

Final Evaluation Report

Your Details	
Full Name	Milica Jaćimović
Project Title	Mass removal of the black bullhead (<i>Ameiurus melas</i>) – Possibilities for self-sustaining commercial farming in Serbia
Application ID	31053-2
Grant Amount	£6000
Email Address	mpucar@imsi.rs
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1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Establishment of zero-point state of the fish community in Lake Markovačko				This activity took place at the very beginning of the project during June and July 2020 and was fully realised.
Selective removal of the black bullhead individuals from the Lake Markovačko				This activity was fully implemented in three localities.
Laboratory analyses for a certain amount of fished out black bullhead specimens at the Institute for multidisciplinary research				Given the pandemic caused by the Covid 19, indoor work was not recommended. Therefore, we decided to do the analysis of the sampled black bullhead individuals on the shores of Lake Markovačko. That is why we did some of the planned laboratory analysis, but not all.
Water sampling to obtain qualitative data using multiparameter probe and quantitative data on phytoplankton, to provide insights on changes in phytoplankton assemblage and in ecosystem metabolism associated with the black bullhead removal.				This activity was fully implemented at three localities.
Establishment of post-removal state of the fish community in Lake Markovačko				This activity took place at the very end of the project during October 2021 and was fully realised.
Experimental rearing of black bullhead individuals at the Centre for Fisheries and Applied Hydrobiology "Mali Dunav" of the ODPF				The black bullhead individuals have reached consumable and commercial size and thus the goal of the project has been fully met. Unfortunately, a large number of reared individuals died during the

"Radmilovac" Faculty of Agriculture (CEFAH)				summer months.
Laboratory analysis of black bullhead specimens at CEFAH				This activity has been fully and successfully realised.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.

Once again, this project showed that ichthyological research must have greater support from the local fisheries service (in this case Fisheries Guard Service of the Lake Markovačko). Field research on Lake Markovačko was carried out according to plan, although without the expected and agreed help from the Fisheries Guard Service. The entire field work was realised with great effort and enthusiasm of the research team of the Institute for Multidisciplinary Research of the University of Belgrade. The fisheries guards only gave us their old boat and never once appeared on the lake during our research. Although the "Fisheries management plan for the fishing area "Great Morava 2" for the period 2019–2027" recommended mass removal of black bullhead, this management measure was never implemented during 2020 and 2021. We had to solve all the difficulties we encountered (such as the loss of the fyke and multimesh nets, the failure of the electric motor, conflicts with certain fishermen). Fortunately, we had help from most local fishermen, who appreciated and supported our efforts.

Due to the pandemic caused by the Covid-19, the planned laboratory analysis was performed on the shores of Lake Markovačko, because indoor work was not recommended. Therefore, we measured the removed black bullhead individuals (length and weight), dissected them, and determined their sex and diet. Analyses that required more precise measurements (measurements of the gonads and liver to obtain values of gonadosomatic and hepatosomatic index) could not be done.

Every month, colleagues from the Faculty of Biology, University of Belgrade (Department of Algology, Mycology, and Lichenology) collected water samples for qualitative and quantitative analysis of phytoplankton, while colleagues from the Institute for Multidisciplinary Research collected data on physicochemical parameters of water with the multiparameter water probe. Natalija Pajović and Mina Dunjić, students from the Faculty of Biology also joined the research team. They wrote master theses on the topic of spring and summer dynamics of phytoplankton in Lake Markovačko. Natalija defended her master's thesis on October 7, 2021, while Mina is still processing samples from the lake.

Given that Lake Markovačko suffers from high anthropogenic pressure (numerous recreational fishermen, farms near the shore, and a large apple plantation that is regularly treated with pesticides and insecticides), we decided to expand fish population research to assess surface water quality based on microbiological parameters and ecogenotoxicological and histopathological tissue analysis of native and non-native fish species. The genotoxic potential was assessed by using an alkaline comet and a micronucleus test to quantify DNA damage in blood cells. Additionally, concentrations of metals and metalloids in the liver, gills, and muscle was monitored, using the ICP-OES method. Colleagues from the Institute are doing this analysis.

