

Contributions to the knowledge on the biocoenotic characteristics of temporary water-bodies from Northern Dobrogea (Romania)

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SUMMARY. In the present study, we analyzed the algae assemblages and environmental condition in four temporary ponds located in Dobrogea mainland (Babadag Plateau) aiming to reveal the role of those habitats as a support to the breeding period of the amphibians. The outcomes of this study were that the algae community structure and its diversity were high given the changing conditions, with a total number of 75 taxa. The highest number of taxa belonged to the phylum Bacillariophyta and Chlorophyta, driven by: temperature and local hydrology conditions. Some of the native amphibians also use this type of habitats during the breeding period, the local climatic conditions having as effect longer juvenile stages in case of each species (in comparison with the ones of populations, of the same species, present in permanent water bodies from the studied region). The novelty of our study resides in the fact that we obtained data on algae assemblage distributed in temporary ponds, information regarding the type of diversity and a better understanding of ecological value of these habitats.

Keywords: algae, amphibians, ponds, Romania

Introduction

Ponds are important habitats within many landscapes, because of the diversity of wildlife they support and their value in terms of biodiversity and socio-economic benefits (Boix *et al.*, 2012). These habitats are harboring singular flora and fauna that are often exclusively or infrequently found in permanent ponds (Jeffries, 2008; Florencio *et al.*, 2014). On a global scale, temporary ponds (TPs) are often neglected due to their ephemeral character, even if on a global scale, they cover a greater total area than lakes (Boix *et al.*, 2012). These ecosystems have been classified as endangered all over

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the world and many of them are at risk, mainly due to human activities, such as agricultural pollution, expansion of cropland and water resources over-exploitation (Dimitriou *et al.*, 2006). No information was recorded on the historic evolution of the TPs of Dobrogea region. Most of those habitats seemed to be not a priority in Romanian nature-conservation policy and thus there are very few information regarding their status. Nevertheless, although Romania adopted in its environmental legislation (O.U.G., 2007) the European directives that are dealing with environmental protection (Council Directive, 1992; Council Directive, 2006), there is not a clear strategy for management and conservation efforts that should be applied for protecting these habitats and avoiding their disappearances.

The temporary ponds of Dobrogea Plateau are important habitats that could be characterized by their variability in size, hydrological functioning, having a total dependence on local hydrology and in most of the cases being linked with permanent aquatic habitats in the spring time. Those ponds that are not linked with permanent water bodies usually occupy small depressions which are an important environment for amphibians and birds (Török *et al.*, 2015).

Material and methods

Field surveys, in situ measurements of water temperature, dissolved oxygen concentration (DO), electrical conductivity (EC) have been performed using HANNA instruments (portable HI 98290 Multiparameter with GPS). Algal biomass using bbe- Moldaenke environmental techniques and evaluation of amphibian populations have been made from July to October 2014.

The biomass of every algae group is given by the concentration of chlorophyll *a*. The bbe- Moldaenke environmental techniques uses the fluorescence extinction spectrum performed at 470 nm LED for green algae, 610 nm LED for blue-green algae (cyanobacteria), 525 nm LED for diatoms and 570 nm LED for cryptophyceae.

Water samples (50 to 250 ml) preserved with Lugol solution, were taken for subsequent analysis of algae taxa.

Quantitative studies have been carried out using a light microscope at low and high magnification. Filaments, colonies and coenobies were counted individually. At least 200 cells were enumerated per each sample.

To assess the trophic state of the temporary ponds, the following indices were used in the present study: delta-eutrophicity index, epsilon-eutrophicity index (Oltean, 1977); Nygaard compound index (1949), Thunmark's chlorophycean index. To determine the level of pollution of the water, the organic pollution index (Palmer, 1969) was used. The saprobic indicator values of certain algal species were considered, following Rott (1997), Hindak (1978), Sládeček (1973). Identifications were made to the species level using more identification keys (Krammer and Lange-Bertalot, 1986, 1988, 1991; Ettl, 1983).

Descriptive statistics using PRIMER 6 statistical package was applied to derive information about common characteristics of the Cerbu TPs. The spatial pattern of algal population was analyzed using Bray Curtis index of similarity.

