

## 1. Contestant profile

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## 2. Project overview

Title:	An impact of habitat changes on arthropod communities and ideal reservoirs for biodiversity in sandpit
Contest: (Research/Community)	Research
Quarry name:	Planá nad Lužnicí

## Abstract

In this study, we compared the biodiversity of selected invertebrate groups of five water bodies and ten delineated terrestrial sites of the Planá nad Lužnicí sandpit (Czech Republic, South Bohemia Region). The sampling sites were chosen on the basis of their distinct appearance and specific characteristics. Using our results, we attempted to explain the differences in the species composition of the communities at the sites. From these data, we evaluated the biological value of the sites and suggested future management practices to maintain local diversity. Thanks to the earlier research of Vácha and Zeman (2018), we had a unique opportunity to compare the results of terrestrial invertebrate surveys in relation to succession. In our work, we focused primarily on selected groups of larger arthropods and plankton. In addition, other taxonomic groups (Aves, Reptilia, Amphibia) were also recorded for the overall current inventory survey. In total, there are nearly a thousand species (taxa), including 465 species of larger arthropods, 366 species of aquatic microorganisms, and 60 species of vertebrates. A total of 69 species are listed on the Red Lists of the Czech Republic and 61 are of faunistic and floristic importance. The results of our analyses suggest that different factors play a role for each of taxonomic groups. In our opinion, habitats with different cover of aquatic macrophytes have the greatest conservation potential. However, equally important lake habitats are now threatened by the fish stocks present. In terms of terrestrial habitats, those in the early stages of succession are the most valuable. Technical reclamation appears to be highly inappropriate for maintaining the diversity of endangered species. Our other outputs are a popularization film introducing the public to importance of the sandpit for local nature and a multimedia virtual tour allowing visualization of our sampling sites and the entire sandpit area.

# Introduction

Restoration ecology became a separate scientific discipline in the second half of the 19th century and the first scientific publications in the Czech Republic were written in the 1980s (Prach, 1987; Osbornová et al., 1989). Except of theoretical study on ecological restoration, a process that helps to restore an ecosystem which has been degraded, damaged or destroyed (Martin, 2017), restoration ecology has far-reaching overlaps directly into ecological practice. An essential part of this discipline is the study on succession in quarries and sandpits, which are important features in our landscape. Not only does the mining of sand or gravel modify the overall landscape, it also greatly affects the plant and animal communities of the site. In the Czech cultural landscape, sandpits take over the role of habitats that have almost disappeared in our territory due to anthropogenic changes, especially during the last century, and thus became refugia for species of many taxonomic groups naturally related to such habitats (e.g. Heneberg et al., 2013; Heneberg & Řezáč, 2022; Roháček 2020). These include, for example, gravel river alluvias or vertical riverbanks largely destroyed as a result of water management and flood control modifications (riverbed regulation and reinforcement), oligotrophic lowland wetlands or quicksand habitats (Chytrý et al. 2001). These habitats are characterized by frequent disturbances caused by dynamic material transfers as a result of environmental factors (fast-flowing water, floods, wind), which in sandpits are represented by the movement of heavy equipment and routine manipulation of the terrain.

For the proper functioning of sandpits as refugia for endangered species after the end of mining, it is crucial to set up an adequate method of reclamation. The principles of so-called technical reclamation are still often applied on former mining sites, where the site is returned to agrarian (mostly forestry) use by planting *Pinus sylvestris* or even non-native tree species (red oak - *Quercus rubra*). Such a reclaimed sandpit provides shelter for only a relatively small group of generalist species (Řehouňková et al. 2012) and is many times more financially demanding in comparison with nature-oriented restoration methods (Kavina, 2004). In the case of sandpits, to maintain the highest diversity of stenovalent and endangered species, we should provide regular disturbance in all types of local environment and prevent the spread of pioneer plant species (Řehouňková et al. 2016).

## Objectives

Our work focuses mainly on the study of communities of selected groups of larger arthropods and plankton at aquatic and terrestrial sites in the Planá nad Lužnicí sandpit. Our results have been analyzed and on their basis we have tried to develop a series of recommendations for practical ecological measures in the sandpit. In our project we have seven main objectives:

to analyse samples of selected groups of plankton and larger arthropods obtained from 5 water reservoirs and 10 delineated terrestrial sites and to describe the species richness and composition of the local communities, and consequently to assess the value of the habitats found there.

to assess which environmental factors determine species variability and which factors have a positive or restrictive influence on the biodiversity of aquatic, wetland and terrestrial sites in the sandpit

to compare the results of the terrestrial invertebrate survey with the study of Vácha and Zeman (2018) and thus

- monitor changes in arthropod communities in relation to succession
- to record all found species of selected groups of organisms
- to develop a proposal for the future management of the mined sandpit in order to preserve the specific biodiversity
- to produce a summary popularisation film for local citizens to make the public aware of the importance of the sandpit in Plana nad Lužnicí for local nature
- to create a multimedial virtual tour allowing the public to visualize our sampling sites and sandpit area.

## Methods

Our study took place from March to August 2022 in the sanpiti of Planá nad Lužnicí. We have partially followed the methodology of Vácha and Zeman (2018), who worked at the sandpit four years ago, in order to compare their data with ours and track the dynamics of the local arthropod communities over time. We set up the rest of our the methodology independently.

### Collection of biological material in the field

A total of 5 collections of microscopic aquatic organisms were made at selected sites from March to July 2022. A four-week interval was maintained between sampling days. Samples were collected in 250 ml bottles using 2 types of plankton nets. Samples were collected either by vertical dragging through the water column or by dragging the plankton nets diagonally between the bottom and the surface. Where these collections were not possible, a telescopic rod with a 1 l container was used, the content of which was then strained through the plankton nets. The phytoplankton (and most of the ciliates) were sieved using a 10 µm mesh plankton net, while the zooplankton with using an 80 µm mesh net. Collected samples were then transferred to a transport cooler until they could be placed to a fridge with 4°C.

