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Rearing Danube salmon, *Hucho hucho* (L. 1758), in controlled environment during early juvenile stage

A dunai galóca, *Hucho hucho* (L. 1758), nevelése ellenőrzött körülmények között az ivadék korai stádiumában

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Kulcsszavak: növekedés, fajlagos növekedési sebesség, kondiciófaktor, *Artemia salina*

Abstract

Two groups of Danube salmon (*Hucho hucho*) fingerlings obtained from artificial propagation conducted in April/2011 on the fish farm "Perućac" in Perućac were compared. The first group was fed with a combination of *Artemia salina*/commercial trout feed, and the other group was fed with a combination of *Gammarus* sp./fish meat. Length and weight growth were monitored during the research period from 22. 06. - 14. 09. 2011. The group fed with *Gammarus* sp./fish meat had statistically higher weight and length gains. Specific growth rate (SGR) of the *Gammarus* sp./fish meat fed group was 39.06% higher than that of the group fed by brine shrimp and trout feed. The weight of fingerlings had most effect on complete transfer to commercial trout food diet, without addition of *Artemia salina* nauplii, and was average 0,846 g. Condition factor in the group fed with the *Gammarus* sp./fish meat combination was 15.55% higher than one in the group fed with the *Artemia salina*/commercial trout feed combination. Less positive correlation between weight and total length and greater coefficient of variation shows that growth was more unstable in the group fed with the *Artemia salina*/commercial trout feed combination.

Kivonat

E munka két csoport dunai galóca ivadékának a növekedését vizsgálja, melyek a dunai galóca 2011. évi szaporításából származtak a Perućac-i pisztrángtelepen, a Drina menti Perućacban (Szerbia). Az első csoport *Artemia salina* nauplii/pisztrángtáp, a második csoport *Gammarus*/halhús kombinációjával volt etetve. A vizsgált időszakban (2011. július 22-től szeptember 14-ig) a testhossz és a testtömeg gyarapodását követtük. A *Gammarus*/halhús kombinációjával etetett csoport egyedeinél a tömeg és a testhossz középértékei jelentősen magasabbak voltak. A fajlagos növekedési sebesség (SGR) 39,06%-kal volt nagyobb a *Gammarus*/halhús kombinációjával etetett csopot egyedeinél. A tápraszoktatás döntő pillanatának a 0,846 g testtömeg elérése bizonyult. Ekkor az ivadék már tápra szokott, és etethető csak pisztrángtáppal. A *Gammarus*/halhús kombinációjával etetett csoport kondiciófaktora 15,55%-kal volt nagyobb az *Artemia salina* nauplii/pisztrángtáppal etetett halak kondiciófaktoránál. A pozitív korreláció kisebb a testhossz-testtömeg viszonyában, és a kondiciófaktor értékei egyenletlen növekedésre utalnak a *Artemia salina* nauplii/pisztrángtáppal etetett halak csoportjában.

Introduction

Danube salmon (*Hucho hucho*) is an endemic fish species inhabiting rivers of the Danube drainage. This species is classified as a representative of salmonid fishes, family Salmonidae, in which there are three subfamilies, 11 genera and 66 species (Nelson 2007). Systematic position of this species is given in *Table 1*. In the *Hucho* genus, besides Danube salmon there are 4 more species: *Hucho perryi*, *H. bleekeri*, *H. ishikawae* and *H. taimen* (Lucas & Baras 2001).

