

Detailed Scientific Report

"Testing of Experimental Methods for Restoration of Natural Population of Endemic Fish Species of Lake Sevan - Sevan Trout"



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INTRODUCTION

As known, endemic species Sevan trout (ishkhan) is one of the most economically and culturally valuable fish species 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 as well as anthropogenic impact on their 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, Kessler) and Bojak (Salmo ischchan danilewskii, Iakowlev), which are also differed by their reproduction ecology. Even before 1930's, where 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) usually not migrated to rivers for spawning (Fortunatov, 1927). Because lake's water level periodically decreased from 1930's up to 2000's, 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. 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 (Pavlov, 1951). According to Smoley, spawning areas of Summer trout in the littoral zone dried out and their spawning migration to the rivers became significantly harder due to poaching in the river mouths (Smoley, 1968). Thereby, because such processes continue, unfortunately Winter trout and Bojak 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 (Asatryan et al., 2016) and as a result they were registered in the Red Data Book of Armenian Animals (Government decree, 2010) 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 (Gabrielyan, 2010; Pipoyan & Malkhasyan, 2014; Lake Sevan, 2016; Barseghyan & Vardanyan, 2015; Barseghyan et al., 2011; Barseghyan et al., 2014; Barseghyan et al. 2016). Activities for rehabilitation of Lake Sevan ecosystem were launched parallelly in 2001. So, since that, the Government of Armenia realizes 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 has shown that this programme doesn't ensure any significant result as natural reproduction of Sevan trout is hardly occurs (Barseghyan et al., 2016), and their survival is still supported by the periodical replenishment of trout resources. Thus, the aim of the project was to reveal the efficiency of different approaches to restoration of Sevan trout natural population through incubation of eggs in the spawning rivers. The project was based on past studies on revealing the state of spawning sites of Summer trout and Gegarkuni.

Project Site. Lake Sevan is one the biggest (1260sqr.km) high mountain lakes (1900m a.s.l.) in the world and the biggest reservoir of freshwater (36billion cub.m.) in South Caucasus region. It is located in the eastern part of central Armenia (Gegarkuniq province). Due to its unique ecosystem and number of habitats Lake Sevan and its tributaries are included in the territory of Lake Sevan National Park. Tributaries are providing the spawning ground for endemic fish species of the lake as well as different provisioning and regulative ecosystem services such as agricultural and domestic water use, fish, water quality etc. Project site is located in the southern part of Lake Sevan and involves two of main tributaries - Rivers Lichq and Masrik. The objects of case study – rivers Lichq and Masrik are located in South-West and South-East parts of Lake Sevan and both flows into Big Sevan (fig. 1). The River Masrik originates from 2880 meter above sea level from the eastern part of the northern slopes of Vardenis range, which consists of mainly igneous rocks and Eastern Sevan mountain range which consists of sedimentary rocks, that's why physicochemical parameters of different tributaries are slightly different. Masrik is one of the largest rivers of Lake Sevan basin (45 km). Drainage basin of Masrik river is the biggest (685 km²) among all 28 rivers in flowing into the lake. It's carrying the impact of Vardenis city and 14 villages situated in the middle and downstream parts of Masrik River and its tributaries. Water resources of the river are mainly serving for hydropower generation and irrigation, but the river also exposed to various anthropogenic pressures like gold mining, sewage and agricultural wastewater discharge. River has V-shape valley in the upper stream part and flat floored valley in the middle and downstream parts which influences on quantity of physical load and hydraulic radius. Due to wide spreading of permeable rocks in the basin precipitations filling the resources of groundwater and many clues feed r. Masrik, which is very important for natural reproduction of Sevan trout. Natural flow is relatively stable during the year and this is also the advantage for the river as spawning area for Sevan trout (Ecology, 2010). River Lichq is one the most saturated river like clues of Armenia and intakes into Big Sevan in the south-west part. Its length is 8km and the drainage basin area is 34sqr.km. Lichq has a flat form valley and very

stable flow regimen during the whole year with mean discharge of 2.21cub.m/sec. Groundwater composed the 96% of its feeding. Water abstracted from the river mainly goes for irrigation purposes (Mnatsakanyan, 2007). Currently about 5200 dwellers live in the basin of Lichq river (National, 2018) 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 (Ecosystems, 2005).

