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# The degree of the formation of protective and food-procuring behavior reactions in the wild – natural and hatchery juvenile fishes of sturgeon (*Acipenser persicus*) at different ages

**Rafiq Y Kasimov, Chingiz A Mamedov, Ulduz F Hashimova and Rauf V Hajiyev**

**Abstract**
The present work explores the effect of environmental conditions on the food-procuring and protective behavioral skills and the identification of the most sensitive periods of early ontogenesis in the formation of these behavioral skills by the example of the comparative studying of the juvenile fishes of Persian (Kura) sturgeon (*Acipenser persicus*) grown in hatchery for 70 days and the wild juvenile fishes of the same age caught from Kura river.

**Keywords:** Behavior, food-procuring reaction, protective reaction, Persian sturgeon, wild sturgeon, hatchery-reared sturgeon

## 1. Introduction
Conservation of biodiversity in modern conditions is one of priorities of science. There has been a dramatic decrease in abundance of the most ancient fish species – sturgeons in the Caspian Sea in recent years.
The reasons of the decrease of Caspian sturgeon stocks in natural water bodies can be different factors: the poaching disregarding age and size, the pollution of their habitats, the decrease of effectiveness of natural spawning, the decrease of a number of juvenile fishes released from hatcheries into the Caspian Sea [1, 4-7, 9, 11, 14].
Currently, the main place of accumulation of sturgeons in the wild is the Caspian Sea. Most species of this family are anadromous - fishes in term of migration, i.e. mature fishes migrate from a sea to a river to spawn, and their offspring born in a river slips down into the sea and lives there until the age of sexual maturity.
However, the natural reproduction of sturgeons since the 50-ths of the last century greatly reduced due to the construction of hydro-power station on their spawning routes in the rivers. At the present time their natural reproduction occurs only at small areas remained below these structures.
In view of the above and compensate for the loss of natural spawning in the lower reaches of rivers, sturgeon hatcheries were built in which by the hormonal effects offspring is gotten from mature individuals of sturgeon and grown to the so-called viable stage (2-3 months), after which they are released into natural reservoirs to maintain inventory numbers of these species in the Caspian Sea. The principle of reproduction of sturgeons in the hatchery is getting more offspring and releasing larger and more formed individuals into natural water bodies in order not to let predators to eat them and to make them better-adapted to new environmental conditions. However, these principles did not have a scientific basis. Although a lot of work was dedicated to the study of morpho-physiological and biochemical indices of hatchery juvenile fishes [16-20], the survival rate and the degree of adaptation of hatchery juvenile fishes in natural conditions are poorly understood [5, 21, 29].
However, previously the degree of adaptation of this juvenile fishes to very harsh conditions of a river was not taken into account and has not been investigated. Probably for this reason, the supposed commercial return of sturgeons from the number of released juvenile fishes was in the 100 and 1000 times lower than expected.

The fact is that during the determining the time of the raising of offspring of sturgeons in the hatchery, their biological characteristics and development conditions in a particular region were not taken into account as well as the capabilities of deterministic reactions ensuring their survival in the wild. According to the published data of A.N. Derzhavin [10], Y.I. Ginzburg [8], R.V. Hajiyev and R.Y. Kasimov [6] the juvenile fishes of sturgeons in Kura river slip from natural spawning areas at earlier dates (larvae or when body weight is between 50 to 800 mg). Larger individuals get into catches very seldom. Therefore, it was important to study adaptation possibilities of wild and coeval juvenile fishes of sturgeons grown in the hatchery. It is well known that an important role in adaptive reactions of organism plays a level of formation of protective and food-procuring reactions, which largely depend on the environmental conditions. In this regard, it was necessary to study the degree of manifestation of these reactions in hatchery and wild juvenile fishes of different ages comparatively, which allowed revealing the influence of environmental conditions on the degree of formation of these reactions in early ontogenesis. Considering this, we studied the degree of formation of protective and food-procuring behavioral reactions and the effect of environmental conditions on these reactions in juvenile fishes of sturgeons grown in the hatchery and fished out of the river at different ages. In addition, the resistance to the starvation of juvenile fishes of sturgeons of natural and artificial generation was studied in comparative terms in order to identify adaptive possibilities of juvenile fishes grown in the hatchery to the natural conditions.

