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Original Scientific Article

COMPARISON OF MORPHOLOGICAL CHARACTERS BETWEEN WILD AND CULTURED STERLET (*ACIPENSER RUTHENUS* L.)

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Summary: Sterlet (*Acipenser ruthenus* L.) populations in the Danube River had experienced severe decline during the 20th century, and have become dependant on stocking measures in significant area of their distribution. Despite the current wide use of stocking, there are few studies dealing with efficiency of stocking efforts, especially with impact of rearing conditions on fitness of released individuals. This study tried to assess existence of morphological changes in reared sterlet that could impact their swimming performance and thus reduce survival of stocked fish. Wild sterlet (*n=45*) from the Danube River were compared with sterlet from aquaculture (*n=20*), originating from wild Danube spawners. Statistical comparison of 15 morphological traits revealed that samples significantly differed in 11 traits, as well as that reared sterlet had significantly shorter pectoral fins and stockier body than those from the wild. Additional investigation is needed to determine if these morphological differences could affect adaptability and survival of reared sterlet after their release into the river. Period of adaptation in lotic environment, prior to stocking, could probably alleviate the influence of aquaculture rearing on the fitness of released fish.

Key words: sturgeon; *Acipenseridae*; Danube River; Fulton factor; pectoral fin; biometry

Introduction

Populations of sterlet (*Acipenser ruthenus* L.) in the Danube River have experienced serious decline during the 20th century (1), and sterlet has been almost extirpated from the German and Austrian section of the Danube River, where its presence depends on continuous stocking efforts (2). It has a limited distribution in the basin of the Middle and the Lower Danube (3), and there are ongoing stocking activities with the majority of fish released by Hungary, and to a small extent by Slovakia and Bulgaria (4-6). Estimated quantity of stocked juveniles in Hungary declined from 10-100 thousands of annually released specimens during the 1980s to only sporadic stocking activities in 1990s and 2000s (3). According to Guti and Gaebele (3), there were 60.000 specimens released in 2002 in the Hungarian section of the Danube River.

While the stocking has been employed as a sturgeon management and rehabilitation tool for several decades, only limited information exists about the long-term effects of stocking on natural sturgeon populations (7). Significant attention of the scientific community was recently focused on the negative genetic effects of stocking on the wild sturgeon populations (8, 9), while the impact of aquaculture rearing conditions on fitness of

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stocked individuals was not addressed in greater extent. Hatchery-cultured fish typically encounter conditions very different from those encountered by their wild counterpart, which may result in behavioural, morphological, and physiological differences (10). Hanson et al. (11) provided one of the first evidences from the wild that the morphology is correlated with the swimming activity. Swimming performance is probably the main trait determining fitness in many species of fish and other aquatic animals (12).

Although the comparisons of the morphology between the reared and wild salmon stocks have been already conducted by a number of authors (13-15), such investigations are still lacking for sturgeon species. The effect of the hatchery rearing on body morphology tends to increase with the time the fish spent in the hatchery (14). In this study, we tried to determine possible morphological differences between the reared and wild juvenile sterlet.

Materials and methods

Sample origin and rearing conditions

A total number of 45 sterlet were caught by professional fishermen during November 2002 in the Serbian part of the Danube River, near Belgrade (44° 50' 36.85"N, 20° 25' 15.83"E), with the average total length 32.3±2.0 cm. Age of sterlet was determined from pectoral fin spine sections using a method of Stevenson and Secor (16), modified further by Lenhardt et al. (17). Average monthly temperatures of the Danube water during that period were: April 11.4° C, May 19.2° C, June 22.4° C, July 25.1° C, August 21.6° C, September 19.3° C, October 13.3° C and November 8.6° C. Sterlet were frozen immediately following the capture and biometric measurements were performed on defrosted material. Analysis of pectoral fin sections showed that all wild specimens were young-of-the-year. Since the late April and early May is the spawning period for sterlet in the Danube, specimens were approximately 6 months old at the time they were captured. Diet analysis, performed by Lapkina et al. (18), showed that in July it consisted mainly of chironomids, while the leeches were dominant in August and September (70% and 100%, respectively). Daily growth in weight was 1.8 g / day in July, and 2.2 g / day in September (18).

