

STRUCTURE OF ZOOCENOSES IN THE EPHEMERAL POND LILOV VIR, WESTERN BULGARIA

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Abstract

This study aimed to observe the development of zoocenoses in an ephemeral karst water body providing a dynamically changing habitat and unstable hydrological regime with an emphasis on assessing the main factors influencing the structure of the macrozoobenthos and zooplankton communities. The study was conducted in Pond Lilov Vir near Karlukovo Vil., Western Bulgaria. The main physicochemical parameters and the concentrations of nutrients were recorded. The macrozoobenthos (MZB) and zooplankton samples were taxonomically determined and statistical analyses of the obtained results were performed. The results have shown that the pond supports a variety of species and species typical to temporary waters. The variation in hydroperiod (HP) duration over the years leads to the formation of different communities. Longer HPs suggest greater biodiversity. In drying pools, especially those with a short HP, the leading factor for structuring communities is the abiotic environmental factors-HP and water volume (WVOL). Biotic factors, such as competition and predation, are not leading, due to the lack of fish and insufficient time for the development of invertebrate predators.

Key words: abundance, macrozoobenthos (MZB), ephemeral pond, temporary pool, zooplankton, hydroperiod (HP), water volume (WVOL), community

Introduction. Temporary water basins are widespread throughout the world and are becoming increasingly prevalent with climate changes, hence, they are gaining greater importance to biodiversity. Temporary ponds are habitats with a

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predictable annual dry phase of 3–8 months, usually in summer and autumn [1]. Seasonal ponds are those formed by snowmelt and precipitation in late winter and early spring in the temperate latitudes of the Northern Hemisphere [1].

In recent years, research on ephemeral waters has increased significantly due to climate change and drought, in which ephemeral waters are becoming more abundant. In Bulgaria, similar studies have been conducted on river floodplains, as well as on some surface and underground basins [2, 3], but intracontinental temporary reservoirs have not been studied.

The present study focuses on the structure of the zooplankton and MZB communities. The structure is expressed by the communities' species composition and the pond's settlement periodicity [4].

The specific water balance of ephemeral water bodies is mainly represented by the changes in HP and WVOL.

This study is aimed at observing the development of the zooplankton and MZB communities in Pond Lilov Vir as a model of an ephemeral hydroecosystem by assessing the main factors influencing their structure under limited environmental conditions.

Material and methods. Pond Lilov Vir is a seasonal karst water body fed mainly by spring snowmelt and rainwater collection from the surrounding hills. It usually fills up in April or early May and exists for between 5 and 15 weeks. Lilov Vir is located in the Karlukovo Karst Area, which is located near the gorge of the Iskar River crossing the area in Western Bulgaria. The climate is temperate continental, with a large seasonal amplitude of air temperature, spring-summer maximum and winter minimum of generally insufficient precipitation, and relatively stable snow cover. It is approximately 120 m long and 100 m wide or 1.2 ha. With a maximum depth of 2.2 m, it reaches a water volume of almost 15 600 m³. The geographic coordinates are: 43°10'37.6854" N, 24°4'40.3572" E, 228 m altitude. Pond Lilov Vir, as well as the similar inland temporary water basins in Bulgaria, have not been studied so far.

The samples were collected from April to August 2012 to 2015. They were collected every 14 days from the filling to the pond drying up. From 2012 to 2015, a total of 37 samples were collected, of which 20 were planktonic and 17 benthic. Main physical and chemical parameters like temperature (°C); dissolved oxygen content (mg/l) and oxygen saturation (%); active reaction (pH) and electroconductivity (µS/cm³), were measured in situ using a WTW Multi 1975i multi-parameter system. The nutrients recorded were: nitrate nitrogen (N-NO₃²⁻), nitrite nitrogen (N-NO₂⁻); ammonia (NH₄⁺); phosphates (P-PO₄²⁻); total phosphorus (TP(PO₄)). Their concentrations were measured photometrically by a WTW PhotoFlex portable photometer according to WTW methodology. The transparency was measured by a Secchi disk (m).

The MZB samples were collected according to EN ISO 9391:1995; EN 28265:1994; EN 27828:1994 standards. The laboratory processing of the MZB materials

included sorting samples by weeks, months, and years and taxonomic determination on an MBS-9 stereomicroscope. Taxonomic determination of benthos was done after KUTIKOVA and STAROBOGATOV [5]. The current systematic status of the organisms is consistent with Taxonomic hierarchy ver. 4.1.