## 2. Material and Methods

The objects of the study were larvae and juvenile fishes of Persian (Kura) sturgeon (*Acipenser persicus*) of different ages and sizes, grown in the hatchery and fished from Kura River. The works were carried out in 2005-2007 years.

Hatchery juvenile fishes were taken from the pools of Kura Experimental Sturgeon Fish Hatcheries. Natural juvenile fishes were caught with trawl net using motor boat in late May and early June. The species, the number of fishes were determined as well as the length and weight of body were measured in caught fishes.

Trawl surveys were conducted at 40-50 km above the mouth of Kura River, near Uzunbabaly and Abbasaly villages of Neftchala region of Republic of Azerbaijan. These areas are above 10-15 km from Kura Experimental Sturgeon Fish Hatcheries and are selected because there are no hatchery juvenile fishes in this area since juvenile fishes released from hatcheries do not rise to these sites against the flow of the river. During this period they have the instinct to slip downstream in the estuary. Therefore, all larvae and juvenile fishes of sturgeons caught in this part of the river, which slip downstream from remained dams of the natural spawning areas, are the result of natural spawning. Generally, within 3 days 10 trawling's were conducted every day. The duration of trawling was 20-35 minutes. In general, 44 individuals of juvenile fishes of natural generation were caught for 3 days.

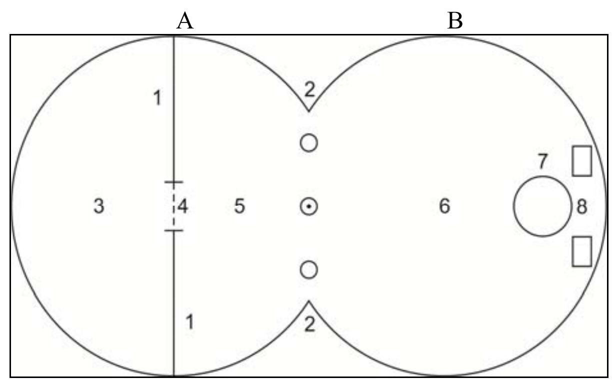
Caught larvae and juvenile fishes were transplanted into special baths and transported to the experimental hatcheries. Then they were transplanted into pools with running water and were divided into three age groups in view of their size and body weight (Table 1).

Natural juvenile fishes of Persian sturgeon were kept in pools for adaptation during 4-5 days as well as the juvenile fishes of hatchery generation. They were fed daphnia (*Daphnia magna*) and white flour worms (*Enchytraeus albidus*). Food-procuring behavioral reactions of juvenile fishes of sturgeon were studied under conditions of free movement in circular tanks (Pic. 1). The diameter of each tank was 80 cm.

The experiment is based on two tanks (A and B), connected by a passage. The left tank (A) is divided into two parts by the partition (3 and 5). The partition has a door (4) for the passage of fishes from one part (3) to another (5). Fishes were fed in the tank B on ordinary days. Fishes were in this tank out of the experiment, they did not see experimental fishes as the partition was opaque.

**Table 1:** Body weight and length of juvenile fishes of different generations

| Growing conditions | Age, in days | Fluctuation limits | |
|---|---|---|---|
| | | Weight, mg | Length, cm |
| Hatchery-reared fish | 18-20 | 140-150 | 2.8-3.0 |
| | 28-35 | 350-360 | 4.5-6.1 |
| | 50-55 | 720-1.100 | 6.2-7.0 |
| | 70-80 | 3.900- 4.500 | 13.9-14.0 |
| Wild fish | 18-20 | 90-130 | 2.9-3.0 |
| | 28-35 | 280-310 | 4.3-6.2 |
| | 50-55 | 610-900 | 6.2-6.9 |
| | 70-80 | – | – |

