4.15 ^A	37.71±4.38	ns	25.15±1.01 ^B	30.81±4.04	ns
C18:1n7	2.24±0.10 ^B	2.32±0.20	ns	2.04±0.49 ^B	2.51±0.54	ns	4.72±0.77 ^A	2.20±0.09	ns
C20:1n9	1.83±0.07	1.97±0.23 ^b	ns	2.75±0.32	2.60±0.23 ^a	ns	2.90±0.79	2.27±0.28 ^{ab}	ns
C20:1n11	0.11±0.00 ^B	0.11±0.01	ns	0.10±0.02 ^B	0.12±0.01	ns	0.34±0.16 ^A	0.12±0.01	ns
C22:1n9	0.52±0.09 ^B	0.40±0.10 ^b	ns	0.23±0.05 ^B	0.32±0.11 ^b	ns	4.33±0.03 ^A	0.60±0.14 ^a	*
C22:1n11	-	0.16±0.02	ns	0.17±0.03 ^B	0.18±0.01	ns	0.93±0.07 ^A	0.15±0.01	ns
C18:2n6	15.86±1.97 ^A	13.94±2.67	ns	11.08±1.20 ^B	13.03±2.13	ns	11.89±0.90 ^{AB}	16.08±1.62	*
C18:3n6	0.70±0.09 ^B	0.93±0.25 ^{ab}	ns	1.38±0.02 ^A	1.17±0.18 ^a	ns	0.97±0.16 ^B	0.75±0.22 ^b	ns
C20:2n6	0.72±0.09	0.77±0.06 ^{ab}	ns	0.96±0.33	0.90±0.16 ^a	ns	0.73±0.05	0.73±0.04 ^b	ns
C20:3n6	0.50±0.06 ^B	0.59±0.12	ns	1.27±0.49 ^A	1.08±0.53	ns	0.22±0.05 ^B	0.63±0.13	*
C20:4n6(ARA)	1.03±0.03 ^A	1.03±0.19 ^a	ns	0.66±0.16 ^B	0.64±0.05 ^b	ns	0.33±0.04 ^C	0.65±0.08 ^b	*
C18:3n3	1.62±0.21 ^A	1.54±0.18 ^{ab}	ns	0.9±0.07 ^B	1.01±0.24 ^b	ns	1.72±0.23 ^A	1.86±0.32 ^a	ns
C20:3n3	0.52±0.06 ^B	0.49±0.05 ^{ab}	ns	0.36±0.05 ^C	0.39±0.10 ^b	ns	0.89±0.05 ^A	0.58±0.10 ^a	*
C20:5n3(EPA)	3.07±0.46 ^A	2.83±0.72 ^a	ns	0.76±0.21 ^B	0.94±0.29 ^b	ns	2.72±0.43 ^A	2.48±0.62 ^a	ns
C22:5n3	1.00±0.08 ^B	1.06±0.11	ns	0.51±0.18 ^C	0.74±0.48	ns	1.31±0.05 ^A	1.02±0.09	*
C22:6n3(DHA)	7.30±1.04 ^A	7.27±1.41 ^a	ns	3.63±0.30 ^B	3.06±0.88 ^b	ns	6.62±0.27 ^A	6.30±1.13 ^a	ns
SFA	29.33±0.43 ^A	28.14±0.29	ns	30.72±0.28 ^A	29.81±2.33	ns	27.35±0.93 ^B	27.37±0.54	ns
MUFA	38.34±4.12 ^B	42.23±4.84	ns	47.78±3.28 ^A	47.07±4.22	ns	45.26±0.35 ^{AB}	41.55±3.94	ns
PUFA	32.33±3.95 ^A	31.61±3.38 ^a	ns	21.51±2.97 ^B	22.96±3.36 ^b	ns	27.4±1.26 ^{AB}	31.09±3.75 ^a	ns
n-6PUFA	18.82±2.23	18.42±1.01	ns	15.35±2.15	16.82±2.31	ns	14.14±0.78	18.84±1.55	*
n-3PUFA	13.5±1.80 ^A	13.19±2.45 ^a	ns	6.16±0.82 ^B	6.14±2.00 ^b	ns	13.26±0.48 ^A	12.25±2.24 ^a	ns

*Data are presented as mean ± SD. Values of the same fatty acid in each row with different superscripts showed significant difference ($p < 0.05$): Lower cases were in females and upper cases were in males. The list of *P*-values shows the independent sample *t*-test result of the fatty acid in the same tissue between males and females: "ns" indicated no significant difference, and "-" indicated no detected, whereas "*" indicated $p < 0.05$. SFA is total saturated fatty acids; MUFA is total mono-unsaturated fatty acids; PUFA is total poly unsaturated fatty acids; n-6PUFA including C18:2n6, C18:3n6, C20:2n6, C20:3n6, ARA; n-3PUFA including C18:3n3, C20:3n3, EPA, C22:5n3, DHA.

