#### **REVIEW**

By Associate Prof. Dr. Vesela V. Evtimova, Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences (IBER-BAS)

Of a dissertation entitled

### "Structure and functions of hydrozoocenoses in temporary dry water bodies",

presented for awarding the educational and scientific degree "Doctor" in scientific field of Hydrobiology, code 01.06.11, professional field 4.3. Biological Sciences, higher education field 4. Natural sciences, mathematics and informatics

by Pencho Danchev Yordanov,

part-time PhD student with the Department of Aquatic Ecosystems, IBER-BAS.

This review is prepared in accordance with article 9 of The regulations for the terms and conditions for acquiring scientific degrees and for occupying academic positions in IBER-BAS, voted by the SC on 22.07.2014, last additional amendment from 25.11.2022 and in compliance with Order N $_{2}$  18/ 26.01.2024 by the Director of IBER-BAS and in relation to the decisions of the Scientific Council (SC) of IBER-BAS, Protocol N $_{2}$  17/ 19.01.2024, article 4 and of the Scientific Jury from its first meeting, held on 15.02.2024.

The PhD candidate Pencho Yordanov graduated from the Faculty of Biology, Sofia University St. Kliment Ohridski in 1994. In January 2013 he has begun his part-time PhD studies with IBER-BAS under the supervision of assoc. prof. Dr. L. Pehlivanov. Pencho has been working in the field of ecology and biology, including participation or initiation of various educational courses for children and students since 1996.

#### (1) General characteristics of the dissertation - volume and structure

The dissertation of Pencho Yordanov aims at exploring "key processes characterising intermittent water bodies with varying hydrological regimes and origin, as well as the main factors, affecting the development, structure and functioning of the two main faunal communities inhabiting these water bodies, namely zooplankton and macroinvertebrates. Four main tasks have been formulated: (1) Exploring "taxon composition, structure and functioning" of invertebrate communities in intermittent water bodies (IWB); (2) Determination of the main environmental factors affecting zooplankton and benthic communities in IWB; (3) Description of trophic relationships in these water bodies and (4) Exploring some limiting environmental factors related to water balance. The working hypothesis rests on the statement that "the structure of hydrozoocenoses in intermittent water bodies is primarily influenced by their specific water balance, which is the main ecological and structure-determining factor".

The dissertation contains the required chapters and it has a total volume of 181 pages, 168 of which represent the body of the dissertation. The work includes the following parts: Contents - 3 pages; Abbreviations and definitions used – 2 pages; (I) Introduction – 2 pages; (II) Literature review – 35 pages; (III) Aim and objectives – 1 page; (IV) Studied sites – 7 pages; (V) Materials and methods – 7 pages; (VI) Results and discussion – 96 pages; (VII) Concluding remarks – 2 pages; (VIII) Conclusions, contributions and recommendations – 3 pages; (IX) References – 10 pages; (X) Appendices – 12 pages.

The abstract of the dissertation consists of 31 pages (including a summary in English) and reflects the structure, main results, conclusions and contributions of the PhD thesis. It also includes a reference list related to the topic of the dissertation, as well as a list of the participations in scientific forums with reports reflecting research within the dissertation.

#### (2) Literary awareness and theoretical preparation of the candidate

The PhD student shows a good literary awareness. The reference list covers 111 titles, of which seven are in Cyrillic and 104 are in Latin; only a bit less than 15% have been published over the last decade.

A critical review of the terminology related to the studied type of water bodies in different parts of the Earth has been made already in the Introduction. Challenges related to the selection of a suitable and generally-accepted term are outlined; for the purposes of the dissertation work, the term "temporary waters/ intermittent water bodies" was chosen. Emphasis is also placed on the probability that the number of different water bodies with a dry phase will increase as a result of the projected climate changes. The presented research is

also relevant due to the limited number of studies on intermittent water bodies in Bulgaria and the absence of studies regarding the influence of the temporary nature of the water bodies on the formation of their plankton and benthic communities.

In chapter Literature Review, different classifications of IWB are presented. Examples of IWB on the territory of Bulgaria are given; as well as of organisms or environmental factors within these water bodies; etc.. The information presented could be organised better and more concisely. There are also technical errors: for some subsections, additional numbering is used (could be confusing), missing/wrong punctuation or words, etc. Not all literature sources are cited everywhere where needed.

# (3) Methodical approach

The studies described within the PhD thesis are methodologically sound. The intermittent water bodies included in the study were selected so that they have different durations of the water phase, are representative of different regions, as well as of different categories of IWB, incl. "seasonal" and "almost perennial" standing IWB, as well as two rivers.

