

THE FIRST SETTLERS OF NEWLY BUILT POOLS: ZOOPLANKTON AND PHYTOPLANKTON CASE STUDY IN SOUTHERN MORAVIA

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Abstract

The biodiversity of pool ecosystems is nowadays fully dependent on building of new ones or reconstructing the damaged ones. Among the factors influencing the potential of being colonised are the habitat's local characteristics including abiotic and biotic factors. One of the most important key roles in the food chains of freshwaters play zooplankton and its high dispersal rate makes them successful colonists of new habitats. Together with phytoplankton and nutrient content development, the state and following evolution of pool ecosystem can be assessed. The aim of this study was to survey initial zooplankton succession of newly built pools and to assess the main influencers on its colonisation success. Two newly built pools (Pool 1 and Pool 4) with different morphometric characteristics were monthly sampled for zooplankton, phytoplankton and physico-chemical characteristics. Zooplankton individuals were sorted according to taxa and stage as cladocerans, copepods (adults), nauplii and rotifers; and according to size structure as follows: <0.5 mm, 0.5–1 mm, 1–2 mm and >2 mm. Phytoplankton species were sorted in five categories: cyanobacteria, cryptomonads, green algae, diatoms and other algae. Basic physico-chemical parameters were measured and nutrient analysis were carried out. In Pool 1, first colonists were rotifers, followed by various cladoceran taxa. Rapid increase of large cladoceran species occurred in late spring. Till the end of the survey, rotifers together with nauplii predominated. Larger copepods were constantly present since late spring. In Pool 4, first colonists were rotifers, followed by copepod nauplii which predominated till the end of survey. Larger zooplankton species peaked in summer. In the first season after inundation, the presence of a massive biomass of charophytes and subsequently green filamentous algae was crucial for the development of the communities in both pools - significantly reduced the development of phytoplankton, caused high water clarity and affected the development of zooplankton. Because there were nutrients released from the sediment nutrient pool inflicted by fertilisation of intensively farmed field, significant fluctuations in pool ecosystem were observed. Also the morphometric characteristics of the pool, such as size, shape, depth and slope of the shores indicated the suitability of the habitat for successful zooplankton colonisation.

Keywords: physico-chemical parameters, phytoplankton, pool, succession, zooplankton

INTRODUCTION

Small standing waters, including pools, are widely defined as shallow flooded wetland areas. Not far in the past, wetlands were target of systematic destruction in favour of increase of agriculture,

infrastructure and building development. Regarding the importance of wetlands for support of the functioning of the other ecosystems, biodiversity and nature conservation (Austin and Schriever, 2023; Dixon *et al.*, 2016), it is terrifying to find out,

that historical reports state the loss of 87% of natural wetland area since the beginning of 18th century (Davidson, 2014). Recently, global wetlands cover more than 12.1 million km² (Gardner et al., 2018). Nowadays, wetlands are the most endangered biotopes in the Czech Republic and furthermore, all over the world. To protect wetlands of all kinds in global scale, the Ramsar Convention on Wetlands was created (The Convention on Wetlands, Ramsar, Iran, 1971). The biodiversity of pool ecosystems is nowadays fully dependent on building of new ones or reconstructing the damaged ones (Williams *et al.*, 2008). The creation of new habitats is followed by a phase of community assembly, which should lead to a gradual increase of local species richness (Loreau, 2000). Among the factors influencing the potential of being colonised are the habitat's local characteristics. This biotic and abiotic conditions of the habitat itself may facilitate or inhibit colonisation by new species (DeMeester *et al.*, 2005). It has been shown that species could easily colonise the areas with low local biodiversity, such as new pools (Shurin, 2000).

One of the most important key roles in the food chains of freshwaters play zooplankton (Vad *et al.*, 2012) which show very high dispersal rate (Louette and De Meester, 2005; Scheffer and Van Geest, 2006). Such ability makes them successful colonists of new niche (Bilton *et al.*, 2001). Usually, they have a strong passive dispersal capacity in which are the individuals dispersed by biotic and abiotic vectors (Gilbert *et al.*, 2023). Within the abiotic vectors we include wind and rain (Moreno *et al.*, 2016), as well as the flowing surface waters (Havel and Shurin, 2004). Among the biotic vectors belong animals as insects, fish, amphibians, mammals (Bilton *et al.*, 2001) and human mediated mechanisms (Waterkeyn *et al.*, 2010; Wejnerowski *et al.*, 2022). The structure of an entire zooplankton community can be dependent on the order in which the pioneer species have arrived at the locality (Frisch and Green, 2007; Allen *et al.*, 2011). According to in situ studies (Pithart *et al.*, 2007), we can assess the state of aquatic ecosystem and predict the way of succession based on the zooplankton species composition.