Experimental rearing of black bullhead in the Centre for Fisheries and Applied Hydrobiology (CEFAH) of the University of Belgrade – Faculty of Agriculture was completed as planned. The black bullhead individuals have reached consumable and commercial size and thus the goal of the project has been fully met. Unfortunately, a large number of reared individuals died during the summer months. Although there is no reliable evidence for this, it is assumed that the mortality was caused by a European catfish virus. We have concluded this on the basis of the data from previous studies. In the summer of 1990, European catfish virus was reported as the main cause of mass mortality in a black bullhead population introduced into a 7-ha pond in France, leaving other fish species unaffected. Furthermore, the same disease caused the mortality of 6 t of black bullhead at a single common carp farm in north-western Serbia over a 1-month period (mid-August to mid-September) in 2008, with no cases of common carp mortality recorded at the time.

3. Briefly describe the three most important outcomes of your project.

- Selective removal has proven to be extremely effective, as in 9 months a total of 15,921 black bullhead specimens (424.622,00g) were fished out from only three localities (from August to October 2020 – 11,487 specimens (269.473,00g) and from April to September 2021 – 4,434 specimens (155.149,00g))
- When it comes to the impact of selective removal on indigenous ichthyofauna, very interesting results were obtained, quite different from the ones we got in the Ponjavica Nature Park. In October 2021 (when the post-removal state was determined), two species appeared in the sample for the first time: European perch (*Perca fluviatilis*) and chub (*Squalius cephalus*). The abundance of bleak (*Alburnus alburnus*) increased three times, while the abundance of Prussian carp (*Carassius gibelio*), another very numerous invasive non-native species reduced eight and black bullhead 16 times. The abundance of roach (*Rutilus rutilus*) decreased 11 times, rudd (*Scardinius erythrophthalmus*) 10 times, pikeperch (*Sander lucioperca*) four and freshwater bream (*Abramis brama*) two times. When it comes to other non - native species, the abundances of pumpkinseed (*Lepomis gibbosus*) increased 10 times, monkey goby (*Neogobius fluviatilis*) three and Amur sleeper (*Pseudorasbora parva*) 2.5 times.
- In addition to field research, laboratory analysis was also conducted for 1,271 black bullhead specimens and the data on their length-weight relationship, food and gender ratio were obtained.
- Colleagues from the Chair of Algology, Mycology and Lichenology of the Faculty of Biology sampled water to obtain qualitative and quantitative data on phytoplankton. Phytoplankton sampling was performed during each field work. At the same time, using the multiparameter probe we got the following data: water temperature, pH value, conductivity, TDS, DO (luminescence time based optical sensor), DO saturation and Chlorophyll *a*.
- This project will quantify DNA damage in blood cells and show the differences between individuals of black bullhead from the natural ecosystem and experimental rearing by using an alkaline comet and a micronucleus test.

Additionally, concentrations of metals and metalloids in the liver, gills, and muscle will be measured, using the ICP-OES method. Such research has not been conducted anywhere in the world so far

- A total of 221 black bullhead specimens were transported to CEFAH. The results of experimental rearing have shown that in the cage breeding system the mass yield has increased 4,6 times when the specimens were fed by commercial-trout feed and four times when the specimens were fed by commercial carp feed. In the recirculating aquaculture system (RAS) the mass yield has increased four times when the specimens were fed by commercial - trout feed and three times when the specimens were fed by commercial carp feed
- Two master's theses are written by students from the Faculty of Biology, University of Belgrade, entitled: "Phytoplankton dynamics during the spring in the Markovačko Lake" (Natalija Pajović) and "Changes in the state of phytoplankton in the summer months during the 2-year mass removal of black bullhead in Markovačko Lake" (Mina Dunjić)
- One defended master theses of student Sanja Zdravković from the Faculty of Agriculture, University of Belgrade, entitled: "Effect of different systems of black bullhead (*Ameiurus melas*) growing on production characteristics".
- This project connected three institutions: the Institute for Multidisciplinary Research (Department of biology and inland water protection), Faculty of Biology University of Belgrade (Chair of Algology, Mycology and Lichenology and Chair of Animal Ecology and Zoogeography) and Faculty of Agriculture (Centre for Fisheries and Applied Hydrobiology), University of Belgrade.
- Results of this research was presented within Educational and Scientific Programme of the Serbian Broadcasting Corporation (Radio Television of Serbia, RTS) in the show "Science is in fashion". Also, the short film called "Black bullhead Project" was broadcast on the cable channel "Hunting and Fishing". Both of these shows can also be found on YouTube. In this way, The Rufford Foundation received huge publicity. Documentary movie "Black bullhead Experiment" won the award for ecological approach to life in the water at Documentary Film Festival "Zlatna buklija" (Golden Flask)
- Another documentary (made from footage from Lake Markovačko and CEFAH) will be created and broadcast on RTS. Like the previous one ("Black bullhead Experiment"), it will be sent to many film festivals dedicated to environmental topics.