The selection of the studied physical, chemical and biological parameters of the water bodies is in accordance with the requirements of WFD 2000/60/EU and Bulgarian (Regulation H4) and European legislation/standards. The main parameters of the water environment are supplemented by determining the depth, area and approximate volume of the water bodies, as well as indicators related to the hydroperiod/drought (HP). Adequate statistical methods and visualisations have been implemented using the software programs PRIMER v6, PAST 4.03 and PowerPlay. Some of the presented data regarding the number of identified invertebrate taxa would be better placed in chapter VI Results and Discussion.

#### (4) Significance of the obtained results, interpretations and conclusions

The topic of the thesis is extremely relevant, especially in the context of the increasing frequency of extreme climatic events in temperate latitudes, which would lead to a possible increase in the number of IWB in Europe and in Bulgaria. The duration of the study (2012-2016), as well as the 155/4? samples (there is a discrepancy in their number, see pp. 51 and 53) of aquatic invertebrates allow obtaining a sufficiently large sample size and imply a good interpretation and significance of the obtained results.

PCA analyses based on the studied environmental factors indicated that the first two main axes explain more than 80% and more than 90% of the total variation in the environmental condition of lentic and lotic IWB, respectively. Statistically significant differences were recorded only when comparing the conditions in the studied rivers, owing to the higher precipitation in 2014 as compared to 2015. As a drawback, I consider the absence of similar analyses for the lentic IWB.

A total of 107 taxa of aquatic invertebrates have been recorded in the standing and 87 in the flowing IWB. The presented data convincingly testify that, despite the dynamic environmental conditions in such water bodies, some general patterns regarding invertebrate hydrobionts can be outlined:

 $\checkmark$  In the absence of anthropogenic stress, a longer HP implies a higher number of established aquatic invertebrate taxa, but often with lower densities.

✓ Most zooplankton samples are dominated by crustaceans of superorder Cladocera and less often of order Cyclopoida. Benthic ones are often dominated by "colonisers" of class Insecta (especially in lakes with a shorter HP) or less often of class Branchiopoda. Juvenile stages of class Copepoda dominate river sites.

- $\checkmark$  When subjected to a similar duration of HP, similarities have been established in:
- taxonomic composition and density/ biomass of zooplankton or zoobenthic communities;
- trophic interactions as suggested by canonical correspondence analysis (CSA: the first two axes explain over 95% of the variation in zooplankton and macrozoobenthos communities of lentic IWB and over 90% in macrozoobenthos of lentic IWb) and
- $\circ$  the observed seasonal succession for both communities.

The complexity of the trophic structure, as well as the number of different "stages" of the seasonal succession, are largely determined by the duration of the HP. The shorter HP, for example, determines a high proportion of phytoplanktonivorous invertebrates in the absence of macrophytes and predators.

 $\checkmark$  Loop analyses also indicate that the complexity of trophic networks, as well as the number of links therein, are proportional to the duration of the HP and the water volume of the water body.

In conclusion, the results and their interpretation aided by the applied statistical analyses and logically reflected in the drawn conclusions, confirm the working hypothesis that

HP is among the environmental factors of essential importance for the formation of zooplankton and zoobenthic communities in the IWB. Local annual climate features are also important.

#### (5) Critical comments and suggestions

The information presented here should be considered rather as a set of recommendations that the candidate could take into account in his future work in case he considers them relevant.

#### Some notes on the structure and content of the PhD thesis:

✓ There are inaccuracies in the used terminology ("rotatorians" is an outdated term for "rotifers") or an inaccurate translation ("detritivores", as well as "temporary dry" in the English summary, where I would use "intermittent").

- ✓ Literature review:
- o point 8 it would be preferable to link these four concepts specifically with IWB;
- point 4 "Biota" contains "Higher plants" and "Some of the main organisms in IWB" (the latter also includes higher plants), as well as "Comparison of different habitats of intermittent water bodies", where for some of the described types the focus is not biota.
- There are a number of repetitions. Example: the classification of IWB based on their origin is given under II.1 and II.2.
- There are whole paragraphs with few or lacking citations. This misleads the reader that the provided information is based on speculations and not on results of previous studies. Example: preferences for specific environmental conditions of dominant hydrobionts.

✓ Important publications from recent years have been omitted; instead they should be included in the literature review and/or in the discussion of the obtained results, as they are directly related to the topic of the dissertation: concerning monitoring (Ninov & Karagiozova 2019, *Proceedings of the XXVIII Conference of the Danubian Countries on Hydrological Forecasting and Hydrological Bases of Water Management*), diatoms (Isheva & Ivanov 2016, *Botanica Serbica*; Isheva & Uzunov 2020, *Comptes rendus de l'Acade'mie bulgare des Sciences*) and macroinvertebrates in intermittent rivers in Bulgaria (Evtimova et al. 2021, *Ecologia Balkanica*) or on the Balkans (Vilenica et al. 2023, *Diversity*).

