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## Morphometric characteristics of return hybrids of kaluga *Huso dauricus* and sterlet *Acipenser ruthenius* (Acipenseridae)

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**Abstract.** The sturgeon fish species cultivation is a necessary activity for the preservation of natural populations in critical condition as well as in a market economy, makes it possible to obtain food products with high added value. Hybridization of sturgeon allows you to create promising cultivation facilities, and introduce them into farms in order to increase the yield of marketable products. Due to the heterosis effect the hybrid inherits useful economic characters of the intial species and also repeats them in the external structure. The degree of heritability of traits of parental species can be estimated by the relative values of body parts and counting characteristics. In addition to the degree of similarity and difference of the hybrid between the parent species it is also possible to assess the degree of heritability of economic traits, thereby conducting selection. A comparative analysis of six-year-old hybrids  $(K \times St) \times K$ ,  $(K \times St) \times St$  and  $(St \times K) \times K$  was carried out. 21 morphometric indicators were used in the work. The patroclinal nature of inheritance of some traits has been revealed.  $(K \times St) \times K$  significantly differed according to the Student's criterion compared to other hybrids in terms of relative head indicators. The diagnostic characters of the studied hybrids turned out to be relative indicators of the head length and the number of lateral scutes.

**Keywords:** morphometry, recurrent hybrid, kaluga, sterlet, confidence interval, Student's criterion, transgression, Luchegorsk

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Научная статья

## Морфометрическая характеристика возвратных гибридов калуги *Huso dauricus* и стерляди *Acipenser ruthenius* (Acipenseridae)

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**Аннотация.** Выращивание осетровых видов рыб является необходимой деятельностью для сохранения естественных популяций, находящихся в критическом состоянии, а также в условиях рыночной экономики, и дает возможность получить пищевые продукты с высокой добавленной стоимостью. Гибридизация осетровых позволяет создавать перспективные объекты выращивания и внедрять в хозяйства с целью повышения выхода товарной продукции. Благодаря эффекту гетерозиса гибрид наследует полезные хозяйствственные признаки исходных видов, а также повторяет их во внешнем строении. Степень наследуемости признаков родительских видов можно оценить по относительным величинам частей тела и счетным признакам. Помимо степени сходства и различия гибрида между родительскими видами, также можно оценить степень наследуемости хозяйственных признаков, тем самым проводить селекцию. Проведен сравнительный анализ шестилеток гибридов  $(K \times St) \times K$ ,  $(K \times St) \times St$  и  $(St \times K) \times K$ . В работе использован 21 морфометрический показатель. Выявлен патроклиний характер наследования некоторых признаков. Гибрид  $(K \times St) \times K$  достоверно отличался по критерию Стьюдента, по сравнению с другими гибридами, по относительным показателям головы. Диагностическими признаками исследуемых гибридов оказались относительные показатели от длины головы и количества боковых щучек.

**Ключевые слова:** морфометрия, возвратный гибрид, калуга, стерлядь, доверительный интервал, критерий Стьюдента, трансгрессия, Лучегорск

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## Introduction

Currently, there is no production of sturgeon fish on an industrial scale, only a quota is allocated for the catch of producers to ensure the operation of sturgeon fish breeding plants and scientific purposes. An alternative to catching sturgeon is commercial sturgeon farming. One of the ways to increase the efficiency of growing sturgeon species of fish is the introduction of hybrids into fish farming practice [1].

Experimental cultivation of sturgeon hybrids has been carried out for a long time in Primorsky Krai at the Luchegorskaya scientific research station (NIS) Pacific Branch of “VNIRO” (“All-Russian Scientific Research Institute of Fisheries and Oceanography”, TINRO) [2–4]. One of the objects is a caster (a hybrid of kaluga × sterlet and sterlet × kaluga) – the Far Eastern analogue of the western beluga (beluga × sterlet). After confirming the fertility of custer [5], returnable hybrids were obtained, and the first results of cultivation [6] will contribute to the creation of industrial hybrids by analogy with beluga breeds: “Vnirovskaya” (beluga × (beluga × sterlet) and “Aksayskaya” (sterlet × (beluga × sterlet).

The advantages of hybrids are manifested in accelerated growth, increased resilience, sometimes in early puberty, and adaptability in cultivation. In practical terms, another feature of hybrids is also important – increased adaptive plasticity [7].