The zooplankton samples were collected by filtering a volume of 100 l of water through an Apstein plankton net, with a 76 μm mesh size. Materials with a working volume of up to 100 ml are processed in the laboratory. Quantitative processing: by counting the organisms in 10 ml in a Bogorov chamber at 16 $\times$ magnification under a stereomicroscope. Quality processing: on the entire volume. An Olympus BX5 compound microscope was used for genus and species determination. The abundance and biomass were represented for m^3 . Taxonomic determination of Cladocera was done according to Kutikova and Starobogatov [5]; Copepoda – according to BLEDZKI and RYBAK [6].

The Shannon Biodiversity Index was used to assess biodiversity. Correlation using Excel 2007 was used to estimate the statistical probability of a relationship between biodiversity and hydroperiod and biodiversity and water volume.

Results and discussion. *Physical and chemical water parameters.* Lilov Vir is a karst pond with a small area and depth greatly varying depending on the temperature and amount of precipitation. As a result, the values of transparency, dissolved oxygen amount, and concentration of nutrients greatly vary (Fig. 1).

From 2012 to 2015 Lilov Vir had an HP of 35, 45, 120, and 110 days, respectively. In the years with short HP, 2012 and 2013, the depth of the pond at the beginning was only 0.35 m and 0.60 m, respectively. In March, the precipitation in these years was between 4 and 39 l/m^2 (according to the National Institute of Meteorology and Hydrology), respectively. In the years with long HP – 2014 and 2015 – the pond filled in May and its depth was 2.0 m and 1.90 m, respectively. In April, the precipitation in these years was 70 and 94 l/m^2 (NIMH), respectively. As solar activity and evaporation increase, the volume of water begins to decrease to 25–30 cm. And at the beginning of August, the pond dried up. Transparency decreased in 2012 from 0.3 m to 0.05 m; in 2013 from 0.5 m to 0.1 m; in 2014 from 0.45 m to 0.15 m; in 2015 from 1.0 m to 0.05 m at the end of HP. The water temperature during HP measured around 12 h, increased in 2012 from 18.4 $^{\circ}\text{C}$ to 26.8 $^{\circ}\text{C}$; in 2013 from 18.2 $^{\circ}\text{C}$ to 28.0 $^{\circ}\text{C}$; in 2014 from 25.0 $^{\circ}\text{C}$ to 33.1 $^{\circ}\text{C}$; in 2015 from 19.6 $^{\circ}\text{C}$ to 29.0 $^{\circ}\text{C}$. The water volume decreased from 143 m^3 to 23 m^3 in 2012; from 143 m^3 to 70 m^3 in 2013, from 4066 m^3 to 52 m^3 in 2014, and from 2687 m^3 to 34 m^3 in 2015. The lack of shading vegetation also contributed to the constant temperature increase during the HP. The wind and the small depth of the pond determined the lack of temperature stratification which is typical for a holo-polymictic water body. As the HP progressed and the water volume decreased the insolation also increased. The wind-induced aeration as well as the uptake of dissolved oxygen by the soils and sediments changes the concentrations