Amphibians were assessed using the methodology developed for nation-wide monitoring of amphibians and reptiles of Community Interest (Török *et al.* 2013).

Description of the Study Area

The research-site within which ponds were naturally dug is located in south-eastern Romania, respectively, in the central part of northern Dobrogea (a historical region delimited by the Danube River and the Black Sea), at the western limit of the forested area of the Babadag Plateau.

There are four small ponds in the landscape of the area, two of them having a significant spatial and temporal variation which mirrored the changes in water level during the summer - autumn period and the pond life during the year (Fig.1).

The taxonomy and natural history of these ponds are unknown. The ponds have no aquatic plant communities; there are fish-free habitats which supported at least four species of amphibians - during the investigations being recorded *Pelophylax ridibundus*, *Rana dalmatina*, *Bufo viridis* and *Pelobates* sp. (the tadpoles of the later taxa were assumed to belong to *Pelobates syriacus*).

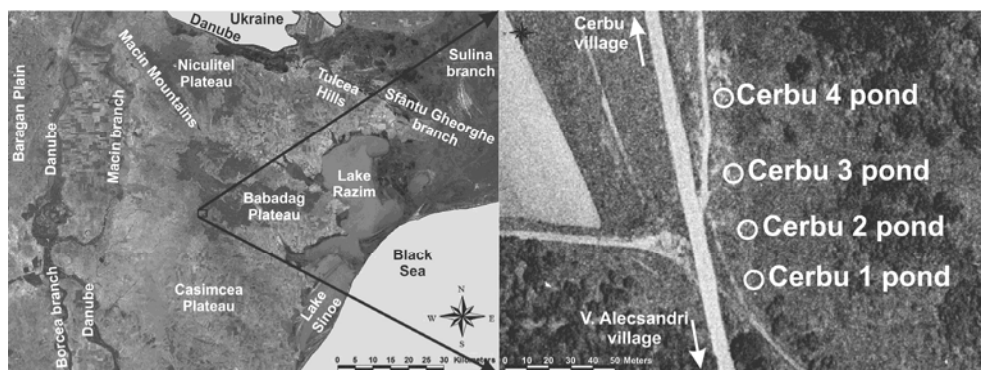


Figure 1. Location of the studied temporary ponds.

Results and discussions

Environmental characteristics of the TPs

An extensive survey of ponds during summer times has identified the ranges of temperature, DO and EC, which create the distinctive patterns of the local variation of those environmental variables that could influence the colonization rate, species richness

and composition/ structure of the algal communities. In this respect, the temperature of water ranged between 25.4°C to 31°C; DO between 4.4 to 22.46 mg/L; EC 151.8 µS to 469 µS. During autumn the temperature dropped at 17.4°C and EC ranged between 256.3 and 411 µS.

Ponds Cerbu 1 and Cerbu 3 (Fig. 1) have dried up and refilled after precipitations during the two seasons.

Phytoplankton communities of the TPs

The algae assemblage of a TP is a good indicator of the ecological status and could be an important tool to explore the biodiversity pattern of those kinds of vulnerable systems.

According to the similar quality of photosynthetic pigments of the Chlorophyceae and Euglenophyceae, and of Bacillariophyceae and Dinophyceae in case of evaluation of the algal biomass using fluorescence extinction spectrum, it was not possible to distinguish between the amounts of biomass produced by these taxonomic groups. Only by additionally analysis, performed by using microscopy techniques, it was possible to differentiate the contribution of those taxonomic groups to the community of the primary producers of the four TPs.

The total recorded concentration of algae biomass reached the maximum of 936 µg/L has been recorded in Cerbu 4 pond (pond no. C4 in Fig. 2). The lowest recorded concentration being 31 µg/L recorded in October in Cerbu 3 pond (pond no. C3 in Fig. 2). The results suggest a high degree of trophic conditions and heterogeneity of the pond life across the present landscape.