Also major role in wetland ecosystems play planktonic cyanobacteria and algae which provide e.g. the food (Graham *et al.*, 2009). Their dispersal capacity is still unresearched in full range and new ways are discovered (Kaštovský *et al.*, 2010; Stanojkovič *et al.*, 2022). In general, we can sort algal and cyanobacterial transport in two ways: 1. Abiotic and Biotic, 2. Natural and Anthropogenic. As abiotic, airborne transport, storm debris or water currents were described. Biotic transport includes aquatic organisms as insect, fish, birds and mammals (Curren and Leong, 2020). In the Anthropogenic category, there are all man-made ways included, e.g. biofouling on ships, plastic debris, watersampling equipment, footwear, motor vehicles. It is a well-known fact that composition of phytoplankton, especially presence

of cyanobacteria species related to eutrophication of fresh waters (Song *et al.*, 2021) and influence the whole condition of aquatic ecosystem.

Major key role in water trophy play carbon, phosphorus and nitrogen. The potential sources of these nutrients in pools in cultural area are run-offs from agricultural land (Kopp *et al.*, 2016). Such occasions make a nutrient pool in the bottom sediments and influence the evolution of the whole ecosystem (Teissier *et al.*, 2012). As a rich source, these sediments are continual long-term providers for phytoplankton and despite the grazing pressure of zooplankton, they are transforming the environment to higher levels of trophy (Sommers and Ryder, 2023).

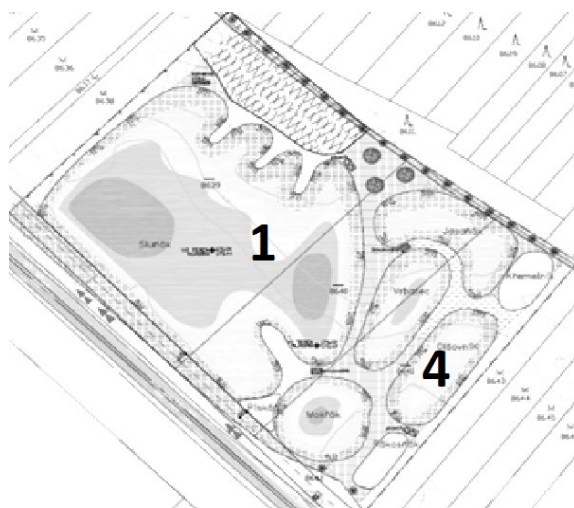
The aim of this study was to survey the initial succession of zooplankton in newly built pools and to assess the main factors of the colonisation success.

MATERIALS AND METHODS

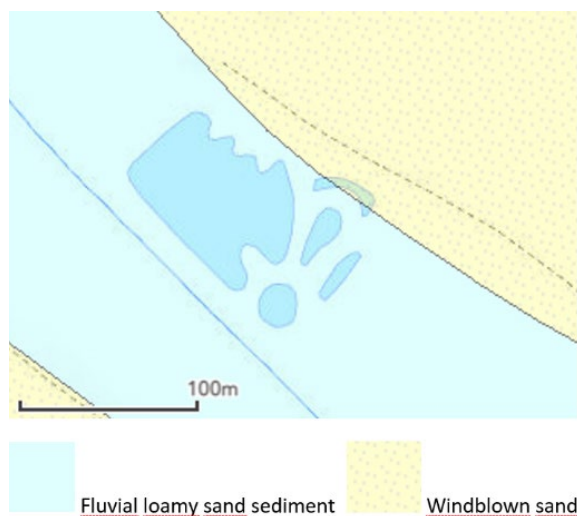
The Area of Studies

Sampled site is situated in the Southern Moravia, near the village Šardice (48.9478169N, 17.0568681E). As former agricultural field, it used to be intensively farmed, as well as the surrounding fields. Since 2012, the private investor decided to buy this area and built a system of five pools (Fig. 1) with the purpose to extend the regional Territorial System of Ecological Stability (TSES) and to increase the local biodiversity by creating suitable habitats for indigenous organisms. The purpose was to utilize the long-time waterlogged fields with character of degraded wetland to renewing the function of the significant landscape element.

Geological substrate is mainly fluvial loamy sand sediment, in north-eastern part there are windblown sands (Fig. 2). The soil for the whole area is classified as carbonated chernozems.



1: The position of Pool 1 and 4 on the site



2: Geological substratum of the site (© Czech Geological Survey, 2022)

Sampled Pools

Within this study, the succession of two newly built pools was assessed. Pool no. 1 had the area of 4796 m² and maximum depth of 2 m, the shores were indented with both slow and steep slopping. One side of the pool was planted by reed. Pool no. 4 had the area of 421 m² and maximum depth 1 m, the shape was regular and slopping of all shores was slow. Both pools were flooded by groundwater and precipitations only. During the whole season, both pools were partially overgrown by submerse vegetation. Building processes, including planting the plants, were finished at August 2013.

Sampling Methods

The first samples from the site were taken at the end of September 2013 to obtain information about very first succession. Main sampling was realised in season 2014, monthly from March to September. Samples were taken regularly at 8 am.

Zooplankton samples were taken and processed according to Hadašová and Kopp (2014). Dominant species were identified with the light microscope. The zooplankters were sorted according to taxa and stage as cladocerans, copepods (adults), copepod nauplii and rotifers; and according to size as follows: <0.5 mm (small), 0.5–1 mm (medium), 1–2 mm (large) and > 2 mm (extra large).

