EXTERNAL SEX SPECIFIC SIGNS IN THE STRUCTURE OF DERIVATIVES OF STERLET (*ACIPENSER RUTHENUS*, LINNAEUS, 1758) CORIUM

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Only females are used in technology of caviar aquaculture, and the males shall be rejected as soon as possible. It is believed that the sturgeons have no clearly defined external morphological sex differences. Because of dark color of most sturgeon, has been previously unnoticed external morphological characteristics, the structure and form of which depends on the sex. The aim of our work was to study the morphological structure of the corium derivatives of the (dorsal scutes) of sterlet different ages and identify their structure depending on the sex. The object of this study was starlet broodstock of Volga population, at the age of 3 years, with the gonads of the second stage of maturity, as well as the three-month larva and year-old juvenile sterlet. Cultivation was carried out in recirculating aquaculture system at an average water temperature 16 ± 2 ° C. Wintering is not used. We were the first in the practice of ichthyology and Aquaculture found that dorsal scutes of sterlet maturing have significant morphological differences that depending on sex. To evaluate the morphological structure of the dorsal scutes is invited to define two groups of indicators that characterize the scutes shape, as well as the structure of the cusps of scutes. We found that dorsal scutes of males is more extended in width and have a flattened shape. Dorsal scutes of males have a long and thin cusps, the number of which is larger than that of females. We first found that established dependence to the structure of dorsal scutes of sterlet, saved as in juveniles with average length 24.8 \pm 1,5 cm and larvae with average length 70.3±3.6 mm. Our results create the methodological basis for the global aquaculture practices to develop systems of early sex identification all Acipenseridae, including the early stages of ontogeny.

Key words: sturgeon, *Acipenser ruthenus*, recirculating aquaculture system, sex determination, external morphological characteristics, dorsal scutes.

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INTRODUCTION

Caviar sturgeon breeding has recently become a popular trend in fisheries. In the technology of caviar aquaculture, only females are used, males should be discarded as early as possible (Barulin 2015). Theoretically, early identification of males and their culling can reduce technological financial costs in the process of industrial cultivation up to 4 times. It is believed that

sturgeon fish do not have pronounced external morphological gender differences, even during puberty, in contrast to salmonids in which it is possible to determine the sex according to external characteristics i.e. in the shape of the anal fin (Zhivotovsky & Kim, 2015).

Currently, many researchers are attempting to develop a method for early identification of sturgeon sex. However, universal methods have not yet been developed. Methods of biopsy and laparoscopy injure fish (Falahatkar & Poursaeid 2014). Ultrasonic (ultrasound) and endoscopic methods are used at a relatively late age (Chebanov & Galich 2010, Hurvitz et al. 2007). Biochemical (Webb et al. 2002), hormonal (Du et al. 2016) and genetic methods (Vizziano-Cantonnet et al. 2016), as well as the method of infrared spectroscopy (Lu et al. 2010) are difficult to apply in aquaculture practice and have a high cost.

We believe that due to the dark coloration of most of the sturgeon, an external morphological feature, whose structure and shape depends on sex, was not previously noticed.

We first discovered that the derivatives of the sterlet corium (dorsal scutes) in *Acipenser ruthenus* (Linnaeus, 1758) have poly specific features of the structure. This has prospects for the development of a method of intravital identification of sterlet and other sturgeon sex.

The purpose of our work was to study the morphological structure of sterlet dorsal bone (dorsal scutes) of different ages and to reveal the dependence of their structure on the sex.

MATERIAL AND METHODS

The research was carried out from 2012 to 2016 at the Department of Ichthyology and Pisciculture and "fish farm complex" of the Belarusian State Agricultural Academy (Gorki, Mogilev region, Republic of Belarus). The fish-breeding industrial complex operates a recirculating aquaculture system (RAS), using mechanical and biological filtration, ozonation and oxygenation of water. The average water temperature was 16 ° C, with short fluctuations \pm 2 ° C during the year. Sterlet wintering was not carried out. High-protein feeds of Coppens were used to feed the sterlet, taking into account the recommendations of the specified company. In the studies, different aged sterlets of the Volga population were used: adult specimens (age 3 years, average length 61.2 ± 1.3 cm); young fish (1 year old, average length 24.8 ± 1.5 cm), larva (age 3 months, average length 70.3 \pm 3.6 mm). There were no statistically significant differences between the lengths of the studied sterlet. For the study of adult sterlet, specimens with gonads were selected in the second stage of maturity according to Trusov's classification (Chebanov & Galich 2010).