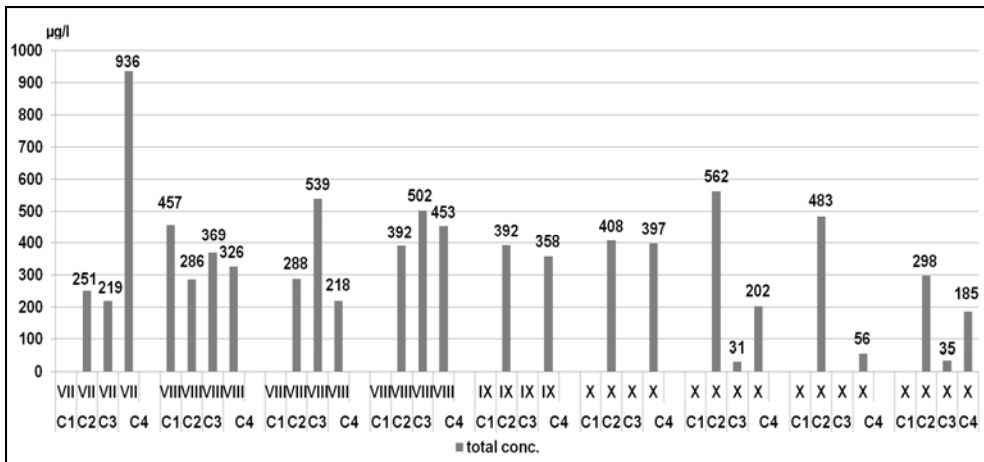


Figure 2. Variation of algae biomass during investigation period

The ponds mostly affected by the spatial and temporal variation of the hydrological events are Cerbu 1 pond (C1) and Cerbu 3 pond (C3). However, the local conditions allowed a rapid development of algal communities. A new water supply and a temporary fill up creating the opportunity to a rapid species colonization of the ponds. The first inhabitants being the groups of Cyanophyceae (blue green algae) (Fig. 3), but the most significant contribution to the maintenance of the diversity through species replacement is given by the green algae through its genera *Scenedesmus* and *Kirchneriella*.

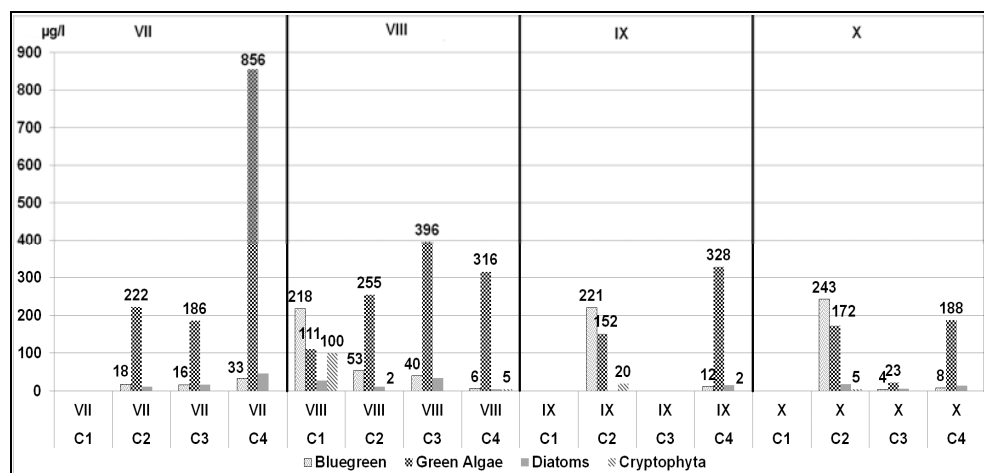


Figure 3. Biomass variation of the taxonomic groups

Algal taxonomical survey

A total number of 75 taxa were identified, belonging to the following phylum: Cyanophyta, Euglenophyta, Bacillariophyta and Chlorophyta (Tab. 1). Numerous taxa identified in the TPs are known to be found in ponds, as: *Euglena hemichromata*, *Phacus acuminatus*, *P. orbicularis*, *Trachelomonas intermedia*, *T. volvocina*, *Cyclotella meneghiniana*, *Scenedesmus intermedius*, *S. protuberans* and *Tetraëdron caudatum*. True planktonic species were also found, as *Aphanothece clathrata*, *Trachelomonas scabra*, *Didymocystis planctonica*, *Kirchneriella subcapitata*, *Scenedesmus bicaudatus* and *S. longispina*. Other taxa have a cosmopolitan distribution, as *Diatoma tenuis*, *Navicula pygmaea*, *Monoraphidium contortum* and *Scenedesmus opoliensis*.