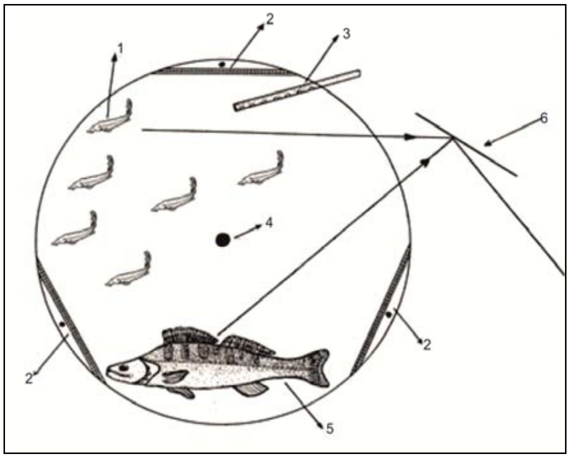



**Picture 1:** The scheme of experimental pisciculture tanks for development of food conditional reflexes in larvae and fry of sturgeons

Experiments were carried out as follows: each fish was supplied through the door (4) from the part «3» into the part «5». Fishes were not fed on the day of experiment. In order to develop the conditional food skills it was necessary to wait untill the juvenile fish's swimmed to another tank (B) and approached the place of conditional feeding (7). When the juvenile fishes were approaching this place they were given 2-3 worms and then a conditional signal (light illumination of 40 lux) was swiched on (Pic. 1). In the future, the conditional signal was gradually advanced from the supply of food. Finally, the conditional signal was switched on when the fishes were in the part «5» (tank A).

The manifestation of food conditional skill was considered a time when fishes which were in the part «5» (tank A) reacted positively to conditional stimulus and moved towards the place of food reinforcement (tank B). The formation of stable conditional food skill was considered a moment when the juvenile fishes correctly responded 5-6 times in a row to the conditional signal and swam to the feeding place. During the experiment the conditional signal was applied 7-8 times with an interval of 2 minutes. The duration of the conditional signal was 15-30 seconds.

The manifestation of a defensive response to the predator was studied by transplanting a few individuals to the area with a predator (river perch) and their behavior was monitored during 30 minutes (Pic. 2). Piscicultural tank was used to study the behavior of a predator and juvenile fishes of different generations: 1 - young Persian sturgeons; 2 - the source of the conditional signal - illumination; 3 - flute for supplying water; 4 - pipe for the outflow of water; 5 – a predator River perch (Stizostedion lucioperca); 6 - a mirror for the observing objects.




**Picture 2.** The scheme of experimental pisciculture tank for investigating of behaviour of juvenile Persian sturgeon fishes at different ages and predator: 1- young sturgeons; 2- the source of the conditional signal - illumination; 3- flut for supplying water; 4- pipe for the outflow of water; 5- a predator – River perch (Stizostedion lucioperca); 6- a mirror for the observing objects.

The time of avoidance and lurking of juvenile fishes was registered in the presence of a predator in their habitat. 2 individuals of perch fish (sizes of 22 and 36 cm) and 10 individuals of wild or hatchery juvenile fishes of the same age were transplanted to one tank and the next day, i.e. 24 hours later the number of fish survived and eaten by a predator was counted. Experiments with each age group were repeated 4-5 times.

### 3. Results and Discussion

The study of food skills of sturgeon juvenile fishes of wild and hatchery generations at different ages showed that at the age of 18-25 days both hatchery and wild juvenile fishes of sturgeon managed to develop strong conditional skills on a combination of changes in light intensity with food (Pic. 3). Hatchery juvenile fishes in this age have stronger food skills than wild ones. We explain that by a high level of orienting responses (responses to the situation). The point is that the new hatchery conditions cause anxiety in wild juvenile fishes and it is hardly possible to them to adapt to the new conditions.