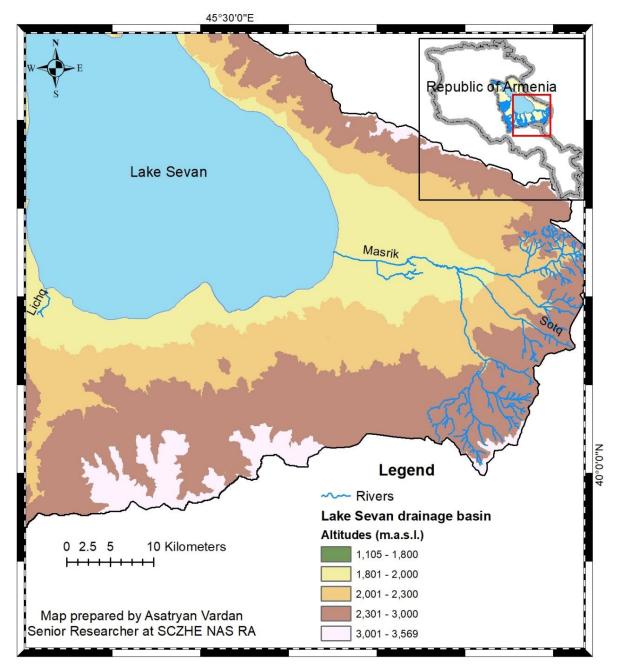


Fig. 1 Location of Lichq and Masrik rivers

The following **goals** were proposed to:

- To reveal the most effective methods for incubation of Sevan trout eggs in natural conditions and restoration of wild stocks of this fish by research and experimental methods;
- To reveal the features of Sevan trout egg development stages dependent on different natural conditions of the rivers;
- To reveal the efficiency of incubation dependent on the differences in bio-conditions and methods for chosen parts of the rivers Masrik and Lichq;
- To develop the most efficient methodology for artificial breeding and further growth of alevins in natural conditions;
- To reach the homing reflex towards the rivers of origin by incubation of Sevan trout eggs in natural conditions;
- To raise the public awareness to the problem of Sevan trout conservation and restoration of its natural population through publications and dissemination activities.

MATERIAL AND METHODS

Taking into account complexity of the aim of the study and the variety of objectives, several two days long field trips were carried out to the basins of rivers Lichq and Masrik during the periods of natural spawning of Summer trout and Gegarkuni at 2018 and 2019.

The following activities was carried out during the field campaigns:

- 1. Catch of mature individuals of Sevan trout migrating for spawning
- 2. The incubation of fertilized eggs by different methods in the chosen parts (habitats) of rivers Masrik and Lichq through the installation of Whitlock-Vibert boxes (WVB) of different constructions in the most appropriate for spawning areas as well as by creation of artificial nests similar to natural nests of Sevan trout from the gravel and cobble
- 3. Artificial incubation of "eyed" eggs.
- 4. Monitoring of the biological state of eggs installed in the rivers as well as of physicchemical, hydrological parameters and water quality.

The next group of activities like laboratory processing of material and data as well as dissemination activities were implemented in the Institute of Hydroecology and Ichthyology of SCZHE NAS RA and local villages.

Hydrophysical, -chemical, -biological studies. Measurements of temperature, pH and dissolved oxygen (DO) have been done by Hanna HI9813-5N pH/EC/TDS and Hanna HI9147-10 DO meters, respectively (fig. 2).

Fig. 2 Hydrochemical measurements in River Masrik



Besides primary data collected, long-year secondary data of Masrik river water temperature (Ecology, 2010) has been used for the comparisons. Presence of smell and foam on the water has been observed empirically across 100m long stretches at each sampling site. Several hydro-morphological parameters like channel form, level of direct morphological alteration (%), presence of natural riparian vegetation, shading level (0-25%; 25-50%; 50-75%; 75-100%) and presence of lurking for the fish have been observed empirically. For the remaining hydro-morphological measurements typical transects across the width of the river sections have been chosen. Width of the stream parts have been measured by preliminary marked rope. Systematic measurements of depths for the interval of 50cm across transects have been carried out using metre stick. Velocity in each sampling site has been measured using float for the 10m transect along river course by 3 replications. Two measurements have been done near the right and left banks accordingly and one measurement at the central part. In case of presence of both riffle and pool parts, the measurements have been done for both of them. The average velocity of the stream part has been calculated by arithmetic mean of all measurements in the stretch. Ruler has been used to measure the substrate components. For more accurate assessment of ground type's distribution, sampling of substratum has been carried out randomly. Ground type has been determined according to Wentworth classification (tab. 1).