Standardised individual collection of larger aquatic arthropods was carried out at sites (especially in the littoral) at least once a month (always on the same day) using a colander (mesh diameter approx. 1 mm). In addition, two floating drop traps made of a cork board measuring 20 × 12 × 1 cm and a plastic cup with saline solution and detergent serving as a surfactant (Ruzicka, 1982) and two live-bait traps made of 5-L plastic barrels using poultry liver as bait were installed at each site. At the same time, a piece of polystyrene was placed on the top to prevent trapped aquatic insects from drowning and to allow access to the water surface (Aiken & Roughley, 1985). Material from the water traps was collected once every three weeks. As for material collection at terrestrial sites, we followed the methodology of Vácha and Zeman (2018), who made monthly 20-min individual collections using tweezers and an exhaustor and a standardized number of swipes with a sweep net (100 at each site) at five study sites. They also installed two ground traps with saline and surfactant at each site. Traps were collected on a monthly basis. Wild boars and vandals regularly destroyed installed traps during the course of the survey, but at least one functional trap was always present at a site. In this case data from one trap from each site where both traps remained intact during the same collection were also excluded from the statistical analysis - these data were also processed as a part of the faunal survey of the sites. We followed a similar procedure if aquatic traps were damaged. The methodology for collecting material at the riparian sites was different. Here, we made monthly 15-min individual collection using tweezers and an extractor and 5-min collection using a modified leaf vacuum ("suction-sampling"; see Helbing et al. 2020).

## Sample processing, fixation, preservation

Before fixation of the collected material, the samples were partially examined under a microscope and binocular loupe. Thus, some of the material was examined fresh, whereas material that could not be analysed in a short period of time was fixed. The zooplankton had to be strained onto a concentrate containing only the bodies of crustaceans, most rotifers and some other larger organisms by using a 70 µm mesh sieve due to the chosen preservation method (Kořínek 1999). Before sieving, 5 mL of water was taken from the sample using a pipette, half a milliliter each time, for analysis of rotifers (the same was done for algae), which were preserved separately. The mass on the sieve was rinsed gently using a wash bottle with distilled water and then rinsed into the glass samplers using a wash bottle with alcohol. These samples were fixed with 80% alcohol, to which a small amount of glycerol was added. To the unpipetted samples for the recording of rotifers and phytoplankton kept in autoclavable tubes, 2 ml of distilled water was added, to dilute the sample and to rinse the pipette tip, and then Lugol's solution was poured as fixing medium to a final concentration of about 6% (Komárková, 2006). All preserved samples were stored in a fridge.

Representatives of larger arthropods, for which laboratory determination was necessary, were placed in 50 ml plastic tubes (falcons) using entomological tweezers or an exhaustor and killed there and preserved in 70% ethanol (spiders and smaller insects) or in ethyl acetate vapour (larger insects). Material from ground and floating traps was sieved through a fine strainer in the field and placed in microtene bags, which were stored in a freezer until later determination and further processing.

## Determination

Species determination was realized in laboratory conditions using magnification optics (BRESSER Advance ICD 10x - 160x Zoom Stereo-microscope, Carl Zeiss Amplival, PZO MST-131) and determination literature focused on individual groups: zooplankton: Brandl (2010), Błędzki & Rybak (2016), Amoros (1984), Kořínek (2005), Příklad (2006, 2014, 2017, 2017b, 2018a, 2018b, 2018c), Illyová & Baláži (2004); phytoplankton: Kaštovský et al. (2018), Šťastný (2010), Starmach (1983) and Coesel & Meesters (2007). Some web galleries were used as comparative photographic material (Kaštovský et al., 2018; Geest, 2009; Plewka; Coesel, 2002; Westen); Araneae: Netwig et al. (2022), Heimer & Netwig (1991), Miller (1971); Coleoptera: Hůrka (1996), Bezděk & Mlejnek (2016), Boukal (2017), Nedvěd (2015), Smetana (1958), Lompe (2010); Hemiptera: Biedermann & Niedringhaus (2009), Kunz et al. (2011), Wagner (1966, 1967, 1974a, 1974b, 1975), Savage (1989), Wachmann et al. (2004, 2006, 2007, 2008), Péricart (1983, 1989a, 1989b, 1989c); Odonata: Dolný et al. (2016); Orthoptera: Kočárek (2013). The correctness of the determination of difficult taxa to determine was consulted, or revised, by specialists on the subject, and representatives of some groups were identified solely with their help (see Acknowledgements). Individual representatives of plankton were classified under the microscope into three semi-quantitative categories based on their relative abundance in the sample: 3 - dominant species (>66%); 2 - frequent (33-66%); 1 - rarely occurring (<33%) (Zelený, 2013). For larger arthropods, absolute numbers of individuals in the sample were recorded. Information on the distribution, ecology and potential threats of specific species was drawn mainly from available finding aids (NDOP AOPK, Czech Arachnological Society database), checklists and catalogues of specific groups (Buchar & Růžička 2002; Boukal 2007; Hůrka 1996), red lists of endangered species (Hejda et al. 2017; Řezáč et al. 2015) and consultations with experts. The degree of threat for all species is based on the small area of their range, the fragmentation of their distribution, the number of potentially suitable sites or the small number of known sites, not on the current or predicted decline of populations. Curiosity and rarity were also assessed for planktonic representatives in relation to habitat type (Příklad, pers. comm.).

