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APPLICATION OF NEW METHOD OF ARTIFICIAL INCUBATION OF SEVAN TROUT (*SALMO ISCHCHAN*, KESSLER) EGGS IN NATURAL CONDITION OF TRIBUTARIES OF LAKE SEVAN[1]

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Abstract. Endemic fish species of Armenian ichthyofauna – the Sevan trout (*Salmo ischchan,* Kessler, 1877) is registered in the Red Book of Animals of the Republic of Armenia as critically endangered (corresponds to IUCN category: CR A2cd). Its natural reproduction hardly occurs as a result of numerous problems related to the use and management of water and bio-resources in the drainage basin of Lake Sevan. The Lichq River is formerly known as a spawning river for two ecological races of Sevan trout, but because of different factors there are no more wild populations of Sevan trout spawning. The aim of the study was checking the efficiency of two methods of egg incubation in the Lichq River for restoration of Gegarkuni population. The experiments were carried out with newly fertilized and eyed eggs of Gegarkuni in the same periods and in the same areas to avoid the unforeseen effects of environmental factors on assessment. The results showed that the mortality rate of green eggs in the natural conditions is quite high before reaching eyed egg stage (65-68%), while the mortality rate of eyed eggs planted in the natural conditions is only 9-17%. This means that the effectiveness of planting eyed eggs is higher for the artificial restoration of Gegarkuni stocks in the nature.

Key words: Sevan trout, Gegarkuni, egg incubation, Lake Sevan.

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Introduction

As known, endemic Sevan trout (*Salmo ischchan* Kessler, 1877) is one of the most economically and culturally valuable representative of Armenian ichthyofauna. Being one of the symbols of Armenia, however, it's endangered nowadays due to mismanagement of Lake Sevan water and bio-resources and anthropogenic impact on the fish habitat.

Originally, there were 4 races of Sevan trout: Summer trout (ishkhan) or Summer bakhtak (*Salmo ischchan aestivalis*, Fortunatov), Gegarkuni (*Salmo ischchan Gegarkuni*, Kessler), Winter trout (*Salmo ischchan ischchan*, Kessler) and Bojak (*Salmo ischchan danilewskii*, Iakowlev) that differ by their reproduction ecology. Even before 1930's, when neither Lake Sevan nor Sevan trout stocks had faced the current problems, these races were isolated by spawning areas. According to Fortunatov, lacustrine forms (Winter trout and Bojak trout) usually did not migrate to rivers for spawning [1]. Because the lake water level periodically decreased in the period from 1930s to 2000s, many clues in the shore zone of Lake Sevan became dry and races of Sevan trout, which spawn in the littoral zone of the lake, lost their spawning areas. According to Pavlov, part of Winter trout shoal started to migrate to the rivers of Lake Sevan basin for spawning [2]. The area appropriate for spawning of Bojak has also reduced dramatically and spawning kept going only in the deeper parts of the lake (up to 35m deep) in January-March [2]. According to Smoley, spawning areas of Summer trout in the littoral zone

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dried out and their spawning migration to the rivers became significantly harder due to poaching in the river mouths [3]. Thereby, as such processes continue, unfortunately Winter trout and Bojak trout have been completely extinct. Two other races of Sevan trout: Summer trout and Gegarkuni, which spawn in Lake Sevan basin rivers, have survived, but have become rare [4] and as a result they were registered in the Red Data Book of Armenian Animals [5] as "Critically Endangered" species (IUCN category: CR A2cd). During the last decade several studies have been implemented to reveal the current state of Sevan trout ecology and biology due to Lake Sevan water level rise [6-12]. Activities for rehabilitation of Lake Sevan ecosystem were launched parallelly in 2001. Since that, the Government of Armenia has been realizing the programme of releasing artificially grown in hatcheries Sevan trout fries into the lake aiming at preventing complete extinction of the species. However, studies carried out by us in the last several years have shown that this programme doesn't ensure any significant result as natural reproduction of Sevan trout is hardly occurs [12], and their survival is still supported by the periodical replenishment of trout resources.

Gegarkuni spawning habits are well known. It prefers the temperatures from +5–+8°C. Thus, the optimal season begins at late October and lasts till February) [6]. The incubation of eggs lasts about 2-3 months. Gegarkuni create redds mainly of pure gravel, pebble and cobble at the optimal depths from 15 to 50 cm and optimal water velocity from 0.15-0.55 m/s [13, 14].

As known, egg planting is one of the common methods for increasing salmonid densities [15]. This is often used in rivers with a very small spawning stock, when opening new river stretches for salmonids and when restoring degraded habitats. Eggs are placed in incubating boxes buried in the gravel bed in river or freely in the bottom substratum, imitating a natural redd [15]. Newly fertilized eggs and eyed eggs are the two developmental stages typically used:

1) newly fertilized eggs (green eggs) are planted between 24 and 48 h after fertilization and water hardening. After that (but before the eyed stage), the eggs are very sensitive to handling stress and are easily killed if move;

2) eyed eggs are robust and tolerate substantial handling and are often used for planting [16]. Thus, the aim of the study was to reveal the efficiency of both methods of egg planting for restoration of Gegarkuni natural population on example of Lichq River.