Ground type	Size (mm)
Boulder	>256
Cobble	64-256
Pebble	4-64
Gravel (granule)	2-4
Sand	1/16-2
Silt	1/256-1/16
Clay	<1/256

Table 1. Classification of ground types by Wentworth grain size scale

According to EU WFD (2000/60/EC) benthic macroinvertebrates are one of the four groups of hydrobionts proposed to use as bioindicators for rivers. Taking into consideration that benthic macroinvertebrates are sensitive to different consequences of land use like eutrophication, worsening of habitat conditions due to sedimentation and growing of toxicity due to using of pesticides, different studies shows that for small mountainous rivers they become the most

valuable and broadly used indicators of water quality when long time-scale influences is obvious (Springe et al., 2006; De Pauw et al., 1993; Johnson et al., 2006). Within field campaigns the multi-habitat sampling principles of macrozoobenthos were carried out (Manual, 2002). Surber sampler with a frame of 0.3x0.3m was used (Nitex, 500µm) for benthic macroinvertebrates collection. Sampling of macrozoobenthos in each sampling site was done by 5 to 10 "replications" dependent on the width and depth of the river and diversity of habitats (fig. 3). The content was placed into containers clearly marked with the sampling site's geographical coordinates. Samples were fixed by 70% solution of ethanol and further processing of samples was realized in the laboratory. Samples were separated from substratum and determined up to family level using different keys (Waringer & Graf, 2011; Elliott et al., 2010; Taxonomie, 2010) under the AmScope 20x-40x-80x binocular stereo dissecting microscope with 2MP USB camera.



Fig.3 Fish catching and sampling of benthic macroinvertebrates in Masrik River Assessment of water quality was done by BMWP (Biological Monitoring Working Party) and ASPT (Average Score per Taxon) indices (Semenchenko & Razluckiy, 2010), which are based on diversity of benthic macroinvertebrates families and their tolerance to pollution. BMWP and ASPT are calculating by the following formulas:

$$BMWP = \sum_{i=1}^{n} T_{i} \qquad ASPT = \frac{BMWP}{N_{taxa}}$$
(1) (2)

where T_i is a tolerance score and N_{taxa} is the number of macrozoobenthos taxa in the sample Based on calculated scores, water quality interpreted as follows (tab. 2, 3).

Water quality	Excellent	Very good	Good	Not high	Poor
Score	>150	101-150	51-100	26-50	<26

Table 2. BMWP scores and water quality

Table 3. ASPT scores and water quality

Water	Excellent	Very	Good	Moderate	Rather	Poor	Very poor
quality		good			poor		
Score	>=5	4.5-4.9	4.1-4.4	3.6-4	3.1-3.5	2.1-3	0-2

For the calculation of feeding base for trout fries the number of representatives of each family of benthic macroinvertebrates and their mass were calculated as well as recalculation of the both parameters in 1 square metre was realized based on the number of replications done during the sampling (1 frame area equals to 0.1m^2).

Egg planting. Field trips in order to catch mature individuals of Sevan trout was launched in July of 2018 which is coincided with spawning season of Summer trout. Before Lake Sevan water level decrease the spawning of Summer trout was during April-June period when average water temperature was +8+10 °C. The main spawning grounds was located near the clues (Smoley, 1968). But, because of unusual dry and warm winter of 2017-2018 water temperature in July was above the average and probably the spawning of Summer trout if yet occur in this river has ends up before the first campaign. Thus, in July the experiments with planting of just fertilized eggs was based on only eggs fertilized in nearby fish farm. These eggs were planted using Whitlock-Vibert Box (fig.4):



Fig. 4 Whitlock-Vibert Box used in experiments

The boxes were buried in those parts of the rivers where ground is appropriate for the Summer trout. Particularly the areas of dominancy of pebble and cobble were chosen. The boxes were covered by metal net (fig. 5).



Fig. 5 Fertilized eggs in the boxes ready for planting

For the experiments in Lichq River the river source part was chosen where the clue is feeding the river and in Masrik River the boxes were installed in lower course part (fig. 6).