Sterlet (*n*=20) reared in aquaculture (Rideg & Rideg fish farm) at Homokmegy, Hungary (46° 29' 43.28"N, 19° 04' 03.75"E), with the average total length 33.4±1.4 cm, were also used for the analysis. These specimens originated from artificially fertilized eggs of adult specimens (6 females and 4 males), which were taken from the natural population in the Danube River, near Budapest. Hatching was performed between 15 and 17 April 2004, and specimens were reared on the average temperature of approximately 20° C, due to the use of underground water of the same temperature for basins supply. Fish were initially fed with tubifex worms and, thereafter, only dry feed was used. Specimens were fed *ad libitum*. In early October sterlet were moved to an outdoor basin with the ambient temperature. The main purpose for sterlet rearing at this farm is ornamental fish production for aquariums and ponds. Before the biometric analysis was performed on 3 November 2004, live specimens were placed in a water tank and anaesthetized with few drops of oleum caranfilium in water. These specimens were about 200 days old.

The distance between the sites of origin of the two fish samples (i.e., the wild fish and the broodstock that the aquaculture specimens originated from) was around 460 km of the river flow. Nevertheless, sterlet are known to move regularly over long distances of 300 km or more (19), and the recent genetic studies confirmed that the Danube sterlet should be considered as a single, panmictic population (20). As a result, we believe that the distance between the two locations produced no bias with regard to the genetic background of the studied specimens.

Since the cultured sterlet were measured fresh, while those from the wild were frozen prior to measurements, additional experiment was conducted to determine if the freezing has an impact on morphological measurements. Fifteen specimens were kept frozen for one month, and morphological measurements were performed on both fresh and defrosted material. Mann-Whitney U test showed that there were no significant differences ($p>0.05$) in any of the measured characteristics.

Laboratory and statistical analysis

In addition to body mass (M) and total length (L_t), biometric analyses of wild and cultured sterlet included 15 morphometric traits, nine of them in the head region, and three meristic traits (Figure 1). Morphological variable distributions were evaluated using the Kolmogorov-Smirnov test for normality, as well as with the Shapiro-Wilk test, due to to the small sample size. As they lacked normality of distribution, Mann-Whitney U test was applied.

It is usual to standardize morphometric measurements related to the head region as a proportion relative to head length, and those not related to the head region as proportions of the total length, if the growth is isometric (21-24). Therefore, a regression analysis was performed on studied specimens using allometric growth formula $y = ax^b$, described by Huxley (25), where a and b (slope - relative growth rates of variables) are constants. In isometric growth, the growth curve has a slope b=1. When slope b is smaller than the isometric slope, it is identified as a negative allometric growth, while it is identified as positive when b>1.

Hypothesis about the equality of slopes of the pectoral fin length related to L_t, between wild and reared specimens, was determined by formula $t = (b_1 - b_2)/S_{b1-b2}$ where b_1 and b_2 are regression coefficients of the two samples, and S_{b1-b2} is a standard error of the difference between regression coefficients (Figure 2) (26).

Fulton's body condition (FC) was estimated as FC = (M / L_t^3) x 100 and Kolmogorov-Smirnov test was used to compare FC between groups (26, 27).

Results

Wild specimens ranged in M between 83.5 and 278.2 g and in L_t between 30 and 37.7 cm. Cultured specimens ranged in M between 80 and 136 g, and in L_t between 30.2 and 36.7 cm. Allometric growth formula $y=ax^b$ (25) showed that wild sterlet displayed negative allometric growth in seven morphometric traits and positive allometric growth in one trait, "Head width at barbel base". Reared sterlet displayed negative allometric growth in 10 morphometric traits (Table 1). As a result, four traits that had isometric growth within both groups were standardized prior to statistical analysis, while the comparison of the remaining traits was performed using original measurements (see Table 1).

Wild and cultured specimens differed significantly (P<0.05) in eight morphometric traits, as well as in one meristic trait (Table 1). Wild sterlet revealed a significantly higher value of the regression slope for pectoral fin length (L_t; Figure 2), and the average length of pectoral fin, expressed as a percentage of the total body length, was 16.1% and 14.1% for wild and reared sterlet, respectively. Six length related traits (measurements 4-9 in Figure 1) were significantly larger in wild specimens, while two width related traits (distance between the eyes and head width at the base of the barbel) were significantly larger in cultured specimens.