On the one hand, hybridization refers to resource-saving technologies, on the other hand, it solves theoretical problems of compatibility of genomes of cultivated species and hybrid forms of sturgeon. The nature of genetic material inheritance is manifested in the morphology of the body structure. Using mathematical statistics it is possible to assess the effect of the influence of parental species.

The aim of the study is to give a morphological characterization of the return custer hybrids.

## Materials and methods

Morphometry data were collected during the annual bonitation in October 2020. The object of the study were six-year-olds (5+) of the return hybrids (K × St) × K, (K × St) × St and (St × K) × K generation 2015 (in the left part, the mother's material is used, and in the right, the father's. For example: (K × St) × K; (K × St) – ♀, K – ♂. All the individuals were grown under the same conditions in the cages of the research station (NIS) of the Pacific Branch of TINRO in the village Luchegorsk (Primorsky Krai).

Morphological studies were carried out according

to existing schemes and techniques on live fish on the left side of the body [8, 9]. The number of abdominal and lateral scutes was calculated from both sides of the body. There were studied 17 plastic and 4 meristic characters. In total, 20 copies of each hybrid were measured.

The confidence interval was calculated using the formula:

$$D = x \pm Sx \cdot t_p,$$

where  $D$  – average of the general population;  $x$  – sample average;  $Sx$  – standard error;  $t_p$  – Student's criterion for probability  $P = 99.9\%$ .

The similarity of features was evaluated using transgression according to the formula:

$$T = \frac{n_1[0.5 \pm \varphi(x_1)] + n_2[0.5 \pm \varphi(x_2)]}{n_1 + n_2},$$

$$\text{where } x_1 = \frac{(\bar{x}_2 - 3S_2) - \bar{x}_1}{S_1}; x_2 = \frac{(\bar{x}_1 - 3S_1) - \bar{x}_2}{S_2}, \bar{x}_1, \bar{x}_2 -$$

average values of the attribute,  $S_1, S_2$  – standard deviations of these features, 3 and 0.5 – numeric values provided by the formula;  $n_1$  and  $n_2$  – total number of observations in the first and second distribution (samples);  $\varphi(x)$  – the second function of the normalized deviation.

Using the Student's criterion, the existence of differences between the two samples was determined:

$$T_{st} = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{S_{\bar{x}_1}^2 - S_{\bar{x}_2}^2}},$$

where  $S_{\bar{x}_1}^2, S_{\bar{x}_2}^2$  – squares of the standard error.

Using the difference coefficient CD which is the ratio of the difference of the averages to the sum of the standard deviations of the samples and is determined by the formula:  $CD = \frac{\bar{x}_1 - \bar{x}_2}{S_1 + S_2}$ .

Statistical processing was performed using the MS Office Excel program.

## Results

The studied parameters (Table 1) for all objects according to the coefficient of variation ( $Cv$ ) varied from 1.86 (K × St) × St – SL (body length from the end of the snout to the end of the middle rays) to 23.39 (K × St) × K – W (body weight) %.

