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Evaluation of the physiological state of the Volga pikeperch (*Sander volgensis*) fingerlings raised in a closed water system

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Abstract
In this study we conducted an experiment to determine the time of ripening of pikeperch (*Sander volgensis*) broodstock. The article presents the period of incubation of eggs of pike perch depending on the water temperature. The experiment of growing of pikeperch fingerlings was described until the age of yearlings with the combined method. The novelties of the work lie in the use of new feeding rations and assess the impact of elevated temperatures on the life activity of pike perch fry, including physiological indicators. The study evaluated the rate of linear (longitudinal) and weight growth, the coefficient of condition factor of the Volga zander fry cultivated in the industrial conditions, spawning-nursery farms and fish caught from the natural habitats were discussed.

Keywords: *Sander volgensis*, pikeperch, yearlings, spawning nests, maturation rate, feeding ration, physiological indicators.

Introduction
Fish produced using various systems and methods i.e. in ponds, cages and tanks of closed water systems. The decline in wild fish populations as a result of overharvest and water pollution has promoted the farming of fishes grown in contaminant-free, indoor recirculating aquacultures systems. Recirculating Aquaculture Systems (RAS) are differs by a high stocking density and high net production in comparison with open aquaculture systems [1]. This technique had spread worldwide for majority fish species, including the European pikeperch, as happened in experiments of Kaliningrad (Russia), Hungary and Western Europe [2, 3, 4, 5, 6].
A pikeperch is one of the most important species of freshwater aquaculture in many European countries. In some of them, pikeperch are cultured up to a commercial size, while in others as seed: Fries either fingerling [7]. Fingerlings of pikeperch reproduction in the southern regions of Russia practiced by spawning and nursery farms, but due to a number of factors (scarce number of broodstock in the enterprises, lack of pond reclamation activities of facility, etc.) currently is not very effective for the conservation of the species. The artificial cultivation of the Volga pike perch in commercial purposes in Russia is practically absent. As a result, is necessary to develop new technologies of cultivation, promoting both the conservation of valuable species, and the intensity of its implementation in aquaculture. Steady decline in commercial stocks of the Volga pikeperch contributes by the anthropogenic factors, namely, poaching, exceeding several times the commercial fishing [8].
According to some authors [9, 10, 11], in the early stages of development of this species of fish it is possible to allocate a number of restrictive features, which largely affect the possibility of its cultivation under controlled conditions: extremely small body size; a small dimensions of the mouth; weak resistance to various types of hatchery processes; susceptibility to various pathogens; increased demands in environmental conditions; and tendency to cannibalism. As a result of studies conducted by Polish scientists [12] in the post-embryonic period of pike perch larvae it is detected three critical stages: The transition to exogenous feeding (mortality up to 99%); Filling of the swim bladder with air (mortality 5-90%); Tendency to cannibalism (mortality 30-70%) (Zakes, 2007) [9]. the aim of this study was to assess the physiological status of the Volga pike perch grown in a closed water system.

2. Materials and methods

Investigations were carried out in the period 2010-2015 at the fish hatchery enterprises within the Astrakhan region to (Figure 1):

- Determine the timing ripening of the Volga zander spawners and the period of incubation of eggs;
- Conduct a cultivation of young of pikeperch from larvae to fingerlings under RAS. Evaluate fish breeding, biological and physiological characteristics of pike perch fingerlings;
- A comparison of juvenile of pikeperch grown in RAS and under natural conditions.

Fig 1: Conducting experiments region

Harvesting of pikeperch broodstock carried out in the Volga-Caspian channel in spring 2013, when the water temperature 8-12 ºC;

Pre-spawning pikeperch retention were carried out in trays types LPL-2m (ЛППЛ–2), as well as in concrete basins sized 4x4,5 m, depth 0.5 m. Ready to spawning fishes have previously been separated by sex.

Spawning procedure performed as with spawning nests, and by decanting of sexual products.

The first version of the study included the injection of pikeperch spawners in artificial nests. As a drug that stimulates the maturation we used a carp pituitary gland dried by acetone. Dosage for females was 2.5 mg / kg, for male as well - 1.5 mg / kg [8]. Injected spawners are seated in the fish tanks. Trays and basins with spawners covered with reed mats (Fig. 2).