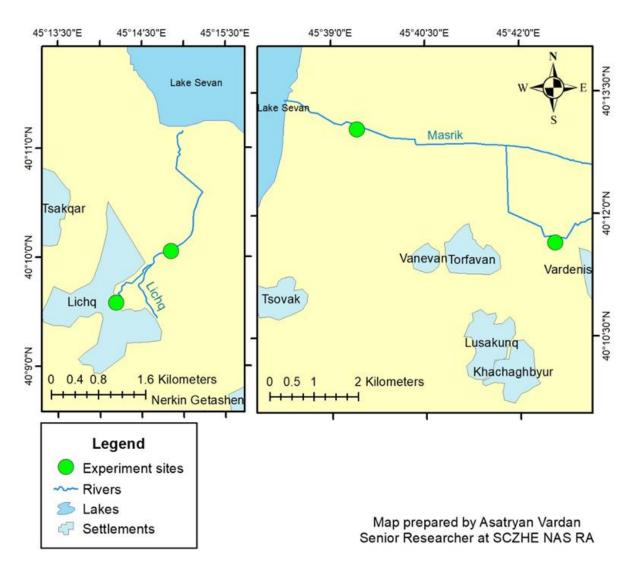


Fig. 6 Project sites for experiments

Because the eggs were gained from the farm, the amount of eggs was revealed by weight method. For example, the average weight of the egg of Summer trout in eyed stage was 0.068g. Totally 6125 eggs of Summer trout were planted in the rivers. This number is significantly more than we planned initially and the mostly provided by the Foundation for restoration of Sevan Trout Stocks and Development of Aquaculture. Transportation of fertilized eggs was organized by foam refrigerators (SARAB) by clearly following all the necessary procedures for that.

Eggs were planted by 16 boxes. As a result, 8 boxes were used in each of the rivers. Each box was filled with 350-450 eggs. To protect the boxes, the metal net was used and

externally stabilized in the water by cobbles and boulders. These constructions were installed in the parts where water column was from 50 to 60cm.

4 boxes were installed in artificial nests buried in pebble of 5 to 10cm depth, and with physical dimensions of 46-60cm x 20-30cm. 2 Boxes was installed in Lichq River and 2 boxes in Masrik River. Remained 12 Boxes were installed just in metal cases. To estimate the success of the experiments the monitoring activities were initiated as planned.

Since the December of 2018 the same activities were launched for Gegarkuni trout. 600 just fertilized eggs were planted only in Lichq River as the state of Masrik River was not appropriate for this action. In January the planting of eyed eggs was began in Lichq and Lusakunq tributary of Masrik River. 2280 eggs were planted by six boxes in Lichq River and 3125 eggs – by 8 boxes in Lusakunq tributary.

RESULTS AND DISCUSSIONS

Summer trout

The experiments with artificial incubation of Summer trout eggs were launched immediately after receiving the funding at July. It's been planned to start the experiments from the most possible early stage – caught and milking of mature individuals, in situ fertilization of eggs and planting. But, these group of experiments has been failed as no mature individual prepare for spawning was caught from lower course part of the river. Even though the literature data proposed that spawning of Summer trout begins in April and ends up in August, but the most significant parameter for determination of spawning period is the water temperature. The most preferable temperatures for Summer trout spawning ranges from +8 to $+10^{\circ}$ C (Savvaitova et al., 1989). Thus, we come to conclusion that if natural spawning of Summer trout yet occurs in the River Masrik, it was finished till the beginning of our works because the average temperature at the early stage of our works was 13.6°C and during the day even the temperatures of +19.8°C was registered. This can be due to unusual dry and warm winter of 2017-2018 and as a result rapid warming of river water at summer of 2018. One more experiment which success rate is hard to estimate was the incubation of eyed eggs of Summer trout in the artificial nest. Prepared nest in Masrik River was covered by fine sediments carried by the water within two weeks after installation which lead to the complete mortality of eggs. Such volume of fine particles in the period of installation of just fertilized eggs are not so common for that part of the river, that's why we can't state that this method has absolutely no potential for restoration of Sevan trout wild population in River Masrik. Thus, from installed in the nest boxes no egg was reached the stage of yolk-sac fry and mortality rate of the experience was measured as 100% (fig. 7).

The next group of experiences was regarding to installation of boxes with the eyed eggs. This method has been launched at the end of July and success rate of it were again 0, which allows us to conclude that artificial incubation of Summer trout in Masrik river mother bed can't be a rational method for restoration of wild stocks of this endemic fish. The main constraining factors revealed in the lower course part of the river for the season when eyed eggs were installed were high water temperature (in average $+15,5^{\circ}$ C), and water quality (annex 1, 2).



Fig. 7 One of the boxes with Summer trout eggs from drilled nest. As it seen all the eggs were died before reaching yolk-sac period

The same measurements for River Lichq have shown that the average temperature was 8.1° C and the murkiness was significantly lower.