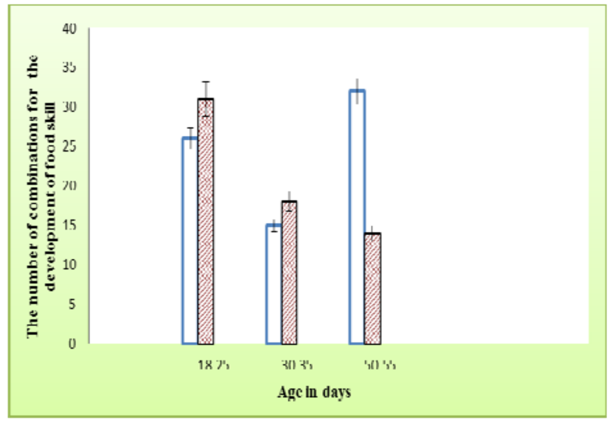
At the age of 30-35 days conditional food reactions in juvenile fishes of both wild and hatchery generations develop faster than at the age of 18-25 days and there is no difference in the rate of development of conditional food skills between wild and hatchery juvenile fishes.

At the age of 50-55 days in the rate of development of food conditional skills between wild and hatchery juvenile fishes there are significant differences and these responses of the wild juvenile fishes develop 2 times faster than in hatchery juvenile fishes. In addition, conditional food skills develop in hatchery juvenile fishes at the age of 50-55 days with difficulty in comparison with earlier ages (Pic. 3).

Probably, these differences are assosiated with the habitat conditions, which are reflected in the formation of brain nerve cells of individuals grown in different conditions.

The point is that wild juvenile fishes of sturgeon, caught from the wild and transplanted into the hatchery pools, differed from the hatchery juvenile fishes also in the manifestation of the protective response to any foreign objects.

To identify the nature of the differences in the responses of wild and hatchery juvenile fishes of different ages to a predatory fish we studied their behavior in the presence of a predatory fish. As the predatory fish we took individuals of river perch of 22-36 cm. The experiments showed that (Table 1) at the age of 18-25 days juvenile fishes of sturgeon of different generations (hatchery and wild) get into the jaws of a predator in almost the same amount.




**Picture 3:** Development of food skills in juvenile fishes of Persian sturgeon: ☐ hatchery- reared fish ▨ -wild fish

For 24 hours a predator manages to catch only 1-3 individuals of wild juvenile fishes (on average, 5%). At the same age the predator eats 20% of individuals of hatchery juvenile fishes for the same time, i.e. 4 times more than wild juvenile fishes.

Experiments with the juvenile fishes of different generations at the age of 50-55 days showed that the predator on all experimental days could not catch any individual of wild juvenile fishes (Table 1). At the same time, on average 30% of hatchery juvenile fishes became victims of the predator.

At the age of 70-80 days 25% of the hatchery juvenile fishes of sturgeon became victims of the predator. The juvenile fishes of natural generation at this age were absent in the river catches. Perhaps, at this age juvenile fishes of sturgeon complete the slipping and then move to sea pastures.

Our observations after juvenile fishes of sturgeon of different generations at the age of 50-55 days (Pic. 2) showed that the manifestation of defensive responses of wild and hatchery juvenile fishes had significant differences in the presence of the predator in the environment. Whereas wild juvenile fishes in the presence of the predator hide or at a distance of 20-25 cm immediately turn and leave abruptly this area, the hatchery juvenile fishes have absolute indifference to the predator; sometimes even they nearly get into the jaws of the predator. All this suggests that hatchery juvenile fishes have very weak defensive responses in comparison with wild ones.

The study of food-procuring activity of hatchery and wild juvenile fishes of sturgeon at different ages also showed that (Table 2) 18-20 and 28-35 day juvenile fishes of sturgeon, grown in the hatchery and caught from the river, find food at almost the same rate. However, at the age of 50-55 days wild juvenile fishes find food 2 times faster than hatchery ones.