4. Briefly describe the involvement of local communities and how they have benefited from the project.

During the field research we had the opportunity to meet a lot of local anglers, explaining the purpose of our work. We experienced help and support from most of them. They saw positive changes in the fish community, and we tried to explain the purpose of our work to each of them. Since we did not have any help and support

from the Fisheries Guard Service, we turned to the local anglers for all the problems. Many times, they came to our aid.

5. Are there any plans to continue this work?

In the coming months, firstly we expect to sort out a huge amount of data and write scientific papers.

Given that high mortality has been detected in reared black bullheads, it is necessary to think carefully and determine why this happened. Although the surviving individuals have reached commercial size, this method of rearing cannot be applied in fishpond systems because of the high mortality. The only cultivation system in which no deaths occurred was recirculating aquaculture system (RAS), but it is very expensive and not economically viable and justified. Therefore, colleagues from the Faculty of Agriculture must give a final conclusion on whether the rearing of black bullhead can be possible and profitable.

When it comes to the application of mass selective removal of black bullhead, the results showed both positive and negative effects on the fish community. It would be useful for continuous removal to be applied for several seasons and years, but this requires great support from companies that manage fishing areas. Namely, the present situation in Serbia is not optimistic in sense that the selective removal will continue to be regular practice of the companies that manage fishing areas.

Due to all that, it is very difficult to apply this idea to a larger number of lakes and reservoirs. Scientific institutions, such as the Institute for Multidisciplinary Research, can provide professional assistance and expertise, but they cannot perform such a large and comprehensive process on their own, such as the selective mass removal of an invasive non-native species.

6. How do you plan to share the results of your work with others?

- The plan is to publish several scientific papers in international journals about: 1) the impact of selective removal of the black bullhead individuals on native and non-native fish species, 2) the relationship between seasonal dynamics of phytoplankton and the fish community, 3) experimental rearing of black bullhead, 4) comprehensive management plan for selective removal of black bullhead and opportunities for self-sustaining commercial farming in Serbia, 5) ecological status of Lake Markovačko by assessment of genotoxicity (comet and micronucleus test) and accumulation of metals in black bullhead tissues, 6) is the difference in the size of black bullhead individuals from natural populations and breeding individuals genetically based?, and 7) is there a repair of DNA chain damage during the process of black bullhead rearing?
- During the course of the experiment approximately 4500 primary and high school children visited CEFAH, while senior pupils (around 5000 of them) were familiarised with the experiment.

- Throughout the project we were accompanied by a team of Serbian Broadcasting Corporation (Radio Television of Serbia, RTS). They documented our activities during field work at Lake Markovačko and experimental rearing at CEFAH, and they will make another documentary. They were broadcasting television report about our previous project many times. Also, they uploaded this report on the official YouTube RTS channel and on the platform RTS Planet. Results of this research were presented within Educational and Scientific Programme of the Serbian Broadcasting Corporation (RTS) in the show "Science is in fashion". Also, the show called "Black bullhead Project" was broadcast on the cable channel "Hunting and Fishing" and You Tube.

7. Timescale: Over what period was the grant used? How does this compare to the anticipated or actual length of the project?

We used The Rufford Foundation grant throughout the entire project. We were able to fully realise all the goals of the project, by rational spending and planning of all activities.

Rufford Foundation grant was used for all experimental costs: material for the installation of different rearing systems and supplies, concentrated fish feed for black bullhead, Electric energy used for the operation of pumps for water supply and operation of aerators in tanks of RAS.

8. Budget: Provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

Item	Budgeted Amount £	Actual Amount £	Difference £	Comments
Value-added tax	1200	1200		The Republic of Serbia taxes each project in the amount of 20% of the total project value.
Overhead for the Institute for Multidisciplinary Research	480	480		6000 – 1200 = 4800£ 10% of this amount was taken by the Institute for Multidisciplinary Research for overhead.
1200 £ + 480 £ = 1680 £. 6000 £- 1680 £ = 4320 £. This amount was divided into two equal parts - 2160 £ each for field research on Lake Markovačko and experimental rearing of black bullhead in CEFAH				
Jeep fuel	623	514	-109	This difference was used to pay per diems to colleagues from