Study Area. The Lichq River is one the most saturated riverlike clues of Armenia and intakes into Big Sevan in the south-west part. Its length is 8km and the drainage basin area is 34 km^2 (Fig.).

Satellite image of the Lich River basin and distribution of sites for the experiments
(Source: Google Earth Pro)

Lichq has a flat valley and very stable flow regimen during the whole year with the mean discharge of 2.21 m^3/s. Groundwater composed the 96% of its feeding. Water abstracted from the river mainly goes for irrigation purposes [17]. Currently about 5 200 dwellers live in the basin of Lichq River [18] and leaving their ecological footprint by using and consuming different provisioning and regulative ecosystem services such as agricultural and domestic water use, water quality which forms due to filtration, decomposition of organic wastes and pollutants in water, and the assimilation and detoxification of compounds [19].

Material and methods

Taking into account complexity of the aim, several two days long field trips were carried out to the basins of the Lichq River during November of 2018 and February of 2019, which coincides with the most optimal spawning period of Gegarkuni in the rivers. Whitlock-Vibert boxes were used for the experiments.

The monitoring activities for the hydro-physical and hydro-chemical optimal parameters to launch the experiment were started from the November of 2018. The installation of the first two Whitlock-Vibert boxes with newly fertilized eggs of Gegarkuni was carried out at December 15th of 2018. The place was chosen at the middle course part of the river due to initial assessment of the grounds and other hydro-physical and hydro-chemical parameters necessary for natural spawning of Gegarkuni [4]. The results of that study showed that the water in the upper course part of the river is nearly stagnant which is not favorable for the chosen method of incubation. Thus, we have predicted higher mortality rate of eggs planted from the earliest stage and consequently launch the first experiment only in the middle course part. Eggs were fertilized in one of the fish farms nearby the Lichq River and immediately transported to the area of experiment in the special refrigerator to avoid any harm to eggs. Number of fertilized eggs put into the boxes was revealed by mass method. The average mass of one green egg of Gegarkuni was 0.065 g. During the first experience, two Whitlock-Vibert boxes with totally 600 green eggs of Gegarkuni were installed into the middle course part of the Lichq River.

The second campaign was carried out on January 19, 2019 aiming at installing the Whitlock-Vibert boxes with already eyed eggs in the upper course part of the Lichq River nearby the source of the river. As there is relatively short period of time remained for fry release after this stage and the resistance of eggs is higher in eyed stage of development, the risk of mortality of eggs due to low velocity is minimal. Number of eyed eggs was also revealed by mass method. The average mass of a single eyed egg of Gegarkuni was 0.068 g. So, totally 2 280 eggs were planted by six Whitlock-Vibert boxes in Lichq River.

The transportation of eyed eggs was also performed by the special refrigerator to avoid mortality of eggs. Eyed eggs were separated by 6 Whitlock-Vibert boxes in the farm and transported under the 6°C of temperature. Each box was filled with 350-450 eggs.

During the installation, to protect the boxes, metal net was used and externally stabilized in the water by cobbles and boulders. These constructions were installed in the parts where water column depth was from 50 to 60 cm.

In order to assess the efficiency of both planting methods, weekly based monitoring of biological state and hydro-physical and hydro-chemical parameters was performed in the Lichq River. In particular, the mortality rate of eggs was measured during each visit by counting the number of died eggs. the measurements of temperature and pH level at each site were done by Hanna HI9813-5N pH/EC/TDS meter and of dissolved oxygen (DO) and Oxygen Saturation (%) by Hanna HI9147-10 DO meter. Temperature was measured twice per day manually: in early morning and late afternoon in order to reveal the average value from diurnal range.

Descriptive statistics for collected data was performed by Statistica 8 package. Spatial data was collected in situ by Garmin eTrex20 GPS receiver and then processed in Google Earth pro software.

Results and discussions

Weekly based monitoring of hydro-physical and hydro-chemical parameters has shown that the most optimal period for launching the first experiment was at the middle of December, when oxygen saturation parameter was regularly exceeding the 100% parameter. In the period of beginning of the experiment, the average water temperature at the middle course part of the Lichq River was 7.2°C which also corresponds to the most preferable conditions for this race of Sevan trout (Table 1).