**Table 2:** Food-procuring activity of hatchery-reared and wild juvenile fishes of Persian sturgeon at different ages

| Age, in days | Length, mm. | Averages in seconds (m ± p) of food finding by juvenile fishes of Persian sturgeon (p = 5) | |
|---|---|---|---|
| | | Wild fish | Hatchery-reared fish |
| 18-20 | 2.8-3.0 | 42.4 ± 6.18 | 47.9 ± 5.95 |
| 28-35 | 4.4-6.0 | 34.2 ± 3.59 | 32.5 ± 1.98 |
| 50-55 | 6.2-6.8 | 16.2 + 2.04 | 39.6 ± 3.11 |
| 70-80 | 12.0-13.5 | – | 35.2 ± 4.23 |

Analysis of the data shows that at the age of 18-20 days the juvenile fishes of both generations find food later than at the age of 28-35 days (Table 2). It is probably associated with the incomplete maturation of the central nervous structures of these generations.

One of the distinguishing indicators of habitat of hatchery and wild juvenile fishes of sturgeon in the Kura region is turbidity of water. It should be noted that hatchery individuals, since the hatching from eggs till the releasing from hatcheries, live in clear water, but after releasing into a river they get into very muddy water with zero visibility. Wild juvenile fishes, on the contrary, in these periods live in muddy water. The fact that the wild juvenile fishes hatch under the dam of Mingachevir reservoir and the first few days of life live in clear water and then slipping down the river fall into the turbid environment. Water of Kura River becomes muddy after the flowing of water of Araz River into it. In areas, where hatcheries release juvenile fishes, the water is muddy. Therefore, the comparison of the behavior of hatchery juvenile fishes with wild ones in muddy water at different ages was important for the clarifying the question of the behavior of juvenile fishes, getting into the muddy river water, and whether the intensity of feeding and the behavior indicators change in these conditions.

Our research, dedicated to the study of behavioral indicators of wild and hatchery juvenile fishes at different ages in comparative aspect in the laboratory, showed that regardless of the age, juvenile fishes grown in the hatchery were not moving within 60-100 seconds during the transplanting from clear to muddy water, as if they were in stressful condition. At the same time they did not react to tactile stimulation, switching of light and other factors. Only after 2-3 minutes they were gradually beginning to take slow swimming movements in different directions. On 8-10th minute the movement and orientation in space normalized, but juvenile fishes did not always respond to a knock on the aquarium and the changing of the light conditions. At the same time, the juvenile fishes, remained in clear water, clearly respond to a knock and changing of the light conditions. Our observations showed that the behavioral reactions of the hatchery juvenile fishes transplanted from clear to muddy water normalized only after a day.

Considering such changing of the behavioral reactions of the hatchery juvenile fishes in muddy water, it was important to study the intensity of feeding of the hatchery and wild juvenile fishes in muddy and clear water.

Our research showed that the intensity of feeding of both the hatchery and wild juvenile fishes in a fact decreased during the changing of conditions of water turbidity in comparison with the conditions from which they were transplanted (Table 3).

**Table 3:** The intensity of feeding of the wild* and hatchery-reared juvenile fishes of sturgeon in clear and muddy water

| Age, days | Length, mm | The average amount of food eaten (mg) in 2 hours by 5 juvenile fishes of sturgeon (experiments were carried out 3 times) | | | |
|---|---|---|---|---|---|
| | | Clear water | | Absolutely muddy water | |
| | | Wild fish | Hatchery-reared fish | Wild fish | Hatchery-reared fish |
| 18-20 | 2.8-3.0 | 14.6 ± 0.88 | 25.3 ±0.42 | 34.6 ± 0.42 | 4.0 ± 0.11 |
| 28-35 | 4.4-6.0 | 276.3 ± 2.18 | 291.0 ± 3.04 | 302 ± 1.06 | 14.3 ± 1.33 |
| 50-55 | 6.2-6.8 | 312.6 ± 3.16 | 424.3 ± 1.43 | 545 ± 1.99 | 18.6 ± 4.05 |

* After the catching from the river wild juvenile fishes were transplanted into the pools with muddy water and after 1 day were taken to the experiment.