Fig. 8 Milking of Sevan trout

During the monitoring activities in River Lichq lifeless eggs of Summer trout were regularly eliminated from the boxes and counted in order to reveal the success rate of experiment in different stages of development.



Fig. 9 Installation of fertilized eggs in River Lichq

The experiments were successful in River Lichq and during the final campaign only the last portion of lifeless eggs as well as the remained membranes were recorded in the boxes. The fries have leaved the boxes freely. One of the reasons why we hadn't revealed the fries after leaving the boxes could be the presence of macrophytes as lurking for fries and it were hard to find them in the "bushes". But another reason could be as well. The Gammaridae crustaceans could attack the fries as doing in the period of yolk-sac (fig. 10).



Fig. 10 Gammarids in the box

In Lichq river lifeless eggs were 407 from planted 3060 eyed eggs and consequently the remained 2653 fries have been released from the boxes successfully. The average mortality rate of Summer trout in natural conditions of Lichq River from two different parts was 13, 3 %: In metal nests the average mortality rate was 8, 59 %, and in artificial nests drilled in ground - 27, 50 %. From these figures we can conclude that the efficiency (success rate) of metallic nests was 91, 41%, and the efficiency of artificial nests drilled in the ground - 72, 50 % (tab. 4).

Table 4. Mortality rates	of Summer	trout eggs	in natural	conditions	of Lichq	River d	luring
different experiments							

Nest type	№ of incubator box	Mortality rate %
Nest from metal net	1	8.75
	2	5,53
	3	9,17

	4	5.12
	5	10.95
	6	5,03
Artificial net in the ground	7	25.03
	8	29.95

One of the reasons of difference between the efficiency of both experiments could be the amount of sediments transported by the water to the nests, and as a consequence the changes in oxygen conditions.

Gegarkuni trout

Because the same experiments were proposed for Gegarkuni trout, the first field trip has shown that the mother bad is inappropriate for the incubation of eggs in it due to high murkiness (Fig. 11) which probably comes from exploitation of Sotq gold mine. Thus, the decision to change the experiment area to a not impacted nearest tributary which satisfied the general demands by water quality was made. That's why all the experiments with Gegarkuni egg planting were made at Lusakunq tributary.



Fig 11. Masrik river mother bad during the period of Gegarkuni reproduction. Water is absolutely murky

2 boxes with just fertilized eggs of Gegarkuni were installed in Lichq River. In the period of beginning the experiment the average water temperature at Lichq River was 7.3°C.

The results showed that the mortality rate of eggs before reaching the eyed stage was 66.3% (fig. 12). From eyed period to fry release the mortality rate was only 17.82%. Usually some mortality has to be observed also between yolk-sac and sac-fry periods, but we have not observed any and probably this is because the gammarids have fed by died yolk-sacs. Thus, the effectiveness of experiment with just fertilized eggs in River Lichq was only 27, 67% (Tab. 5). We have included the scene of attack of gammarids to the boxes in created short video about the process of project implementation shared by social media (https://www.youtube.com/watch?v=-5atuprCiYM).

№ of incubator box	Mortality, %		
	Before eyed period	from eyed to fry period	
1	67.97	17,34	
2	65,08	19,00	

Table 5. Mortality rates of just fertilized Gegarkuni eggs of Lichq River



Fig. 12 The box with Gegarkuni eggs reached the eyed stage

The experiment with eyed eggs incubation launched in December was finished in January and the rates of success are as following (Tab. 6).

№ of incubator	Lichq river	Lusakunq tributary		
	Mortality rate, %			
1	15.96	19.42		
2	14.89	22.73		
3	8.76	18.14		
4	10.28	16.85		
5	16.54	17.36		
6	11.43	19.01		
7	-	17.37		
8	-	15.28		

Table 6. Mortality rates of eggs from Lichq and Masrik rivers

Totally 325 eyed eggs have not reached the sac fry period and remained 1955 have reached in Lichq river. In natural conditions, the average mortality rate of Gegarkuni eggs was 14.25 %:

In Lusakunq tributary the success rate was quite low. The number of lifeless eggs was 578 and remained 2547 was reached the fry stage. Thus, in natural conditions the average mortality rate of Gegarkuni trout in Lusakunq tributary was 18.50 %.

