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EARLY DEVELOPMENT AND GROWTH OF STERLET (*ACIPENSER RUTHENUS*) IN THE CZECH REPUBLIC

J. Rybnikár, M. Prokeš, J. Mareš, M. Cileček

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Abstract

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Growth rate of sterlet larvae and juveniles during 2008 and 2009 was studied under experimental and farming conditions in the Czech Republic. The embryos hatched when reaching a mean total length (TL) of 9.0 mm. Larvae were fed by living food, with a gradual transition to dry diet. The exogenous feeding and the larval period of ontogeny started at DAH 9 (day after hatching) reaching TL of 15–17 mm accompanied by melanin plug exclusion. Towards the end of larval period (DAH 39–43, TL 50–58 mm), the embryonic finfold disappeared and the formation of fin apparatus was nearly completed. During the larval and early juvenile development, daily increments of TL and weight (w) ranged between 0.33–4.23 mm.d^{-1} and 0.0018–1.6400 g.d^{-1}, respectively. The specific growth rate (SGR) ranged from 25.65 to 2.73 %.d^{-1}. Growth intensity and length parameters are similar to the Starry sturgeon, lower than those of the Siberian sturgeon and Russian sturgeon and significantly lower than at Beluga sturgeon. Sterlet's Fulton weight condition factor (FWC) was higher than in the Siberian and Starry sturgeon. The development was also observed on the basis of morphological changes. The larval development could be divided into six steps.

early ontogeny, specific growth rate, length–weight relationship, factor of weight condition

The sterlet (*Acipenser ruthenus* L.) is the smallest native freshwater species of the family Acipenseridae, subfamily Acipenserinae for the Czech Republic (Baruš, Oliva *et al.* 1995). It ranges in the Danube Slovakian stretch, from where it spreads, or spread into the Morava River on the territory of the Czech Republic. The following experts studied the growth of sterlet in the Slovak Danube natural stretch – Kovrižnych (1988), Stráňai (1992) and Kováč (1997). Baránek *et al.* (2004, 2006); Jirásek *et al.* (1997); Krupka *et al.* (2000); Mészáros *et al.* (2004) and Prokeš *et al.* (1997a, 1999, 2000a,b, 2003b) examined, under experimental and intensive production of the Czech and Slovak Republics, the early ontogeny, larval and juvenile growth and rearing. The current status of sterlet in the Slovak Republic, including information on its pisciculture and stocking in the Danube, was stated by Holčík *et al.* (2006). Current sterlet farming, rearing and production in the Czech Republic has been conducted since 1995 in the Fishery Unit Pohořelice, Inc. (Rybníkářství Pohořelice, a. s.) (Baránek *et al.,* 2004; Prokeš *et al.,* 2003b). Initial import of fertilized eggs and larvae and juvenile rearing of six sturgeon species, including sterlet (*Huso huso, Acipenser stellatus, A. baerii, A. ruthenus, A. gueldenstaedtii* and *Polyodon spathula*) was carried out in the Mydlovary hatchery, Hluboká n. Vltavou, a. s. (fishery enterprise) in 1994–1996 (Hohausová *et al.,* 1996; Jirásek *et al.,* 1997; Klívar, 1996; Prokeš *et al.,* 1996, 1997b,c, 1999, 2000a,b, 2002, 2003a) and later in the Research Institute of Fish Culture and Hydrobiology (RIFCH) in Vodňany (Linhart *et al.,* 2000, 2003, Policar *et al.,* 2004, Gela *et al.,* 2008, Flajšhans *et al.,* 2009, Pšenička *et al.,* 2010 and others).

The aim of this study was to monitor the ontogeny and growth of embryos and larvae of sterlet reared under controlled conditions in the Czech Republic

and to compare collected data with the relevant data, available both in this country and abroad. The data on development and growth of sterlet larvae in the Czech Republic, not yet published, should be used mainly for improvement of fry and fingerlings rearing of this species, as well as for purposes of their introduction into natural aquatic ecosystems. The natural sterlet population is currently endangered in the upper Danube and its tributaries.

