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Review of Dissertation

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Dissertation title: "Structure and functions of hydrozoocenoses in temporarily drying water bodies".

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The present thesis in its form as a volume, aim and objectives, formulated working hypothesis, results, conclusions and presented contributions, meets the requirements for a dissertation of the Philosophy Doctor degree.

The dissertation is focused on a topical issue - it deals with questions concerning hydrobiological studie of four different in origin and character water basins with unsteady - drying hydrological regime. They are - Lilov Vir in the area of the Karlukovo village, Aldomirov swamp in the area of the Slivnitsa town, Dragomansko swamp located near by Dragoman, and the artificial lake Ariana in the Borisova Garden in Sofia. Also in the the dissertation are included two small rivers what inflows into the Black Sea. These rivers are Silistar and Butamyata. They also have an erratic regime. They have visible inflow in the Black Sea only during the extremely high rainfalls. Otherwise they are separated from the sea by so-called sand bars. It is no coincidence that the focus is on the study of water bodies with drying periods and those with higher or lower water levels and volumes. The study sites are important - on the one side, most of them are protected sites with high biodiversity. On the other side they are located in several Natura 2000 sites.

The volume of the work is 182 pages. The dissertation is structured according to the standard scheme adopted in our country with chapters dealing with the main types of studied water bodies. It includes the standard chapters for this type of work – such as Introduction of 2 pages. Literature review of 35 pages. In it, the main place is given to the studies conducted in different countries and places, in previous periods, by many authors, investigating the hydrobiological features of water bodies with unsteady hydrological regime. The review is successfully related to the subject of the thesis. In this part of the dissertation, the genesis of zooecenoses - zoobenthos and zooplankton in this type of water bodies were discussed.

In my opinion, it would be good to attach the texts of the papers what are part of the procedure of defense, in order to see additional information supporting the results of the thesis.

The aim of the dissertation is clearly stated - "To observe and describe the development of the macrozoobenthos and zooplankton communities, their species richness and functionality in relation to the hydrological regime and changes in the physico-chemical parameters of the water bodies, and to identify the factors that govern the development of these cenoses. "

Regardin to this goal, the four tasks were formulated:

1. Study of the species composition, structure and functionality of benthic and planktonic invertebrate communities in different categories of temporary water bodies in the different parts of the country: karst lakes and swamps, artificial ponds and drying rivers.

2. Identification of the leading environmental factors what influencing the formation of planktonic and benthic invertebrate communities.

3. Qualitative description of trophic relationships in the communities of the studied aquatic ecosystems through model trophic chains and networks.

4. Study of the influence of limiting conditions of the specific water balance of the studied temporary ponds on the trophic structure of the communities.

I think that In relation to the aim and objectives, a well prepared working hypothesis was formulated: 'The structure of hydrozoocenoses in drying water bodies is primarily influenced by their specific water balance, which is a major ecological and structural determining factor. The effect of water balance is mainly expressed by the hydroperiod and changes in water volume. Different HPs and inconsistent water volume in different years imply the formation of different communities. Nevertheless, even under the severely limited living conditions of drying ponds, the communities within them can be viable and important for maintaining local and global biodiversity."

The chapter Material and Methods is detailed and clearly describes the used field and laboratory methods, indices, and numerous statistical methods used in the dissertation. Its volume is 6 pages. The period in which the samples for the dissertation were collected is from 2012 to 2016 years.

It is indicated that a total of 155 samples were collected and processed during the sampling period, of which 79 zooplankton samples and 76 macrozoobenthos samples. The samples were collected during the period April - August of the respective years. In parallel, water physico-chemical indices were measured.

The figures/pictures presented in the thesis and appendices with marked sampling points help to orientate the location of the respective stations and water bodies under study. The geographic coordinates of the sampling stations presented, indicates the precision of data collection for the work. I would recommend that the colleague, in subsequent publications, report not only the coordinates but also the datum on which the positioning device was set. This would avoid a deflection of several hundred meters at times when working with devices set to different datums.

Also very useful is the table in Annex 4 what indicatas the sampling stations/points and the type of samples collected from sampled water bodies - planktonic and benthic.

The focus of this dissertation is on studies of zooplankton and zoobenthos in temporarily drying ponds. Their evolutionary stages are eutrophic or even hypereutrophic. The hydrological and hydrochemical conditions in the studied water bodies are drastically influenced by periods of low volumes and complete drying and also by belts of semi-submerged vegetation.

The most important part of the dissertation is the Results and Discussion chapter. It is set out on 96 pages. In the chapter there is a subchapter dealing with the physico-chemical parameters

of the studied water bodies, including geomorphological and hydrological data from different periods and the different hydrological regimes at the study sites. Particularly appropriate to me is the use of the aridity index and the statistical analysis applied to interpret the data collected on water physico-chemical parameters.

A total of 38 taxa were found in the zooplankton assemblages of the standing water bodies studied, at the level of order, genus and species. It was found that the different developmental timing of the hydrobionts implies some consistency in their occurrence in the respective water body. Highly variable percentages of similarity in plankton complexes associated with highly dynamic and divergent hydroperiods were also found.