CONCLUSIONS

Based on the results of intensive experiments we can make a conclusion that the incubation of both races of Sevan trout - Summer trout and Gegarkuni is more efficient in the River Lichq compared with River Masrik and its Lusakunq tributary. As the experiments in river Masrik has failed or provide the 0 success rate the Lusakunq tributary has becoming the only appropriate area for further use of the methods tested but with some restrictions as the incubation of Summer trout has not been tested in that tributary. Also, the same results have shown that Lusakunq tributary has enough potential for natural reproduction of Gegarkuni in it.

It's been proven experimentally, that the process of artificial incubation of eggs in the rivers is mostly constrained by the water temperature and the amount of sediments transported by the rivers. The development of eggs of Summer trout was constrained in Masrik River by high average temperatures. It means that if it would be possible to regulate the reproduction in the farms to start the process of milking earlier as it happening in the natural conditions, maybe the experiments would have more success. Taking into account that Summer trout has preferred the lower course parts of the rivers for reproduction, in current state Masrik River is inappropriate for that purpose but some tributaries could still support the process.

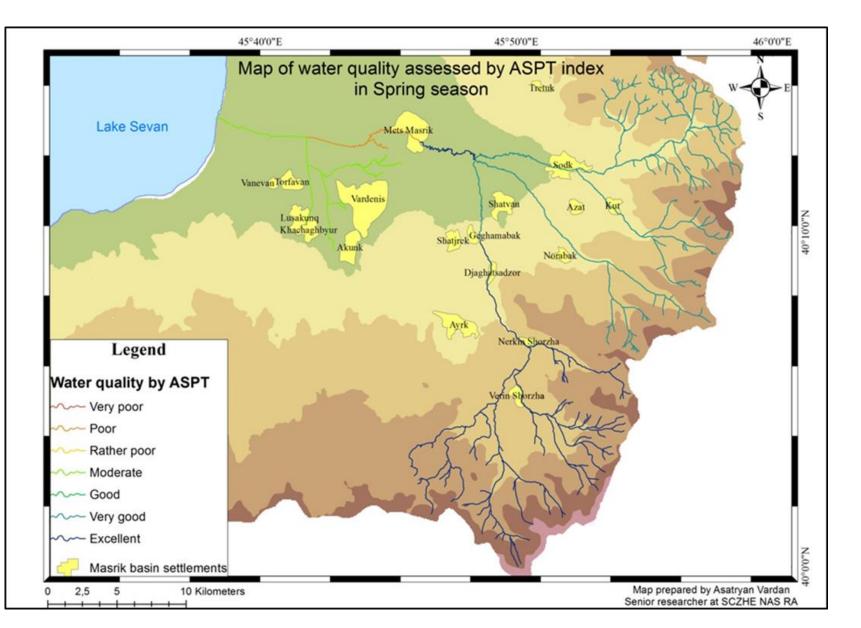
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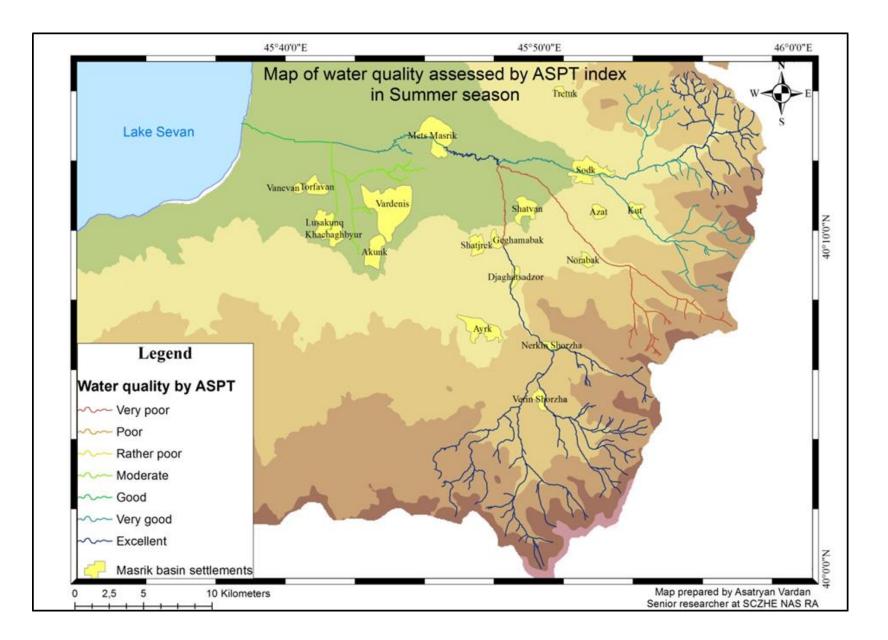
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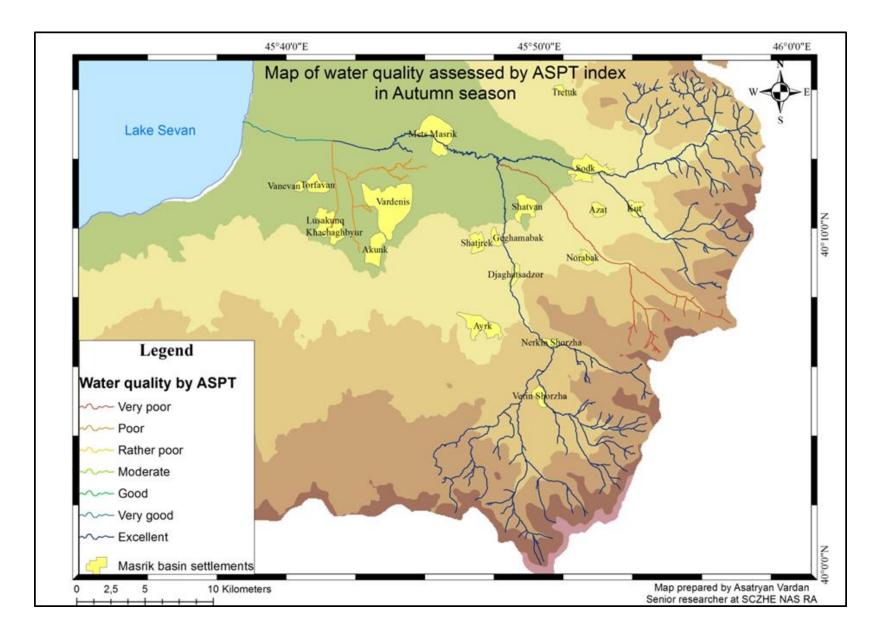
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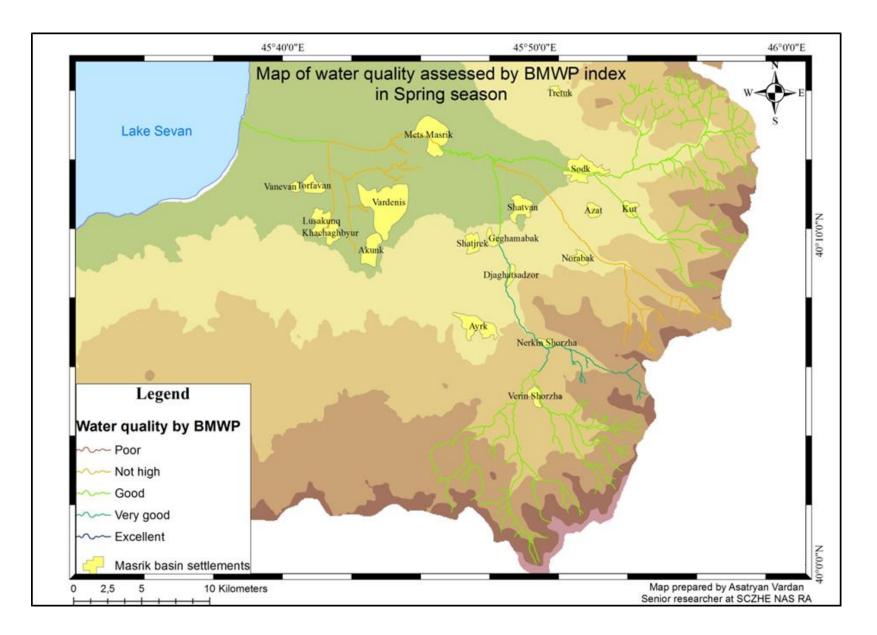
Maps of water quality of r. Masrik in different seasons