Table 1

Algal taxa identified in the samples from the four temporary ponds studied in this work (abbreviations: SU-summer, AU-autumn)

TAXA	SU_C1	SU_C2	SU_C3	SU_C4	AU_C2	AU_C3	AU_C4
Phylum Cyanophyta							
<i>Aphanocapsa cf. holsatica</i> (Lemmermann) G.Cronberg & Komárek 1994					+		
<i>Aphanothece clathrata</i> West & G.S.West 1906		+					
<i>Aphanothece minutissima</i> J.Komárková-Legnerová & G.Cronberg 1994					+	+	
<i>Merismopedia tenuissima</i> Lemmermann 1898				+			
<i>Microcystis pulchra</i> (Kuetzing) J. Stein				+			
Phylum Euglenophyta							
<i>Euglena gaumei</i> Allorge & Lefèvre 1931				+			
<i>Euglena hemichromata</i> Skuja 1948					+	+	
<i>Phacus acuminatus</i> Stokes 1885		+		+			
<i>Phacus orbicularis</i> K.Hübner 1886				+			
<i>Trachelomonas intermedia</i> P.A.Dangeard 1902					+		
<i>Trachelomonas oblonga</i> Lemmermann 1899					+		
<i>Trachelomonas scabra</i> Playfair 1915					+		
<i>Trachelomonas volvocina</i> Ehrenberg 1834					+	+	
Phylum Bacillariophyta							
<i>Achnanthes minutissima</i> Kützing 1833					+		
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen 1979	+			+			
<i>Caloneis molaris</i> Krammer in Krammer & Lange-Bertalot 1985	+	+		+	+		
<i>Cocconeis pediculus</i> Ehrenberg 1838	+	+		+	+		
<i>Cocconeis placentula</i> Ehrenberg 1838	+	+		+	+		
<i>Cocconeis placentula</i> var. <i>euglypta</i> (Ehrenberg) Grunow 1884	+	+	+	+	+		
<i>Cyclotella meneghiniana</i> Kützing 1844	+			+	+		
<i>Diatoma tenuis</i> C.Agardh 1812				+			
<i>Diploneis oculata</i> (Brébisson) Cleve 1894				+			
<i>Fragilaria ulna</i> (Nitzsch) Lange-Bertalot 1980	+						
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot 1980				+			
<i>Gomphonema parvulum</i> Kützing 1849	+			+		+	