As evidence of the strong influence of the dynamic hydrological regime of the basins on plankton, it highest abundance was found in Lilov Vir during 2015 and at the same time the lowest was registered in the same pond in 2013. The results are richly illustrated with numerous figures in the text and in the appendices. Logically, the calculated species diversity indices have the highest values in Lilov Vir and Aldomirovsko swamp. The work reports highly dynamic changing in different seasons and years quantitative parameters of cladoceran and copepod zooplankton and zoobenthos in the studied basins. The phenomenon is typical for the water bodies with a highly variable hydrological regime including complete drying for shorter or longer periods, sometimes year-round. In my opinion a successful attempt has been made, to assess the trophic structure and relationships in the study sites. I think that this is one of the strengths of the thesis. It has been established that poor connectivity and fewer trophic relationships in a system such as Lilova vir do not necessarily lead to instability of the system. It has been suggested that having fewer taxa with weak connectivity between them creates a stable community, making the most of the limited conditions of drying ponds.

A total of 69 taxa of zoobenthos were identified from the surveyed four stagnant ponds. The zoobenthos found was predominantly insect in nature. As expected, the highest zoobenthos quantitative parameters were recorded in the more extensive and longer hydroperiod water bodies.

The trophic structure of the zoobenthos in the studied reaches of Silistar and Butamiata was found to be characteristic of lower reaches and estuarine sections. The values of the structural parameters for both rivers are relatively high and suggest good conditions for the development of the zoobenthos community. The development of macrophyte overgrowth also favours the development of zoobenthos. An interesting finding, in my opinion, is that there is no stress from the interruption of the studied river courses due to drought. According to the author, intermittent river currents are favourable for zooplankton development in them. My recent personal observations indicate the presence of relatively rich zooplankton complexes in all estuaries of the Bulgarian rivers, what inflows in Black Sea.

I would say that the results concerning aquatic communities are not only indicated, have been processed by numerous statistical methods, but have also been interpreted in detail by PhD student. The reasoning and conclusions sound logical and convincing. The application of relevant biodiversity indices has also contributed to the discussion of the results. In my opinion, this is also a major positive aspect of the thesis.

Finally, the dissertation presents 10 conclusions and recommendations of a scientific and applied nature. In my opinion, they are in line with the reflections made in the dissertation.

They focus on the composition and structure of the zooplankton complexes studied the dominant species and their changes over the years of study and the other components of zooplankton in this specific type of basins.

1. Temporary inland reservoirs have a large dynamics of physico-chemical parameters and nutrients, which depends mainly on the duration of HP and their water volume.

2. The zooplankton and zoobenthic communities are mainly formed by three components:

a) species with cosmopolitan distribution;

(b) species that occur exclusively or predominantly in temporary ponds;

(c) colonising species, mainly of the class Insecta.

3. In view of the existence of two periods in temporary ponds - dry and aquatic, a significant factor in structuring the communities is the succession of pool colonization after filling. From this point of view, species can be classified as follows:

a) initial colonization of species of the order Cyclopoida (Acanthocyclops spp., Thermocyclops spp.), Fam. Chironomidae and class Oligochaeta (genera Lumbriculus, Nais and family Enchytraeidae);

(b) followed by species of the order Calanoida (genus Arctodiaptomus) and species of Cladocera mainly of the genera Daphnia, Moina and Scapholeberis;

c) a week or two after filling, organisms of the class Ostracoda, order Harpacticoida, some aquatic worms (genera Sigara, Notonecta), sometimes dragonflies (genus Libellula) appear;

d) 4-5 weeks after filling, dragonflies of the genus Lestes and water beetles from the genus Dytiscus;

e) if the HP is long enough, 10 weeks after filling up, various organisms of the orders Coleoptera (genus Poecilus, Gyrinus, Haliplus) and Ephemeroptera (genus Cloeon) 156 may appear.

4. As the HP increases, the number of taxa included in the zooplankton and zoobenthos communities increases. Hydroperiods longer than 100 days suggest greater biodiversity and species richness, while shorter periods suggest fewer taxa but higher abundance.

5. The permanence of the water volume of the basin, in temporary water bodies, e.g. in a flow-through lake, provides more stable physico-chemical and hydrological parameters of the environment, which can largely counteract the effect of the temporary nature of the basin in terms of community structuring.

6. The unstable hydrological regime of the temporary inland ponds does not allow the development of a full trophic structure. However, despite short trophic chains and poor connectivity, systems can be stable and resilient.

7. The trophic complexity of temporary ponds is related to their temporal heterogeneity. The greater the predictability of the hydrological regime of the basin, the greater the trophic complexity will be.

8. Short CPs, below 100 days, are not conducive to the inclusion of predators in the zoobenthos community.

9. The unstable hydrological regime and the dynamics of physico-chemical parameters of temporary ponds associated with short HPs and low water volume suggest that the hydrobionts of the zooplankton and benthic communities will be adapted to abiotic rather than biotic factors. With very short HPs, biotic relationships will be virtually absent.