Discussion

The nutrients distribution in 3-year old subadult Dabry's sturgeon with the stage II gonad was reported in the present study. The protein and lipid

contents of muscle in this study were slightly higher than those reported in 5-year old fish, which were 15.41% and 5.71%, respectively (Gong *et al.*, 2012); and the moisture content in this study

was somewhat lower. The results imply that the contents of lipid and protein stored in the muscle might be used as energy substrates or transferred to ovary for gonad development as the age of Dabry's sturgeon increased, which is similar to the result that lipid and protein levels decreased in muscle as the gonad of silver pomfret developed (Huang *et al.*, 2010).

Lower moisture content in the gonad means higher energy and nutrient reserves for gonad development (NRC, 2011), the relationship between lipid and moisture either in muscle, liver or gonad shows negative correlation and the sum of lipid and moisture is almost stable (Zaboukas *et al.*, 2006). Our results supported this, which partly suggested that female subadult Dabry's sturgeon started to deposit lipid for gonad development. The nutrients distribution displayed in this study also agree with those in Chinese sturgeon (Zhou *et al.*, 2017), northern pike *Esox Lucius* (Linnaeus, 1758) and sockeye salmon (Idler and Bitners, 1959). In addition, the higher lipid reserves in the liver and gonad suggest their important roles in the onset of puberty, and diverted energy distribution pattern from somatic growth to reproduction during gonad development (Webb and Doroshov, 2011; Hajizadeh and Shinn, 2016).

Zaboukas *et al.* (2006) also stated that the energy cost on reproduction is higher in females than in males. In this study, the higher lipid level of gonad in females compared with that in males indicates that the females need more

energy than the males for gonad development at the same stage, and more lipid from liver is transferred to gonad in metabolic processes, which was also illustrated by the lower lipid level in liver of females than that in males.

The difference in the contents of some fatty acids among muscle, liver and gonad may suggest their respective physiological functions in gonadal development, among which EPA and DHA are especially important for early development of fish (Lund *et al.*, 2007). It has been reported that the contents of EPA and DHA in broodstock diet affected the quality of their eggs (Zhuang *et al.*, 2002), even the early development and growth of larvae (Hajizadeh and Shinn, 2016). In the present study, the contents of DHA and EPA in gonad were the most abundant n-3 PUFAs, which may imply that DHA and EPA were selectively transferred to the gonad for later reproductive activity (Rakhi *et al.*, 2015). Similar results were also reported in silver pomfret broodstock (Huang *et al.*, 2010) and Chinese sturgeon (Zhou *et al.*, 2017). ARA is a precursor of eicosanoids, which are a part of the important regulatory factors for the production of steroid hormones (Van Der Kraak and Chang, 1990) to promote gonad development (Wade and Van Der Kraak, 1993). When fed with higher levels of dietary ARA, Atlantic halibut *Hippoglossus hippoglossus* (Linnaeus, 1758) exhibited higher fertilization and hatching rates (Mazorra *et al.*, 2003), and European sea bass showed higher

quality of eggs, and correspondingly promoted the survivability of larvae (Bruce *et al.*, 1999). However, the ARA contents in muscle, liver and gonad of females in this study were lower when compared with the data reported in Senegalese sole *Solea senegalensis* (1.29%, 1.08% and 1.97%, respectively) and silver pomfret (1.64%, 1.07% and 2.11%, respectively) (Huang *et al.*, 2010; Norambuena *et al.*, 2012). Amino acids are vital to early fish ontogeny, which act as fuel molecules, signaling factors and major substrates for synthesis of a range of bioactive molecules and proteins (Wright and Fyhn, 2001). Liver can supply essential components of amino acids during fish ontogeny (Finn and Fyhn, 2010). In the present study, some amino acid ratios in liver of female were higher than those in muscle and gonad which may suggest their important functions for further metabolism in gonad development. Furthermore, the higher ratios of Ser, Ala, Tyr, Cys and Thr in liver and gonad than those in muscle of males might suggest that these amino acids are important for testis development, meanwhile the higher ratios of Ser, Ala, Tyr, Cys and Thr in gonad of males than females indicate that more amino acids are required during gonad development in males than in females, as supported by the differences in the composition between sperm and egg (Huang *et al.*, 2009).

In conclusion, the present study obtained the nutritional status of muscle, liver and gonad of subadult

Dabry's sturgeon and the difference between sexes. The results suggested that lipid and HUFAs, especially EPA and DHA, may play pivotal roles in gonad development of Dabry's sturgeon which should be fortified in the diets for this sturgeon. Whether the lower ARA contents in this study suggest that the deposition of ARA was insufficient for gonad development. Metabolic differences between fish species and sexes needs further investigation. Future studies should focus on the regulation process of fatty acids and amino acids on the gonad development of Dabry's sturgeon for the success of gonad maturation in controlled rearing systems.

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