## Statistical analysis

The data for the analyses and some statistical methods were processed in Microsoft Excel (2022). From the available analyses of this software, Pearson correlation was used to find linear correlations and linear regression to describe correlations of interesting variables graphically. It was also used to produce simple bar, pie and some other graphs showing basic interpretations of various data. We also used it to calculate and then plot the values of the Jaccard index describing differences in species composition between two communities (Delawska 2013) in a matrix table (Tab. 1). More complex statistical calculations were performed in R studio version 4.2.1 (Rstudio team 2022) using the packages vegan (Oksanen et al. 2022), cluster (Maechler et al. 2022) and devtools (Wickham 2022). Species composition variability was analysed using non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity matrix. Passively projected significant variables with significant linkage to the axes ( $p < 0.1$ ), tested using the envfit function, were tested with 5039 permutations. The NMDS plot also shows the species with the best fit, i.e. occurrence at more than two sites, in relation to site variability along the first and second axes. We generated the same for rare species. All NMDS plots were plotted separately for all groups. Comparison of site similarity was tested by agglomerative clustering using Ward's method and plotted on a dendrogram via the clust function. In addition, a boxplot was rendered for some of the data. Significant correlations that came out in Pearson's rank correlation coefficient were further tested by Spearman's correlation test using the cor.test function.

## Measurement of environmental variables

Penetrability, sand grain size, and cover of vegetation were measured at all terrestrial sites (including banks). Only on the banks, we measured bank slope and bank roughness. At other terrestrial sites, we also surveyed substrate moisture. Penetrability was measured four times during the study using a penetrometer from GEOTEST Inc. The result of each of the four measurements is equal to the median of the four values measured at a given site on one day. The grain size was measured on certified sieves (fractions 32, 16, 4, 2, 1, 0.5, 0.25, 0.125 mm) at the MUNI Úgv and is expressed as the mass fraction of each fraction in the sample. Plant cover (and plant litter) at the sites was determined using three phytocenological images (1 × 1 m). Phytocenological imaging was conducted in July. Slope was measured at the bank line level and 1 m from the water using a slope gauge. To measure relative bank roughness, we developed a custom methodology that expresses the difference of the straight-line distance between two selected points on the shoreline and the length of the bank line between them. For this measurement we used a string and a tape measure. Substrate moisture was measured using the gravimetric method (Schmugge et al. 1980). Other environmental variables that we considered at terrestrial sites are their age, which we determined mainly by consulting the sandpit manager and former contest participants, and the successional stage indirectly dependent on it (following Rehounkova & Pracha 2006).

During each collection of plankton, aquatic environmental parameters such as pH, conductivity, oxygen concentration, subsurface temperature, transparency and depth were measured simultaneously. A boat was used to achieve sufficient distance from the lake or pond shore for sampling. Water temperature and pH were measured using GMH 3530 device, conductivity using

GE104 probe and outdoor temperature using Pt100 sensor. Transparency determination was carried out by Secchi disk. Furthermore, supplementary factors such as barometric pressure, humidity, wind velocity, air temperature, cloudiness scale and rainfall rate were included in the sampling protocol and taken into account in case of extreme deviations in the measured values of water parameters. The water samples for the analysis of nitrate, phosphate and ammoniacal nitrogen concentrations were submitted to the Orlická laboratoř, Ltd. Other environmental variables included the presence of fish, aquatic vegetation cover, depth, morphometrics, age, and reservoir area data. Fish occupancy was not directly studied at the sites by a quantifiable method, but by observation or catching of individuals. Furthermore, we deduced this factor retrospectively from the quantitative and qualitative species composition of zoo and phytoplankton, as well as from the transparency and presence of macrophytes (following Hazuková 2016). The age was determined using the Mapy.cz map server. We used GIS software application (ArcGIS) and a drone to measure the site areas and vegetation coverages.

## Results

### Research sites characterization

**Loc. W1** – the smallest aquatic habitat studied in terms of area, which is also the shallowest with its average depth of 15-20 cm (max. depth 40 cm). It is thus an aquatic site without any distinct zone of free water. There is a very dense cover of *Utricularia* (association *Utricularietum australis*) throughout its entire part. The other accompanying vegetation are sedges stands roughly in the middle, which are supplemented by occasional *Juncus effusus* and *J. articulatus*, *Alisma plantago-aquatica*, *Lythrum salicaria* and very scattered *Lycopus europaeus*. The sandy bottom is covered by a layer of organic material and in some places by periphyton. High values of median conductivity (610  $\mu\text{mS}\cdot\text{cm}^{-1}$ ) were measured here. The median pH value is 7.4. The median of O<sub>2</sub> concentration here is the second highest (8.62). The bank of this site (W1rip) has the highest average plant cover (91%) and ruggedness (860/400 cm). It is continuously covered with mosses (*Polytrichum* sp.) and there are very frequent clumps of *Juncus* in direct contact with water. There is the lowest penetrability (57.5 kPa) and the lowest bank slope at the shoreline (4°). The major substrate fraction here is 0.25-0.5 mm, which makes 24.1 %.

**Loc. W2** – pool with a distinctly smaller proportion of littoral vegetation towards the limnetic zone and, on the contrary, more evident submerged vegetation of unspecified elodeoids. It is a pool with a greater depth in places (max. values up to 120 cm) and an undulated bottom, and therefore the depth varies greatly, often from 25 cm to a metre. The surface is covered by occasional floating *Potamogeton natans* and only sporadically by *P. crispus*. In shallower areas, there is also the occasional *Alisma plantago-aquatica*. The bottom is sandy and often covered with larger stones. The highest values of conductivity (690  $\mu\text{mS}\cdot\text{cm}^{-1}$ ) were measured here, and the median is also the highest (650  $\mu\text{mS}\cdot\text{cm}^{-1}$ ) compared to the other sites (330  $\mu\text{mS}\cdot\text{cm}^{-1}$ ). The fluctuations in conductivity were also the highest here, reaching 150  $\mu\text{mS}\cdot\text{cm}^{-1}$  (the median of the others is 90  $\mu\text{mS}\cdot\text{cm}^{-1}$ ). Furthermore, the median of pH value is the highest here (8.15), and its fluctuations during the measured season are also among the highest. The shore (W2rip) is relatively bare, occasionally covered with graminoids, mainly *Calamagrostis epigejos*, and possibly rarely with *Lycopus europaeus* and *Bidens* sp.. The littoral is fringed by larger scattered stands of *Typha latifolia*. Further along the banks there is occasional *Utricularia* and only sparse *Lemna* sp.. The average plant cover here is very low (24 %), with a main grain size fraction of 0,25-0,5 mm (32 %). The slope here varies from 10,5° at the shoreline to 6,5° 1 m from the water. The ruggedness measured here is 550/400 cm.

