OPINION

by Professor Boyko B. Georgiev, PhD, DSc, Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences, regarding the dissertation entitle *Study of the breeding and feeding habitats of sturgeon fish from the Bulgarian section of the Danube River* for the acquisition of the Scientific and Educational Degree Doctor by Borislava Kostadinova Margaritova, PhD candidate in hydrobiology at the Department of General and Applied Hydrobiology, Faculty of Biology, University of Sofia "St. Kliment Ohridski", supervisors Assoc. Prof. Dr Eliza Uzunova and Assoc. Prof. Dr Lyubomir Kenderov

The dissertation under consideration has an actual topic because it is aimed at studying a group of fish with high conservation importance. In the past, sturgeon species in the Danube River had an important economic importance, which today has been completely lost due to human activities. Moreover, in the Danube River and in the Black Sea, they are on the verge of extinction – not only as a resource but also as biological species. The author presents a brief but well-thought-out and convincing literature review. From it, we can conclude that the information on the feeding and breeding of Danube sturgeons collected more than 50 years ago, in the conditions of hydrological, hydrochemical, morphological and biological changes in the river ecosystems of the Danube, is no longer up-to-date. Therefore, it is not sufficient to be the fundament of the modern management and conservation of sturgeon populations. Knowledge of feeding and breeding sites is crucial for the conservation and recovery of sturgeon populations in the Danube. For this reason, the studies included in this PhD project are mainly in this direction.

I find the research tasks set are precisely formulated and subordinate to the proposed general research objective. The research aims to determine the breeding and feeding habitats of the sturgeon fish in the Bulgarian section of the Danube with a view to their protection and restoration. Research work was carried out on three main research tasks:

(1) Determining the status of sturgeon fish in the Bulgarian section – research on the species composition and numbers of sturgeons as well as their size structure and condition.

(2) Survey of potential breeding habitats historically documented as spawning grounds. This includes surveying migration of different size-age groups upstream and downstream, surveying for spawning in areas of potential or past known spawning grounds and analysis of abiotic factors in spawning habitats.

(3) Survey of potential foraging habitats by identifying the food contents and characterizing the macrozoobenthos composition in the foraging habitats.

I find the general objective and research tasks of the dissertation formulated in this way to be clear and with a high potential for collecting the basic information needed for the protection of sturgeon populations in the Bulgarian section of the river.

The studies were carried out with methods corresponding to the main objective and the research tasks; applying them, a huge amount of data was collected. To investigate potential breeding habitats, field surveys were conducted over 102 days and 531 samplings were made using ichthyoplankton nets for eggs, pre-larvae and larvae. To study migration, demersal catches were carried out for 231 days, surveying 1420 transects. Up-to-date methods were used – marking with external and internal markers with the application of individual codes in order to analyse the frequency of catch, the period of stay in the studied section, the rate of growth, survivability and speed of movement along the stream. The nutritional spectrum was studied by means of a non-invasive method

through stomach lavage. The methods for studying benthic communities as well as the physical and chemical characteristics of the river also meet modern standards.

The results are presented methodically and clearly. The discussions presented are convincing and logically lead to the main conclusions of the study. They demonstrate the ability of the doctoral student to analyse and summarise scientific information.

The conducted research has a contributing character, both from a purely scientific and from a practical point of view, especially for the protection and restoration of sturgeon fish populations in the Danube River. I consider the following contributions to be the most significant:

- Updating information on the status of sturgeon species and their habitats in the Bulgarian section of the Danube River. A persistent downward trend in populations was identified and an extension of the fishing ban was recommended, as well as continued support of populations through stocking and anti-poaching.
- The role of by-catch was assessed and recommendations were made for working with local fishing communities in order to minimise its negative impact.
- Information was collected on the behaviour in the river of the cultured sturgeons imported as stocking material and on their rate of growth in the river ecosystems. It confirms the effectiveness of activities for the maintenance and restoration of sturgeon populations in the Danube.
- The first data on the length-weight relationship and on the condition factor for the zero-year-old and young specimens of three types of sturgeon fish were obtained.
- The speed of movement of zero-year sturgeons of 4 species during their migration to the Black Sea was determined.
- The dependence of the reproductive period on temperature and water level was analysed. On this basis, practical recommendations have been developed for improving the methodology for monitoring sturgeon fish in the Danube.
- The potential breeding habitats of the four species of sturgeon fish in the Bulgarian section of the Danube have been determined. This information is extremely important for focusing future conservation and monitoring efforts on them.
- The nutritional spectrum of the four species of sturgeon fish in the Bulgarian section of the Danube was studied; these are the first data in nearly 60 years and after the dramatic changes in the river ecosystem.
- A habitat for the growth and feeding of small sturgeon fish was identified in the waters of the Danube near the village of Vetren and it was proposed for protection due to its extreme importance for the populations of the four sturgeon species.

On the subject of the dissertation, Borislava Margaritova has published 3 research papers. These included one article is in a journal included in the Web of Science database (i.e., a journal with an impact factor), one is a journal in the Scopus database and one as a full-text report in a thematic collection of works published by the University of Sofia. The results of the dissertation have been presented with four reports at scientific forums in Bulgaria (3) and abroad (1).

I have no critical remarks about the thesis. When reading the final version of the dissertation, I noticed that B. Margaritova took into account the few critical notes I had when opening the examination procedure several months ago.

I have two questions to the PhD candidate:

1. Are there any data on whether interspecific competition among zero-year sturgeons is not a negative factor for any of the four species studied? I believe that the answer to this question is extremely important, because favouring one or two species (for example, by stocking with cultured sturgeon) can be a strong negative factor for the populations of the remaining species in conditions of interspecific competition.

2. Are there any data on how stocking with cultured zero-year-old sturgeons affects the intraspecific genetic diversity of the population of the respective species in the Danube River?

In conclusion, I believe that the dissertation corresponds to the requirements of the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria. A comprehensive reading of the dissertation shows that Borislava Margaritova has developed as an excellent hydrobiologist, ichthyologist and conservationist. She is familiar with the literature and has used a variety of methodological approaches. She collected and processed a large volume of materials. The presented texts demonstrate logic and analytical approach as well as an excellent handling of terminology. The conclusions and scientific contributions are clearly stated and I accept them.

This gives me reason to support the awarding of the educational and scientific degree "Doctor" to Borislava Kostadinova Margaritova.

Sofia, 15/12/2022

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