Observation of sterlet was carried out from three months of age, with further supervision at the age of 1 year and with confirmation of sex at the age of 2 years. Observed individuals were labeled from the age of 3 months which allowed later, after confirming the sex in 2 years, to establish sex at the age of 3 months and 1 year. To determine the sex of sterlet, the ultrasounddiagnostic method was used on the portable veterinary scanner MindrayDP-6600, followed by a visual check of the gonads in the killed individuals. In complex cases, gonad specimens histological were sent for examination. Ultrasound diagnosis was carried out by the author of the studies, which has a confirmatory certificate of training and experience with this method for more than 5 years (more than 15 000 sturgeon sighted fish).

The killing of fish was carried out in accordance with the principles of humane treatment of animals. All the mortified specimens of adult sterlet were destined for slaughter for commercial purposes. In the euthanized specimens, a layer of dorsal scutes was cut from the head to the beginning of the dorsal fin. The first dorsal scutes, closely adhering to the scutes of the skull, was not cut due to the complexity of its rapid extraction without deformation. The study took into account the dorsal scutes from the second

(starting from the head, in our study having the first number) to the last major dorsal scutes in front of the dorsal fin or LSDR (Last Scute of Dorsal Row), which looks like "Spears". Small dorsal scutes after LSDR were not taken into account. After cutting, the dorsal scutes were cooked, cleaned, washed and photographed on a Canon EOS 500D camera in a macro mode. The resulting images were measured in the Image J soft using the "Straight Line", "Polygon selections", "Elliptical selections" tools using the Trust Canvas Widescreen Tablet. In the process of studying the dorsal scutes, the following parameters were measured: the length of the dorsal scutes; width of dorsal scutes; length of the left and right lobes; area of dorsal scutes; the area of the conditional circle in which the dorsal scutes was placed; the length of the maximal tooth of the dorsal scutes; The width of the base of the maximal tooth: Number of teeth. The diameter of the conditional circle, in which the dorsal scutes was placed, was equal to its width or length, depending on the removal of the dorsal scutes from the head (or its number). Dorsal scutes between No. 10 and LSDR were not taken into account, since their number varied greatly in all specimens studied, regardless of sex.

Based on the measurements obtained, the following coefficients were calculated: "coefficient W/L" is the ratio of the width of the dorsal scutes to its length; "average coefficient L/L" is the average ratio of the length of the left and right lobes of the dorsal scutes to its total length; "filling ratio" - the ratio of the area of the dorsal scutes to the area of the conditional circle in which it was placed; "coefficient L/W" - the ratio of the length of the maximum tooth to the width of the dorsal scutes; "coefficient L_t/ W_t" - the ratio of the length of the maximum tooth to the width of the base of the maximum tooth.

R soft (R Core Team 2016), including packages R Commander (Fox 2005), PMCMR (Pohlert 2014), MASS (Venables & Ripley 2002), etc., were used for statistical processing of results. To determine the level of statistical reliability, we used the Student's parametric test, provided the data distribution was normal (estimated by the Shapiro-Wilk test) and homogeneity Group variances (estimated Levene test). If these conditions were not observed, the nonparametric Mann-Whitney U test was used (for the two groups studied).

RESULTS AND DISCUSSION

As a result of our studies, we found that between the dorsal scutes of adult males and sterlet females there are many statistically significant differences. However, the differences in absolute parameters such as the lengths of the dorsal scutes, the left lobe of the dorsal scutes, the right lobe of the dorsal scutes, the area of the dorsal scutes, the length of the maximal tooth of the dorsal scutes, the width of the maximal tooth of the dorsal scutes, will only be relevant if the comparisons are carried out in fish of the same age and size otherwise the individual sizeweight indicators will distort these parameters and lead to errors in the diagnosis of sex. Therefore, the relative parameters (coefficients) are of interest for accurate sex diagnostics: the effect on individual dimensions and weights will be minimal. These relative parameters also include the number of teeth of the dorsal scutes.