Phytoplankton samples were collected and processed according to Kopp *et al.* (2016). The identified species were sorted in five categories: cyanobacteria (Cyanobacteria), cryptomonads (Cryptophyta), green algae (Chlorophyta), diatoms (Bacillariophyceae) and other algae (Dinophyta, Chrysophyceae, Xanthophyceae, Euglenophyta).

Basic physico-chemical parameters were measured according to Kopp *et al.* (2016) at the site. Oxygen saturation, pH and temperature were measured by HACH HQ40d (Hach Lange, USA), conductivity was

measured by conductivity meter Hanna Combo HI98130, water transparency was assessed using a Secchi disc.

Samples of water for chemical analyses were sampled and processed according to APHA (1998) and Lorenzen (1967) in Kopp *et al.* (2016). Water was taken into plastic bottles and stored in the cooling box until processed.

The presence of fish predators was surveyed by electric aggregate catches (aggregate Honda 2.0, rectifire box BMA Plus).

Statistical Analysis

Differences between both studied pools were assessed using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity measure. Environmental variables used in NMDS analysis were temperature, oxygen saturation, pH, conductivity, transparency, TOC, TN, TP, chlorophyll-a concentration, N-NH₄, N-NO₂, N-NO₃, P-PO₄, CODCr, ANC, Cl⁻ and Ca²⁺. NMDS was done using Canoco 5 (Ter Braak and Šmilauer, 2018).

RESULTS

Physico-chemical Parameters and Chemical Analyses

Water temperature was characteristic for season when it was measured in both pools. The content of dissolved oxygen corresponded in both pools with increasing of macroscopic green algae (*Chara vulgaris* and filamentous types of *Zygnemophyceae*) due to their photosynthesis activity, as well as pH. Except the first sampling in Pool 4 (in September 2013), the water transparency was up to the bottom for the whole survey in both pools. Low values of total nitrogen and total phosphorus confirm the consumption by macroscopic green algae. Higher values of N-NH₄ at the beginning season were significant probably due to the nonmineralized residuum of organic matter at the bottom of both pools.

Values of physical and chemical parameters are presented in Tab. I.

In general, Pool no. 1 showed more stable values during the sampling season than Pool no. 4.

Phytoplankton

The composition of phytoplankton in sampled pools during the season changed very quickly. The development of phytoplankton in pools is in Fig. 3 and 4, shown as proportion (%) of main groups and number of cells.

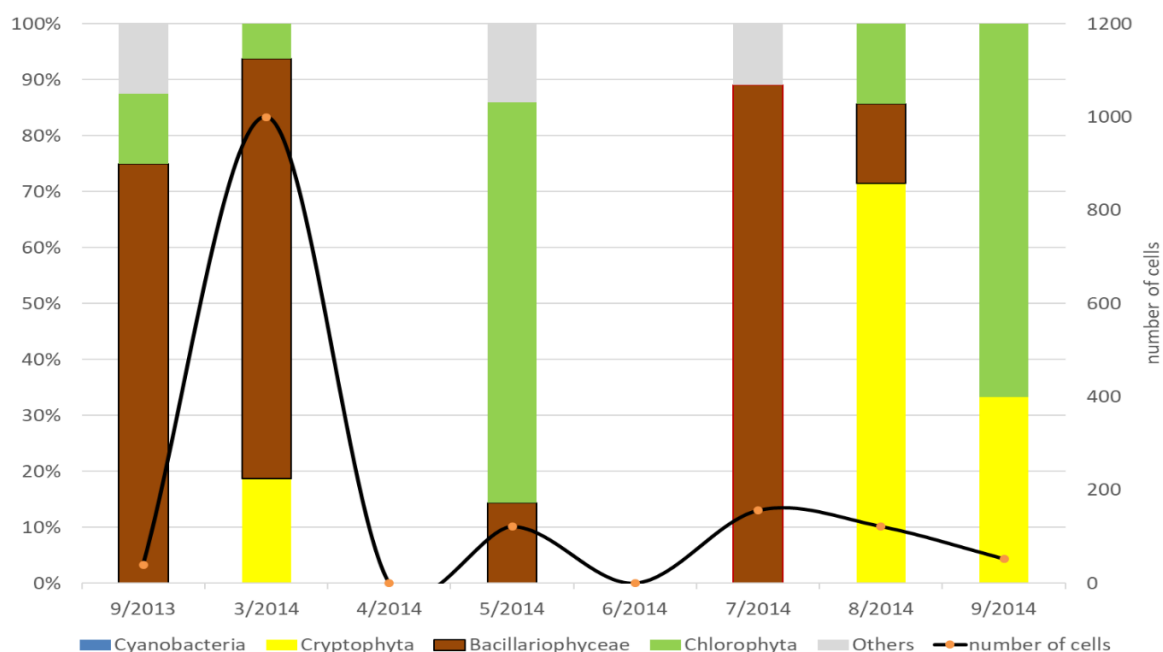
In the spring period, only smaller-sized species of pennate diatoms (genera *Achnanthes*, *Nitzschia*), green algae (genera *Chlamydomonas* and *Tetraselmis*) and cryptomonads (genera *Cryptomonas* and *Rhodomonas*) were found in both pools. In the period from April to June, small phytoplankton species were essentially absent in