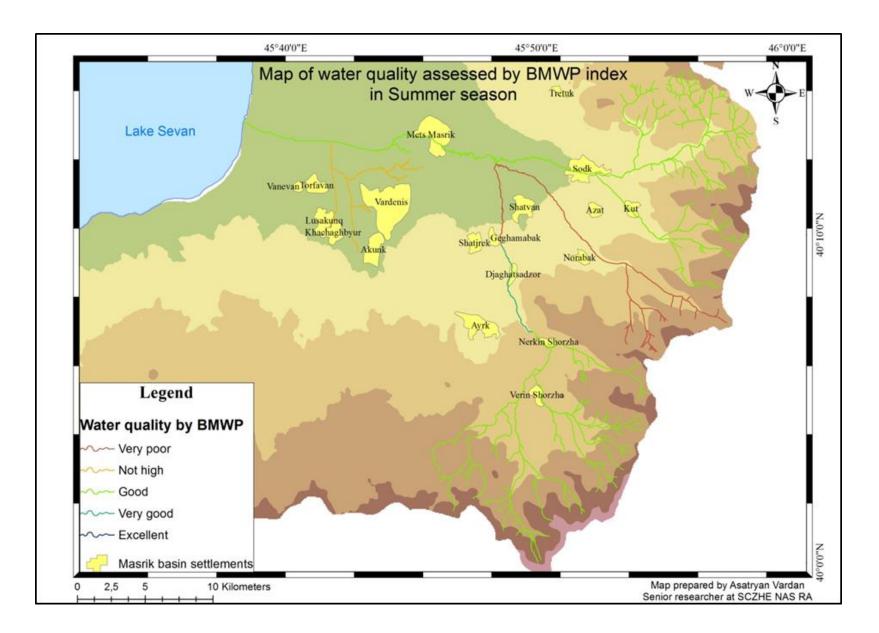


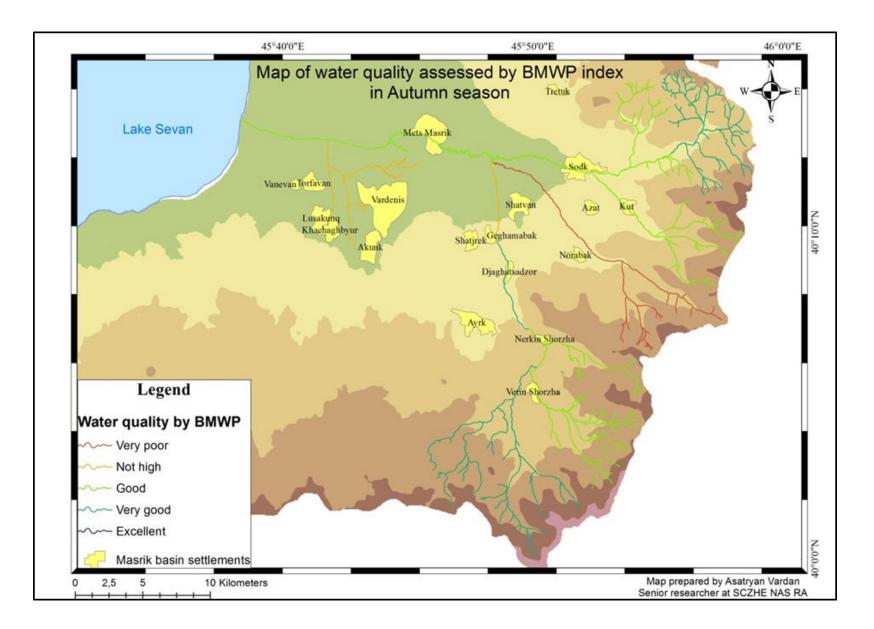
Annex 1











Sampling site	Average	Average	Average	Average		
	Dissolved	Oxygen	temperature	value of pH		
	Oxygen (mg/l)	saturation (%)	(^{0}C)	-		
		July				
Lichq middle-course	9.9	92	+8.2	7.3		
Lichq upper-course	9.7	90	+8.0	7.1		
Masrik mother bad	8.2	85	+13.6	8.2		
		August				
Lichq upper-course	9.9	92	+7.9	7.07		
Lichq middle-course	10.7	98	+8.5	7.2		
Masrik mother bad	8.4	89	+15.5	8.4		
Lusakunq tributary	10.5	85	+14.6	8.5		
]	November				
Lusakunq tributary	8.5	90	+7.5	7.95		
Lichq middle-course	10.35	94	+7.3	7.25		
Lichq upper-course	9.96	92	+7.3	7.17		
		December				
Lusakunq tributary	8.9	91	+7.1	7.83		
Lichq upper-course	12.3	104.3	+7.1	7.23		
Lichq middle-course	12.51	104.7	+7.2	7.3		
January						
Lichq upper-course	11.4	100.1	+7.1	7.4		
Lichq middle-course	12.7	105	+7.2	7.1		
Lusakunq tributary	9.3	92	+6.8	7.9		

Measured average monthly physical-chemical parameters of the experiment areas