MATERIAL AND METHODS

The monitoring of sterlet early ontogeny and growth took place in 2008 and 2009. The material of embryos (after hatching called also eleuteroembryos or prelarvae) and larvae (after DAH 9) was obtained from artificial brood fish stock reared at hatchery Velký Dvůr, Fishery Unit Pohořelice, Inc. (Rybníkářství Pohořelice, a.s.). In order to determine the Daily Feeding Ratio (DFR in % of fish weight), larvae were weighted regularly in two or three–day intervals. Along with the larvae measuring, samples were collected for fixation in the 4% formaldehyde solution. Three months after, once the mass stabilization had finished, all fixed fish were measured and weighted. The following features were determined: total length (TL in 0,1 mm) and weight (w in 0,0001 g).

2008

For experimental purposes, sturgeons hatched on 9 May 2008 at the hatchery Velký Dvůr, Rybníkářství Pohořelice, Inc. at water temperature of 18 °C. In the age of DAH 5 these were placed at the Section of Fishery and Hydrobiology MENDELU (Mendelova univerzita v Brně) in a recirculation system consisted of plastic trough, filter, aeration and heater. Trough volume was 100 l and brood density at the beginning of the experiment was 17 pcs/l. Measurements of water temperature, oxygen saturation percentage and pH were performed every morning before feeding. Used devices: WTW Multi 340i and WTW pH 315i. The temperature ranged from 17 °C on the initial day towards 19 °C at the end of experiment, without fluctuation. The oxygen content ranged 63%–91% throughout the whole monitoring. The minimum pH value was 7.5, maximum 8.4; without high fluctuation. Feeding was not initiated until the DAH 5. We applied nauplii brine shrimps (*Artemia salina*) SANDERS (50% protein and 20% fat) brands. Brines *were* incubated at the Section of Fishery and Hydrobiology MENDELU. After incubation, the larvae were either fed immediately or the nauplii kept refrigerated at 4 °C and fed during maximum 12 hours storage. Along with brine shrimp, the perla larva proactive 5 (62% protein and 11% fat) was fed, as well.

2009

The monitoring was performed at hatchery Velký Dvůr, Rybníkářství Pohořelice Inc. Fish embryos began to hatch on 20 April 2009 at water temperature of 14.5 °C. After hatching, they were transferred to flow–through troughs. Trough water source was a small pond free of fish brood. Foam filter prevents the intrusion of organisms and contaminants. Larvae and juvenile rearing took place in the hatchery, from which the samples were taken. First, the larvae were fed *ad libidum* with sorted zooplankton (200–2000 µm), later on with dry diet, which was applied to advanced larvae and juveniles by means of automatic feeder over the period of 24 hours. Early development and intervals of ontogeny were classified after Lange *et al.* (1974); Detlaf *et al.* (1981); Balon (1986); Peňáz (1995, 2001) and Pavlov (2007). The monitoring included the final part of embryonic period (E), the whole larval period (L) divided into 6 steps (L1–L6), and the initial part of juvenile period (J). Growth was analyzed via absolute and relative increments (DI), specific growth rate, SGR = [(ln w_1 – ln w_0). 100], Fulton's weight condition factor, FWC = (w.10^5) . TL^{-3}. Calculations of length–weight relationship ($w = a.\ TL^b$) and polynomial growth curves (t:TL and t:w). All abovementioned parameters always used total length (TL). For needs of production indicators, the Daily Feeding Ratio (DFR) was calculated. The Microsoft Excel program was used for graphs, regression curves and calculations.

RESULTS AND DISCUSSION

The following changes were found in the length, weight and morphology of sterlet during subsequent age (DAH) of early ontogeny:

2008

DAH 5, E, TL = 13.1–5.3 mm, w = 0.0122–0.0185 g, FWC = 0.4007–0.6390.

Still no food was visible in the digestive tract of free embryos; melanin plugs were apparently formed at six individuals in the spiral part of their intestines. There were apparent the pectoral and ventral fins. Individuals of different size had approximately the same weight – therefore the relationship between length and weight was not significant (Table I, Fig. 1A). Values of Fulton's weight condition factor (FWC) decreased with increasing total length (Fig. 1A).

DAH 10, L1, TL = 14.5–18.6 mm, w = 0.015–0.0327 g, FWC = 0.3699–0,6517.