#### **Technical errors:**

✓ In Literature review, part III there is a subsection "Dissolved  $O_2$  and  $CO_2$ ", within which, however, there is no mentioning of the dynamics and importance of  $CO_2$ .

 $\checkmark$  Different levels of numbering rather than starting from 1 within different parts of the same chapter triggers confusion.

 $\checkmark$  I recommend consistency in using units, preferably use them according the international SI system (example: volume).

 $\checkmark$  When citing figures, published in previous studies, the original paper should be referred to (example: Figure II.5). All appendices should be cited within the relevant place in the main text of the PhD thesis.

 $\checkmark$  It would have been useful to provide detailed information on some of the analyses in chapter Results and discussion. Example: the individual weights of each of the environmental factors included in the PCA analyses (as an appendix).

 $\checkmark$  There are inconsistencies in the formatting of the list of cited literature: some journals are abbreviated, others are in italics; "&" and "and" are also used; for some the authors and the year are separated by commas, while for other references they are not; missing year (#3) and other details, which make it difficult to find where a paper is cited; for references #30 and 31 some of the authors are written in all caps.

 $\checkmark$  There are spelling, punctuation and grammar errors.

# Questions:

 $\checkmark$  What is the reason for the values describing the environmental factors to be averaged for Ariana, but not for Lilov vir Lake, for which there are data for the same time period (Fig. VI.5.)?

 $\checkmark$  Have the correlations among the environmental factors themselves been explored, including e.g. relationship between (length of) HP or drought index SRI and % macrophyte cover? Data on the relationship between macrophyte development and dynamics of various hydrological indicators exist in the literature, but these publications seem to be missing/ underrepresented in the interpretation of results.

 $\checkmark$  When and how are trophic networks in the studied water bodies assessed as stable and resistant? A resistance gradient is also mentioned, can the candidate provide some definition of this term?

 $\checkmark$  Based on the presented results and analysis and the candidate's accumulated experience, what are the interrelationships between the complexity or connectivity, in functional terms, of a system and its resistance? Specifically for IWB and in general?

#### (6) Contributions to science

A total of seven contributions are presented, all of which I accept as being original. I would like to make two clarifications here. The first concerns contribution #1, which states "for the first time in Bulgaria, animal communities in ... intermittent water bodies were studied". There is at least one earlier study which focuses on benthic invertebrates in intermittent Bulgarian rivers (Evtimova et al. 2021, *Ecologia Balkanica*) so rewording is needed. In my view, as formulated, contributions 6 and 7 are essentially making the same claim.

In general, the contributions adequately reflect the obtained results. The insufficient study on intermittent water bodies in the country is among the main reasons that there is little data on the subject, therefore, the contributions are significant namely due to obtaining new data in these understudied field.

## (7) Assessment of the quality of scientific contributions reflecting the dissertation

The doctoral student is presenting three scientific publications and two reports on the topic of the dissertation. Two of the articles are in thematic collections based on the two oral presentations given at the *Seminar of Ecology* (in 2015 and 2016) on the topic of the dissertation. The third scientific publication was published in *Comptes rendus de l'Academie bulgare des Sciences* (an international journal with an impact factor). The results, presented in two of the publications, concern two of the studied IWB, Aldimirovsko blato and Lilov vir. Pencho is the first author of both, among five or two co-authors, respectively. In the third publication, with four co-authors, Pencho is the second author. This last article presents first data on the oligochaete fauna of IWB in Bulgaria.

The papers and oral presentations on the topic of the PhD thesis are important contributions enriching our knowledge of aquatic invertebrate communities in IWB within the region.

# (8) A motivated answer to the question to what extent the dissertation research is primarily the personal work of the doctoral candidate

As far as I know, the vast majority of the results presented are the personal work of the PhD student who has been conducting research on the dissertation topic for years. Help with the

identification of zooplankton organisms was provided by Dr. Stefan Kazakov, as stated on page 55 of the thesis.

# (9) Conclusion

The PhD thesis meets the requirements of the Law on the development of the academic staff in the Republic of Bulgaria, the Regulations for its implementation, as well as the Regulations for acquiring scientific degrees and occupying academic positions in IBER-BAS.

All the provided information and namely the publications in specialised journals, participation in scientific forums and courses, literary awareness and the mastered methods for field and chamber work, the demonstrated independence are proof that Pencho Ivanov is, to a large extent, able to conduct an independent research and his dissertation work is a serious contribution in the study of intermittent water bodies in Bulgaria.

Given the above, I propose to the honourable jury to award P. Ivanov the educational and scientific degree "Doctor".

Sofia, 22.04.2024

Signature:

(Assoc. Prof. Vesela Evtimova)