Fig 2: Packaged trays and basin for spawning of pikeperch

The second experiment consisted in complete control of the process of reproduction of pikeperch. The injection was carried out by double and threefold system.

Stripping reproductive products from matured brooders and insemination is carried out by dry fertilization method and degumming by tannin solution at a concentration of 0.5 g per 1 liter of water (Fig. 3).

Fig 3: Decanting the sexual products from spawners of pikeperch

Growing of pikeperch juveniles was carried out in trays (Yeisk hatchery) sized 300 x 55 x 40 cm, fabricated from fiberglass. Water exchange was 0.2 l/s, which ensures complete replacement of water for 50 minutes. Oxygenated water was carried out using an aerator. Feeding in the first phase of cultivation was carried out manually, in the future - by twelve hours automatic feeders, correspondingly was loaded by food twice a day.

The construction of water recycling system consisted of six trays for rearing fish larvae, mechanical treatment unit, water heating, aerator, electric pump, the settler and discharge channel. Water subjected mechanical cleaning and heated to the required temperature, gravity flow acted on the plumbing in the fish containers. The water supply was carried out in each tray through the foam filter for an additional purification from fine suspended matter.

From the trays water by gravity flow through drainage canals was poured into a settling tank, from which pumps again was fed into the storage device. Added fresh water was 10% in a day to maintain an optimal state of hydro-chemical indicators. Basins cleaning were carried out 2 times per day, and as well as polluted. Washing the filter foam and clayite filters was carried out once a day.

In determining the basic physical and chemical parameters that affect the growth process of juvenile fish, we were guided by the requirements for members of the genus of freshwater perch to complex environmental conditions [13].

Switching to mixed feeding took place by 5-6 days. After hatching, larvae feeding in this period in Artemia nauplii were carried out based on 50% of the larvae biomass. On the twelfth day after hatching in the diet of juvenile we began to add starter trout feed «Aller Futura» fraction "00", starting with 3%, and gradually, as the individuals growth, increasing the rate of feeding up to 6% of the total weight of the larvae. Feeding is carried out manually.

The second stage of growing juvenile of pikeperch was carried out in continuous-flow water in the trays. Initial stocking density of individuals of average mass of 0.169 mg was 533 pieces / m 3. Full water exchange was carried out in half an hour.

Water supply to the basins was carried out from settler. Upon reaching the temperature of water in the settler 24 °C the transition to the flow water directly from the water source was carried out, i.e. from the river with supplemental oxygen and mechanical cleaning. This manipulation was due primarily to the ecological features of pikeperch juveniles, namely it's stenothermal [14, 15]. To evaluate the physiological state of

juveniles of zander we determined the concentration of total serum protein (STP), the number of red blood cells and hemoglobin. The analysis was performed at the age of 70 and 130 days. The amount of hemoglobin was determined by electrophotometric method on KFK-3, the number of red cells in chamber of Goryaev.

3. Results and discussion

The physiological indicators of the broodstock individuals were as in table (1).

Table 1: Size and weight, physiological parameters and indices of internal organs of females and males of pike perch in the Volga-Caspian channel.

Indicators	females			males		
	M ± m	S	CV,%	M ± m	S	CV,%
Mass, kg	1.7 ± 0.1	0.39	23.25	1.2 ± 0.1	0.45	37.24
Standard length, cm	48.4 ± 1,04	4.16	8.6	45.5 ± 1.27	5.54	12.16
Fork length (FL), cm	52.6 ± 1.06	4.26	8.09	49.5 ± 1.47	6.42	13.07
Fulton's condition factor	1.48 ± 0,05	0.21	14.09	1.26 ± 0.06	0.24	19.4
Hemoglobin, g / l	58.5 ± 1.89	8.04	12.58	53.5 ± 2.7	12.65	23.67
Serum Total Protein (STP), g / l	42.4 ± 5.14	21.82	51.47	50.9 ± 3.37	11.1	21.92
GSI, %	7.74 ± 0.28	0.11	14.3	1.9 ± 0.034	0.13	6.9
HSI, %	1.3 ± 0.09	0.34	26.36	0.15 ± 0.014	0.056	36.7
Heart index, %	0.013 ± 0.01	0.3	22.3	0.075 ± 0.01	0.018	37.3
index of spleen, %	0.05 ±0.003	0.012	25.93	1.2 ± 0.1	0.45	37.24

Hydrochemical indicators during growth of juveniles of pikeperch at this stage as a whole were in the normal ranges. However, it should be noted that in the period from mid-July to the second decade of August, the average temperature does not fall below 27 ºC. This value of the temperature index is not optimal for a given age group; therefore, potential opportunities of growth were not realized to the full extent.