There was a food visible in larvae digestive tract, 3% of specimen had not received any food, so far. The differentiation of unpaired finfold was more pronounced. Ventral fin margin did not exceed the pre–anal finfold. The end of the notochord was straight. The melanin plugs were floating in water at the trough bottom. The individuals found themselves in the so–called critical period, with respect to the need for suitable food. (Table I, Fig. 1B).

I: *Total Length (TL in mm), Weight (w in g), Fulton's Factor of Weight Condition (FWC), Length–Weight Relationship and Specific Growth Rate (SGR) in free embryos and larvae of sterlet (Acipenser ruthenus) in 2008 and 2009. Explanations: DAH = Day After Hatching, TL = Total Length, w = Weight, FWC = Factor of Weight Condition, a,b = Regression Coefficients, R^2 = Determination Coefficient, SGR = Specific Growth Rate, SD = Standard Deviation*

Date	DAH	TL (mm)		w (g)		FWC (K_F)		w = aTL^b			SGR	n
		average	SD	average	SD	average	SD	a	b	R^2		
14 May 2008	5	14.4	0.5531	0.0144	0.0013	0.4886	0.0699	0.0071	0.2637	0.0134		30
19 May 2008	10	16.5	0.8278	0.0223	0.0051	0.4879	0.0751	2.00E-06	3.3024	0.5480	10.93	32
22 May 2008	13	17.5	1.4457	0.0276	0.0092	0.4945	0.0724	3.00E-07	3.9552	0.8629	7.11	30
25 May 2008	16	20.2	1.9418	0.0469	0.0162	0.5426	0.0544	6.00E-07	3.7348	0.9515	17.67	30
27 May 2008	18	21.4	2.9382	0.0577	0.0303	0.5444	0.0539	1.00E-06	3.5437	0.9997	10.36	30
30 May 2008	21	25.4	3.9629	0.0974	0.0514	0.5437	0.0672	2.00E-06	3.3082	0.9497	17.45	20
2 June 2008	24	28.9	3.6282	0.1308	0.0583	0.5146	0.0440	3.00E-06	3.1581	0.9553	9.83	17
5 June 2008	27	32.1	5.0369	0.1991	0.0918	0.5505	0.0612	2.00E-06	3.3604	0.9706	14.01	20
8 June 2008	30	37.2	6.0848	0.2927	0.1313	0.5249	0.0387	4.00E-06	3.0623	0.9819	12.84	22
11 June 2008	33	43.7	3.5137	0.4508	0.1025	0.5299	0.0203	5.00E-06	3.0067	0.9778	14.40	20
15 June 2008	37	45.3	8.1131	0.5968	0.2930	0.5926	0.0510	6.00E-06	3.0054	0.9769	7.01	21
6 May 2009	16	17.I	0.4706	0.0269	0.0041	0.5339	0.0552	2.00E-07	4.0935	0.5413		31
10 May 2009	20	18.0	1.1756	0.0300	0.0081	0.5051	1.1495	4.00E-07	3.8624	0.9040	2.73	31
14 May 2009	24	22.1	1.7211	0.0717	0.0213	0.6413	0.0545	6.00E-07	3.7399	0.9371	21.78	31
18 May 2009	28	24.0	3.4375	0.0863	0.0420	0.5679	0.0632	6.00E-07	3.6980	0.9782	4.63	31
21 May 2009	31	26.9	3.7111	0.1261	0.0543	0.6098	0.0447	5.00E-06	3.0495	0.9694	12.64	27
25 May 2009	35	35.0	4.6234	0.2887	0.1307	0.6363	0.0463	4.00E-06	3.1672	0.9732	20.71	20
29 May 2009	39	50.7	4.3731	0.8053	0.1776	0.6097	0.0370	2.00E-06	2.7216	0.9534	25.65	21
5 June 2009	46	61.7	7.3537	1.5259	0.5376	0.6233	0.0549	3.00E-06	3.2008	0.9544	9.13	7
18 June 2009	59	62.5	12.3700	3.4137	1.3025	0.5896	0.0660	3.00E-05	2.6333	0.9455	6.19	4
23 June 2009	64	95.1	9.9454	4.9300	1.6900	0.5581	0.0511	1.00E-06	3.3104	0.9586	7.35	5
30 June 2009	72	103.6	10.7470	6.1563	1.6274	0.5455	0.0412	4.00E-05	2.5893	0.9553	2.78	5

DAH 13, L2, TL = 14.8–20.5 mm, w = 0.0131–0.0466, FWC = 0.3594–0.5928.