				the Institute for Multidisciplinary Research
The bread we used as bait	110	110		
Fees for the researchers	1163	1762	+599	When the budget was made, the plan was that it was enough for two researchers to go to the field, because we were expecting the agreed help from the Fish Guard Service. Since we were alone during the field research, two researchers were not enough to realize everything that was planned. Also, due to a pandemic caused by the Covid 19 virus, laboratory work could not take place on the institute's premises. Therefore, we had to do all measurements and dissections on the field, i.e., on the shores of Lake Markovačko. And because of that, it took more colleagues (from 4 to 5) to be able to do everything. These are the reasons why this budget item is higher than planned.
Translation of the contract between the Institute for Multidisciplinary Research at the Rufford Foundation		34	+34	In the original planning, this item was not even included because we didn't know we were going to need it
Fuel for the boat of Fish Guard Service	264		-264	We did not use this budget item because we used an electric motor during field research on Lake Markovačko.
Material for preparation pond system: Waterproof cord (length 140 m)	140	140		
Material for preparation pond system: Fish nets	280	280		
Material for preparation pond system: Integrated pillars (24 pieces)	107	107		
Preparation small cages: Disinfectant	20	20		
Preparation RAS: Disinfectant	20	20		

Preparation RAS: Transportation costs	180	180		
Concentrated experimental fish feed for black bullhead (400kg)	473	473		
Electric energy used for the operation of wells pumps for water supply and operation of aerators in tanks	940	940		
	6000	6260	-260	The difference of 260 £ was reimbursed from the material costs intended for scientists in Serbia, which are provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

9. Looking ahead, what do you feel are the important next steps?

In the coming months, the most important task is summarising the results, making comparisons with data from the Ponjavica Nature Park and Lake Markovačko, analysing data from experimental rearing of black bullhead, processing a large number of samples and writing scientific papers. Only after that plan can be made for the future.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

Yes, we used the Rufford Foundation logo during presentations of our work, on promotional t-shirts, as well as during the recording of reports for RTS.

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Dr. Milica Jaćimović (Project Leader)

Prof. Dr. Zoran Marković (Team Member - responsible for experimental rearing of black bullhead)

Prof. Dr. Jasmina Krpo-Četković (Team Member - advisory role)

Dr. Božidar Rašković (Team Member - responsible for laboratory analysis of black bullhead specimens at CEFAH)

Dr. Marko Stanković (Team Member - assistance during experimental rearing of black bullhead)

Dr. Marija Smederevac-Lalić (Team Member - assistance during field activities at Markovačko Lake)

Dr. Gorčin Cvijanović (Team Member - assistance during field activities at Markovačko Lake)

Dr. Dušan Nikolić (Team Member - assistance during field activities at Markovačko Lake)

Dr. Stefan Skorić (Team Member - assistance during field activities at Markovačko Lake)

Dr. Željka Višnjić-Jeftić (Team Member - assistance during field activities at Markovačko Lake)

Dr. Jovana Kostić–Vuković (Team Member - assistance during field activities at Markovačko Lake)

Dr. Karolina Sunjog (Team Member - assistance during field activities at Markovačko Lake)

Dr. Dragana Predojević (Team Member - phytoplankton sampling and data analysis)

Marija Pečić (Team Member - phytoplankton sampling and data analysis)

Vojislav Sokolović (Team Member, student of the Faculty of Biology University of Belgrade)

Dorđe Gajić (Team Member, student of the Faculty of Biology University of Belgrade)

Teodora Radović (Team Member, student of the Faculty of Biology University of Belgrade)

Natalija Pajović (Team Member, student of the Faculty of Biology University of Belgrade)

Mina Dunjić (Team Member, student of the Faculty of Biology University of Belgrade)

Dalibor Vukojević (Team Member, PHD student of the Faculty of Agriculture, University of Belgrade)

Sanja Zdravković (Team Member, student of the Faculty of Agriculture, University of Belgrade)

Vukosav Golubović (Team Member, PHD student of the Faculty of Agriculture, University of Belgrade)

Stefan Marjanović (Team Member, PHD student of the Faculty of Agriculture, University of Belgrade)

Igor Dučić (Team Member, student of the Singidunum University – Environment and sustainable development studies)

Jovana Dragić May (Team Member, WWF Adria)

12. Any other comments?

I think the answers to the previous questions were detailed and clear enough, so there are no additional comments.