Table 1. Systematic position of Danube salmon

Phylum	Chordata
Subphylum	Craniata
Superclassis	Gnathostomata
Classis	Actinopterygii
Subclassis	Neopterygii
Divisio	Teleostei
Subdivisio	Euteleostei
Superordo	Proacanthopterygii
Ordo	Salmoniformes
Familia	Salmonidae
Subfamilia	Salmoninae
Genus	*Hucho*
Species	*Hucho hucho* (L., 1758)

Systematics acording to Nelson, J. S. 2007

With the maximum recorded length of 1650 mm and weight of 60 kg this is one of the largest representatives of this family (Kottelat & Freyhof 2007). Danube salmon is differentiated from other members of family Salmonidae by: 180-200 scales in lateral line, absence of red dots and white margin on fins, dorsoventrally flattened head which length is 22-24% of standard body length, deeply cut-in caudal fin and proportionally large adipose fin (Kottelat & Freyhof 2007). Sexual dimorphism is not specially expressed, but during spawning period it is possible to determine gender by size of genital papilla and by darker coloration of males. Danube salmon life expectancy is approximately 20 years, and sexual maturity is reached at the age of 3-4 (males) or 4-5 (females). Spawning period is during March and April, with water temperatures reaching from 6-10 °C. Combination of temperature and photoperiod is main limiting spawning factor (Holčík et al. 1988). Within the Danube salmon, as with other Salmonid species, phylopatry is strongly expressed, thus, before the breeding season, they migrate upstream to the upper reaches of rivers or smaller tributaries of rivers in which they are found. During the breeding season pairs are formed and they make nests at 0.5-1.5 m depth (Holčík et al. 1988). Nests are made with fins movements and measure 1.2-3.0 m in diameter and 10-20 cm in depth (Kottelat & Freyhof 2007). Fecundity varies according to different authors. It averages to 1000 eggs per kilogram of body mass. Diameter of eggs depends on the female's age, so that it measures 4.26 mm in case of the 5 year old females, and 5.01 mm in case of the 7 year old females (Bartel et al. 1999). Danube salmon inhabits rivers and streams in upland areas (200-600 meters above sea level), which are characterized with a fast water flow, moderate temperature (usually up to 15 °C) and higher amount of dissolved oxygen (8-9 mg/l) (Simonović et al. 2011). Even though it is salmonid, Danube salmon is primarily piscivore during the adulthood and its ecological niche resembles that of large lowland water predators like pike (*Esox lucius* L., 1758) and pikeperch (*Sander lucioperca* (L., 1758)). In Serbia, Danube salmon is found in the rivers of western Serbia: Drina, Lim, Beli Rzav, Poblaćnica, Uvac, Vapa, Moravica and Đetinja (Mijović-Magdić 2007). Because of its size and life cycle, this species is under great anthropogenic pressure, and its populations are reducing in the whole natural distribution (Jungwirth 1979). The greatest influence on the populations drop have overfishing activities, dams built on the most rivers inhabited by Danube salmon, and pollution (Lelek 1987). Witkowski et al. (2013), state that habitat degradation, overfishing and pollution are the main threats for Danube salmon. Dams, usually constructed without a fish ladder, stop natural breeding migrations and irretrievably degrade natural habitats of Danube salmon. Because of relatively small depth at which this fish makes nest and long incubation period, approximately 300 day-degrees, fluctuating water level caused by dams poses a great threat for incubating eggs. Adult specimens are also easily visible and unwary during breeding period, meaning they are an easy target for poachers. Like all salmonids, Danube salmon is also extremely sensitive to pollution and there have been many massive dyings in last few decades caused by pollution.

Danube salmon is globally endangered and protected by the World and European conventions: IUCN (endangered - EN), Bern convention (Appendix III), EU Habitat directive (Anex II and IV). In Serbia, Danube salmon is protected by the Law of protection and sustainable use of fish fund ("Sl. glasnik RS", br. 36/2009) and its subordinate files. Order on protection and sustainable use of fish fund ("Sl. glasnik RS", br. 104/09) establish fishing ban between the 1st of March and 31st of August and issue minimal keeping length of 100 cm.