I: The physical and chemical characteristics of the sites from September 2013 to September 2014 (mean \pm standard deviation)

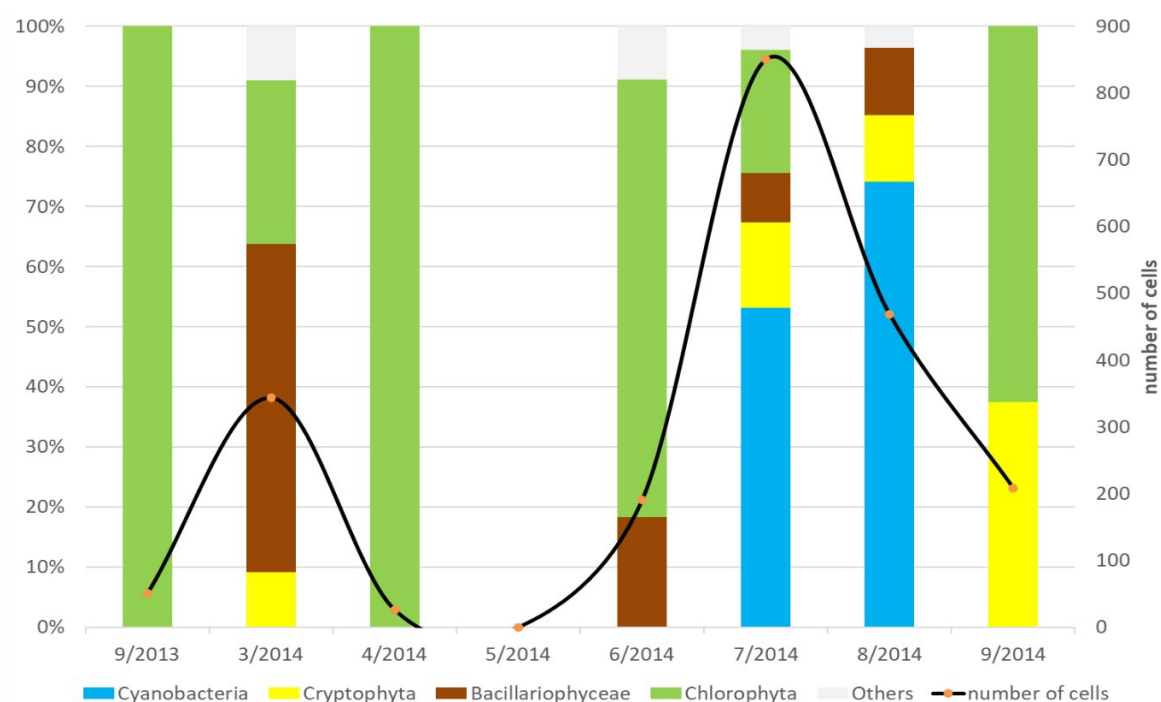
Variables	Unit	Pool 1	Pool 4
Temperature of water	°C	16.65 \pm 5.1	15.98 \pm 5.0
O ₂ saturation	%	97.51 \pm 7.0	89.65 \pm 14.8
pH		8.92 \pm 0.5	8.74 \pm 0.4
Conductivity	$\mu\text{S}\cdot\text{cm}^{-1}$	546.37 \pm 78.1	596.13 \pm 89.4
Water transparency	cm	200 \pm 0	93.8 \pm 16.5
TOC	$\text{mg}\cdot\text{l}^{-1}$	7.24 \pm 3.4	9.41 \pm 5.5
NTOT	$\text{mg}\cdot\text{l}^{-1}$	1.013 \pm 0.9	1.438 \pm 1.3
PTOT	$\text{mg}\cdot\text{l}^{-1}$	0.04 \pm 0.01	0.06 \pm 0.04
Chlorophyll <i>a</i>	$\mu\text{g}\cdot\text{l}^{-1}$	4.44 \pm 2.7	4.94 \pm 3.2
N-NH ₄	$\text{mg}\cdot\text{l}^{-1}$	0.03 \pm 0.01	0.01 \pm 0.00
N-NO ₂	$\text{mg}\cdot\text{l}^{-1}$	0.007 \pm 0.002	<0,001
P-PO ₄	$\text{mg}\cdot\text{l}^{-1}$	0.008 \pm 0.004	0.026 \pm 0.026
N-NO ₃	$\text{mg}\cdot\text{l}^{-1}$	0.138 \pm 0.103	0.24 \pm 0.065
COD	$\text{mg}\cdot\text{l}^{-1}$	17.34 \pm 2.33	40.8 \pm 36.9
ANC	$\text{mmol}\cdot\text{l}^{-1}$	2.46 \pm 0.94	2.12 \pm 0.83
Cl ⁻	$\text{mg}\cdot\text{l}^{-1}$	34.46 \pm 2.40	30.98 \pm 6.25
Ca ²⁺	$\text{mg}\cdot\text{l}^{-1}$	64.87 \pm 16.66	67.78 \pm 29.99

the water of both pools. This corresponds with the beginning of the development of the charophyte green algae in both pools (April) and macroscopic filamentous algae (May). The very low abundance of planktonic algae in Pool 1 persisted until the end of the growing season. Phytoplankton also included the diatom *Rhopalodia gibba* and representatives of the genus *Synedra*.