The oxygen content in the water at this stage of growing was within the recommended optimum for perch fish and averaged 110% saturation. Nitrite, nitrate, ammonium, do not exceed the recommended maximum concentration limit.

The obtained results of the physiological state of juveniles of zander are summarized in Table 2.

Table 2: Hematological parameters of zander's juveniles

Weight, g	Serum Total Protein (STP), g / l	Hemoglobin, g / l	The number of red blood cells, thousand units / micro liter
5±0.17	50.7±2.1	34.5±5.1	1690±190
25±1.8	52.5±1.3	37.1±4.3	1700±150

The average weight of the first batch of fish that was investigated 5-6g, the second batch - 25.0 g. Thus, of pikeperch fingerlings in an interval between the studies (about 62 days) significantly had grown.

As a result of the experiments the following parameters were obtained.

In the first experiment stripped eggs was detected through five days. The percentage of fertilization was 90, and eggs survival rate did not exceed 40%.

Males matured for 20-28 hours. Schedule of speed of ripening of females depending on temperature are shown in Figure 4.

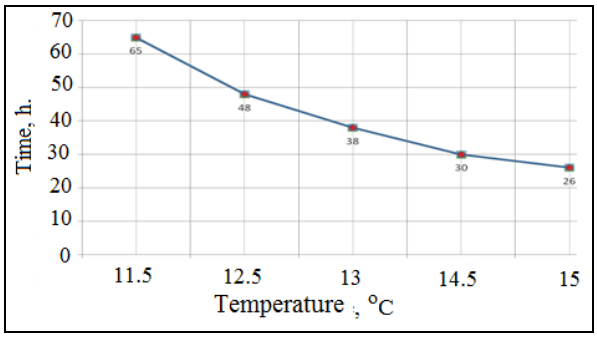

Fig 4: The dependence of the rate of maturation on water temperatures at nesting spawning

From pro injected broodstock 90% of the pikeperch matured and gave benign eggs. In the fiberglass trays females chose to lay eggs in the nest, located at the floodgate. In concrete basin with an area of 18 m^2, spawning passed uniformly over the entire area on the established nests, but the largest number of eggs was laid on the outlet. Nests with fertilized eggs were transferred to the incubation apparatus of Tray type (Fig. 5). Fertilization percentage was 95%. The survival rate of eggs was - 80%.

Fig 5: The incubation apparatus Tray Type

The best pikeperch spawners with the most obvious signs of readiness for spawning were selected. After the injection, the matured 85% of females were given benign eggs. The amounts of extracted eggs were averaged 10% of the female's weight. Graph of maturation rate depend on the water temperature is shown in Figure 6.

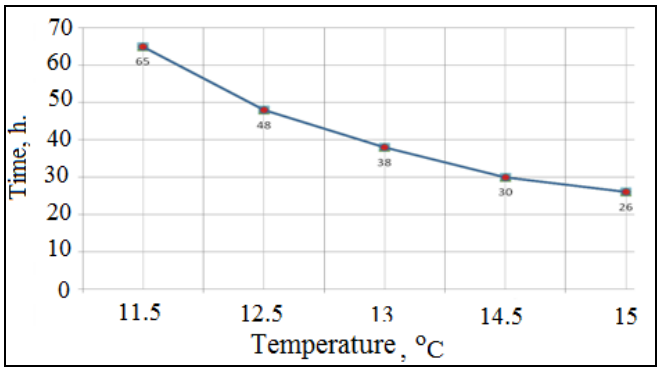

Fig 6: The dependence of the rate of maturation of producers on the water temperature when receiving sexual products "decanting" method

By the method of semi-dry, ovulated fertilized eggs of pikeperch, the un-sticking of fertilized eggs were by solution of tannin, and then we lay to incubate in apparatus "Weiss" (Fig. 7).