10. Reduction of water runoff, stoppage of water flow and water body incision, in rivers with unstable hydrological regime, have no significant impact on the formation and functioning of the zoobenthos, but favour the development of the zooplankton community.

The specific characteristics of inland temporary ponds create diverse and stable zooplankton and zoobenthic communities that contribute to local biodiversity. They can be an important resource and reservoir for other ponds in the area and also provide habitat for a range of amphibians, reptiles and birds. The extremely wide distribution of temporary ponds, even more so in view of the global increase in temperatures, makes them an important component for biodiversity conservation. It is therefore necessary to survey, map and classify them.

The recommendations made by the author are directed towards future research on similar water bodies and, in my opinion, have a contributory nature.

I think the dissertation would also be benefit from mentioning contributions of a confirmatory nature that are related to previous research on these epicyclic in nature bodies of water. In this way it would be emphasised that a link is being sought with knowledge gained from previous studies of various eutrophic water bodies in this country.

In connection with this interesting dissertation I have some questions and some critical notes, some of them, I will share here:

1. Why there are no data on Rotifera organisms presented in the dissertation? These organisms are widely distributed in the pools, what where the subject of this thesis? In water bodies where species of the type are abundant and often have high quantitative parameters and forming dominant complexes, studies of this group are without alternative.

2. Why there was not mentioned as used for determination of organisms from order Cladocera such basic guide books, such as those of Manuilova, (1964), Flossner, (1972), Smirnov, (1974), Alexeev Tsalolikhin, (2010) etc.? The use of only one guide book of - Kutikova, Starobogatov, (1977) - Determinant of freshwater invertebrates of the European part of the USSR (plankton and benthos), as well as that of Bledzki, Rybak (2005) - Freshwater Crustacean Zooplankton of Europe Cladocera & Copepoda (Calanoida, Cyclopoida) - Key to species identification, with notes on ecology, distribution, methods and introduction to data analysis may lead to inaccuracies in determinations. Also, the use of only one source for the determination of species of the subclass Copepoda is, in my opinion, insufficient for their precise determination. Why was such important guide book for the determination of the group such as that of Monchenko, (1974) not used?

3. How would the dissertant explain the founding of the cladoceran *Daphnia rosea* in Lilov Vir - in the area of Karlukovo village, as well as in Aldomirovsko and Dragomansko swamps. The first water basin is located at 230 m a.s.l and the rest are at approximately 500 m a s l. The species is a glacial relict, highly abundant in glacial lakes in Rila, Pirin, Alps, High Tatras. It is a cryostenothermic organism, and at the same time is polystenooxybiont. It is an inhabitant of oligotrophic water bodies, and even in such reservoirs like Iskar the species has been not found.

Finding it in low elevation water bodies, that are in the eutrophic category, such as the two swamps mentioned, is debatable in my opinion. Also interesting to consider is the question of how, and by whom, the species would possibly have been transported to these water bodies from the respective glacial lakes?

4. I think that, more explanations are needed regarding the establishment of another Cladocera species in some of the study ponds - *Euricercus lamellatus*. The species has a northern zoogeographic characteristic according to Flossner, (1972), Smirnov, (1972), Najdenow, (1994), (2000), Kozhuharov et al. (2011) etc. The southern border of its range is believed to have passed through our territory. It was common in the glacial lakes in Rila and Pirin until several stocking campaigns were carried out, as a result of which it disappeared. In the last 60 years no alive specimens of this species have been found. How would the author explain its occurrence in water bodies with low altitude and thermal regime too different from the typical one?

5. Why in 2012 samples were collected only from Lilov Vir and from Ariana Lake, and in 2016 only from Dragomansko swamp?

6. Figure VI 19 lacks abundance and biomass designations for zoobenthos, this is also noticeable in other figures in the text.

7. In future research I recommend that the colleague use absolutely quantitative methods for sampling zoobenthos such as the Surber frame, Hess bottom sampler, etc. This will increase the comparability of the results obtained with those from other studies.

8. In my opinion, the work would only benefit from adding data on Rotifera species and their quantitative parameters. Subsequently, the processing of the results obtained by using indices examining the proportion of different major groups of zooplankters such as Rotifera, Cladocera, Copepoda, in different trophic status water bodies. This is, by the way, what I would suggest to my colleague in his future research.

Three publications have been cited in connection with this dissertation - two full papers published at a workshop on Ecology with international participation and one in the journal Proceedings of the BAS. I did not find them in the reference list at the end of the thesis, and I think they should be listed there. This highlights their relevance to the thesis.

The remarks what I give and questions raised by me do not detract from the contribution of the thesis under review and do not call them into question. Their purpose is to give the colleague the opportunity to discuss them, and to help him in further building up publications, which I hope will follow in the near future in the scientific papers.

Conclusion:

On the basis of the briefly mentioned and discussed results and conclusions contained in the dissertation, I believe that it meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for its implementation. My opinion of the work presented by Pencho Ivanov is entirely positive. I believe that it can be admitted in this form to defence and can be successfully defended before a scientific jury. I believe that in the obtained results there are still a lot of materials for processing and discussion which I wish to my colleague Pencho Ivanov to publish in the scientific journals.

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