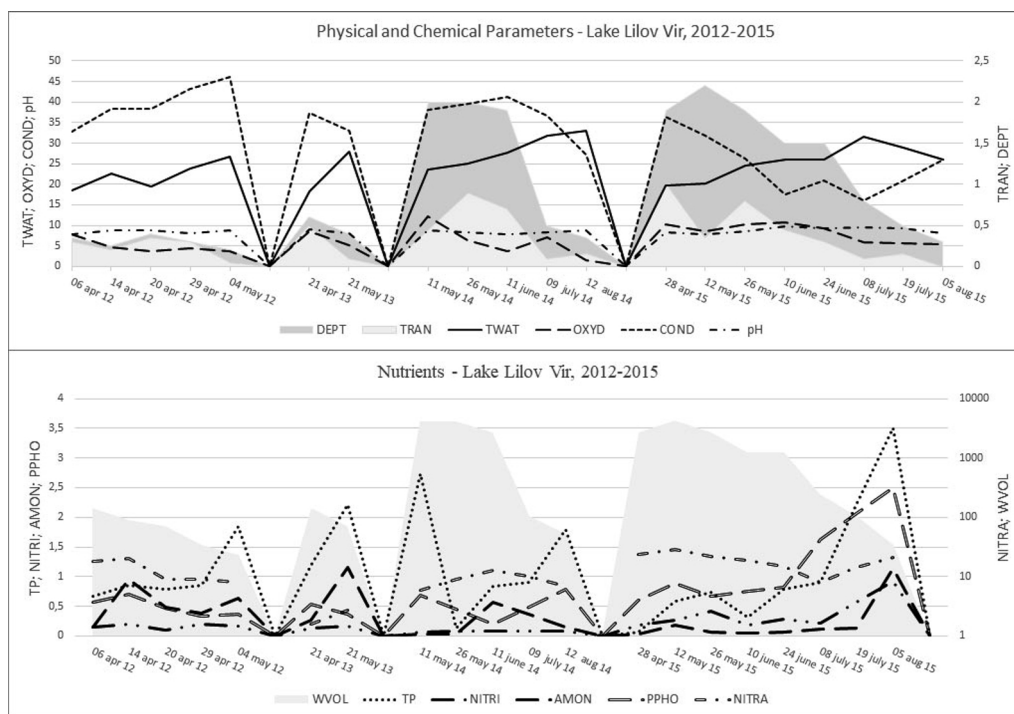


Fig. 1. Pond Lilov Vir, 2012–2015. Physical and chemical parameters: DEPT–Depth (m); TRAN–Transparency (m); TWAT–Water Temperature ($^{\circ}\text{C}$); OXYD–Dissolved Oxygen content (mg/l); COND–Electroconductivity ($\mu\text{S}/\text{sm}^3$); pH–Active Reaction. Nutrients: WVOL–Water Volume (m^3); TP–Total Phosphorus; NITRI–Nitrite Nitrogen; AMON–Ammonia; PPHO–Phosphates; NITRA–Nitrate Nitrogen (mg/l)

of the water and dissolves gases as well [7]. The dissolved substances' concentration dynamics were also observed for the nutrients (nitrates, nitrites, ammonium, and phosphorus) in Pond Lilov Vir. Nutrients come mainly with runoff from the surrounding arable lands and grasslands in the watershed. Moreover, the grazing cattle regularly enter directly into the pond.

The concentrations of nutrients in Lilov Vir vary, forming one or two peaks. This is due to the processes of hydration and drying up. There are relatively high levels of nutrients at the beginning of the hydration with the initial release of nutrients from the mineralization of dead tissue accumulated during the dry phase. This forms the first peak, which is the only one in the years with very short HPs. As the HP progresses the soil absorbs some of the precipitated nutrients, but the evaporation of water, which leads to the concentration of nutrients, keeps their levels relatively constant. At the end of the HP the concentrations are high and we observe a second, greater peak since the soil is oversaturated, the evaporation is higher and the water volume significantly decreases. In the longer HPs, the concentrations are less dynamic and their values are more stable over time, which is also in agreement with the results of MAGNUSSON and WILLIAMS [8].

Structure of communities. The HP in Lilov Vir varies from year to year and we observed diverse communities with a different number of taxa and different species. A total of 22 zooplankton taxa and 33 MZB taxa have been identified in Lilov Vir. The highest number of taxa was found in 2015 when the HP was 110 days. The lowest number was in 2012 when HP was 35 days. Zooplankton communities are represented mainly by calanoid and cyclopoid copepods, cladocerans and ostracods. MZB communities consist mainly of branchiopods and chironomids, and occasionally Ephemeroptera, Heteroptera, Odonata, Dytiscidae beetles, and nematodes. Widespread species (*Daphnia longispina*, *Chironomus pulmosus*, *Cyclops strenuus*, *Chydorus sphaericus*) were observed, according to the results of TISHEVA and KOZUHAROV [2]. Here, however, there is significant participation of large-sized forms of Cladocera, in contrast to the reservoirs studied by them with the presence of predatory pressure from planktivorous fish [2]. Species-specific to drying water bodies (*Triops longicaudatus*) are also observed. The structure of the communities in temporary waters according to BOIX et al. [9] and MENGE and SUTHERLAND [10] are mainly influenced by the HP. Short HPs imply a small number of taxa. This is in agreement with the studies of many authors according to whom the longer HP suggests the larger number of taxa [9,11]. This is also shown by the results of the Lilov Vir pond. Different species develop at different stages of HP. This creates a certain sequence in their appearance. According to JEFFRIES [4], the important biological factor for the formation and structure of the communities in temporary waters is the sequence of appearance of the species. In Lilov Vir the cyclopoid copepods are the first to develop (Fig. 2) and have numerical domination in the community during the first weeks (except for 2015).