Fig 7: Incubation of pike perch eggs in the "Weiss" apparatus

15% of females have not responded to hypophyseal injections been pro injected; we give it additional third injection and are replanted to nesting spawning in the trays. The injection dose was 20% of the resolution. After 48 hours, 80% of females ripened, lay eggs on the nest.

On average, the relative fecundity of the females was 100 thousand eggs. Number of eggs in 1 gr ranged from 1000 to 1100 eggs, in average 1050 eggs. The average weight of eggs - 1 mg. Fertilization percentage was 25%. The survival rate of eggs constituted to only10%.

Incubation in the first and second options of the experiment lasted for 4 - 6.5 days, depending on the water temperature.

Hydrochemical indicators during growth of juvenile of pikeperch under the whole installation of water recycling were within the normal range. Oxygen ranged from 7.3 to 8.4 mg / L, the saturation percentage was 80-110%. The water temperature was in the range of 18-21 ^{o}C. Nitrates, nitrites, ammonia and ammonium at certain moments exceeded the boundaries of the recommended optimum, but do not exceed the limiting concentrations.

Research has shown that of 5 grams juveniles concentration of STP achieves high average value at the amplitude of the individual oscillations from 48.6 to 52.8 g / L, indicating the active feeding of juveniles.

A similar orientation on the dynamics observed in juvenile of pikeperch when studying the concentration of hemoglobin and the number of erythrocytes. Hemoglobin level in juveniles 5-6 g has an amplitude of individual variability 26.6-44.2 g / l, the number of red blood cells ranged from 1 315 to 2 085 thousand units / microliter. On reaching the juveniles mass of 20-30 gr the values of these parameters had an individual variations from 30.8 to 42.0 g / l, and from 1625 to 1875 thousand units / microliter, respectively.

Information about the accumulation of matter and energy in young fish during the rearing period, which is an integral reflection of the growth and metabolic processes, are presented in Table 3.

Table 3: The total chemical composition of muscles of juveniles of zander (in% of the raw material)

Weight, g	sampling date	Dry matter	Protein	Lipids	Minerals
5.0±0.17	15.07.10	26.4	24.2	0.7	1.5
9.0±0.33	01.09.10	18.7	16.9	0.9	0.9
20.0±1.6	13.09.10	22.0	19.8	0.9	1.3
33±2.15	14.10.10	22.8	20.8	0.9	1.2
52±3.01	14.10.10	22.5	20.4	0.8	1.3

Analysis of the data indicates that juvenile of Volga pikeperch, growing by industrial method in the trays until fingerlings was characterized by a fairly high level of metabolic processes. Feeding fish with artificial food with additives of minced fish ensured the accumulation of dry matter and protein in the muscles of pikeperch in the second decade of July to 26.4 and 24.2%, respectively. Then there is some reduction of organic and mineral substances that have reached the minimum values to the beginning of autumn, due to the seasonal rearrangement of an organism in anticipation of the fall active feeding, marked in pikeperch in nature in October and November. During this period in the conditions of controlled growing in fingerlings it is observed an increase of the protein and mineral substances in the body.

On the 18 th day of cultivation high coefficient of variation in body weight was noted, which led to mass cannibalism. In order to reduce it been carried out a partial grading of individuals and lowered the water temperature up to 18 ^{0}C. Linear and weight growth rate is presented in Figure 8.

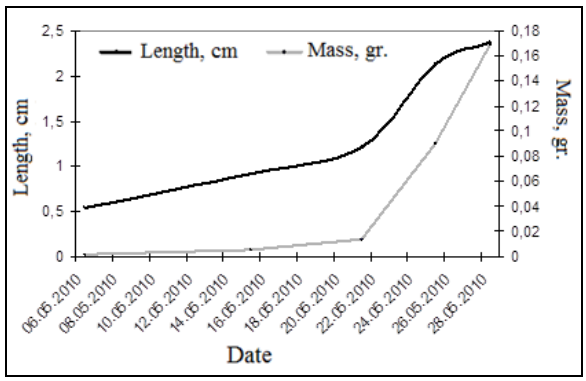

Fig 8: The linear and weight growth rate of larvae of the Volga zander

The content of fat throughout the study period remained almost unchanged and ranged from 0.7 to 0.9% in the raw material. This is primarily due to the fact that the accumulation of fat reserves in the body of pike perch occurs mainly not in muscle, but in the form of strands on internal organs in the abdominal cavity. For comparison: the fat

content of zander fingerlings from natural bodies of water amounted 0.96% [16].