Table 1 continued

<i>Gomphonema truncatum</i> Ehrenberg 1832						+
<i>Hantzschia amphioxys</i> Grunow in Cleve & Grunow 1880		+		+	+	+
<i>Navicula cincta</i> (Ehrenberg) Ralfs in Pritchard 1861				+	+	+
<i>Navicula cryptotenella</i>						
Lange-Bertalot in Krammer & Lange-Bertalot 1985	+	+		+	+	
<i>Navicula gregaria</i> Donkin 1861						+
<i>Navicula lanceolata</i> Ehrenberg 1838				+		
<i>Navicula cf. mutica</i> Kützing 1844	+	+				+
<i>Navicula phyllepta</i> Kützing 1844				+		
<i>Navicula pygmaea</i> Kützing 1849	+			+	+	
<i>Navicula radiosa</i> Kützing 1844				+	+	+
<i>Nitzschia intermedia</i> Hantzsch in Cleve & Grunow 1880				+		
<i>Nitzschia palea</i> (Kützing) W.Smith 1856	+					+
<i>Nitzschia paleacea</i> Grunow in Van Heurck 1881	+	+			+	+
<i>Nitzschia paleaeformis</i> Hustedt 1950	+				+	+
<i>Nitzschia perminuta</i> (Grunow) M.Peragallo 1903	+				+	
<i>Pinnularia borealis</i> Ehrenberg 1843	+					
<i>Pinnularia brebissoni</i> (Kützing) Rabenhorst 1864	+				+	
<i>Pinnularia microstauron</i> (Ehrenberg) Cleve 1891	+					
<i>Stauroneis anceps</i> Ehrenberg 1843	+	+			+	+
<i>Surirella angusta</i> Kützing 1844	+	+			+	
Phylum Chlorophyta						
<i>Closteriopsis acicularis</i>						
(Chodat) J.H.Belcher & Swale 1962						+
<i>Cosmarium subcostatum</i>						
Nordstedt in Nordstedt & Wittrock 1876					+	
<i>Crucigeniella rectangularis</i> (Nägeli) Komárek 1974					+	
<i>Didymocystis planctonica</i> Korshikov 1953					+	
<i>Kirchneriella microscopica</i> Nygaard 1945					+	+
<i>Kirchneriella subcapitata</i> Korshikov 1953					+	
<i>Monoraphidium contortum</i>						
(Thuret) Komárková-Legnerová in Fott 1969	+	+			+	+
<i>Monoraphidium griffithii</i>						
(Berkeley) Komárková-Legnerová 1969					+	+
<i>Scenedesmus acutus</i> Meyen 1829					+	
<i>Scenedesmus acuminatus</i> (Lagerheim) Chodat 1902					+	
<i>Scenedesmus bicaudatus</i> Dedusenko 1925	+	+	+	+	+	+

Table 1 continued

<i>Scenedesmus danubialis</i> Hortob. 1970								+
<i>Scenedesmus ecornis</i> (Ehrenberg) Chodat 1926	+	+			+			
<i>Scenedesmus gutwinski</i> var. <i>heterospina</i> Bodrogsközy 1950						+		+
<i>Scenedesmus intermedius</i> Chodat 1926			+	+	+			+
<i>Scenedesmus longispina</i> Chodat 1913								+
<i>Scenedesmus cf. obtusus</i> Meyen 1829	+	+						
<i>Scenedesmus opoliensis</i> P.G.Richter 1895								+
<i>Scenedesmus opoliensis</i> var. <i>mononensis</i> Chodat 1926								+
<i>Scenedesmus pecsensis</i> Uherkovich 1956								+
<i>Scenedesmus protuberans</i> F.E.Fritsch & M.F.Rich 1929						+		+
<i>Scenedesmus protuberans</i> var. <i>danubianus</i> Uherkovich 1956								+
<i>Scenedesmus quadricauda</i> (Turpin) Brébisson in Brébisson & Godey 1835					+	+	+	+
<i>Scenedesmus quadricauda f granulatus</i> Hortob. 1960								+
<i>Scenedesmus quadrispina</i> Chodat 1913								+
<i>Schroederia setigera</i> (Schröder) Lemmermann 1898						+		
<i>Tetraëdron caudatum</i> (Corda) Hansgirg 1888							+	
<i>Tetraëdron minimum</i> (A.Braun) Hansgirg 1888	+	+			+	+		
<i>Tetraëdron muticum</i> (A.Braun) Hansgirg 1888							+	
<i>Tetrastrum triangulare</i> (Chodat) Komárek 1974								+
TOTAL TAXA	25	22	9	27	36	8	22	

The phylum Bacillariophyta dominated the algal communities, reaching a percentage of 42%, followed closely by Chlorophyta with a 40%, Euglenophyta and Cyanophyta with less percentage (11% and 7%, respectively).

The total number of taxa differed from the two seasons, the highest variation being recorded in pond C3, ranging between a maximum of 36 taxa (recorded in autumn) to a minimum of 9 taxa (identified in the summer samples). Two of the ponds are extremely vulnerable due to the variation of water level: C1 dried out in at the end of summer (and remained dry the autumn) (Fig. 4), respectively C3 which was dry for a short period at the middle of the summer, then again in different weeks of the autumn (depending on the amount of rains).