**Loc. W3** – deep pond with muddy bottom. Farther from the shore, the bottom is at about 180-190 cm. In the limnetic zone there are more extensive stands of *Potamogeton natans*. At specific hours, the pond is shaded on one side by a denser birch stand. As at Loc. 4 (lake), there is a fish occupancy of unknown exact weight. In addition, the species composition of the fish probably changed after the formation of the connecting channel with the lake sometime in June. The median of conductivity at this site is 340  $\mu\text{mS}\cdot\text{cm}^{-1}$  and the pH median is 7.26. The median of O<sub>2</sub> concentration is about 7.04. During the March sampling, the highest O<sub>2</sub> concentration was measured here (in littoral) (10.93), while during July sampling, the lowest O<sub>2</sub> concentration was measured in the pelagial (6.38). The joint mean of the difference between the highest and lowest O<sub>2</sub> concentrations in littoral and pelagial was the highest here (4.4) compared to the other sites (median 3.15). There was a large oscillation in transparency, with a difference of less nearly 150 cm between the maximum (193 cm in March) and minimum (45 cm already in April) value, and a median of transparency equalled 88 cm. After the lowest values in April, transparency slowly increased until it reached 132 cm in July. The banks (W3rip) here are very steep (50°) for the first 20-40 cm from water and then change to almost horizontal. Around 40% of weight of the sampled substrate here consists of fractions > 32 mm. It has the highest median of penetrability (250 kPa) and a relatively high plant cover (84.3%). The littoral vegetation is dominated by reeds (association *Phragmitetum australis*) and frequently occurring *Juncus effusus*. Less frequent graminoids are present, with *Calamagrostis epigejos* predominating and scattered and isolated *Lycopus europaeus*, *Stachys palustris* and *Bidens* sp.. Away from the water, the bank is covered with *Trifolium repens* and *T. medium*.

**Lok. W4** – large lake with not very well developed continuous littoral vegetation. The overall depth of the lake is around 2 metres, maximum up to 3 metres. Scattered throughout the lake are small populations of *Potamogeton natans* and reeds, with a larger reed formation (association *Phragmitetum australis*) only near the eastern shore, where littoral sampling took place. The littoral sampling sites contain frequent *Utricularia* and only sporadic *Potamogeton natans*. This is a hard-bottom habitat, muddy bottom was found only in the place with more reed formation. The conductivity median was 315  $\mu\text{mS}\cdot\text{cm}^{-1}$ . During April sampling, pH measured here (in the pelagic) was the most alkaline of all sites (9.01), which resulted in the highest difference between the highest and lowest pH values. Thus, the labelling of the lake as the site with the highest measured pH fluctuation is only due to the significantly deviated pH values, as the pH values were already stable in the rest of the sampling, whereas at Loc. 2 with the second highest fluctuation values, the pH changed constantly. Overall, transparency oscillated here, with a difference between the max. (more than 170 cm in March) and min. value (77 cm in July) of more than 90 cm, which is close to the median of transparency (93 cm). Scattered at the water/shoreline boundary were *Juncus effusus*, *Bidens* sp., and graminoids dominated by *Calamagrostis epigejos*. Furthermore, alder and willow occur where water and bank meet. Along the shores of the entire lake there is *Juncus effusus*, scattered graminoids and very rarely *Alisma plantago-aquatica*. The shore is lined with tree stands. It is initially very steep (34,5°) and becomes less steep to some extent - a few centimetres above water surface (25°). Vegetation

cover has been measured here at 82 % and the average penetrability is 120 kPa. The bank roughness here was very low compared to other sites (420/400 cm).

**Loc. W5** – the largest examined wetland site with an average depth of about 30-40 cm, further from the eastern bank there is a not very large depression with a zone of maximum depth up around 120 cm. There are 3 islands on the site area, consisting mainly of stands of *Eleocharis* (association *Eleocharitetum palustris*). Along the shoreline, there are isolated patches of *Juncus articulatus* and *J. effusus*, and sporadically *Carex* spp. Scattered and sporadic *Alisma plantago-aquatica* grows throughout the area, and in the north-eastern part there is a scattering of *Potamogeton natans*. It is clearly a less nutritious site, with a sandy bottom poorly covered with variously sized stones and, in some places periphyton. We measured the lowest median of conductivity values here, reaching only 100  $\mu\text{mS}\cdot\text{cm}^{-1}$ , compared to the highest value of 690  $\mu\text{mS}\cdot\text{cm}^{-1}$  at site 2 and the median of the rest of the sites (330  $\mu\text{mS}\cdot\text{cm}^{-1}$ ). At the same time, the conductivity here was the most stable, with fluctuations of just 20  $\mu\text{mS}\cdot\text{cm}^{-1}$  compared to those of the other sites (median 95  $\mu\text{mS}\cdot\text{cm}^{-1}$ ). The pH median was 7.5. The difference between the max. and min. pH values then resulted as the lowest (0.29 pH) compared to the other sites (0.875), and therefore this wetland is also the most stable in terms of this parameter. The median of O<sub>2</sub> concentration (8.74) was again the highest, and the variation of O<sub>2</sub> values was also the lowest (2.14) compared to the other sites (median 3.35). The sand sample from the shore of this site had the highest fraction <0.125 mm (19.3%) compared to the other sites. At the same time, a rather high degree of ruggedness was measured here (570/400 cm). The vegetation here was rather insular and tussocky and the surrounding surface was bare (59 % total cover).