The food was visible in digestive tract of all larvae. Differentiation of unpaired finfold continued, there were slight rudiments of dorsal and anal fins. Notochord was slightly bent upwards. The critical period continued; the minimum FWC value was still the lowest, the average value was, however, slightly higher (Table I, Fig. 1C).

DAH 16, L2–L3, TL = 16.6–24.1 mm, w = 0.0195–0.081 g, FWC = 0.3815–0.6272.

All the morphological changes detected in previous sample were more conspicuous. Ventral fin exceeded the edge of unpaired finfold (Table I, Fig. 1D).

DAH 18, L2–L4, TL = 16.7–28.8 mm, w = 0.0235–0.1437 g, FWC = 0.4563–0.6346.

Ventral fin exceeded the edge of unpaired finfold significantly. Pre–anal finfold remnants still were present. Mesenchyme condensation in the place of formation lower lobe of caudal fin was first noticed (Table I).

DAH 21, L3–L5, TL = 20.8–35.3 mm, w = 0.0423–0,2452 g, FWC = 0.4329–0.6817.

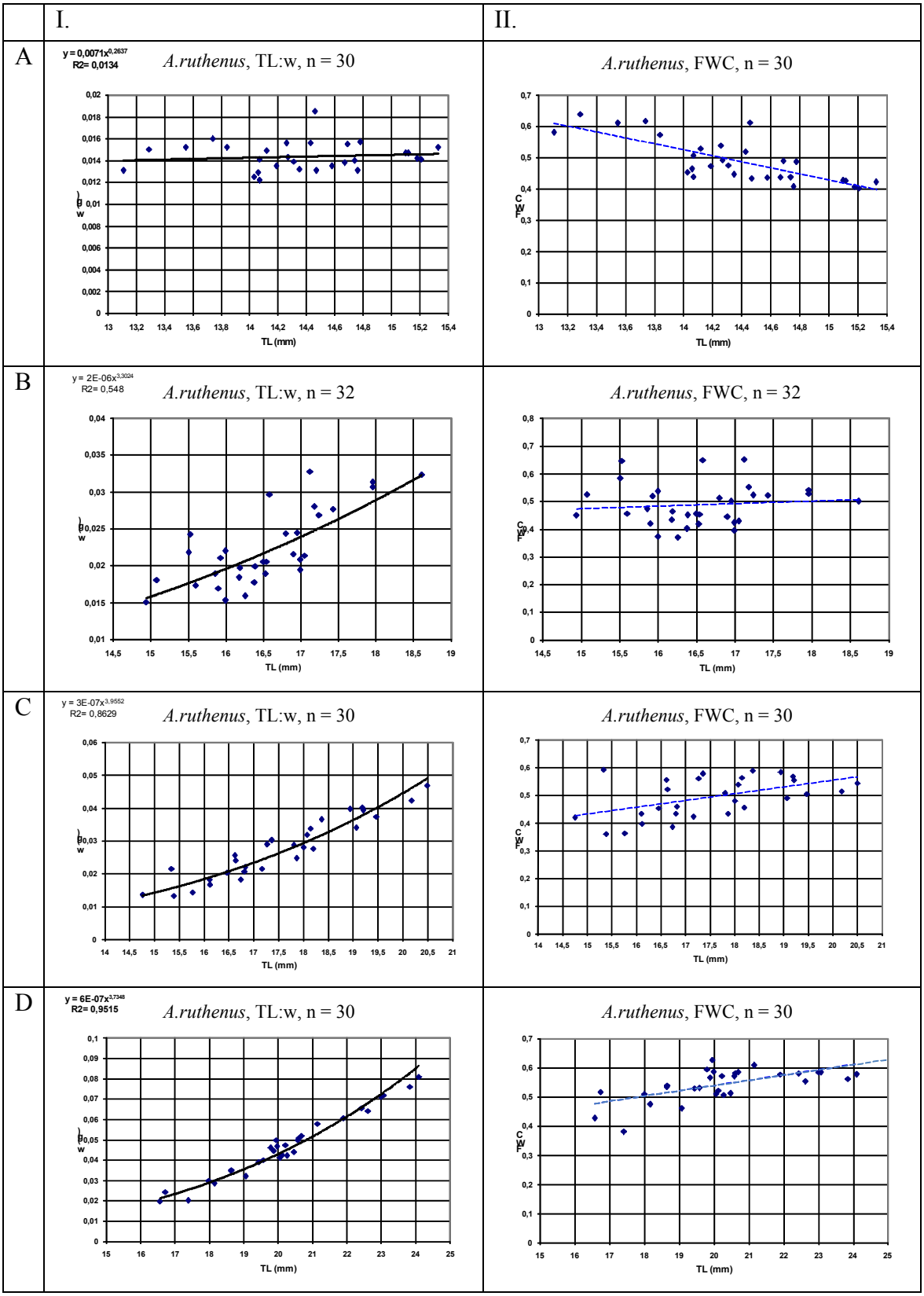
Vestiges of the finfold in front of the anal fin are no longer detectable, they are still present only between the dorsal plates. Mesenchyme condensation occurred on the future lower lobe of caudal fin (Table I).