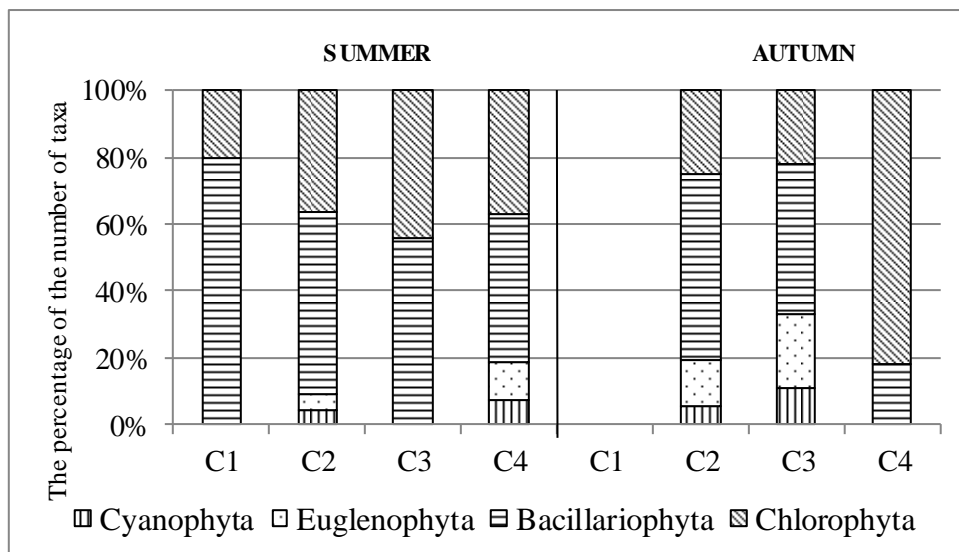


Figure 4. The distribution of phylum based on the number of species / season

A number of 25 taxa were identified in C1 in the samples collected in summer. In pond C2 the highest number of taxa was identified in autumn (36 taxa), whereas in summer only 22 taxa were recorded. In pond C3 the number of taxa was low, 9 in summer and 8 in autumn. In the TP C4 the number of 27 taxa in summer samples, dropped to 22 taxa in autumn (Fig. 5).

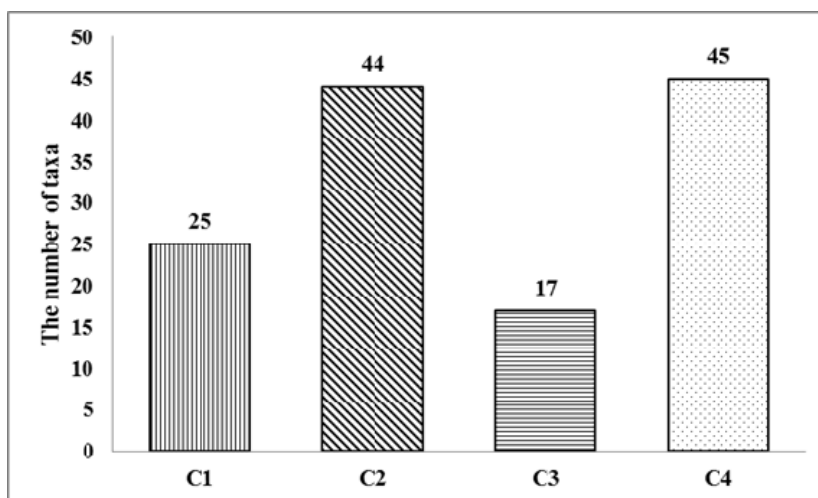


Figure 5. Number of taxa identified in each pond

The algal composition similarity between the sites from the four TPs, based on the presence – absence of the species at each site, using Bray-Curtis similarity performed with PRIMER 6 statistical package, shows that the special pattern of the algal population had a high similarity between ponds C1 and C2 (Fig. 6). Also in case of biomass, small variations were registered between ponds in late summer and autumn (Fig. 7).