**Loc. T1** – the most humid terrestrial habitat near the wetland (average substrate moisture is 10.1%) with clearly visible succession. Most of the area is covered with mosses and small pines are relatively abundant. The vegetation cover here is 87 %, which means that it has increased by 36 % (from 51 %) since the time of the Vácha and Zeman study. Grains of all fractions are relatively abundant, especially the 0,25-0,5 mm fraction.

**Loc. T2** – a lit up sandy site with a coverage of 31 %. Apart from open sandy areas, there are locally *Calamagrostis epigejos* and pine stands. At a specific time of day the site is partially shaded by *Robinia* trees. The most abundant grain size fraction here is 0,25-0,5 mm (58,7 %) and grains of other fractions are rather rare. The site has the lowest average penetrability (30 kPa).

**Loc. T3** – a technically reclaimed area with a dense stand of pine trees (about 3 m high) and *Calamagrostis epigejos*. *Calluna vulgaris* also grows abundantly here, and more rarely *Vaccinium* or *Centaureum*. The vegetation cover is relatively the highest here and has increased by 16 % over 4 years (from 83 % to 99 %). The soil moisture has also increased significantly, more than double (from 3,4 % to 7,2 %).

**Loc. T4** – a former wastepond. The main grain size fraction here is 0.25 - 0.5 mm (52 %), with a large proportion of lower grains. Penetrability (from 19.6 kPa to 50 kPa) and cover (from 22% to 82%) have increased significantly over the past 4 years. Pines about 2 m high are very abundant on the site. Other plants include willows, *abele* and *Robinia*. The surface here is mostly covered by the fall of the named trees and the bare areas are almost exclusively confined to the paths of larger game. There is a newly created wetland habitat close to the site.

**Loc. T5** – This site was not studied in 2018. The original habitat was an intermediate soil deposit that was shaved this year. Site T5 is particularly specific to the proportion of individual grain size fractions - 72% of the sample weight was composed of boulders (fraction > 32 mm). The age of the site (2 years) corresponds to the local composition of plant species. In parts there are mainly ruderal and pioneer species (*Tanacetum*, *Tussilago*, small birches), but the overall cover is very low (21%). The average penetrability was 160 kPa at sites with lower grain size.

## Diversity of surveyed groups

A total of 465 species of larger arthropods were found during the research, of which 194 belong to the order Coleoptera, 122 to the order Araneae, 66 to the suborder Heteroptera and 27 to the suborder Auchenorrhyncha (Fig. 1). Representatives of the other orders are listed in the species list in the Appendix. For terrestrial species, the richest site is T5, where 84 species were found by standardized methods (9 of which are on the Red List of Threatened Species of the Czech Republic, but many other species have some sort of distinct habitat requirements), and the poorest site is T4 (59), but where most of the Red List species are found (10). Of ripicolous arthropods of the groups studied, most species were found at Loc. W5rip (64), which also contained the most redlisted species (8). The lowest diversity of riparian species was observed at W4rip near the big lake, where 32 species were found (3 redlist species).

We also recorded 85 species of zooplankton (Fig. 2). Of this number, 25 belong to the order Cladocera, 20 to the subclass Copepoda, and 40 to the phylum Rotifera. The average number of species per site comes out to 39.4, and the lowest number of species found was 33 in site W4P, while the highest number was found in W4L (48). Wetland W5 (6) hosted the most interesting species, followed closely by littoral of site W3 (5). The fewest such representatives were found at sites W2 and W4L (2 and 3 species). At the same time, we recorded 33 species of beetles and 20 species of aquatic Heteropterans at the water sites. Most aquatic insect species were found at Loc. W5 (36, of which 9 redlisted) and the least at Loc. W4 (14 a 4 redlisted). We found a total of 45 species of arthropods classified in the Red List of Threatened Species of the Czech Republic during the survey - LC (category refers only to spiders and is equivalent to the NT category): 12, NT: 6, VU: 18, EN: 8, CR: 2. The phytoplankton included 223 taxa, of which the most abundant groups are *Zygnematophyceae*, comprising nearly 31%, *Chlorophyceae* 22% and *Euglenophyta* 18% (Fig. 3). The average number of taxa in the sample comes out to 104, with a min. of 88 found at site 2 and the highest again at W4L (121). For this community, we found most interesting representatives in the lake littoral (26) and also in wetland 5 (24). The fewest representatives were then found at site W2 and W3P (15).

In percentage similarity testing of plankton species composition against sites using Jaccard's index, it came out (Fig. 4) that, on average, the sites were less than 40% similar for zooplankton and 42% similar for phytoplankton species composition. The

zooplankton community was most similar ( $>=49\%$ ), but only 54% at most, at sites W1 and W2; W3L and W4L; W4L and W5. While phytoplankton is most similar at a max. of 68%, at W4L and W3L; W4P and W3P; W4L and W4P. For the species composition of insects and spiders associated with aquatic habitats on the sandpit, the average percent agreement between sites was 34% (35% for insects, 28% for spiders) (Fig. 4b). For insects, the highest agreement between sites W1 and W5, W2 and W5, and W1 and W2, and for spiders, the highest agreement between sites W3 and W5, W2 and W5. The terrestrial sites (T1 - T5) have the highest very low percentage similarity in species composition (around 10%), while the average similarity at the riparian sites is less than 28% (27.8% for spiders, 29% for insects). The most similar sites in terms of insect species composition are W2rip and W5rip, W1rip and W2rip, followed by W1rip and W5rip, W2rip and W3rip for spiders.