In our studies, the coefficient W/L in male adult sterlet varied from 1.55 ± 0.08 to 1.17 ± 0.05 ; in females - from 1.41 \pm 0.04 to 1.16 \pm 0.05 (Table 1). The difference between dorsal scutes of males and females in the ratio W/L varied from the first to the fifth dorsal scutes from 15 to 13 percentage points (differences for the first, third and fifth dorsal scutes tes are statistically significant at a confidence level of p <0.05), with further decrease. The average coefficient of L/L in males varied from 0.70 ± 0.02 to 0.61 ± 0.02 ; in females - from 0.76 ± 0.02 to $0,67 \pm 0,01$. The difference between the dorsal scutes of males and females, according to the averaged coefficient L/L, varied from the first to the tenth scutes from 10 to 4.5 percentage points (differences for all dorsal scutes, except for the fourth and fifth, are statistically significant at a confidence level of p <0.05). The average filling

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Table 1. Comparison of the coefficients (C) of the morphological parameters (mean \pm standard error of the mean) of the first six dorsal scutes of males (n = 20) and females (n = 20) of the sterlet of the Volga population (*Acipenser ruthenus* Linneaus, 1758). Age - 3 years. The second stage of gonad maturity. The average length is 61.2 ± 1.3 cm. Growing conditions - recirculating aquaculture system. The average growing temperature is 16 ± 2 °C. Wintering was not carried out

	Number of dorsal scutes / sex					
Parameter	1		2		3	
	male	female	male	female	male	female
C W/L	1.48±0.04*	1.33±0.04	1.55 ± 0.08	1.41±0.04	1.54±0.04*	1.41 ± 0.04
C L/L	$0.67 {\pm} 0.02$	0.76±0.02*	$0.66 {\pm} 0.02$	0.74±0.02*	$0.67{\pm}0.02$	0.77±0.02*
filling ratio	0.51±0.06	0.61±0.01*	0.50±0.08	0.58±0.02*	0.49±0.06	0.58±0.02*
C L _t /W	0.08±0.01*	0.05±0.01	0.09±0.01*	0.06±0.01	0.11±0.01*	0.07±0.01
CL/W	$0.89{\pm}0.06*$	0.51±0.04	0.95±0.05*	0.46±0.05	1.03±0.06*	0.56±0.04
Number of teeth	11.24±1.03*	7.64±0.96	11.55±0.87*	6.50±0.97	10.64±0.62*	6.50±0.76
	4		5		6	
C W/L	$1.50{\pm}0.06$	$1.37{\pm}0.06$	$1.41 \pm 0.04*$	1.26 ± 0.04	1.40±0.04	1.30±0.04
CL/L	$0.70{\pm}0.02$	$0.74{\pm}0.02$	$0.68 {\pm} 0.02$	$0.74{\pm}0.02$	0.66±0.02	0.75±0.03*
filling ratio	0.51±0.06	0.60±0.02*	0.53±0.07	0.61±0.01*	0.53±0.05	0.61±0.02*
C L/W	0.13±0.01*	0.08±0.01	0.12±0.01*	$0.08{\pm}0.01$	0.13±0.01*	0.09±0.01
CL/W	$0.98 {\pm} 0.06 *$	0.54±0.06	$1.00{\pm}0.06*$	0.51±0.04	$1.01{\pm}0.06*$	$0.52{\pm}0.05$
Number of teeth	9.50±0.70*	5.92±0.60	8.86±0.55*	5.50±0.64	8.91±0.62*	5.43±0.58