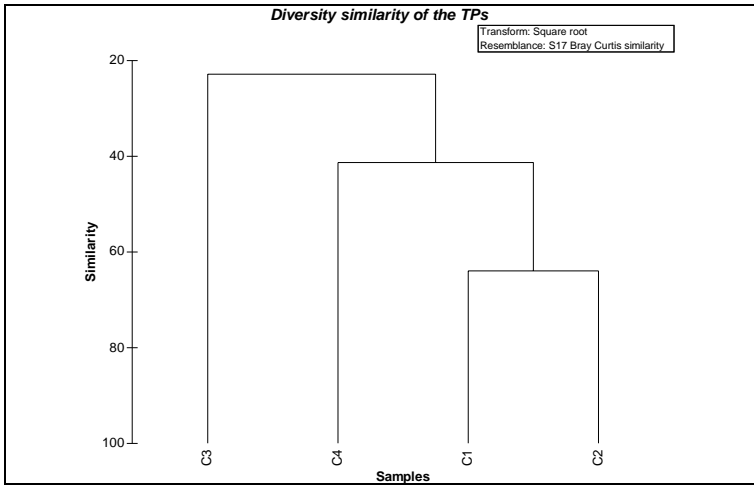


Figure 6. Diversity similarity of the temporary ponds from Cerbu area.

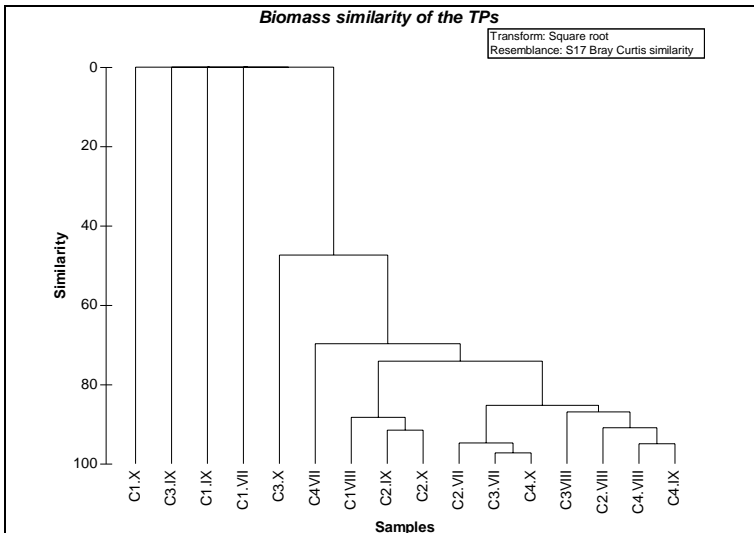


Figure 7. Biomass similarity of the temporary ponds from Cerbu area.

The highest value of phytoplankton abundance was recorded in pond C3 and C4 in summer and C2 and C4 in autumn. In those ponds there were recorded the highest amount of cells / sample.

Generally speaking when this huge development of algae occurs there is so-called algal blooms which are evident when algae color the water and the cell number reaches $5 \cdot 10^6$ (Goldman *et al.*, 1983). In the studied ponds this values were associated with the decreasing trend in algal biodiversity. In summer, in pond C3 the dominating taxa were *Scenedesmus protuberans* and *S. quadricauda* with more than 90% of the total number of cells. In pond C4, in the summer samples, the highest percentage was reached by *Phacus acuminatus* (70%), other small percentages belong to *Tetraëdron* (9%), *Scenedesmus* (7%), *Monoraphidium* (5%) and *Euglena* (3%). In the autumn samples, in both C2 and C4 ponds, *Kirchneriella microscopica* was the dominant taxa (around 70% in both samples). Pond no. C2, in autumn had the highest algal-diversity with 10 species belonging to 9 genera, *Kirchneriella* was followed by *Tetraëdron* with 10% percentage (other small percentages belonged to: *Kirchneriella subcapitata*, *Scenedesmus bicaudatus*, *S. intermedius*, *S. protuberans*, *S. quadricauda*, *Tetraëdron minimum*, *Trachelomonas volvocina*, *Aphanocapsa cf holsatica*, *Monoraphidium contortum*, *Euglena hemisphaerica*, *Aphanothece minutissima* and species belonging to Bacillariophyceae).

Ecological status of the TPs

Each time a pond fills with water, a new episode in community ecology begins as species arrive to take advantage of the opportunity to complete the free-living aquatic stage of their life cycle (Wilbur, 1997).