Representatives of the cyanobacteria group, which formed the dominant part of the phytoplankton during the two warmest months of the year, were also found in the more shallow Pool 4. These were two mainly benthic genera, *Anabaena* and *Komvophoron*, which are also occasionally found in open water. In September, the algae of the cryptomonads algae group (genus *Rhodomonas*) and



3: Proportion (%) and number of cells of phytoplankton development in Pool 1



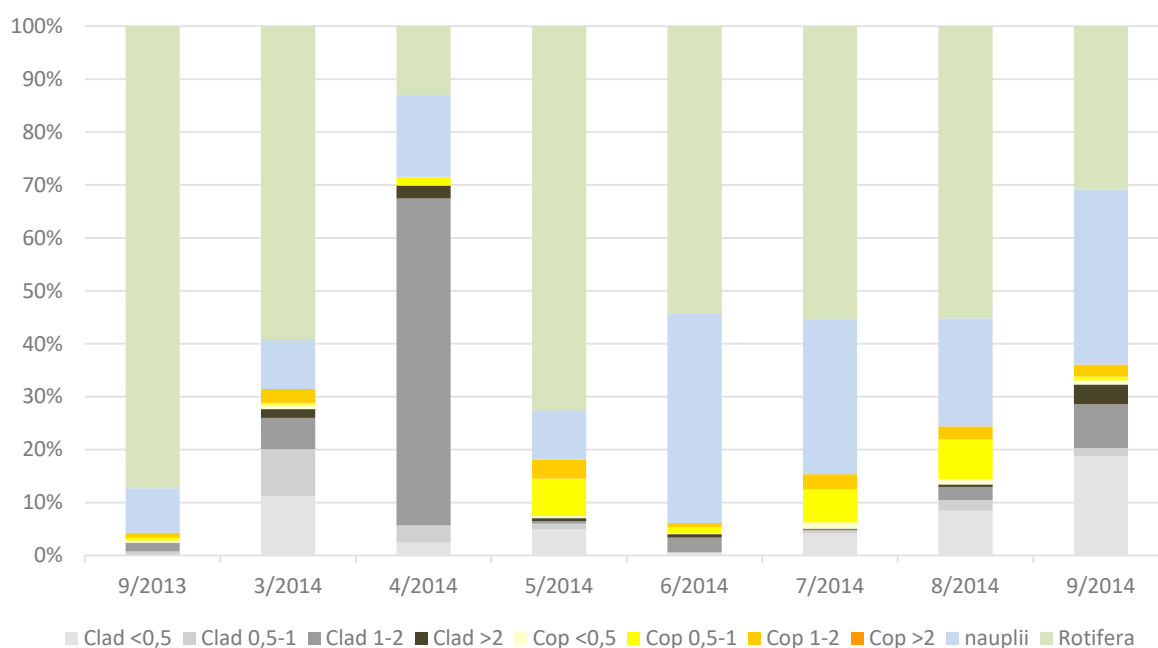
4: Proportion (%) and number of cells of phytoplankton development in Pool 4

the green filamentous alga *Geminella planctonica* were again dominant in Pool 4.

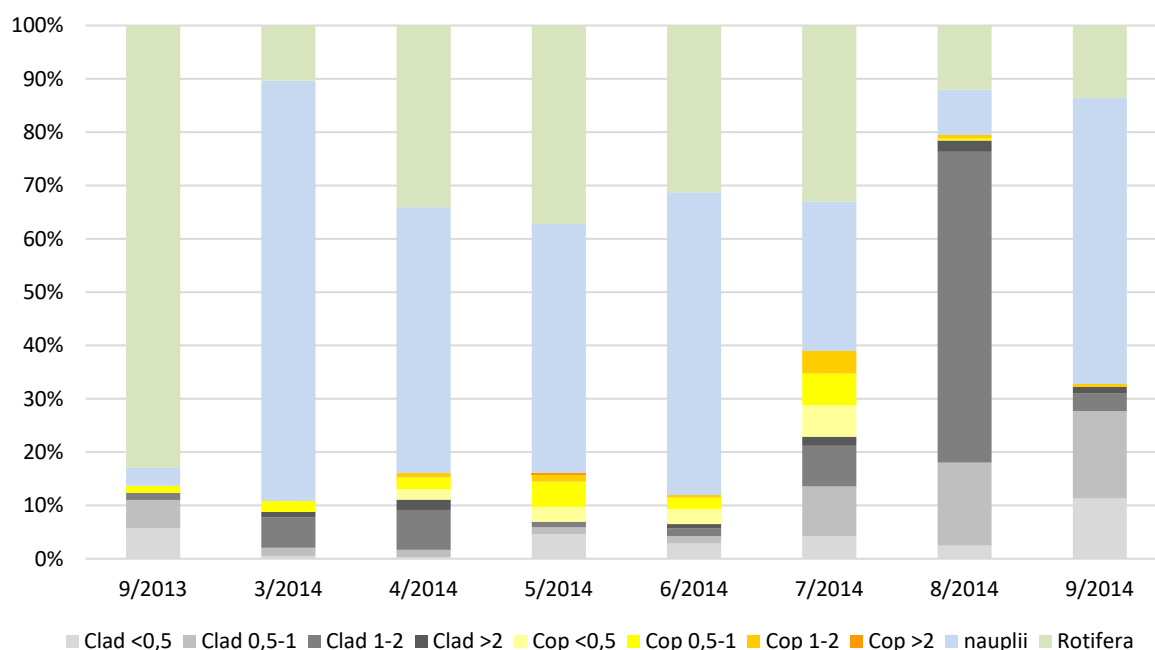
In total, we recorded two species of Cyanobacteria, five species of Cryptophyta, seven species of Bacillariophyceae, 16 species of Chlorophyta and five species of other algae.