## Environmental conditions of different types of water habitats and terrestrial sites

Almost all values of the measured chemical parameters by Hartman et. Ad I. (2005) were within the normal range for surface waters in the studied water bodies (Tab. 2). From the resulting dendrogram of cluster analysis of site similarity (Fig. 5), it can be seen that there are three clusters in terms of physico-chemical water parameters. The first one shows the similarity of sites W1 and W2, which are mainly separated from the others by their high conductivity. The second shows the similarity of the lake habitats, which differ from the other sites mainly in lower O<sub>2</sub> median concentration and mean conductivity. The least similar to all sites are the parameters of wetland number W5, which was characterized by lower and stable variables. Thus, clusters W1 and W2 are more similar to each other than both to site no. W5. In terms of environmental factors (Fig. 6), there are only two clusters where the similarity is more cascaded. The first cluster groups Site W1 and W4L, which are thought to be similar to littoral of Site W3. This is due to the high cover of littoral macrophytes and small area. In the second group, sites W2 and W3P are closest to each other, but are also less similar to wetland W5, and finally W4P. These are linked by a differently sized limnetic zone with a greater max. depth. The graded character of the similarity of latter group is mainly due to different surface area, relative depth, cover of submergent or natant vegetation, but also the presence of fish. The banks of reservoirs are divided by dendrogram (Fig. 7). It can be seen that environmental factors are the most distinguishing features of the W1rip bank. This is due to its low slope, low penetrability and high cover together with the ruggedness of the bank. Thereafter, the W3rip site is separated by higher cover, larger grain content and slope. W2rip and W5rip are very similar to each other, but W4rip differs from them, particularly in its low gradient, higher slope and vegetation cover. For the terrestrial sites, we see the separation of sites T2 and T4, which are linked by similar grain size. Site T5 separates again with a very specific grain size. Sites T1 and T5 form a separate cluster with a similar grain size ratio, higher moisture content and cover.

The water transparency parameter, which was not included in the analyses due to measurements at only 2 sites, shows significantly high oscillations within the sampling season. In general, early spring values were such that it was possible to see the bottom without problems. Conductivity values fluctuated, but not so much within the season, but among sites. Its high values most probably corresponded with higher major ion content. Measured pH was broadly similar for all sites, with minimal variation between months ( $<1.5$  pH points). Overall, it averaged highest in spring and cumulatively its values were in the neutral-alkaline range ( $<7$ ). Measured oxygen values at the surface never fell below 6 mg/L and never below 70% saturation. According to many of the indicators, the aquatic sites investigated are more likely to be mesotrophic or eutrophic habitats. Fish were certainly the most important contributor to the higher trophic level of the lake and pond. According to the manager of the Planá nad Lužnicí gravel pit and the competing team of the QLA 2018, the presence of fish in these habitats was due to the floods of the very nearby Lužnice River in 2002 and 2006. However, no one quite knows whether some species (e.g. brown bullhead) were directly introduced into these habitats for fishing opportunities. Greater fish abundance negatively affected littoral vegetation size (significantly only in linear tests) and was also negatively correlated with lower O<sub>2</sub> median levels. In contrast, a positive relationship emerged with the standard deviation of O<sub>2</sub> and somehow with its max. values.

At terrestrial sites, penetrability was significantly influenced by grain size, vegetation cover and surface moisture. As a result, its values varied dynamically over time depending on the amount and frequency of precipitation. Since 2018, penetrability changed the most at site T4 (from 19.6 to 50 kPa), where, in addition to the gradation of mean moisture, there was an expansion of moss cover. Site moisture depended on its distance from water, site obscure, surface permeability, and the amount of organic material on the site. This was best observed at Loc. T3, which was very dry in 2018, whereas this year it was one of the wettest, probably due to the shading of the site by pine trees and their organic litter (from 3.4% to 10.1%). The successional stage of the sites was most influenced by their age (see Řehounková & Prach 2006), but, for example, the planting of pines at T3 significantly accelerated succession, and we therefore increased its succession index to stage "4" ("Late stage"), although its age does not correspond to this stage (Tab. 4). The other sites took on indices of "1" - "3" ("Initial stage", "Young stage" and "Middle stage"). The successional stage is also related to vegetation cover, which is positively correlated with it. It also appears that cover increases significantly with habitat moisture.

## Structure of plankton biocenoses

We often use several classifications of significance levels below, so we will introduce them first. At the lowest significance level lies  $\alpha < 0.01$ , we indicate a significant result with  $\alpha < 0.05$  and marginally significant with  $\alpha < 0.1$ . In the following, only significant correlations with  $\alpha < 0.1$  are interpreted.

The results of the ordination with fish stock scaled to 3 values are interpreted below. In this model (Fig. 6), the community varies along the depth gradient at the lowest significance level of  $\alpha < 0.01$ . At significance along the gradient of fish occupancy and cover by emerged macrophytes. Marginally significant variables then include age and min. temperature. The O<sub>2</sub> median and standard deviation of temperature lie at the full limit of significance  $\alpha < 0.1$ . The most species-rich community occurred on sites with either no or present but small fish stock, rather younger and not very deep sites, with more littoral vegetation and rather medium or lower values of minimum temperature. No parameters emerged as significant variables in the Pears. correlation to diversity. This was the case for depth, more fish, higher median of temperature and higher max. O<sub>2</sub> values and deviation. Likewise, the positive correlation of the background was not significant. In the Spear correlation test, temperature median, max. O<sub>2</sub> values and obscure were no longer significant, while fish stock, age and depth were stronger and significant. However, the relationships of these correlations cannot be assessed as too strong, but as borderline with moderate and strong correlations. In contrast, a strong positive and significant relationship was found for higher O<sub>2</sub> median. The species composition of sites with a larger limnetic zone