Results of this research affirmed that food had a considerable influence as in pikeperch growth and body composition. Such experiments confirm the purpose of improving the quality of feed by complementing the lacking fatty acids and albuminous fractions, which could have a significant impact on the indices of growth of pikeperch, and their condition and structure of their muscle tissue. These facts are often observed for other fish species [17, 18].

Such close values of lipids in juveniles reared on artificial feeds and natural food resources in the water basin leads to the conclusion that the feeding artificial diet in combination with meat and vitamins did not cause significant changes in fish in lipid metabolism. Zander Juveniles content in the body of nutrients is very close to natural juveniles, enabling it to adapt quickly with the release into the river.

For comparison, according to the weighted growth rate was taken juveniles grown in spawning-culture farms and industrial conditions (Fig. 9).

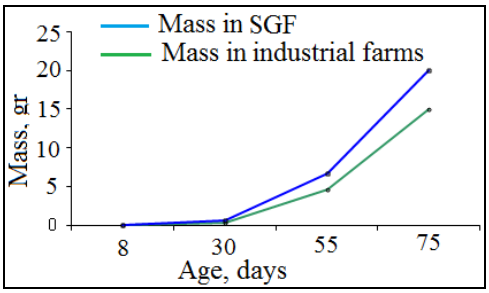

Fig 9: The rate of weight growth of juveniles of pike perch grown in spawning-culture farms and industrial conditions

As can be seen from the graph, the rate of growth in the spawning-growing farm at 55 and 75 days was significantly higher than industrially farmed juveniles, where it amounted 2.03 g versus 4.71 g and 2 g versus 15 g, respectively, and by 30 days the mass of pikeperch was on the same level. This can be explained by the fact that up to one month of age in trays juveniles were fed as artificial feed as well live food organisms that provide a balanced feed, and consequently a high growth rate, but the transference of juvenile to exclusively pelleted feed, the growth rate somewhat slowed, and the retard increased up until the end of the study period.

For comparison, according to the linear growth rate it was taken juveniles grown in hatchery-growing farms and industrial environments, as well as from the natural environment (Fig. 10)

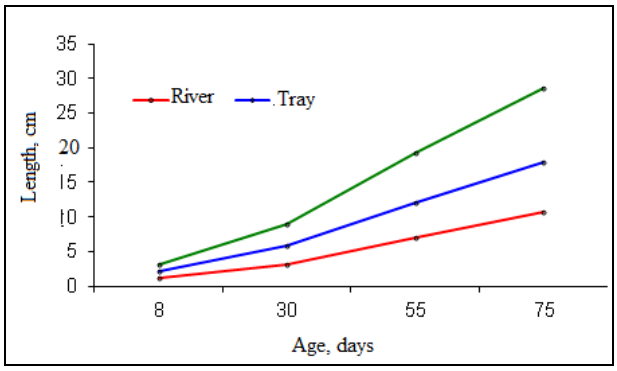

Fig 10: The rate of linear growth of pikeperch fingerlings reared in NVH, in industrial conditions, and caught in the river

The best linear growth was observed in juveniles reared in hatchery-growing farms (HGF), the lowest - in the river. Juveniles, cultivated in industrial conditions, had a slower growth rate, but in contrast to juveniles caught from their natural environment, grew much better. So to 75 days the length of juveniles in trays, spawning and nursery farms and the river was 7.2, 15 and 3.8 cm, respectively. Also notable the fact that the young length gone over to external food, in spawning and nursery farms was higher (1.11 cm), in comparison with juveniles, grown in trays - 9.5, while the juveniles caught in the river - is just 0.6 cm on the 30th day juveniles length, grown in industrial conditions and spawning-nursery farms was 2.6 and 3.15 cm, respectively, which is a good indicator of the rate of linear growth.

Analyzing the data obtained about the length and weight of juveniles of pikeperch we compare condition factor coefficients, grown in an environment close to nature (HGF), and industrial method (Fig. 11).