During the early juvenile stages, fish in the Salmonidae family are under great mortality rates (95% mortality during first 1-2 years of life is common). Combination of high fecundity of adults and mortality of juveniles in nature creates opportunity for high production of salmonid species by application of artificial breeding. Data concerning attempts of artificial reproduction in Europe date up to 14. century (Allendorf & Waples 1996). Today, artificial reproduction of Danube salmon is used as one of the conservation measures which have a goal in strengthening of natural populations and reintroduction to the habitats from where Danube salmon extirpated. Breeding season in wild is relatively short and ovulation period is not synchronized within the females in a population, which represents one of the bigger problems during the artificial reproduction. Consequently, there are low chances of capturing females that have ovulated and not spawned yet. This issue is solved by the use of carp pituary for inducing ovulation (Jungwirth 1979). During the fry rearing, the greatest problem is provision of adequate fish food. Common trout food used at the start of active feeding can cause problems to fry because of their underdeveloped digestive tract.

The aim of this paper was to estimate efficiency of two feeding strategies using growth and condition parameters. In the first approach we used nauplii of *Artemia salina* and common trout food, and in the second we used *Gammarus* sp. and fish meat. In addition, our aim was also to determine the optimum fry size for habituation to common trout food.

Material and methods

Danube salmon fry used in this research were obtained from artificial propagation conducted during the April of 2011 within the project "Artificial reproduction of Danube salmon in aim of strengthening on natural population in the Drina River". Propagation and rearing were done on the fish farm "Perućac" in Perućac. In this experiment, 622 fish were used and kept in a controlled environment during the whole time of the experiment. Two groups were formed, the first group (D group) constituted of 572 fish which were fed with nauplii of *Artemia salina* (Linnaeus, 1758) and commercial trout feed made by Coppens (TroCo Crumble HE) granulated to 0.3-0.5 and 0.5-0.8 mm. The second group (L group) constituted of 50 fish which were fed using natural food. As the natural food, we used *Gammarus* sp. Linnaeus, 1758 caught in nearby stream and fish meat. Fish were monitored from 22nd of June until 14th of September in 2011. Experiment was conducted in elongated pools that measured 4 m in length, 0.3 m in width and 0.3 m in depth. Waterflow during the monitoring period was between 0.3 and 0.5 l/s. Physical and chemical parameters of water were also monitored during the experiment. Concentration of oxygen, saturation, and temperature were measured with WTW Oxi 340i/SET, conductivity was measured with Eutech Instruments Ecoscan Con5, while pH value was recorded using Eutech Instruments pH Tester10. In addition, ammount of suspended solids (TSS), total organic carbon (TOC), nitrate concentration (NO3), surfactants (SUR), biological oxygen demand (BOD) and chemical oxygen demand (COD) were measured using Secomam Pastel UV.

Photoperiod was 20 and 4 hours, light and dark respectively. Light period was from 04 until 00 hours. For the illumination, the Tungsram Daylight neon lamps of 20 W and 60 cm length were used, which were set at 35 cm from surface of water. Measured illumination was 1210 lx. The D group was fed every hour during the whole light period and L group was fed *ad libidum* 2-3 times per day.

Fish were measured every week between 22nd of June and 14th of September in 2011. The Weight and length measurments were conducted using caution because of the fish small size. Weight was measured using scale KERN 440-47N, with the tolerance of ±0.2 g.

Length was measured without a direct contact with fish based on method described by Sidek and Halawani (2010) using digital camera FujiFilm S6500fd and software Image. A reference length defined on ruler, and backcalculation over proportion were used to determin fish length. In the period between the 22nd of June and the 31st of August, average weight and length of fry in both groups were followed. Specific growth rate (SGR) was calculated weekly using formula by Boyer et al. (1994):

$$SGR=100[(\ln W_t-\ln W_0)/t]$$

where W_t represents weight of fish after t days expressed in grams (g), W_0 starting weight of fish expressed in grams (g), t number of days between two measurements

Condition factor was calculated using formula by Ricker (1975):

$$CF=(W/L^3)*100$$

where W represents weight of fish expressed in grams (g), L total length of fish expressed in centimeters (cm).