They could adapt to a wider range of temperatures and this combined with the possibility of a latent period of their eggs allows them to develop faster [12]. Calanoid copepods increase in number significantly in the middle and late HP. The observation is consistent with TOLOMEYEV's findings [13]. The lack of predators and direct competitors allows them to develop quickly and massively when filling the water basin. Cladocerans are more significantly present in the later stages of the HP when water temperatures rise and enough copepods develop to form part of the diet of some predatory cladocerans. Ostracods have a relatively constant share in an HP. The dynamics of the biomass of the studied zooplankton groups during the HP follow that of the number of individuals, especially for Calanoid and Cyclopoid copepods. Ostracods, which are small in size, have an almost negligible contribution to the community's biomass. The cladocerans, on the contrary, have more massive sizes, and even though their number increases evenly during the HP, their biomass is significant already at the beginning of the HP. Then there is an opportunity for the development of larger individuals since there is no competition for food resources. In the middle of the HP, their biomass decreases due to the small size of the individuals and then increases again due to their high numbers.

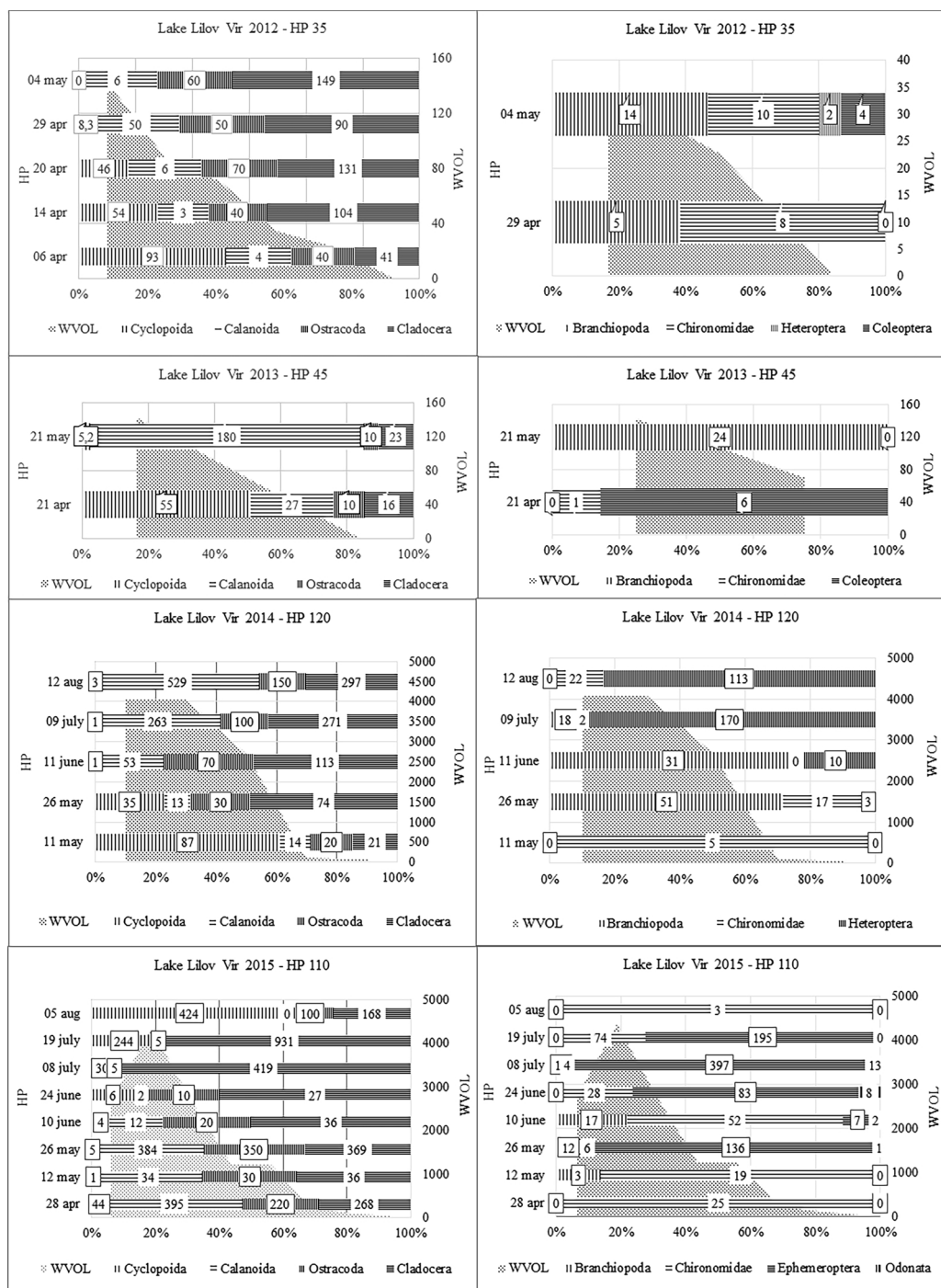


Fig. 2. The sequence of appearance of the species in Pond Lilov Vir during HP, 2012–2015, Plankton (Left side); Benthos (Right side). HP – hydroperiod (day); WVOL – Water Volume (m^3); The figures reflect the number of individuals in the sample

In 2015, calanoid copepods outnumbered cyclopoid copepods in the initial stages of pond filling. This is probably due to the lower temperatures (19.6 °C), the earlier filling of the pond, lower rainfall at the end of April 2015 – 51 l/m² (NIMH) and higher transparency up to 1 m. Whereas in early May 2014, rainfall was 206 l/m² (NIMH) and transparency was only 0.45 m.

The MZB samples in Pond Lilov Vir showed an even more significant difference in the number of identified taxa in the different HPs than those of the zooplankton. Only three taxa (two of which Insecta) appeared in 2013 and 23 taxa (15 of which Insecta) in 2014. In 2012 and 2013 (Fig. 2), when the water volume in Lilov Vir was low and HP was extremely short, the number of chironomids and branchiopods is very high and they almost exhaust the diversity of taxa in the pond. In 2014, the HP in Lilov Vir was significantly longer, and the first chironomids and branchiopods appeared again, with the highest numbers in the 3rd and 4th week of filling the pond. During the 8th, 9th and 10th weeks of filling the pond, a very large number of Diptera, mainly of the genus *Corixa* (imago), was observed, representing over 80% of the specimens in the benthic community. In 2015 there was again a long HP and Ephemeroptera, mainly of the genus *Cloeon* (imago), representing over 80% of the specimens in the benthic community between weeks 8 and 12. Similar observations were shared by WILLIAMS and FELTMATE [14]. According to them, members of the Insecta are widely distributed in temporary waters but do not