Table 1

Measured average monthly physical-chemical parameters of the experiment areas

Sampling site	Average Dissolved Oxygen, mg/l	Average Oxygen saturation, %	Average temperature, °C	Average value of pH
November				
Lichq middle-course	10.35	94.0	+7.3	7.25
Lichq upper-course	9.96	92.0	+7.3	7.17
December				
Lichq upper-course	12.30	104.3	+7.1	7.23
Lichq middle-course	12.51	104.7	+7.2	7.30
January				
Lichq upper-course	11.40	100.1	+7.1	7.40
Lichq middle-course	12.70	105.0	+7.2	7.10
February				
Lichq upper-course	10.58	96.0	+7.2	7.36
Lichq middle-course	10.24	98.0	+7.2	7.25

The results of biologic observation of mortality rates showed that the total mortality rate of eggs in the green stage was 66.3%. From the eyed stage to fry release the mortality rate was decreased more than three times and composed only 17.82%. Usually some mortality has to be observed also between yolk-sac and sac-fry periods, but we have not observed any during both experiments. Probably this is because Gammaridae crustaceans (gen. Gammarus) have fed by died yolk-sacs. Thus, the effectiveness of experiment with green eggs in the Lichq River was assessed by us to only 27.67% (Table 2).

Table 2

Mortality rates of just fertilized Gegarkuni eggs of Lichq River

Incubator box	Mortality, %	
	Before eyed period	From eyed to fry period
1	67.97	17.34
2	65.08	19.00

Compared with the literature data provided for the fish farms where similar experiments were done [20] this rate is very low. In particular, the success rate of planted in the fish farms green eggs of Gegarkuni was 92-95%. Considering the hydro-physical and hydro-chemical parameters of the Lichq River which during the dozens of measurements not fluctuated significantly, such high rates of mortality in the nature could be also explained by some mistakes done during the experiments. The second reason could be the state of eggs fertilized in the factory as the milking peaks were in November and we have used the last portions of milked eggs in December.

The experiment with the eyed eggs incubation launched in January and was successfully finished in February, 2019. The rates of success of the second experiment are as following (Table 3).

Table 3

Mortality rates of "eyed" eggs from the Lichq River

Incubator	Mortality rate, %
1	15.96
2	14.89
3	8.76
4	10.28
5	16.54
6	11.43

Totally 325 eyed eggs didn't reach the sac fry period, so, consequently the remained 1 955 have survived as a result of experience in the Lichq River. Not less important is the fact that during our experiment the overall mortality rate of Gegarkuni eggs was 14.25%.

Literature data also considers variable data on the survival of planting eggs, and experiments exhibit no systematic difference in survival between eggs planted in boxes or placed directly in the gravel or if they are buried as green or "eyed" eggs [21]. However, planting of eyed eggs is often preferred since it allows for proper veterinary health control of the spawners prior to the planting. When using eyed eggs, there is a less-strict time constraint on the egg release.

Conclusions

Carried experiments are shown that the mortality rate of green eggs planted in natural conditions till the eyed egg stage is quite high and reaching 65-68% while the mortality rate of eyed eggs planted in natural conditions is only 9-17%. The average mortality measured separately from each of six boxes was 12.98 ± 3.25%. Green eggs are more sensitive towards environmental conditions, it also contributes to higher mortality rates. Meanwhile, the eyed eggs are more resistant and the mortality rate was low.

This is allowed to have a conclusion that the incubation of eyed eggs in natural conditions could ensure better results for artificial replenishment of natural stocks than the incubation of green eggs.

The results of incubation of Sevan trout eggs by Whitlock-Vibert Boxes were positive, thus, in order to restore its natural population this method can be proposed for implementation in the other spawning rivers of the drainage basin of Lake Sevan, which currently have appropriate conditions for the eggs growth.

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ПРИМЕНЕНИЕ НОВОГО МЕТОДА ИСКУССТВЕННОЙ ИНКУБАЦИИ ИКРИНОК СЕВАНСКОЙ ФОРЕЛИ (*SALMO ISCHCHAN*, KESSLER) В ЕСТЕСТВЕННЫХ УСЛОВИЯХ ПРИТОКОВ ОЗ. СЕВАН (АРМЕНИЯ)

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Эндемичный вид ихтиофауны Армении – севанская форель – был занесен в Красную книгу Армении как исчезающий вид (категория IUCN: CR A2cd). Естественное воспроизводство форелей практически не происходит из-за многочисленных проблем, связанных с управлением водными и биоресурсами водосборного бассейна оз. Севан. Река Личк являлась нерестовой для двух экологических рас севанской форели, но в настоящее время дикая нерестовая популяция в этой реке не встречается ввиду различных причин. Целью исследования являлось тестирование эффективности двух методов инкубации икры форели в реке Личк для восстановления дикой популяции одной из рас – гегаркуни. Эксперименты проводились как с оплодотворенными икринками гегаркуни, так и с икринками на стадии «глазка» в одни и те же периоды и на схожих участках, что обеспечивает схожесть факторов окружающей среды во время оценки. Из результатов экспериментов следует, что уровень смертности зеленых икринок в естественных условиях до достижения стадии «глазка» достаточно высок (65–68 %), в то время как уровень смертности икры на стадии «глазка» составлял всего 9–17 %. Это означает, что при искусственном восстановлении запасов гегаркуни в естественных условиях эффективность инкубации икры на стадии «глазка» выше.

Ключевые слова: севанская форель, гегаркуни, инкубация икры, озеро Севан.

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