Relation between total length (L) and standard length (l) was calculated using formula:

$$L=a*l+b \text{ (in Microsoft Excel)}$$

Relation between weight (W) and total length (L) was tracked using formula by Tesch (1968):

$$W=a*L^b \text{ (in Microsoft Excel)}$$

Comparison of the two monitored groups was performed using software STATISTICA 10.0 and Student t-test of dependant pairs with the confidence interval of 95%.

Results and discussion

Fish farm where fry of Danube salmon were reared gets its water from an underground spring of the river Vrelo. Physical and chemical parameters didn't vary significantly during time period of the experiment and were matching for fry rearing (*Table 2.*). Jungwirth et al. (1989) states that the best growth is achieved at the temperature of 16°C, while on fish farm "Trešnjica", successful rearing was achieved on the temperatures ranging from 10.5 to 14.0 °C (Mijović-Magdić 2007).

Table 2. Average values of water parameters in pools during the experiment

t (C°)	$[O_2]$(mg/l)	$[O_2]$(%)	Con(μs)	pH	TSS	TOC	NO3	SUR	BPK	HPK
10.3	6.5	59	444	8.0	<2.5	<0.5	3.3	0.5	<1	0.4

With the provided conditions on the fish farm, first eyed eggs were observed 23 days after the spawning and activation of eggs, and after 4 weeks first hatching occurred. Hatching lasted for two days (*Table 3.*). Technology of rearing Danube salmon fry at this stage is not different from the technology used in rearing of other salmonid fry. After 48 days since fertilization, three days after the start of active swimming, fry were provided with nauplii of *Artemia salina*, which is in accordance with the data provided by Mijović-Magdić (2007).

Table 3. Dynamics of embryo development

Development stage	Date	Days after fertilization	Day-degree after fertilization
Fertilization	10.04.2011	0	0
Eyed stage	02.05.2011	23	236.9
First hatching	07.05.2011	28	288.4
Swim away	23.05.2011	45	463.5
Start of exogenous feeding	26.05.2011	48	494.4
Full resorption of vitellus	01.06.2011	53	545.9

Two weeks after the start of feeding with nauplii, trout food Coppens (TroCo Crumble HE), with granulation of 0.3-0.5 mm, and later on 0.5-0.8 mm was introduced in the feeding strategy On the 22nd of June, L group was separated from the rest of the fish, and the addition of *Artemia salina* in D group was continued until it stopped being effective. According to Mijović-Magdić (2007), it is safe to stop with nauplii feeding after 15 days from the start of exogenous feeding, while Jungwirth et al. (1989) states that on water temperature of 16 °C it is best to stop with nauplii feeding after 29 days from the start of exogenous feeding. Jungwirth et al. (1989) also reported that at that moment Danube salmon fry had an average weight of 0.750 g. De Verga & Bohm (1992) stated that digestive tract development is completed with formation of piloric caeca 28 days after the start of exogenous feeding. During this period, additional feeding with zooplancton is crucial, because it represents exogenous source of enzyme activity, which improves digestion of common food (Jungwirth et al. 1989). In accordance to the provided data, we tried to exclude nauplii from feeding strategy of D group between the 16th and the 20th of July. This resulted in stagnation of fry growth and in negative SGR recorded on 20th of July (*Table 4.*). After this *Artemia salina* nauplii were introduced back into the feeding protocol and used until the 24th of August (90 days from the start of exogenous feeding), since the measurements perfomend on the 15th and the 24th of August showed that nauplii don't affect fry growth in the D group (*Table 4.*). The 15th of August was taken as the date when nauplii stopped being effective because of the recorded reduction of SGR. Average weight at that moment was 0.846 g which is in accordance with the data stated by Jungwirth et al. (1989).