However, during the transplanting of the hatchery juvenile fishes from clear to muddy water, the feeding intensity decreases very rapidly in comparison with the wild juvenile fishes in the same conditions. Perhaps, such difference is associated with the fact that from time to time in a river the wild juvenile fishes contact with decreased turbidity of water in comparison with to the hatchery ones, which from the first day until the release from the hatchery are grown only in clear water. Therefore, the conditions of muddy water for the hatchery juvenile fishes are a stronger stimulus than clear water for wild juvenile fishes.

The results have led us to believe that the hatchery juvenile fishes, getting into the muddy river water, may starve for a long time and die due to the braking of food-procuring activity. Therefore, it was important to investigate the resistance of the hatchery juvenile fishes to starvation. In addition, it was necessary to identify adaptive capabilities of uneven-aged juvenile fishes of wild and hatchery generations to absolute starvation.

Three repeated experiments showed that all the individuals of both generations at the age of 20 days died 11-12 days after absolute starvation (Pic. 3). The resistance of juvenile fishes of wild and hatchery generations to starvation increases with advancing age, i.e. adultery juvenile fishes (35-50 days) lives for a longer time without food in comparison with younger (20 days) individuals. At the age of 35 days even-aged individuals of the wild juvenile fishes of sturgeon are more resistant to starvation than hatchery ones (Pic. 3).

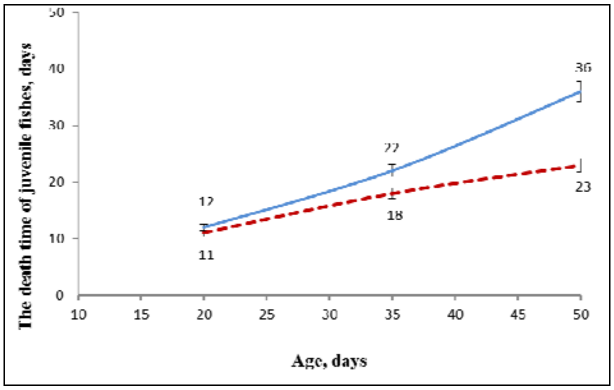
It should be noted that the even-aged wild juvenile fishes are less well-fed in comparison with the hatchery ones, but they are able to starve for a longer time after the complete cessation of food.

Thus, our studies showed that during the release from the piscicultural ponds into the natural environment - the river the juvenile fishes of sturgeon grown in the hatchery differ from the one-dimensional wild juvenile fishes caught from the river in the degree of development of the behavioral reactions.

The fact is that the information content of the hatchery is different from the information content of the natural habitat of sturgeons.

During the hatchery reproduction of sturgeons, unfortunately, the biological characteristics of the species were not taken into account as well as the directed influence of environmental conditions on the formation of the most important functions of the organism and also on the behavioral reactions. It should be noted that the level of structural and functional development of the central nervous system is largely defined by the intensity of sensory stimulation of animals during its formation [3, 22, 23, 25].

In this regard, it was important to identify the sensitive periods of early ontogenesis in the formation and establishment of behavioral reactions of juvenile fishes of sturgeon.




**Picture 3:** The averages of death time of the wild ▬▬ and hatchery juvenile fishes ---- of sturgeon at different ages after complete cessation of food

Our earlier studies showed that the beginning the formation of behavioral reactions of juvenile fishes of sturgeons was observed at the age of 18-22 days and ended at the age of 35-45 days. At that age juvenile fishes manage to develop strong food and protective behavioral responses [14, 15, 26].

The comparative study of behavioral reactions of uneven-aged juvenile fishes of wild and hatchery sturgeons showed that there were no significant differences in the nature of manifestation of food-procuring and protective behavioral responses at the age of 18-25 days. Apparently, after the hatching until the age of 18-25 days the deterministic - innate behavioral acts play a dominant role in adaptive responses of individuals. In later ages the environmental conditions play an important role in the formation of both neuronal structures of the central nervous system and the most important behavioral reactions.

In more sensitive periods (age of 25-55 days) the formation of the most important functions of the organism is strongly influenced by genetically inherent and external environmental factors. Among the latter we should note such environment parameters as temperature, salinity, illuminance, oxygen content, pH of environment, stern organisms, enemies of farmed fish etc.