Zooplankton

Pool no. 1: In the samplings taken just after flooding (September 2013), first colonists occurred immediately. The main group were rotifers, but few cladocerans and copepods in adult and nauplii stages were present. In March 2014, main group were rotifers, abundance of cladocerans increased with the presence of large species. Major part of copepods were present as nauplii. In April, the



5: Proportion (%) of zooplankton assemblage development in Pool no. 1



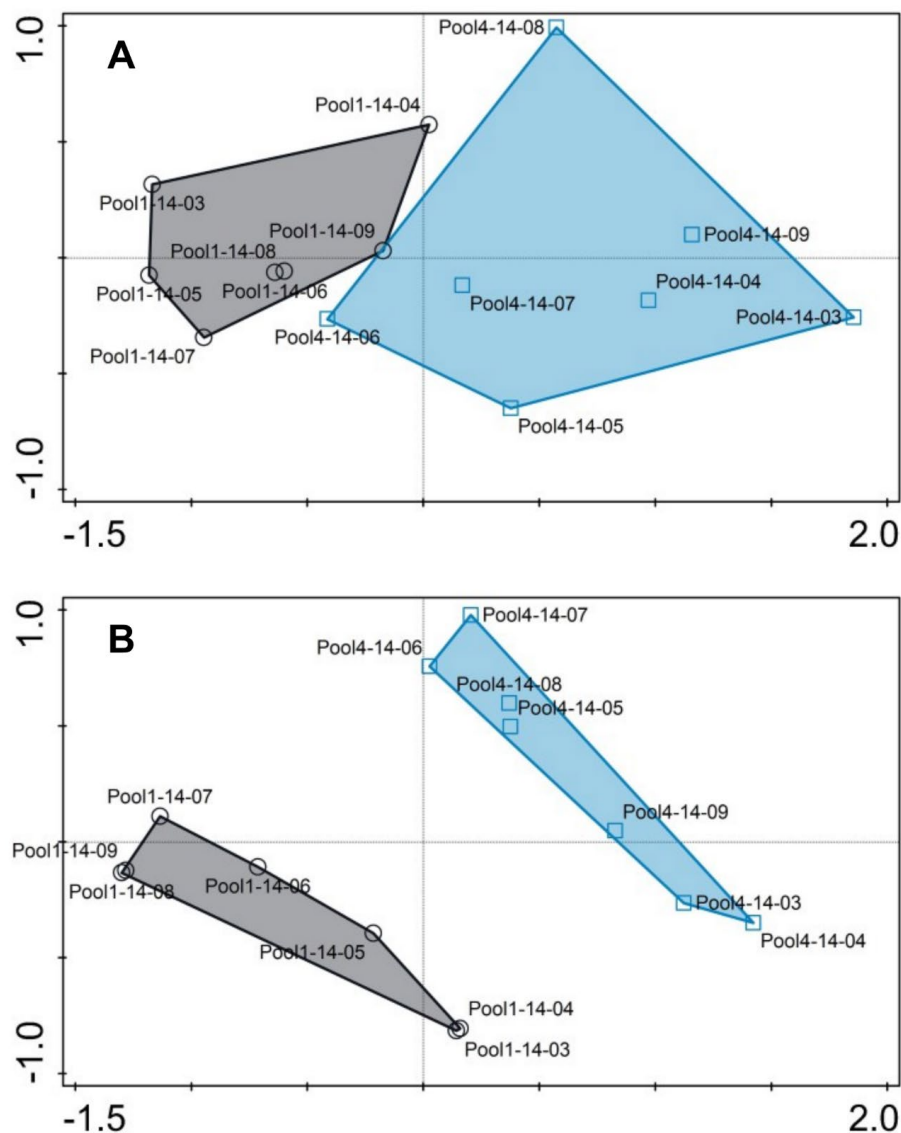
6: Proportion (%) of zooplankton assemblage development in Pool no. 4

abundances of zooplankters rapidly decreased in general. But large individuals of cladocerans were predominant and copepods were present mostly in nauplii stage. In May, the abundances of zooplankters peaked, large species were present, also permanent eggs (ephipia) of *Daphnia* sp. occurred and maintained until autumn. In June and July, rotifers were main group, copepods were mainly as nauplii but other stages were present as well. In general, larger species were more abundant than the small ones. Cladocerans were present evenly in all size groups. In August, abundances of all three groups rapidly decreased. In the autumn, abundances of rotifers and nauplii decreased as well, the main present group were large cladocerans.