Table 1

Morphometric indicators of recurrent hybrids and their descriptive statistics\*

Character	(K × St) × K (n = 20 ex.)			(K × St) × St (n = 20 ex.)			(St × K) × K (n = 20 ex.)					
	X ± m	Confidence interval at P = 99.9%	δ	Cv, %	X ± m	Confidence interval at P = 99.9%	δ	Cv, %	X ± m	Confidence interval at P = 99.9%	δ	Cv, %
W, g	7 762.5 ± 406.01	6 185.98-9 339.02	1 815.71	23.39	7 950 ± 408.51	6 363.75-9 536.25	1 826.92	22.98	6 797.5 ± 3 26.96	5 527.93-8 067.07	1 462.2	21.51
TL, sm	117.96 ± 2.13	109.69-126.23	9.53	8.08	116.83 ± 2.16	108.43-125.22	9.67	8.27	109.61 ± 1.58	103.47-115.74	7.06	6.44
In % of TL												
SL	88.31 ± 0.53	86.25-90.36	2.37	2.68	87.63 ± 0.36	86.72-89.05	1.63	1.86	89.11 ± 0.77	84.98-96.67	3.45	3.87
FL	84.34 ± 0.55	82.22-86.47	2.44	2.90	83.92 ± 0.38	82.44-85.40	1.71	2.04	84.55 ± 0.75	78.51-92.86	3.35	3.96
aD	61.77 ± 0.38	60.27-63.26	1.72	2.79	60.50 ± 0.38	59.04-61.96	1.68	2.78	61.39 ± 0.49	59.07-66.89	2.18	3.55
aV	54.01 ± 0.36	52.62-55.39	1.59	2.95	52.42 ± 0.29	51.30-53.54	1.29	2.46	53.42 ± 0.48	50.46-57.81	2.15	4.03
aA	68.63 ± 0.67	66.01-71.25	3.02	4.40	67.46 ± 0.75	64.54-70.38	3.36	4.98	69.73 ± 1.00	65.40-85.49	4.48	6.43
aP	21.72 ± 0.31	20.53-22.91	1.37	6.31	21.16 ± 0.31	19.96-22.35	1.37	6.48	21.70 ± 0.24	19.88-24.11	1.07	4.92
C	20.05 ± 0.30	18.90-21.21	1.33	6.62	19.61 ± 0.33	18.32-20.90	1.48	7.56	19.85 ± 0.23	18.23-22.32	1.03	5.20
p/	10.42 ± 0.10	10.02-10.82	0.46	4.40	10.46 ± 0.16	9.86-11.06	0.70	6.65	10.58 ± 0.17	9.71-13.33	0.76	7.19
In % of C												
R	34.03 ± 0.84	30.79-37.28	3.74	10.98	39.70 ± 0.60	37.37-42.04	2.69	6.78	40.48 ± 0.59	34.95-43.75	2.65	6.55
OP	46.14 ± 1.00	42.24-50.04	4.49	9.74	51.90 ± 0.52	49.86-53.94	2.35	4.52	52.49 ± 0.67	46.12-57.69	2.98	5.68
O	4.34 ± 0.11	3.92-4.77	0.49	11.20	4.19 ± 0.13	3.69-4.70	0.58	13.77	4.40 ± 0.07	3.60-4.81	0.31	7.13
BC	37.80 ± 0.95	34.11-41.49	4.25	11.25	42.18 ± 0.98	38.36-46.00	4.40	10.44	41.31 ± 0.91	35.60-48.08	4.08	9.87
rc	19.09 ± 0.80	15.97-22.22	3.60	18.85	23.10 ± 0.60	20.77-25.43	2.68	11.62	23.10 ± 0.60	18.52-26.96	2.69	11.63
rr	32.95 ± 0.91	29.43-36.47	4.05	12.30	38.71 ± 0.49	36.82-40.59	2.17	5.60	38.80 ± 0.65	32.52-43.52	2.89	7.44
rl	13.85 ± 0.50	11.92-15.79	2.23	16.09	15.61 ± 0.30	14.46-16.75	1.32	8.46	15.70 ± 0.24	13.59-18.06	1.09	6.95
lc	15.48 ± 0.50	13.53-17.44	2.25	14.55	17.13 ± 0.41	15.55-18.71	1.82	10.63	17.80 ± 0.53	11.63-23.36	2.36	13.26
SO	31.08 ± 0.52	29.05-33.11	2.34	7.53	30.30 ± 0.56	28.11-32.49	2.52	8.32	31.01 ± 0.41	27.91-35.65	1.83	5.89
Meristic characters												
S1	39.95 ± 0.61	37.57-42.33	2.74	6.87	43.9 ± 0.96	40.19-47.61	4.28	9.75	39.5 ± 0.57	37.29-41.71	2.54	6.44
S1/2	40.30 ± 0.67	37.72-42.88	2.98	7.38	44.1 ± 0.75	39.69-48.51	3.35	7.61	38.6 ± 0.64	36.11-41.09	2.87	7.44
Sy1	9.55 ± 0.26	8.56-10.54	1.15	12.00	10.1 ± 0.18	8.71-11.49	0.79	7.80	10.0 ± 0.21	9.20-10.80	0.92	9.18
Sy2	9.35 ± 0.17	8.7-10.00	0.75	7.97	10.2 ± 0.17	8.50-11.90	0.77	7.53	10.1 ± 0.25	9.13-11.07	1.12	11.08