Range of body mass was wider in the sterlet from wild population (145.8±38.4 g) than in the reared specimens (111.2±15.7 g). Fulton's body condition in wild specimens (0.42±0.06) was significantly higher than in the cultured ones (0.30±0.02; Kolmogorov-Smirnov test P<0.01).

Discussion

Significantly smaller number of ventrolateral scutes in cultured sterlet, obtained in this study, could be explained by different water temperatures during early rearing. The scute number in sturgeon may vary due to water temperature during early rearing (28), and retarded growth during larval development (i.e., due to a low temperature) can result in a higher number of serial features, such as scutes, because they are allowed more time to form (29).

Cultured specimens in this study had a significantly shorter pectoral fin (P<0.05). This is in accordance with findings of other authors (30,31) that cultured fish exhibit shorter fins than the wild fish of similar size. Kalmykov et al. (32) found three sterlet subpopulations within the Lower Volga which showed correlation between the water velocity and the pectoral fin length: in the Volga River sections with water velocities 0.36-0.76m/s, 0.76-1.00m/s and 0.84-1.23m/s, length of pectoral fins (expressed as a percentage of the total body length) was 15.59-15.69, 16.32 and 16.51-16.58, respectively.

Fulton's body condition for wild sterlet (with L_t ranging from 14.2 cm to 42.5 cm) varied from 0.27 to 0.79 throughout an annual cycle, with the highest value recorded in June (33). In

Table 1: Mean values ± SD of 15 morphometric characters and three meristic traits, correlation coefficients (R2) and slopes (b) and results of Mann-Whitney U test, for the studied wild and cultured sterlet samples

Morphometric and meristic traits (see footnote)	Wild sterlet (*n*=45)		Cultured sterlet (n=20)		Mann-Whitney U test	Wild sterlet (n=45)	Cultured sterlet (n=20)
	R^2	*b*	R^2	*b*	*P*	mean±SD	mean±SD
Standard length	0.88	1	0.84	0.82	0,702134	26.5±1.7	26.4±1
Fork length	0.91	0.99	0.81	0.84	0,622832	28.5±1.8	28.5±1.1
Preanal length	0.87	1	0.77	0.81	0,000793	19.3±1.3	18.1±0.7
Predorsal length	0.81	1	0.86	0.89	0,000569	20.6±1.3	19.3±0.9
Prepectoral length	0.58	0.92	0.53	0.95	0,000710	7.6±0.6	7.1±0.4
Pectoral fin length	0.91	1	0.81	1	0,000000*	5.2±0.4	4.7±0.2
Head length	0.66	0.89	0.46	0.87	0,000154	7.4±0.4	6.9±0.4
Preorbital length	0.54	0.91	0.59	1	0,000884	3.7±0.4	3.5±0.3
Preoral length	0.75	1	0.76	1	0,098007**	4.4±0.5	4.2±0.3
Prebarbel length	0.56	1	0.57	1	0,120252**	2.9±0.3	2.7±0.3
Mouth width	0.67	0.91	0.64	0.8	0,827056	1.3±0.1	1.3±0.06
Distance between eyes	0.57	0.9	0.7	0.84	0,029066	1.7±0.1	1.8±0.08
Maximum head width	0.68	1	0.68	1	0,332208**	3.3±0.3	3.1±0.2
Head width at barbel base	0.69	1.1	0.72	0.72	0,004776	1.5±0.1	1.6±0.06
Head width at mouth level	0.49	0.84	0.5	0.95	0,213410	2.4±0.2	2.3±0.1
No. of dorsal scutes					0,180403	13.6±0.6	13.1±1.1
No. of lateral scutes					0,837093	61.8±3.9	62.1±1.8
No. of ventrolateral scutes					0,000002	14.2±0.9	13±0.9

* Variable standardized prior to statistical comparison as a proportion of the total length
** Variables standardized prior to statistical comparisons as a proportion relative to head length