DAH 24, L4–L5, TL = 24.1–38.4 mm, w = 0.0770–0.3031, FWC = 0.4421–0.5984.

Still, there were unpaired finfold remnants between dorsal plate tags. Either the lateral line plates way the lower lobe of the caudal fin has been formed, yet. Ventral line plates are already created (Table I).

DAH 27, L4–L6, TL = 22.8–40.3, w = 0.0554–0.3502, FWC = 0.4341–0.6320.

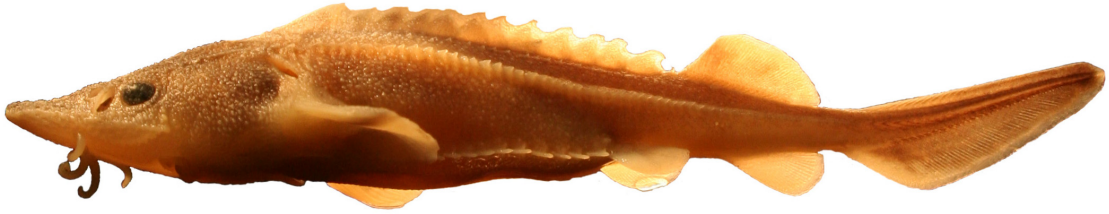
Unpaired finfold still remains between dorsal plate tags. No rays are formed in the caudal and anal fins. Lateral line plates' formation is finished, dorsal fin rays creation began (Table I), (Fig. 2).



1: *Length–Weight Relationship (I) and Weight Condition Values (FWC)(II) for different sized individuals 2008; A – DAH 5, B – DAH 10, C – DAH 13, D – DAH 16*

2: *DAH 27, L3, TL = 30,74 mm, w = 0,1723 g*
- no rays formed in the caudal and anal fins, lateral line plates' formation finished

3: *DAH 30, L4, TL = 35,34 mm, w = 0,2250 g*
- formation of rays in dorsal and caudal fin, dorsal plate tags almost free of finfold remnants



4: *DAH 38, L5, TL = 57,04 mm, w = 1,0108 g*
- dorsal, caudal and anal fin rays extended up to the edge, rays of ventral fins visible, not extended to the edge

5: *DAH 69, J, TL = 111,4 mm, w = 7,3502 g*
- fulcrae reached almost to the end of the dorsal edge of caudal fin, rays in all fins almost created

DAH 30, L4–L6, TL = 24.0–47.6, w = 0.0751–0.5662, FWC = 0.4510–0.5938, L4–L6.

Dorsal plate tags are almost free of finfold remnants, that persist in front of the dorsal fin base. Formation of rays in dorsal and caudal fin. Lateral line plates are already completed in some individuals, measuring of standard length (SL) was possible. Rays formation is less conspicuous in the abdominal fins and had not proceeded in caudal fin, yet (Table I), (Fig. 3).

DAH 33, L5–L6, TL = 35.0–49.3, w = 0.2284–0.6202, FWC = 0.4962–0.5706.

All three plate lines, except of the lateral line, are completed. Dorsal and caudal fin rays extended up to the edge. No rays could be seen in ventral fins, yet.