Table 4. Data of total length (L), weight (W) and specific growth rate (SGR) in the two test groups

fertilisation days/ feeding days	measuring date month.day	L group L (mm)	L group W(g)	D group L(mm)	D group W(g)	L group SGR	D group SGR
75/27	6.22.	35,70	0,260	35,45	0,245	4,65	3,82
82/34	6.29.	39,30	0,360	38,50	0,320	4,58	2,07
89/41	7.06.	42,25	0,496	39,70	0,370	4,13	0,84
99/51	7.16.	47,61	0,750	42,00	0,403	4,36	2,73
103/55	7.20.	51,20	0,893	42,65	0,449	3,16	-0,92
110/62	7.27.	54,91	1,114	43,20	0,421	2,96	3,46
117/69	8.03.	58,05	1,371	45,18	0,536	4,90	3,55
124/76	8.10.	64,19	1,931	47,27	0,688	2,14	4,14
129/81	8.15.	67,37	2,149	51,40	0,846	2,44	2,38
138/90	8.24.	73,64	2,678	54,57	1,048	2,09	1,16
145/98	8.31.	76,41	3,100	56,00	1,136		

Comparing average values of total lengths (L) of L and D group, we can notice that with an increase in size, there is an increase in difference between these two groups in benefit of the group fed with the *Gammarus* sp./fish meet combination (*Fig. 1.*). Using Student's t-test of dependent pairs it was determined that the differences in lengths of the two groups are statistically significant ($t = 4.70$; $df = 10$; $p = 0.0008$). By comparing average values of weights (W) of L and D group, it can be observed that an increase in fry size is followed by an increase in the difference between these two groups (*Fig. 2.*). Significantly higher values were recorded again in the L group, which is also illustrated with the Student's t-test ($t = 3.89$; $df = 10$; $p = 0.0030$).

Comparison of specific growth rate (SGR) between the D and L group shows that SGR is constantly high and has positive value in the L group, while in the D group greater variations between measurements and even negative value in one occasion were recorded (*Fig. 3.*). If SGR is observed for the whole monitored period (W_t – weight at 31st of August, W_0 – weight at 22nd of June and T=70 days), growth rates of 3.18 and 1.97 were observed for the both groups respectively. These data show that L group had 39.06% higher growth rate during the time period of the experiment.