Pool no. 4: In September 2013, the pool was colonised mainly by rotifers, but cladocerans and copepods (nauplii) were present also. In March 2014, rotifers dominated, cladoceran abundances increased with presence of large individuals, copepods were mainly as the nauplii stage. In April and May, all zooplankton abundances increased, mainly represented were large cladocerans, copepods were mainly as the nauplii stage. In June, the abundances of all zooplankton peaked, main group were copepods in nauplii stage. In July, the numbers of rotifers and nauplii decreased, large cladocerans dominated. In August, large cladocerans peaked and represented most abundant group. In September, the most abundant were nauplii.

II: List of zooplankton taxa identified in pools 1 and 4

Cladocera	Copepoda	Rotifera
<i>Alona</i> sp.	<i>Eudiaptomus gracilis</i>	<i>Ascomorpha</i> sp.
<i>Bosmina longirostris</i>	<i>Macrocylops albidus</i>	<i>Asplanchna priodonta</i>
<i>Chydorus sphaericus</i>	<i>Microcylops bicolor</i>	<i>Asplanchna sieboldi</i>
<i>Daphnia gal. x cuc.</i>	<i>Thermocyclops</i> sp.	<i>Bdelloidea</i> gen. spp.
<i>Daphnia magna</i>		<i>Brachionus calyciflorus</i>
<i>Scapholeberis</i> sp.		<i>Brachionus budapestinensis</i>
		<i>Hexarthra mira</i>
		<i>Keratella cochlearis</i>
		<i>Keratella quadrata</i>
		<i>Lecane luna</i>
		<i>Polyarthra dolichoptera</i>



7: The comparison of Pool 1 and Pool 4 in the size structure of zooplankton assemblage (A) and environmental parameters based on non-metric multidimensional scaling. Sample codes include pool number, year and month of sampling (e.g. pond1-14-07 represent Pool 1 in July 2014).

Proportion (%) of zooplankton assemblage during the study in both pools is presented in Fig. 5 and 6.

When comparing zooplankton and environmental variables in both pools, the results clearly show significant differences between Pool 1 and 4 (Fig. 7). Zooplankton in Pool 4 was more variable showing slightly higher dissimilarity among samples compare to Pool 1 (Fig. 7A). Selected environmental variables separated both pools very clearly (Fig. 7B).

In total, 6 taxa of cladocerans, 4 taxa of copepods and 11 taxa of rotifers were observed in both pools (Tab. II).

Fish Predators

The electric aggregate catches proved absence of any fish species during the whole survey in both pools.

DISCUSSION

The succession of the newly created aquatic ecosystem is dependent on the abiotic conditions of the surrounding area, as well as on the biotic interactions among colonising organisms (Kuczyńska-Kippen and Nagengast, 2006), because effective colonisation is not only arriving at a new locality, but also surviving there and successfully reproducing (Keller and Yan, 1998). According to Audet *et al.* (2013) local factors are having a stronger influence than regional factors in shaping the community.

The fluctuation of physico-chemical parameters in the first year after inundation is typical for freshly flooded soil, because there are nutrients released from the bottom nutrient pool inflicted by fertilisation of intensively farmed field (Kopp

et al., 2016). So, we can say, that after arriving to new locality, the zooplankton assemblage is nutritionally dependent on the nitrogen and phosphorus sources from the bottom sediment mediated via phytoplankton. Other author (Teissier *et al.*, 2012) also confirms that the seasonal changes in the structure, abundance and spatial distribution of phytoplankton is natural adaptive response to fluctuation of basic physico-chemical conditions of site during the season, which also our study confirmed. Nevertheless, in next years, stronger stabilisation of physico-chemical conditions in pools can be expected due to the exhausting of freely available nutrients from newly flooded soil and progressive mineralisation of sediment (Kopp *et al.*, 2016).