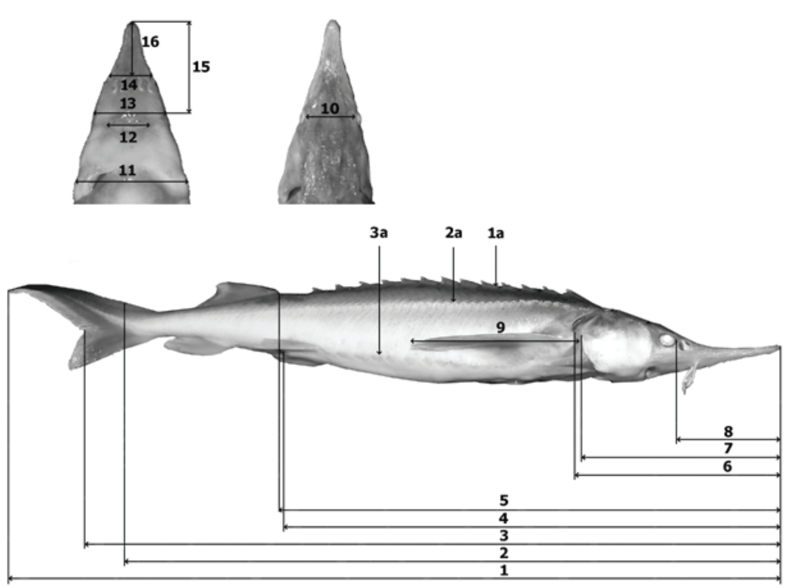

Figure 1: Sterlet morphometric and meristic traits used in analysis: 1 – Total length, 2 – Standard length, 3 – Fork length, 4 – Pre-anal length (from tip of rostrum to anterior margin to anus), 5 – Predorsal length, 6 – Prepectoral length, 7 – Head length, 8 – Preorbital length, 9 – Pectoral fin length, 10 – Distance between eyes, 11 – Maximum head width, 12 – Mouth width, 13 – Head width at mouth level, 14 – Head width at barbel base, 15 – Preoral length, 16 – Prebarbel length, 1a – Number of dorsal scutes, 2a – Number of lateral scutes, 3a – Number of ventrolateral scutes

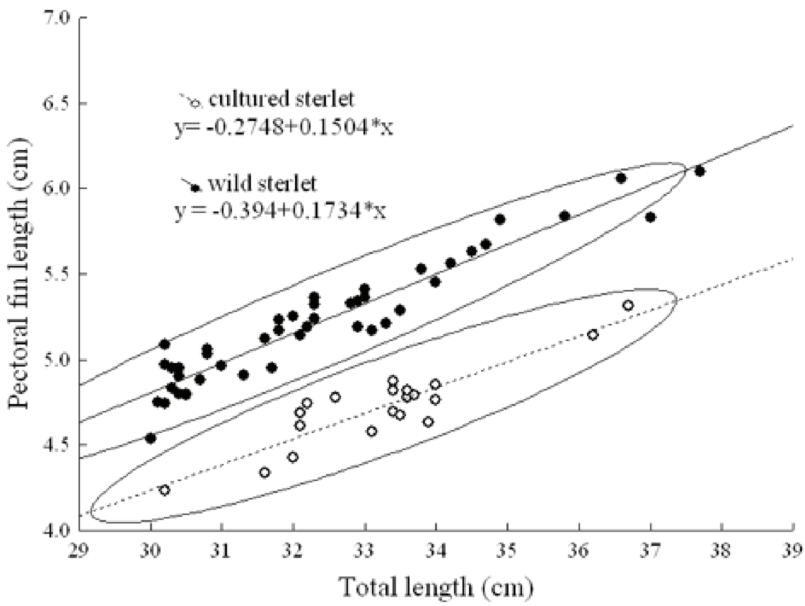

Figure 2: Pectoral fin length of the two groups (wild and reared sterlet), presented as a function of the total length. Regression: $Y_{cultured}$ = -0.2748 + 0.1504 x; Y_{wild} = -0.394 + 0.1734 x; ellipses represent 95% confidence limits

the analysis of six months old sterlet reared in warm water culture, the average value of the FC was 0.45±0.07 (ranging from 0.34 to 0.55; M. Prokeš, pers. comm.). Data obtained within this study showed that the wild specimens had a higher value of FC (0.42) than the cultured ones (0.30). This could be explained by the transfer of cultured sterlet to an outdoor basin with a lower temperature one month before sampling, as well as by a significant daily increase in wild sterlet weight during August and September, caused by a diet based on leeches (18).