DAH 37, L5–J, TL = 26.2–63.7, w = 0.0953–1.2625, FWC = 0.4884–0.7139.

Lateral line was not completed, yet. Fulcral scales could be seen clearly in the–caudal fin. Dorsal,

caudal and anal fin rays extended up to the edge. The rays of ventral fins were visible, however not extended to the edge. The lower lobe of caudal fin had not developed, yet (Table I), (Fig. 4).

Other recorded and calculated parameters, i.e. average values and standard deviation, TL, w and FWC, specific growth rate (SGR), coefficients (a, b, R2), length–weight relationship, sample frequency and date of sampling are listed in (Table I).

2009

As for development, the 2009 samples coincided with 2008 material, however their sampling proceeded until higher development stage. Description of the development in 2009 continued from the point, at which the 2008 samples terminated, however these two partly overlap (see Table I).

DAH 46, L6–J, TL = 50.1–71.9 mm,
w = 0.7356–2.1564 g, FWC = 0.5501–0.6874.

Rays did not fully reach to the edges of caudal, dorsal and anal fins, neither to the edge of ventral fins. Dorsal plate tags are bent backwards. The caudal fin lower lobe is not formed, yet. Fulcral scales are approximately at the half of the caudal fin.

DAH 59, J, TL = 70.6–94.2 mm,
w = 1.9882–4.6724 g, FWC = 0.5205–0.6766.

Rays of all fins reached almost to the edge. The caudal fin lower lobe is formed clearly. Pronounced pigmentation is noticeable throughout the body.

DAH 64, J, TL = 78.4–102.6 mm, w = 2.7015–7.0792 g,
FWC = 0.5487–0.6758.

The differentiation of caudal fin lobes was proceeding. Significant skin pigmentation and pronounced cutaneous formation is perceptible.

DAH 69, J, TL = 89.5–114.7 mm, w = 4.3775–7.7670 g,
FWC = 0.5112–0.6116.

Caudal fin lobes differentiation continued. Fulcrae reached almost to the end of the dorsal edge of caudal fin. Rays in all fins were almost created (Fig. 5).

As in the previous case, all other identified and calculated parameters are listed in Table I.

By the observation of morphological and morphometric changes and its comparison with the data of Krupka *et al.* (2000), we obtained results contributing to a more detailed knowledge of the early development of the sterlet. During its development process, the sterlet undergoes through two larval steps only, according to Krupka *et al.* (2000). As our results confirm and on the ground of fin structure changes determined, the larval period can be divided into six steps. Between TL and w, no significant regression was observed on the fifth day after hatching (DAH 5) (Fig. 1A). Initially, Fulton's weight condition coefficient value decreased with the size of individuals (Table I, Fig. 1A), which is normal, since fish still had the yolk sac, using endogenous nutrient sources for the weight and length growth and these nutrients' metabolic waste and energy consumption reduced fish specific weight, but not the specific length. At the time of decrease, there were still no exogenous food intake in the analyzed sample of the approximate size of 15 mm TL, the specific weight began to rise gradually, afterwards (Table I, Fig. 1B). On the thirteenth day after hatching (DAH 13), significant positive regression between TL and w of larvae was found out (Table I, Fig. 1C). Weight condition factor and length of larvae increased significantly (Table I, Fig. 1C). In terms of larvae morphological development degree, these fell within the second larval step (L2). Particularly, gill respiratory system development was noted, as well as slipping–out of mouth, located at the base of head, during feeding, formation of paired pelvic base and unpaired dorsal and anal fin. Back string end section was slightly bent upwards and the intestines of all individuals were considerably filled with food. End of larval development period was observed at DAH 37–46 (TL 50–56 mm). Except of the complete formation of lower lobe of caudal fin, all remnants of unpaired finfold completely disappeared. Development of rays continued in unpaired fins during this period. The growth intensity of sterlet during the interval from hatching to the end of larval period was considerably high. The original TL average of 9 mm, observed after hatching, increased in this period 6 times on average (to 54 mm), and initial weight of 0.01 g increased almost 100 times (to 0.98 g). It is apparent from the above–stated data, that the maximum length and weight changes occurred in the period after the intake of exogenous food initiated. The lowest weight condition rate was found in individuals, those TL was 15–17 mm. The size and growth intensity of free embryos and larvae in the period from hatching to the end of larval development period was significantly affected by the species–specific growth potential, which is the smallest one among sturgeons reared in the Czech Republic (Gisbert *et al.*, 2000). Under the conditions of the Czech Republic, size and growth intensity parameters of sterlet are, for this reason, similar to those of the Starry sturgeon (Klivar, 1996), lower than the Siberian sturgeon (Prokeš *et al.*, 1996) and Russian sturgeon (Prokeš *et al.*, 1997b) and significantly lower compared to Beluga sturgeon (Hohausová *et al.*, 1996). However, the Fulton weight condition factor was, in case of sterlet, higher than that of the Starry sturgeon and Siberian sturgeon. The observations we found were similar to data presented by Dettlaff *et al.* (1981), Mil'stein (1982), Hochleithner (1993), Jirásek *et al.* (1997), Krupka *et al.* (2000) and Wegner *et al.* (2009).