The comparative studies of food-procuring and protective behavioral responses of hatchery and wild juvenile fishes of sturgeon in these sensitive stages of development showed that the formation and manifestation of these responses differ significantly from each other in the period of ontogenesis.

Juvenile fishes grown in the hatchery without much effort find sufficient food in their habitat during the period of morpho-functional formation and development of the most important functions of organism under the condition of absence of a predatory fishes.

In the same period the juvenile fishes taken from the river still find food despite the fact that there are many different enemies in their habitat. In other words, the hatchery and natural conditions differ sharply in enrichment of sensory stimuli.

Probably, that is why these reactions are less pronounced in the hatchery juvenile fishes in comparison with the wild ones.

Previously, it was shown that the raising of juvenile fishes in the sensory-depleted environment led to the slowdown in the growth of the brain, the decreasing the intensity of the synthesis of DNA/RNA in the neurons, the deterioration of the CNS adaptive properties being evident in the change of development rate of a series of conditional reflexes and in the ability to preserve acquired skills [3, 13, 14, 24].

Our data showed that for better adaptation of the hatchery juvenile fishes for river conditions, it was necessary to release them at the age of 28-35 days, when morpho-functional responses of these fishes begin to take shape. This contributes towards the formation of these functions in accordance with the conditions of their habitat what will provide them with better survival and adaptation to natural conditions.

The raising of juvenile fishes of sturgeons in the hatchery for a longer time (70 days or more) leads to the formation of the type of reactions which ensure their survival in the hatchery and in the conditions of their raising in aquaculture. In those conditions individuals are more well-fed, grow better and have high survival rates due to the better food supply and the absence of predators. However, the juvenile fishes, getting into the natural conditions with a tough selection and wide informative, will have a great loss and will adapt to these conditions with difficulty. Even more well-fed hatchery juvenile fishes compared with wild ones will die sooner than the natural ones under the condition of the absence of feed.

Probably, when food is enough the metabolism of the hatchery juvenile fishes is more accelerated in comparison with wild ones, which find food with difficulty. The metabolism of the wild juvenile fishes forms more efficiently and is used in accordance with the conditions of their habitat.

It was shown that [28] the biochemical indicators of caviar of the wild Black sea perches and hatchery ones are very different.

The significant differences in the level of highly unsaturated fatty acids in muscles, liver, ovaries and eggs of the wild and domestication silver eels were also found by Japanese scientists [27].

It should be noted that the difference in various indicators between the wild and hatchery individuals of different species of fish are noted in a number of other works [2, 30-32].

Our studies showed that during the raising of sturgeons of particular region in the hatchery, their biological characteristics of development in natural conditions and the identification of critical periods of the formation of the most important physiological functions should be taken into account and thus to define the role of environmental factors in the formation of these functions.

Considering this, it is necessary to identify the age periods, when the formation of the morphological and functional indicators begins, which ensure their survival in specific habitat conditions.

We showed that the age period of the Persian (Kura) sturgeon between 28-45 days is more sensitive, because in this period there is the formation of the most important functions of their organisms. In this period of ontogenesis an important role is played by the habitat in the formation and consolidation of these functions, as if they are raised in hatchery, in a more scanty environment (absence of a predator, the monotony of forage organisms and others.), the appropriate morphological and physiological indicators form, which allow them to thrive in these conditions. It is very difficult for such juvenile fishes, getting into natural conditions (differ from the hatchery ones in informativeness), to adapt to these conditions and easily fall as a victim of a predator.

Considering the above, we recommend to release juvenile fishes out of the Kura sturgeon hatcheries into the river from the age of 28-30 days (the body weight of not less than 1 gr.), when the formation of the morphological and functional characteristics begins, to ensure the further formation of these functions in accordance with conditions of their habitat. It ensures their better adaptation to natural conditions and improving the efficiency of the breeding of sturgeons in the Kura-Caspian region.

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