can be described as uniform to pond ecosystems. The percentile agreement of species composition of such sites here was more than 50% compared to sites more overgrown with macrophytes hosting on average 42% identical species. Indeed, pond zooplankton are referred to by Baxa (2018) as being partly similar in species and seasonal changes to the generalized description of zooplankton for shallow lakes. The average Jaccard index between the two types is less than 27%. A second ordination diagram showing the selected species with the best explanatory ability along the first two axes (Fig. 7) showed that sites with large fish occupancy, higher age and depth, are typically characterized by smaller species that can resist fish predation. For smaller values of the previous variables and littoral vegetation already present, larger species of water fleas and cyclopids are typical. Sites with small fish stock, low average depths, and conversely with larger or medium littoral cover and higher values of min. temperatures were inhabited by species tied to aquatic vegetation. With such temperature values and medium or higher O<sub>2</sub> concentrations and completely fishless, littoral features and large daphnia were more likely to occur. With higher median of O<sub>2</sub>, sites not too overgrown with littoral vegetation and with average min. temperature had different communities and abundant large daphnia. For a few taxa, higher or lower values of environmental parameters apparently did not play a major role. The third ordination shows the occurrence of rare or interesting species along the first two axes (Fig. 8). These suitable zooplankton representatives for habitat protection and conservation occur differently in the vector space. It appears that waterfleas *Diaphanosoma mongolianum* and *D. orghidani* prefer more fish and higher temperature gradients. These were sites with a large limnetic zone, greater depth, and an almost absence of aquatic macrophytes (the exception being the abundant *Potamogeton*). *Scapholeberis ramneri* and the rotifer *Stephanoceros fimbriatus* occurred in sites with higher temperature deviations and less fish, typically in littoral vegetation of the lake. In contrast, sites with fish were clearly not suitable for the cyclopoid *Macrocyclops fuscus*. Water flea *Ceriodaphnia quadrangula* did not appear to prosper at these sites and its occurrence was more likely to be associated with moderately high min. temperature values. Its higher numbers and more frequent records were found in more overgrown sites. Sites with lower conductivity were inhabited by species of cyclopoid that are more associated with low-nutrient waters with little or no fish stocking (Přikryl, pers. comm.): *Eucyclops macrurus*, *E. macrurus*, *Mycrocyclops varicans* and the rotifer *Lepadella triba*. This is mainly fulfilled by wetland 5, slightly also by the littoral of pond. On the other hand, sites with high conductivity are inhabited by the copepod *Eudiaptomus vulgaris*, where it was more frequently found in sites with a visible but not very large free water zone with multiple elodeoids.

Phytoplankton community structure varied along a gradient of fish occupancy and O<sub>2</sub> median at the lowest significance level (<0.05). The marginally significant variables then include conductivity median, min. temperature and max. O<sub>2</sub> value and standard deviation. At the full significance level <0.1 lie the lag and min. conductivity (Fig. 9). Among the significant variables in the Pears. correlation to diversity, higher values and deviations of conductivity came out negatively in the strong correlations ( $R > 0.7$  and  $> 0.8$ ) and at the significance limit higher values of median temperature. The lower effect of higher obscure of submerged or natant plants correlated with previous conductivity came out non-significantly. A significant positive correlation ( $R > 0.6$ ) emerged only for larger temperature deviations and at the borderline of significance for higher values of min. pH. In the Spear. cor. test, deviations and higher median of temperature values and higher min. pH appeared weaker and non-significant. In contrast, median conductivity and submersed and natant flora cover came out stronger and as significant variables. The increased correlation of obscure was not significant. The other correlations remained constant, but there was a shift towards the significance threshold. A second ordination plot showing the selected species with the best explanation along the first two axes (Fig. 10) showed that the sites with a fish stock and higher or intermediate O<sub>2</sub> deviations were characterized primarily by Chlorophyceae, desmids *Staurastrum* and *Staurodesmus*, Xanthophyceae, various euglenids and *Dolichospermum cyanobacteria*. Some taxa of these tended to be in low conductivity sites. This is especially true for habitats with more shade. More than the presence of fish, the occurrence of some euglenids is associated with medium O<sub>2</sub> variations and average or higher conductivity. As O<sub>2</sub> median values increase (this is especially true for habitat 1 and less so for 2 and 5), more Dinophyta, Trebouxiophyceae, and less Chlorophyceae. Phytoplankton taxa are most concentrated around the beginning of vectors. The third ordination shows the occurrence of rare or interesting species along the first two axes (Fig. 11). It shows that in places with more fish and higher temperature variation (this is mainly true for lake-like or even 4L habitats), Desmids mainly of the genera *Cosmarium* and *Staurodesmus* appear in mesotrophic or eutrophic conditions. In addition, *Gymnodinium uberrimum*, *Trachelomonas globulosa*, *T. cf. klebsii* and *Phacus cf. raciborskii*, and the green algae *Desmodesmus bicaudatus*, *Micractinium belenophorum* and *Polyedropsis spinulosa* were found at these sites. In biotopes with lower or medium conductivity (conditions mainly in W5), euglenid *Phacus inflexus*, especially the mesotrophic desmids *Cosmarium angulare*, *C. contractum*, *C. crenulatum*, *C. margaritifera*, *Euastrum verrucosum*, *Pleurotaenium archeri*, *Staurodesmus convergens* and *S. patens*, and the green algae *Ducelliera chodatii* and *Tetraëdron trigonum* occurred. Diatoms *Surirella tenera* and *Tabellaria fenestrata* also tended to be present. Diatoms *Eunotia pectinalis*, *Cosmarium botrytis*, *C. reniforme* var. *compressum* and *C. subgranatum* var. *borgei*, *Desmodesmus aculeolatus*, *D. cf. brasiliensis* and *D. cf. magnus*, and *Mallomonas insignis* tended to occur in shallow W1 and W5, sites with higher or medium O<sub>2</sub> median O<sub>2</sub> values and higher min. temperatures. The presence of *Neglectella solitaria* and *Cosmarium impressulum* is associated with sites with higher conductivity and higher O<sub>2</sub> median values and higher min. temperatures. *Daphnia* were abundant at these sites. With even higher conductivity, *Botryococcus territorialis* and the desmids *Cosmarium humile*, *C. turpinii*, the diatom *Surirella roba* and the green algae *Desmodesmus armatus* occurred under similar conditions. Only the conductivity was more pronounced, and even the slightly larger fish stock was related to the occurrence of the alga *Elakothrix genevensis*.