*. – the differences are statistically significant at a confidence level p < 0.05

ratio in males varied from 0.57 ± 0.07 to 0.49 \pm 0.06; in females, from 0.61 \pm 0.02 to 0.57 \pm 0.02. The difference between dorsal scutes of males and females according to the filling ratio varied from the first to the sixth dorsal scutes from 10 to 8 percentage points (differences for all the first six dorsal scutes are statistically significant at a confidence level of p < 0.05), with a further decrease to 2-4 percentage points. The average coefficient of L/W in males varied from 0.08 ± 0.01 to 0.14 ± 0.01 ; in females from 0.05 ± 0.01 to 0.11 ± 0.01 . The difference between the dorsal scutes of males and females varied from 2 to 5 percentage points in the ratio L/W (differences for all dorsal scutes except the seventh and ninth are statistically significant at a confidence level of p < 0.05). The maximum difference was for the first six dorsal scutes. The average coefficient of L_t/ W_t in males varied from 0.89 ± 0.06 to 1.06 ± 0.09 ; in females from 0.46 ± 0.05 to 0.65 ± 0.08 . The difference between the dorsal scutes of males and females varied from 28 to 51 percentage points in the coefficient L_t/W_t . (differences for all dorsal scutes are statistically significant at a confidence level of p <0.05). The highest differences were observed for the first eight dorsal scutes. The average number of teeth of dorsal scutes in males varied from 11.55 ± 0.87 to 7.55 ± 0.5 ; females - from $7,64 \pm 0,96$ to 5.00 ± 0.54 . The difference between dorsal scutes of males and females in the number of teeth varied from 77.7 to 31.3 percentage points (differences for all dorsal scutes are statistically significant at a confidence level of p <0.05). Note that the maximum differences were observed for the first five dorsal scutes.

The presented data in the present study indicate that there are statistically significant differences between the morphological structure of the dorsal scute of adult males and sterlet females with gonads that have reached sexual differentiation (Fig. 1). From the studied relative parameters, two groups of indicators characterizing the visual qualities of the dorsal scutes plates can be distinguished: 1. A group of morphological parameters characterizing the shape of the dorsal scutes: the coefficient W/L, the average coefficient L/L, the filling factor.

2. A group of morphological parameters characterizing the teeth of the dorsal scutes: the coefficient L_t/W , the coefficient L_t/W_t , the number of teeth.

As our studies showed, the largest number of statistically significant differences were observed in the first five dorsal scutes.

As a result of the conducted studies, the question remained about the possible preservation of the observed sexual patterns in the structure of the dorsal scutes plates in the young sterlet. As Wurtz et al. (2011) have shown, in Atlantic and American Atlantic sturgeon larvae, there are specific differences in the structure of scutes, which, as Tiheren et al. (2015) have shown, persist in sexually mature individuals. This allowed us to hope for a positive result in developing a method for identifying sterlet sex in earlier stages of ontogeny. The conducted studies established that the revealed poly specific regularities in the structure of dorsal scutes in adult sterlet, in most of the previously identified morphological parameters, were preserved in the structure of the dorsal scutes of the young sterlet (1 year old, average length 24.8 ± 1.5 cm, Fig. 2).

So the coefficient W/L in male young sterlet varied in the first five dorsal scutes from 1.48 \pm 0.10 to 1.18 \pm 0.07; in females - from 1.30 \pm 0.08 to 1.17 ± 0.05 . The difference between the dorsal scutes of the young males and females from the first to the fifth dorsal scutes varied from 17.8 to 1.4 percentage points (differences for the second and fourth dorsal scutes are statistically significant at a confidence level of p <0.05). The average coefficient of L/L in male varied in the first five dorsal scutes from $0.69 \pm$ 0.03 to 0.58 \pm 0.02; in female s - from 0.80 \pm 0.05 to 0.66 ± 0.03 . The difference between the dorsal scutes of males and females, according to the averaged coefficient L/L, varied from the first to the fifth scutes from 18.0 to 2.6 percentage points (differences for the first four dorsal scutes s are statistically significant at a confidence level of p <0.05). The average filling factor in the young males in the first five dorsal scutes ranged from 0.53 ± 0.09 to 0.42 ± 0.04 ;



Fig. 1. Typical contours of dorsal scutes of males (upper) and females (lower) of adult sterlet. Age - 3 years. The second stage of gonad maturity. The average length is 61.2 ± 1.3 cm.