support a diverse fauna as in permanent waters. They have a longer life cycle and cannot complete it within the short HP. Because of this, insects re-colonize temporary water bodies every year. Therefore, there may be significant differences in the time of onset of these two groups. And this contributes to the strong seasonal sequence characteristic of the temporary aquatic fauna. These observations fully support the results obtained from Pond Lilov Vir. In the first 2 to 4 weeks after filling the pond, crustaceans with a shorter life development period and/or presence of drought-resistant stages in their life cycles appear. They form the first peak in the number of benthic organisms. This peak is the only one for short HP. These are taxa such as Branchiopoda, Cladocera, Copepoda and Chironomidae. The genus *Triops* is a representative especially characteristic of temporary water bodies in temperate latitudes [15]. Due to the specificity of its life cycle, which requires the presence of a dry phase in its development, the presence of *Triops* can be treated as an indicator of temporary waters [11]. Longer HPs allow taxa with a longer life cycle such as predatory beetles, odonates, and other insects to develop and colonize the pond. They form the second peak in the number of specimens in the community, usually after the middle of the HP. There is a relationship between the peaks in the number of hydrobionts and those in the concentrations of nutrients. The MZB community is more strongly influenced by the HP length and water volume than the zooplankton community since the short HP and the water volume decrease lead to shrinking of the water basin boundaries and drying up of those parts of

the benthic zone that remain outside the water volume. This process constantly takes away living space from benthic organisms. The results in respect of the relationship between the HP, the water volume, and the Shannon Biodiversity Index are also confirmed by the evidence about the correlation of the biodiversity index with the HP and the water volume – 0.642 and 0.336 for the benthos, respectively, 0.447 and 0.290 for the zooplankton, respectively.

Short HPs exclude more specialized species due to poorer predictability of resources, on one hand, and insufficient time for their life cycle to develop, on the other. Therefore, invertebrate predators appear either late, as colonizers, or are absent at all. This, as well as the total lack of fish, due to the regular drying up of the pond, leads to the lack of predatory pressure. The sequence of occurrence of the different taxa greatly reduces the impact of competition for resources. As shown above, the plankton is less affected by water volume decrease than the benthos. Therefore, regardless of the paramount importance of the MZB to the functioning of standing water basins, the plankton community has a key role here meaning that the ecosystem functioning in Pond Lilov Vir is focused mainly on the pelagic zone. The absence of predatory pressure in Lilov Vir, especially in short HPs, suggests that the limited environmental conditions and not the biotic relationships will play a more important role in the structuring and functioning of the communities.