There is a need for research that would be focused on the best conditions of sterlet rearing. As stated by Vehanen and Huusko (34), a simple hatchery environment may delay or modify the development of morphometric characteristics that are important in a natural river environment. Differences caused by the aquaculture environment might be probably alleviated if individuals could be released in certain small and confined parts of the natural habitat, before the actual stocking, to enable their adaptation to natural conditions. Chebanov et al. (35) presented a comprehensive system of guidelines for sturgeon hatcheries, including those for the juvenile rearing for release into natural waterbodies. According to these guidelines, the key parameters that would have to be taken into consideration are the illumination regime that mimicks a natural photoperiod, thermal regime, sufficient water flow and the use of live feeds (35). Holčik et al. (6) recommended that all juveniles reared in the fish farms should be adapted to the conditions in natural water bodies before their stocking, mostly through feeding with natural diet and residing in facilities with lotic environment.

Comprehensive literature exists about morphological divergence between cultured and wild juvenile salmon, and results indicated that it may affect the success of cultured specimens after their release into the wild (13, 14, 36, 37). Wild Atlantic smolts differed in the shape from hatchery-reared smolts, and this difference was less pronounced but still statistically significant when wild adults were compared with hatchery-reared adults after a year spent in the sea (14). Furthermore, Svåsand et al. (37) reviewed morphological and behavioural differences between reared and wild individuals of the Atlantic cod and European lobster, while Arechavala-Lopez et al. (38) found a clear morphological differentiation between farmed and wild individuals in a number of Mediterranean fish species. According to Sarà et al. (39), the key parameters that influence morphology of reared fish are stock density, container volume, reduced swimming performance and the food quality. Although there were some attempts to assess the performance of the stocked specimens of sturgeon species, such as the one that compared the growth between the wild and stocked European sturgeon (*Acipenser sturio*) juveniles (40), such studies are unfortunately still scarce. This study was the first attempt to compare morphology of the wild and reared sterlet, and it revealed the presence of morphological differences. Nevertheless, this investigation had a number of limitations, especially when bearing in mind a small, but still realistic possibility that the analyzed specimens from the wild could originate from stocking activities in Hungarian section of the Danube. Such problems could be alleviated by further studies, which would involve assessment of a more representative sample and also include comparisons of adult individuals. Additional research should also deal with the assessment of inter-annual differences between cohorts in a culture system, as well as of differences among wild populations living in conditions with different water velocities.

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PRIMERJAVA MORFOLOŠKIH LASTNOSTI DIVJE IN GOJENE KEČIGE (*ACIPENSER RUTHENUS* L.)

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Povzetek: Populacije kečige (*Acipenser ruthenus* L.) v reki Donavi so v 20. stoletju doživele veliko padcev in postale odvisne od stopnje gojenja v značilnem področju njihove razširjenosti. Kljub trenutni široki uporabi gojenja rib se malo raziskav ukvarja z učinkovitostjo uspeha gojitve, še posebej z vplivom rejskih pogojev na izpuščene osebke. Naša raziskava poskuša ugotoviti obstoj morfoloških sprememb pri gojenih kečigah, ki bi lahko vplivale na kakovost plavanja in tako zmanjšale preživetje gojenih rib. Divje kečige (*n=45*) iz reke Donave smo primerjali s kečigami iz akvakulture (*n=20*), ki izvira iz divjih ikrnic na Donavi. Statistična primerjava 15 morfoloških značilnosti je pokazala, da se vzorci razlikujejo v 11 značilnostih, poleg tega pa so imele gojene kečige značilno krajšo prsno plavut in bolj čokato telo v primerjavi z divjimi. Potrebne so dodatne raziskave, da bi se ugotovilo ali proučevane značilnosti lahko vplivajo na prilagodljivost in preživetje gojenih kečig po izpustu v reko. Obdobje prilagajanja v okolju pred gojitvijo lahko najbrž zmanjša vpliv akvakulturne reje na preživitvene sposobnosti izpuščenih rib.

Ključne besede: kečiga; *Acipenseridae*; reka Donava; Fultonov faktor; prsna plavut; biometrija