Fig. 2. Typical dorsal scutes of males (upper) and females (lower) of young sterlet. Age - 1 year. The average length is 24.8 ± 1.5 cm.

in females - from 0.57 ± 0.03 to 0.55 ± 0.02 . The difference between dorsal scutes of males and females in the filling factor from the first to the fifth dorsal scutes varied from 13.5 to 4.3 percentage points (differences for the first four dorsal scutes are statistically significant at a confidence level of p < 0.05). The average coefficient of L/W in male in the first five dorsal scutes varied from 0.14 ± 0.01 to 0.18 ± 0.01 ; in females - from 0.05 ± 0.01 to 0.08 ± 0.01 . The difference between dorsal scutes of males and females varied from 7.4 to 9.4 percentage points from the first to the fifth dorsal scutes with respect to the coefficient of W/ L from the first to the fifth scutes (differences for the first five dorsal scutes are statistically significant at a confidence level of p < 0.05). The average coefficient of L_t/W_t in young males from the first to the fifth dorsal scutes varied from 0.95 \pm 0.04 to 1.75 \pm 0.10; in females - from 0.53 \pm 0.04 to 1.39 ± 0.09 . The difference between the dorsal scutes of males and females in the first five scutes varied from 13.7 to 101.7 percentage points in the coefficient of L/W, (differences for all the first five dorsal scutes are statistically significant at a confidence level of p < 0.05). The average number of teeth of dorsal scutes in male juveniles varied in the first five scutes from 9.4 \pm 0.7 up to 7.2 \pm 0.5 and from 6.2 \pm 1.0 up to 4, 0 ± 0.6 in females . The difference between the dorsal scutes of males and females in the number of teeth varied from 125 to 60 % (differences for all pasterns of dorsal scutes were statistically significant at a confidence level of p <0.05). Thus, the conducted studies have established that there are statistically significant differences between the morphological structure of the dorsal scutes of males and females of young sterlet of 24.8 ± 1.5 cm size.

As a result of the conducted studies, the question remained about the possible preservation of the observed sexual patterns in the structure of the dorsal scutes in sterlet larvae.

The conducted studies established that the revealed poly specific regularities in the structure of scutes in adult sterlet, in most of the previously identified morphological parameters, were preserved in the structure of the spinal plates of sterlet larvae (age 3 months, average length 70.3 ± 3.6 mm, Fig. 3).

Thus, the coefficient W/L in male sterlet larvae varied in the first five dorsal bony plates from 1.75 ± 0.12 to 1.47 ± 0.09 ; in females - from 1.40 ± 0.07 to 1.08 ± 0.04 . The difference between scutes of male and female larvae from the first to fifth dorsal scutes varied from 42.8 to 15.7 percentage points (differences for the first five dorsal scutes are statistically significant at a confidence level of p < 0.05). The average coefficient L/L in male larvae varied in the first five dorsal scutes from 0.68 ± 0.04 to 0.57 \pm 0.02; in female larvae - from 0.74 \pm 0.05 to 0.68 ± 0.03 . The difference between the dorsal scutes of males and females, according to the averaged coefficient L/L, varied from the first to the fifth scutes from 10.1 to 1.2 percentage points (differences for the first four p dorsal scutes are statistically significant at a confidence level of p <0.05). The average filling factor in male larvae in the first five dorsal scutes ranged from 0.50 ± 0.07 to 0.40 ± 0.02 ; in females from 0.62 ± 0.06 to 0.52 ± 0.03 . The difference between dorsal scutes of males and females in the filling factor from the first to the fifth dorsal scutes varied from 13.9 to 9.5 percentage points (differences for the first five dorsal scutes are



Fig. 3. Typical dorsal scutes of males (upper) and females (lower) sterlet larvae. Age - 3 months. The average length is 70.3 ± 3.6 mm.