Conclusion. The zooplankton and MZB communities in Pond Lilov Vir include two main components: widespread species and taxa which are only or predominantly found in temporary waters (*Triops*). The different HPs offer different conditions and facilitate the development of different communities in temporary waters. A longer HP suggests greater biodiversity. The benthos is more strongly affected by the unstable hydrological regime of the pond than the plankton. Given the absence of predators, Zooplankton and MZB communities in Pond Lilov Vir (especially in short HP) will be formed primarily under the influence of environmental factors, mainly HP and water volume, rather than biotic factors. This suggests that organisms will be adapted primarily to the drying up of the water basin and not to avoid predatory pressure and competition.

REFERENCES

- [1] PÉREZ-BILBAO A., C. J. BENETTI, J. GARRIDO (2015) Biodiversity and conservation of temporary ponds – assessment of the conservation status of “Veiga de Ponteliñares”, NW Spain (Natura 2000 Network), using freshwater invertebrates. In: Biodiversity in Ecosystems: Linking Structure and Function (Ed. J. A. Blanco, Y.-H. Lo, S. Roy), London, IntechOpen, 642 pp.
- [2] TISHEVA M., D. KOZUHAROV (2013) Structure of the zooplankton communities from several water bodies in Natura 2000 zones, Bulg. J. Agric. Sci., **19**(Suppl. 2), 246–249.

- [3] KOZUHAROV D., K. BOGACHEVA (2016) Study of the Rotifera and Crustacea species composition from some surface and subterranean karst water bodies in Godech district, Speleological Studies of Caves in Godech Municipality, Part 1, 17–20.
- [4] JEFFRIES M. J. (2001) Modeling the incidence of temporary pond microcrustacea: The importance of dry phase and linkage between ponds, *Isr. J. Zool.*, **47**, 445–458.
- [5] KUTIKOVA L. A., YA. I. STAROBOGATOV (Eds) (1977) Keys to the Freshwater Invertebrates of the European Part of USSR: Plankton and Benthos, Gidrometeoizdat, 318 pp (in Russian).
- [6] BLEDZKI L. A., J. I. RYBAK (2016) Freshwater Crustacean Zooplankton of Europe: Cladocera & Copepoda, Springer, 918 pp.
- [7] SPOSITO G. (2008) The Chemistry of Soils (2nd ed.), Oxford Univ. Press, 344 pp.
- [8] MAGNUSSON A. K., D. D. WILLIAMS (2006) The roles of natural temporal and spatial variation versus biotic influences in shaping the physicochemical environment of intermittent ponds: a case study, *Archiv für Hydrobiologie*, **165**(4), 537–556.
- [9] BOIX D., J. SALA, R. MORENO-AMICH (2001) The faunal composition of espolla pond (NE Iberian Peninsula): the neglected biodiversity of temporary waters, *Wetlands*, **21**(4), 577–592.
- [10] MENGE B. A., J. P. SUTHERLAND (1976) Species diversity gradients: synthesis of the roles of predation, competition, and temporal heterogeneity, *Am. Nat.*, **110**(973), 351–369.
- [11] WILLIAMS D. D. (2002) Temporary water crustaceans: biodiversity and habitat loss. In: *Modern Approaches to the Study of Crustacea*, Kluwer Academic/Plenum Publishers, NY, 223–233.
- [12] WILLIAMS D. D. (2006) *The Biology of Temporary Waters*, Oxford University Press, 337–369, ISBN 13: 9780198528128.
- [13] TOLOMEYEV A. P. (2002) Phytoplankton diet of *Arctodiaptomus salinus* (Copepoda, Calanoida) in Pond Shira (Khakasia), *Aquatic Ecol.*, **36**(2), 229–234.
- [14] WILLIAMS D. D., B. W. FELTMATE (2009) *Aquatic Insects*, Published online: Cambridge University Press, 360 pp.
- [15] RZÓSKA J. (1984) Temporary and other waters. In: *Sahara Desert* (ed. J. L. Cloudsley-Thompson), Oxford, Pergamon Press, 105–114.

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