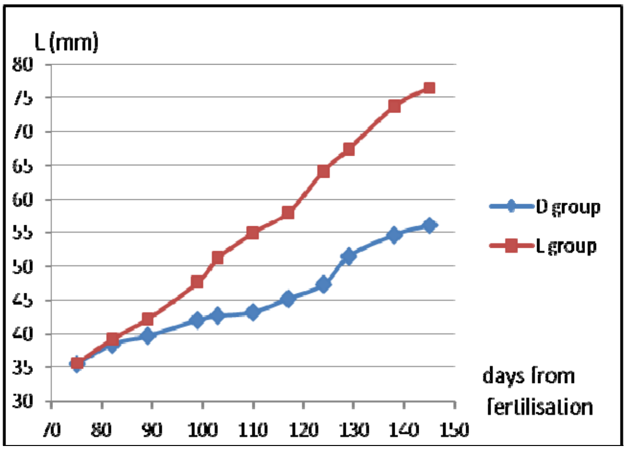

Fig. 1. Changes of total length (L) of both groups during the experiment

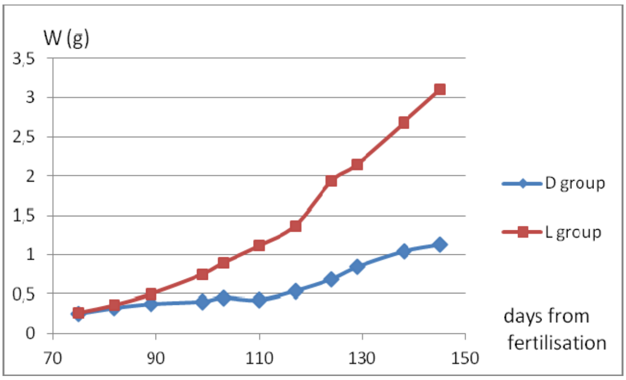

Fig. 2. Changes of weight in both groups (W) during the experiment

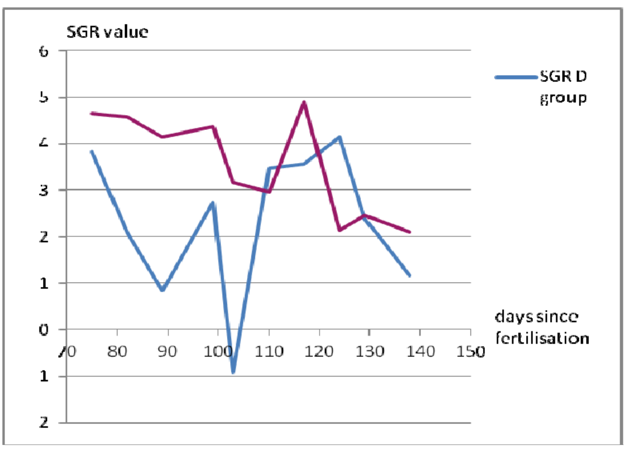

Fig. 3. Specific growth rate (SGR) during the experiment

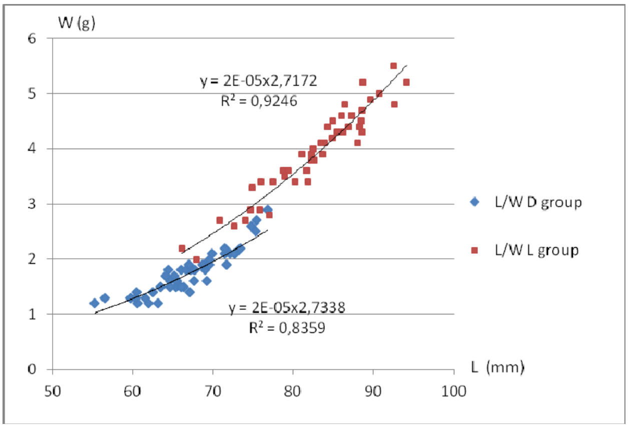

Fig. 4. Relation between total length (L) and weight (W) in D and L group

Interdependence between the total length (L) and weight (W) also shows a high mutual positive correlation in groups D and L. In both groups, b value is less than 3, pointing at the negative allometric growth (length growth is more expressed then weight growth). In D group slightly lower correlation between the total length and weight is observed (R^2=0.8359) in the group D, compared to the group L (R^2=0.9246). This could be a consequence of the weaker condition factor of D group (*Fig. 4.*).

Table 5. Fulton condition factor parameters (CF) on the 14th of September 2011.

	L group	D group
Average CF	0.688	0.581
Standard deviation	0.045	0.053
Coefficient of variation	6.540 %	9.122 %

From this table it is visible that condition factor in L group is 15.55% higher. Presented data indicate that feeding strategy based on combination of *Gammarus* sp. and fish meat was more efficient than combination of *Artemia salina* and commercial trout food. Higher coefficient of variation in D group (9.122%) indicates an uneven condition in D group.

Conclusion

In the period between 22nd of June and the 31st of August observed average values of length and weight gains were significantly higher in L group (fed with combination of *Gammarus* sp. and fish meat) Though, since there were no replications of the treatments and in group D the number of individuals kept in the fish tank was much different from the number in group L the size differences at the end of the experiments may not only due to the feeding regime, but also to the experimental design. Specific growth rate was 39.06% higher in L group, which indicates better weight growth in this group. For transition to complete diet with commercial trout food, without addition of *Artemia salina*, weight of fingerlings was crucial, and in this experiment it was 0.846 g. Less positive correlation between weight and total length and higher value of coefficient of variation in D group indicates an uneven growth in fingerlings fed with *Artemia salina* and commercial trout food combination. Since condition factor in L group was 15.55% higher than in D group we conclude that feeding strategy based on *Gammarus* sp. and fish meat is more efficient than *Artemia salina* and commercial trout feed.