statistically significant at a confidence level of p <0.05). The average coefficient of L/W in male larvae in the first five dorsal scutes ranged from 0.04 ± 0.01 to 0.11 ± 0.01 ; in females - from 0.05 \pm 0.01 to 0.10 \pm 0.01 (there were no statistically significant differences, p> 0.05). The average coefficient of L/W, in male larvae from the first to the fifth dorsal scutes varied from 0.91 ± 0.02 to 1.72 ± 0.10 ; in females - from 0.42 ± 0.01 to 0.92 ± 0.05 . The difference between the dorsal scutes males and females in the first five scutes varied from 1.0 to 79.8 percentage points in the coefficient of L_t/W_t. (differences for the first, second, third, fifth dorsal scutes are statistically significant at a confidence level of p<0.05). The average number of teeth of dorsal scutes in male larvae varied in the first five dorsal scutes from 8 pieces up to 4 pcs.; in females - from 5 pcs. up to 3 pcs. The difference between dorsal scutes of males and females in the number of teeth varied from 133 to 0% (differences for the second, fourth and fifth dorsal scutes are statistically significant at a confidence level of p < 0.05).

Thus, the conducted studies have established that there are statistically significant differences between the morphological structure of the dorsal scutes of males and females of sterlet larvae of size 70.3 ± 3.6 mm.

Our results show that in the males of all ages (adult individuals, juveniles, and larvae) the general poly specific regularities in the morphological structure of the dorsal scutes (mainly on the first five plates) remain. In comparison with females, the dorsal scutes of males are more elongated in width, in relation to length (results of length and coefficient W/L); Due to the smaller size of the blades, the dorsal scutes in males appear more flattened and narrower than in females (the results of the length of the left and right lobe, the results of the coefficient L/L). Based on the results of the filling factor, we observed that the dorsal scutes in females look more rounded or oval than in males. The bright distinctive feature of all male dorsal scutes examined is the presence of longer, thin and pointed teeth that stand out with respect to the width of the plate and their number is larger than in females (the results of the length and width of the teeth, the coefficients L_t/W , L_t/W_t).

Many researchers have repeatedly attempted to find external morphological signs in sturgeon fish, depending on sex and to develop a simple method for identifying the sex of sturgeon. Thus, Falakhatkar and Poursaeid (2014) did not find reliable sex differences in size and weight in the beluga Huso huso. Chiotti et al. (2016) point to the available reliable sex differences in determining the ratio of length to maximum girth in the A. fulvescens lake sturgeon. However, such a significant difference was observed in fish that are in the last stages of maturity. Maltsev and Markulov (2016) pointed out the possibility of early detection of sex in the Russian sturgeon A. gueldenstaedtii using biometric methods. However, as noted by Chebanov and Galich (2010), these morphometric methods have not been fully developed and despite the ease of application they can not be recommended for widespread use in aquaculture. 11.

Vecsei et al. (2003) established sex differences in the shape of the urogenital opening in white *A. transmontanus*, the American Atlantic *A. oxyrinchus* and the short-haired *A. Brevirostrum* sturgeon. However, Chebanov and Galich (2010) were skeptical about this method of sex determination, pointing to the lack of reliable sex differences in other sturgeon species.

In our opinion, despite the prevailing skepticism towards attempts to find external morphological sex differences in sturgeon fishes, such studies should continue. We drew attention to the existing features in the structure of the dorsal scutes, which depend on the sex. Such features may not attract the attention of other authors, because of the dark coloration of the back of sterlet.

CONCLUSIONS

We have established for the first time in the practice of ichthyology and aquaculture that the dorsal scutes of adult sterlet, as well as juveniles and larvae, have significant morphological differences that depend on sex. To assess the morphological structure of the dorsal scutes it is proposed to determine two groups of indices characterizing the shape of the plate, as well as the structure of their teeth. We determined that in male sterlet, the dorsal scutes are more elongated in width, have a more flattened shape, and also have longer and more pointed teeth, the number of which is larger than in females.

We believe that the methodology for determining sex by external morphological features requires further comprehensive research. The observed patterns in the structure of the dorsal scutes. depending on sex, create the methodological basis for the world practice of aquaculture for the development of early and early-early sex determination systems for other members of the Acipenseridae sturgeon family, without the use of expensive equipment (for example, ultrasound scanner or endoscope). This is of practical importance for aquaculture. An interesting question is the possible sexual patterns in the structure of not only the dorsal but also lateral and abdominal scutes. The prospects outlined above, the subject of our further publications.

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