Because there was removed the top soil when digging new pools, we expected that there was no egg-bank present (Frisch and Green, 2007), as well as there is no straight connection with other locality which might be the source of individuals. The arriving of zooplankton individuals was realised by wind (Havel and Shurin, 2004; Vanschoenwinkel *et al.*, 2008), rain (Cáceres and Soluk, 2002) or by birds and insect (Green and Figuerola, 2005; Slusarczyk *et al.*, 2019). These ways can guarantee fast colonisation especially for rotifers (Moreno *et al.*, 2019), which can intensively colonize the new habitat even at local measure (Louette and De Meester, 2004, 2005; Michels *et al.*, 2001), as showed our results. Immediately after flooding, there were predominant rotifers in both pools. Other zooplankton groups were not abundant probably because of the lack of aquatic macrophytes which provide the shelter (Balls *et al.*, 2006; Schriver *et al.*, 1995). But in the spring of next year, cladocerans inhabited Pool 1 and nauplius stages inhabited Pool 4. For explaining such differences, we know that zooplankton groups vary in the dispersal and colonisation capability (Jenkins, 1995; Jenkins and Buikema, 1998; Cáceres and Soluk, 2002) and that dispersal limitation influences the structure and function of the assemblage at all (Havel and Shurin, 2004). Cladocerans, as the most successful colonisers are described by Fryer (1985), Jenkins and Buikema (1998), Bohonak and Jenkins (2003), Cohen and Shurin (2003) and Louette and De Meester (2005). On the contrary, Louette *et al.* (2008) states cladocerans as unsuccessful colonisers due to the strong competitive interaction with the first colonists or inappropriate site conditions which could be present in Pool 4 (smaller size of pool, providing less options to hide due to its regular shape and shallow water). On the contrary, Soto and Hurlbert (1991), Cáceres and Soluk (2002), Yan *et al.* (2004) and Frisch and Green (2007) considered copepods as rapid colonisers in some conditions because female cyclopoids are able to store sperm making mating multiple times, so the habitat can be colonised by only one fertilised female.

During the whole season in both pools, extra large species of zooplankton were more or less present. This comes probably due to absence of predators – planktivorous fish in every pool (Komárková, 1998). We have carried out the electric aggregate catches and none fish was caught. Based on further studies (Kuczyńska-Kippen and Nagengast, 2006; Bielańska-Grajner and Gładysz, 2010), we can expect the increase of species diversity within zooplankton assemblage in few following years.

The phytoplankton of both pools was in very low abundance throughout the whole period under study. This condition was caused by two main factors. The absence of fish allowed sufficient development of zooplankton, which by their predation pressure limited the development of phytoplankton. The second factor was the development of a high biomass of the macroalgae *Chara vulgaris* and filamentous algae of the Zygnemophyceae group (mainly the genera *Zygnema* and *Spirogyra*), which depleted the available mineral nutrients from the water.

Dispersal success of phytoplankton is dependent on the distance, density of origin population, desiccation tolerance and other types of abiotic conditions (Sharma *et al.*, 2007), but it is their very small size which makes the freshwater algae rapid colonists (Finlay and Clarke, 1999). The main role in algae traveling plays wind and the highest colonization potential has Chlorophytes (Chrisostomou *et al.*, 2009).

Planktonic algae are the primary producers of aquatic ecosystems and form the basis of aquatic food chains (Graham *et al.*, 2009). The plankton growth is dependent on various environmental parameters such as temperature, light intensity, and nutrient concentrations. In spring, the phytoplankton composition in pools was similar to that in pools with low fish stocking, where the phytoplankton is composed of a low biomass of fast-growing algae such as the genus *Cryptomonas* (Fott *et al.*, 1974). The development of a phytoplankton community with an overlay of green algae and cryptomonads as in the pools studied here has been observed in microcosms of vernal pools simulating a cold spring after a warm winter without snow (Celewicz and Góldyn, 2021).

Species diversity and total phytoplankton biomass in both pools was very low, as evidenced by low overall cell counts and low chlorophyll *a* concentrations. The main reason for the low phytoplankton biomass is the robust development of Charophytes, which are typically fast colonizers of aquatic ecosystems with a strong positive effect on maintaining high water clarity (van Donk and van de Bund, 2002). Phytoplankton tend to be limited by low phosphorus availability in Charophyta-dominated water bodies. In waters with dense and well-developed submerged vegetation, zooplankton may be less important for maintaining clear waters

(Blindow *et al.*, 2000). Another factor for limiting phytoplankton development is the apparent allelopathic effect of Charophyta on some green algal species (Mulderij *et al.*, 2003). However, this may change over time as pools evolve, especially if fish enter the pools.

The very important roles in succession processes play morphometric characteristics of the pool, such as size, shape, depth and slope of the shores (Standards for nature and landscape management, 2014). From the size of the pool point of view, the small aquatic ecosystems are more vulnerable to

fluctuation of abiotic factors than the large ones (Oertli, 2002). As well as regular shape is less suitable for biodiversity increase than diversified shore, because it provides less opportunities to hide from predators or waves and for finding food. As can be clearly seen at Fig. 7, due to the completely different morphometric characteristics, there are no similarities in the development of size structure of zooplankton assemblages between Pool 1 and 4, so individual development of each pool during the years can be expected.

CONCLUSION

The process of primary succession of newly constructed pools in terms of zooplankton depends on many factors. The most important are the morphometric characteristics of the pool and the chemistry of the freshly flooded soil from which nutrients are released into the water. These factors also influence the development of the cyanobacterial, algal and aquatic macrophyte community, which serves as a suitable (or unsuitable) food source for zooplankton and is crucial for their successful colonisation. In the first season after inundation, the presence of a massive biomass of Charophytes and subsequently green filamentous algae was crucial for the development of the communities in both pools, which significantly reduced the development of phytoplankton, caused high water clarity and affected the development of the zooplankton community. The electric aggregate survey proved that the development of the pools was not adversely affected by the presence of fish in the first year after inundation.

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