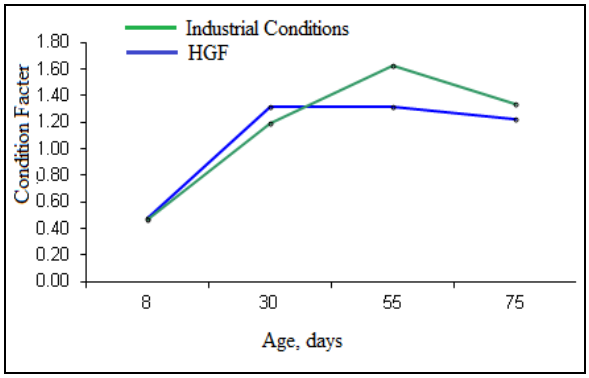

Fig 11: The coefficient of condition factor of pikeperch juveniles grown in HGF and industrial conditions

The condition factor of juveniles reared in different conditions, during the study period of growth is changed. So on the 8th day, it had similar values and was in spawning-growing farms and industrial conditions, the following values: 0.48 and 0.47, respectively. But at the age of 30-day coefficient condition factor of juveniles grown in trays had a lower values - 1.19, and in HGF - 1.31. Later, fatness on Fulton of juveniles grown in trays had indicators higher than the perch from spawning and growing farms. It is connected with mixed feeding, the abundance of food in the trays, a lesser expenditure of energy to find the food.

A special focus of our research was to study the physiological and biochemical parameters of juveniles of pikeperch grown in basins and ponds. Table 4 gives comparative data on the physiological indicators.

Table 4: Comparative estimation of hematological and biochemical parameters of pikeperch juveniles

Indicators	Basins	Ponds
Overall		
Age, days	170	180
Mass, gr	42.5	54.94
Hematology		
Serum Total Protein (STP), г/л	52.5±1.3	21.18±1.63
Hemoglobin, г/л	37.1±4.3	65.75±3.85
Biochemistry (% of wet weight)		
Dry substance	22.65	23.9
Protein	20.6	22.2
Lipids	0.85	0.13
Minerals	1.25	1.6

It should be noted that juvenile of pikeperch was almost completely adapted to feeding on artificial diet, which was used as a trout feed «Aller Aqua» of various granulation. The role of food organisms (Artemia nauplii and Daphnia) in the ration of zander juveniles was minimal and was amounted no more than 10%.

By the end of October, the average mass of fingerlings was 35.5 g (Figure 12), the survival rate - 46%.

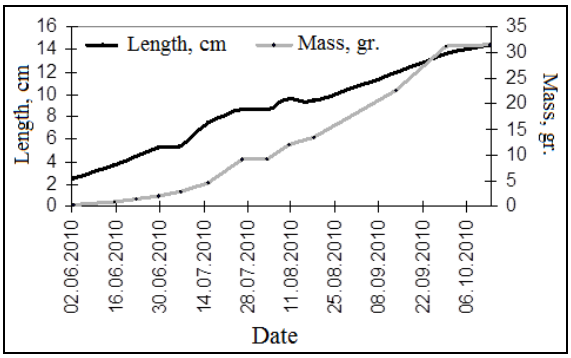

Fig 12: The linear and weight growth rate of Volga zander juveniles

The results of the study in hematology were different, so STP in juveniles grown in basins, has a value 2.5 times higher, so the hemoglobin below 1.8 times. Data on the biochemical analysis had similar value on the percentage of dry matter, protein, minerals and lipid content in the ponds juveniles 7 times less, which could indicate a lack of natural food unbalance.

4. Conclusions

The ripening period of pikeperch spawners, since the introduction of an injection at the nesting spawning amounts to 26-65 hours, depending on water temperature, ripening period from the resolving injection until eggs decanting constitutes 24-48 hours depending on the water temperature. The mean relative fecundity was 100 thousand eggs, which corresponded to 10% by weight of the female body. At the "nesting method" spawning of pikeperch marked the highest percentage of fertilized eggs and the survival rate. The growth rate in the spawning-growing farm was significantly higher than in industrially farmed juveniles. As the rate of linear growth of pikeperch fingerlings reared in industrial conditions, lagged behind the juveniles reared in conditions of spawning-growing farms, but in comparison with the riverine juveniles, it has significant the industrial advantages. Body condition factor had high values, but at certain stages, even exceeded the values of spawning-growing farms juveniles.

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