\* X ± m – average value and the error of the average; n – number of instances; δ – standard deviation; Cv, % – coefficient of variation. Characters designations: TL – total body length; W – body weight; SL – body length from the end of the middle rays; FL – body length from the end of the snout to the roots of the middle rays; aD – anterodorsal distance; aV – anteventral distance; aA – antennal distance; aP – antepectoral distance; C – the length of the caudal stem; R – the length of the head ; p/ – the length of the head from the end of the snout to the line passing through the middle of the base of the middle antennae; rr – the distance from the end of the snout to the cartilaginous arch of the mouth; rl – the distance from the base of the middle pair of antennae to the cartilaginous arch of the mouth; SO – the width of the largest tendril; Sy1, Sy2 – the number of setules of the lateral row, respectively, on the left and right.

The group of parameters expressed as a percentage of  $TL$  varied less unlike the group of features from  $C$  and meristic characters. Among the group of characters from  $TL$ , the head size varied the most in the hybrid  $(K \times St) \times St - 7.56\%$ , and the least in  $(St \times K) \times K - 5.2\%$ .

In the set of characters from  $C$ , changed the most  $rc - 18.85\%$   $(K \times St) \times K$ , and the occlusal space of  $OP$  changed the least  $-4.52\% (K \times St) \times St$ .

Bone scutes are an important diagnostic feature in sturgeon. In this case, the lateral and abdominal rows of scutes are considered. On the side rows, there is a noticeable difference on the average of 4 bone scutes (rounded) between  $(K \times St) \times St$  and  $(K \times St) \times K$ , with

differences in  $(K \times St) \times K$  and  $(St \times K) \times K$  are not noticed.

The confidence interval at the third level of significance ( $P = 99.9\%$ ) allowed us to distinguish only hybrids  $(K \times St) \times K$  and  $(K \times St) \times St$  according to the two characters  $R$  and  $rr$ , most of the considered characters of the two hybrids were included in the confidence interval  $(St \times K) \times K$ .

In Table 2, according to the Student's criterion at  $p < 0.01$ , there were significantly different  $(K \times St) \times St$  and  $(K \times St) \times St$  in characters of  $R$ ,  $OP$ ,  $rc$ ,  $rr$ ,  $Sl2$ , and at  $p < 0.1$ ,  $aV$ ,  $rl$ ,  $Sl1$ ,  $Sv2$  additionally differed.

Table 2

**The value of the Student's criterion when comparing the averages of the three hybrids with each other  
( $p < 0.1 = 2.711$ ,  $p < 0.01 = 3.566$  with 38 degrees of freedom)**

Character	$(K \times St) \times K - (K \times St) \times St$	$(K \times St) \times K - (St \times K) \times K$	$(K \times St) \times St - (St \times K) \times K$
$W, g$	-0.33	1.85	2.20
$TL, sm$	0.37	3.15**	2.70
In % of $TL$			
$SL$	1.05	-0.86	-1.74
$FL$	0.63	-0.22	-0.75
$aD$	2.36	0.60	-1.45
$aV$	3.46	0.98	-1.78
$aA$	1.15	-0.91	-1.81
$aP$	1.30	0.04	-1.41
$C$	0.99	0.54	-0.60
$pl$	-0.20	-0.77	-0.50
In % of $C$			
$R$	<b>-5.51*</b>	<b>-6.29</b>	-0.92
$OP$	<b>-5.08</b>	<b>-5.27</b>	-0.70
$O$	0.88	-0.47	-1.43
$BC$	<b>-3.20</b>	-2.66	0.65
$rc$	<b>-3.99</b>	<b>-3.99</b>	0.01
$rr$	<b>-5.60</b>	<b>-5.26</b>	-0.11
$rl$	<b>-3.03</b>	<b>-3.33</b>	-0.25
$lc$	-2.54	<b>-3.17</b>	-1.01
$SO$	1.01	0.10	-1.02
Meristic characters			
$Sl1$	<b>-3.48</b>	0.54	<b>3.95</b>
$Sl2$	<b>-3.79</b>	1.84	<b>5.57</b>
$Sv1$	-1.77	-1.37	0.37
$Sv2$	<b>-3.55</b>	-2.49	0.33

\* At  $p < 0.01$ ; \*\* at  $p < 0.1$ .