Based on ecological preference of algal species the following characteristics were observed: out of the 75 taxa identified in the samples, 23 taxa are indicators of different saprobic values, a percentage of 44% indicating that the water saprobic value is β -mesosaprobic. The Palmer genus index (which refers to the organic pollution of the water) reached the highest value in pond C4: 6 genera (out of the 20 indicator algal genera) as: *Euglena*, *Phacus*, *Aulacoseira (Melosira)*, *Navicula*, *Nitzschia* and *Scenedesmus* (Palmer, 1969). At genus level, the value of the organic pollution index (form 7 to 18) indicated low to intermediate organic pollution of the water in the temporary ponds studied in the present work.

According to the eutrophic classification of Oltean (1977), in pond C2, delta-eutrophic conditions were detected in autumn 2014, because of the “water blooms” with Chlorococcales (species *Kirchneriella microscopica*); epsilon-eutrophic conditions were identified in pond C4, summer 2014, due to “water blooms” with taxa belonging to Euglenophyta (specifically *Phacus acuminatus*). The values of the indices (2.04 and 10.02, respectively), together with the values of other indices: Thunmark’s chlorophycean index (27) and Nygaard’s compound index (42) indicate that the temporary ponds present

in this study are in an advanced state of eutrophication. This conclusion is supported by the presence of 20 taxa that indicate high levels of eutrophication, as: *Aphanocapsa delicatissima*, *Euglena gaumei*, *E. hemichromata*, *Phacus acuminatus*, *P. orbicularis*, *Trachelomonas intermedia*, *T. oblonga*, *T. volvocina*, *Aulacoseira granulata*, *Cyclotella meneghiniana*, *Diatoma tenuis*, *Monoraphidium contortum*, *Scenedesmus opoliensis* and *Tetraëdron caudatum*.

Amphibian communities of the TPs

Amphibians, having a biphasic life cycle, use for growth and development the temporary ponds. According to our results, the temporal scale of Cerbu ponds with drying periods influenced the life cycle and growth of amphibians by modification of the optimal time of metamorphosis. Previously studies showed, that the regulation of population density and the fitness of individuals are determined by complex interactions among competition, predation, and uncertainty in the length of the time ponds retain water. Anurans can have strong effects on the partitioning of the flow of nutrients through the phytoplankton vs. the periphyton (Wilbur, 1997).

In case of the four temporary waterbodies from nearby Cerbu village, the Marsh Frog (*Pelophylax ridibundus*) was the only species constantly present in the study area. Although (even if only few) adult specimens were recorded each time in the ponds filled with water, tadpoles were also observed at least till the middle of October. In the permanent aquatic systems of the region (most of them located in the valley of the Danube river, in the Danube Delta and in the Razim-Sinoe lagoony area), tadpoles of *Pelophylax ridibundus* are usually recorded only till middle of July – beginning of August.

In case of other amphibian species, practically there were recorded only tadpoles or recently metamorphosed specimens (*Bufo viridis* and *Pelobates syriacus* till August, respectively, *Rana dalmatina* till July). Adults of these species are present in the ponds only in the spawning period (March – April). In the permanent waterbodies of the region the tadpoles of these species reach the end of metamorphosis at least one month earlier. The only adult (a *Rana dalmatina*) was observed one time (at the beginning of August) in Cerbu 2 pond, being probably a vagrant specimen, that accidentally reached, for a short time, the respective pond (in most cases, after the spawning period, the adults of this species occur practically exclusively in terrestrial, forested habitats).

Conclusions

The highest algal diversity has been recorded in pond no. C2. Even though the pond no. C1 dried out in autumn, the colonization rate was much higher than in pond no. C3, this was also dried out frequently in the two seasons. The most abundant taxa belonged to genus *Kirchneriella* and *Scenedesmus*, the environmental conditions being more suitable to their preferred ecology.

The temporary ponds are important habitats that insure the existence of local populations, even if small, of four native species of amphibians. According to the results of the present study, the metamorphosis of the amphibians recorded in the temporary ponds was completed later (with 2 or 3 months delay, depending on the species) than in case of the populations of the same species from permanent water bodies of the region.

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