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References

Allendorf, F. W., Waples, R. S. (1996): Conservation and Genetics of Salmonid Fishes. p. 238–280. In: Avise, J. C., Hamrick, J. L. (Eds.): *Conservation Genetics: Case Histories from Nature*. Chapman & Hall, New York.

Bartel, R., Bieniarz, K., Epler, P. (1999): The relationship between egg size, and the size and age of Danube salmon (*Hucho hucho* L.) females. *Archives of Polish Fisheries* 7/2: 221–226.

Boyer, N. J., Van Toever, W., Jansen, M. E. (1994): Effect of photoperiod on growth of Arctic charr under commercial production conditions. *Progressive Fish-Culturist* 56: 44–46.

De Verga, V., Bohm, J. (1992): The effect of freeze-dried zooplankton as a dry feed additive for Danube salmon (*Hucho hucho* L.) fry. *Aquaculture* 108/1–2: 155–168

Directive 92/43/EEC. (1992) Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of Wild Fauna and Flora. – OJL, 206, 22.07.1992, 7–50.

Holčík, J., Hensel, K., Nieslanik, J., Skácel, L. (1988): *The Eurasian Huchen, Hucho hucho. Largest Salmon of the World*. Joint edition published by Dr. W. Junk Publishers, Netherlands and VEDA, Bratislava.

IUCN 2000. *IUCN Red List Categories*. IUCN Species Survival Commission, Gland, Switzerland; Cambridge, UK.

Jungwirth, M. (1979): Ovulation inducement in prespawning adult Danube salmon (*Hucho hucho*, L.) by injection of acetone-dried carp pituary (cP). *Aquaculture* 17: 129–135.

Jungwirth, M., Kossman, H., Schmutz, S. (1989). Rearing Danube salmon (*Hucho hucho* L.) fry at different temperatures with particular emphasis on freeze-dried zooplankton as dry feed additive. *Aquaculture* 77: 363–371

Kottelat, M., Freyhof, J. (2007): *Handbook of European Freshwater Fishes.* Kottelat, Cornol, Switzerland and Freyhof, Berlin, Germany.

Lelek, A. (1987): *Threatened Fishes of Europe*. Aula-Verlag, Wiesbaden. p. 118–122.

Lucas, M.C., Baras, E. (2001). *Migration of Freshwater Fishes*. Blackwell Science, Oxford, pp. 193.

Mijović-Magdić, J. (2007): Savremeni status mladice *Hucho hucho* (Linnaeus, 1758) u vodama Srbije i mogućnost kontrolisanog mresta i gajenja mlađi. Doktorska disertacija. Poljoprivredni fakultet, Univerzitet u Novom Sadu, Novi Sad

Nelson, J. S. (2007): *Fishes of the World*. 4th ed. Department of Biological Sciences, University of Alberta, Edmonton, Canada. John Wiley & Sons, Inc. p. 199–204.

Ricker, W. E. (1975): Computation and interpretation of biological statistics of fish populations. *Fisheries Research Board of Canada Bulletin* 191: 1–382.

Sidek, Z. M., Halawani, S. M. (2010): Computer vision application in measuring fish length. *European Journal of Scientific Research* 45/1: 47–54.

Simonović, P. D., Nikolić, V. P, Tošić, A. D., Marić, S. P. (2011): Length-weight relationship in adult huchen *Hucho hucho* (L.,1758) from Drina River, Serbia. *Biologia* 66/1: 156–159.

Tesch, F. W. (1968): Age and growth. p. 93–120. In: Ricker, W. (Ed.): *Methods for Assesment of Fish Production in Fresh Waters.* Oxford and Edinburgh.

Witkowski, A., Bajić, A., Treer, T., Hegediš., A, Marić, S., Šprem, N., Piria, M., Kapusta, A. (2013): Past and present of and perspectives for the Danube huchen, *Hucho hucho* (L.), in the Danube basin. *Archives of Polish Fisheries* 21: 129–142.

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