Hybrids  $(K \times St) \times St$  and  $(St \times K) \times K$  significantly differed at  $p < 0.1$  in terms of  $TL$ ,  $R$ ,  $OP$ ,  $rc$ ,  $rr$ ,  $rl$ ,  $lc$ , and at  $p < 0.01$  only  $R$ ,  $OP$ ,  $rc$ ,  $rr$ . Hybrids  $(K \times St) \times St$  and  $(St \times K) \times K$  differed only in lateral scutes.

When comparing a pair of  $(K \times St) \times K$  and  $(K \times St) \times St$  transgressed (Table 3) indicators  $W$ ,  $R$ ,  $OP$ ,  $rr$ ,  $rl$ , respectively 88.06, 80.97, 85.53, 87.01, 86.95%.

Table 3

## Transgression indicator for pairwise comparison of morphological features of three hybrids

Character	$(K \times St) \times K - (K \times St) \times St$	$(K \times St) \times K - (St \times K) \times K$	$(K \times St) \times St - (St \times K) \times K$
$W, g$	88.06	99.81	99.94
$TL, sm$	99.95	99.91	99.92
In % of $TL$			
$SL$	98.28	99.53	91.95
$FL$	99.17	99.42	95.50
$aD$	99.72	100.00	98.54
$aV$	99.69	99.99	95.41
$aA$	98.08	99.94	98.00
$aP$	99.53	99.98	98.67
$C$	99.67	99.93	98.66
$pl$	97.32	98.63	99.72
In % of $C$			
$R$	80.97	85.97	99.66
$OP$	85.53	80.68	99.22
$O$	98.24	99.87	94.90
$BC$	98.41	97.63	99.92
$rc$	93.25	94.34	99.87
$rr$	87.01	78.49	99.34
$rl$	86.95	91.95	99.59
$lc$	97.63	97.60	98.93
$SO$	99.56	99.94	98.53
Meristic characters			
$Sl1$	99.92	92.04	99.88
$Sl2$	100.00	95.98	100.00
$Sv1$	98.86	97.15	99.82
$Sv2$	95.40	96.92	99.21

In hybrids  $(K \times St) \times St$  and  $(St \times K) \times K$  transgressed  $R = 85.97$ ,  $OP = 80.68$ , and  $rr = 78.49\%$ . The remaining indicators did not exceed the value above 91.95%

The index of the CD difference coefficient did not exceed 1.28 for any of the studied parameters.

## Discussion

The return hybrids based on  $K \times St$  significantly differed in terms of indicators ( $R$ ,  $rr$ ,  $OP$ ,  $rc$ ,  $rl$ ,  $Sl$ ) and Table 1 shows how the average relative indicators from the head change when using a male sterlet (they increase with a smaller head size) in comparison with the use of paternal kaluga material.

When comparing the hybrid  $(St \times K) \times K$  with each of the forms of  $K \times St$ , differences appear separately either on the characters of the head or on the side scutes. Apparently, at the sterlet  $\times$  kaluga stage, certain morphological characteristics are fixed, and with the addition of "blood" from the male, only some of them are enhanced. In the work on the morphological features of casters at the age of two years (1+) [10], the differences of reciprocal hybrids are considered, while

the influence of sterlet on the formation of head characters is stronger than that of kaluga. Our work indicates that, in general, the patroclinic nature of inheritance of the studied indicators is preserved at the stage of recurrent crossing. It is necessary to conduct an analysis of recurrent and reciprocal hybrids with an assessment of similarity using a hybrid index.

## Conclusions

1) A couple  $(K \times St) \times K$  and  $(K \times St) \times St$  can be reliably distinguished (student's criterion, transgression, confidence variant) by indicators such as the length of the snout –  $R$  and the distance from the end of the snout to the cartilaginous arch of the mouth –  $rr$ .

2) Hybrids  $(K \times St) \times K$  and  $(St \times K) \times K$  can be reliably distinguished (student's criterion, transgression) by the length of the snout –  $R$ , the orbital space  $OP$  and the distance from the end of the snout to the cartilaginous arch of the mouth –  $rr$ .

3) Pair  $(K \times St) \times St$  and  $(St \times K) \times K$  are reliably distinguishable by the Student's criterion only by side scutes.

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