SUMMARY

The aim was to investigate the early ontogeny and growth of sterlet reared under controlled conditions. The free embryos and larvae hatched at the hatchery Velký Dvůr, Rybníkářství Pohořelice Inc. were used for the monitoring which took place in 2008 and 2009. During 2008 the sterlet embryos, larvae and juveniles were reared at the Section of Fishery and Hydrobiology MENDELU. Rearing took place in trough with recirculation system. Larvae were fed by the brine shrimps (*Artemia salina*) and perla larva proactive 5 dry feed. In 2009, the monitoring took place at hatchery Velký Dvůr, Rybníkářství Pohořelice Inc. The larvae were fed *ad libidum* with sorted zooplankton, later on with dry diet. Samples collected were stored in the 4% formaldehyde solution. Three months after, once the mass stabilization had finished, all fixed embryos, larvae and juveniles year 2008 and 2009, were individually measured and weighed (TL in 0.1 mm, and w in 0.0001g). Sampling was initiated in 2008 in age of 5 days after hatching DAH 5 and ended at the age DAH 37 with eleven samples. The 2009 sampling ended on DAH 69. Each sample corresponds approximately to one periodic development jump. On DAH 10 larvae already fed exogenous food were classified as falling within 1st larval step. On DAH 37, larvae, whose larval features were vanishing gradually, fell within the 6th larval step, i.e. to the last larval step. Transition to the juvenile development period occurred at TL 50–54 mm. The fin apparatus of juveniles was formed completely, except of the lower lobe of caudal fin, whose development continued also during the initial part of the juvenile period of development. On DAH 5, no significant regression was observed in the total length (TL) and weight (w) of free embryos. Exogenous feeding did not occur so far. On DAH 13, significant positive regression between TL and w was detected. Weight condition factor increased significantly along with larvae length. The TL of 9 mm observed after hatching increased during larval period 6 times on average (to 54 mm) and 0.0100 g weight increased almost 100 times (to 0.98 g). The maximum length and weight changes occurred in the period after the exogenous feeding intake initiation. The lowest weight condition factor was found in individuals with the TL of 15–17 mm. Size of free embryos and larvae, as well as growth intensity were significantly affected by the species–specific growth potential, which is the lowest one among sturgeons reared in the Czech Republic. We found that size and growth intensity of sterlet larvae are in the Czech Republic similar to the Starry sturgeon, lower than Siberian sturgeon and Russian sturgeon and significantly lower than that of Beluga sturgeon. However, the Fulton weight condition factor during early ontogeny was, in case of sterlet, higher than the Starry sturgeon and Siberian sturgeon.

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Address

Ing. Juraj Rybnikár, doc. Dr. Ing. Jan Mareš, Ing. Martin Cileček, Ústav zoologie, rybářství, hydrobiologie a včelařství; Mendelova univerzita v Brně; Zemědělská 1, 613 00 Brno, Česká republika, Ing. Miroslav Prokeš, CSc., Ústav biologie obratlovců Akademie věd ČR, v.v.i., Brno, Květná 8, 603 65, Česká republika, e-mail: xrybnika@node.mendelu.cz, mares@mendelu.cz, xcilecek@node.mendelu.cz, prokes@brno.cas.cz