## Evaluation of the conservation of aquatic biotopes (NCV index - index of nature conservation value)

Since Desmids are highly sensitive microorganisms suitable for aquatic habitat conservation, especially in cases where macroorganisms have failed (Popelkova 2014), we have chosen a specially developed nature conservation index especially for these algae. We calculated it for each habitat following Coesel (2001) and found out that all sites take its high values (on average 7.4 out of 10 possible ones). Based on this index, site 5 and 4L and 3L reach the highest conservation potential. These sites also host the most interesting representatives of the entire plankton.



# Discussion

## Environmental factors affecting biocenosis structure

The community structure of zooplankton and aquatic insects of wetland pools and reservoirs varied along several environmental gradients depending on a predetermined range of fish occupancy scale. A model with a larger scale of fish was carefully chosen for the results. Namely, abundance defined only from presence and absence affected the results of the interpretation of species richness of littoral communities. It ignored the fact that many of the species found in these habitats were large representatives, which would contradict the general trend of fish predation pressure to select for smaller representatives. It is the littoral that should protect these species (individuals) by its vegetation, see (Šrámek-Hušek, 1962; Černý & Petrušek, 2022; Volemanová, 2007) and its biodiversity of animal communities is usually higher than in the limnetic zone (Kreidlová 2013). According to Šmejkal (2010), the presence of fish in the littoral depends on their horizontal migration, age, predation and some other factors. Thus, we certainly do not claim that fish are not present in the littoral of lake and pond, but that they do not play a major role as predators. For aquatic beetles, stock rates seem to be an absolutely key factor. For fish, aquatic beetles make up about 70% of the diet, leading to strong predation pressure (Brož 2011). In contrast to beetles, Heteroptera are much less affected by fish occupancy (Kolář 2016). This may be due to their chemical defences, for which they use scent glands. This makes true bugs disgusting to predators (experimentally verified in the project). The results of our research support these theories. It is noteworthy that some species, not only true bugs (*Sigara falleni*, *Laccophilus* spp.), have a certain affinity for sites with higher fish stocks, but it seems that this conclusion may be misleading, as other environmental factors or even combinations of them may play a greater role in the small number of sites studied. Sites W3 and W4, where these species were most abundant, have relatively similar max. depth, transparency or trophic level, for example. Of the other aquatic environmental parameters, we chose to discuss only those that we hypothesized might influence the communities in the case of aquatic insects. E.g. oxygen saturation, with which aquatic beetles were significantly positively correlated, is not expected to have a significant effect on groups of organisms whose representatives breathe atmospheric O<sub>2</sub>. The abundance of submersed plants, which is positively correlated with diversity and abundance of aquatic beetles, also appears to be an important factor in aquatic environments, as confirmed by other work (Kolar 2013). The depth gradient, whose increase does not significantly reduce diversity, was found to be the most important factor affecting the zooplankton community. However, it appears to be shaping the community from littoral to pelagic, along with the decline of some macrophytes, with fish playing an additional role, see below. In contrast, habitats that are too shallow, based on Gvozdjaková (2021) research on the biota of drying field spills, increase the density of the community, do not offer as many refugia for shelter and are at risk of drying out, thus not hosting greater diversity. We did not observe such a decrease in richness at our sites, as shallow sites had often deeper spots, whereas the author's sites were often shallower than the shallowest Loc 1. At the same time, water depth also significantly affects beetle communities (Wellborn et al. 1996). Increasing depth causes changes in a number of other parameters that can negatively affect animal communities in the reservoir (reduction of macrophytes, opening of space for fish, decreasing water temperature and transparency) (Kolář 2013, Kloskowski 2010). Other important indicators that came out were the presence of emerged macrophytes (habitats with them had richer fauna and vice versa) and fish occupancy (discussed below). Species-rich zooplankton had an optimum roughly at the beginning of the gradient or even lower of fish stock, depth, year, min. temperature and littoral vegetation cover. Corresponding with Pliska study of the zooplankton of pools (2022), it can be seen that without distinct aquatic vegetation, the crustacean community in particular was mostly represented by smaller species that are more pelagic and resistant to fish predation. In contrast, more overgrown habitats were dominated by species that were larger and tied to water vegetation. Diversity should then be significantly richer in areas with higher O<sub>2</sub> concentrations. Kosik (2007) mentions that low saturation reduces species richness. The lower O<sub>2</sub> concentration correlated with fish - see below. The most species-rich communities are 50% similar to each other according to Jaccard index values.

Several environmental gradients depending on the predetermined size range of the fish stock also played a role in the variability of the phytoplankton community structure. The scale of the fish occupancy and O<sub>2</sub> median emerged as the most important factors. Several environmental gradients depending on the predetermined size range of the fish stock also played a role in the variability of the phytoplankton community structure. The scale of the fish occupancy and O<sub>2</sub> median emerged as the most important factors. The median of conductivity, min. temperature, and max. O<sub>2</sub> value and standard deviation came out as other significant parameters. Obscure and min. conductivity were at threshold of significance. This community also resembled more of a pond community in sites with fish, see (Heteša et al. 2014). Species richness was highest in sites with either no or present fish, varying O<sub>2</sub> fluctuations, and sites with higher and lower min temperatures. Diversity should be more diverse on sites with conductivity rather below 300 µm/cm (with fewer ions). However, this result may be influenced by predation by large filtrators, as also reported by Zelený (2016), or by higher cover of submerged plants, as both of these factors were abundant under high conductivity conditions. This effect is discussed more below. Furthermore, perhaps in locations with a larger standard deviation of temperature. The latter corresponds to rather larger and deeper habitats and so the relationship may again be affected by the same factors as for conductivity. In general, temperature will play a role in seasonality, i.e. changes in temperature during the year will lead to community reshaping and thus higher diversity, see (Delawska 2013). The lower number of green algae and overall lower diversity of non-filamentous types at Site 2 will be mainly caused by higher numbers of the consumer *Daphnia longispina*. The occurrence of filamentous algae here may in turn be a response to predation, see (Zelený 2016), as filamentous forms are not fully exploited by zooplankton for food (Musil 2016). This is also supported by the result that filamentous types were not significantly present in the limnetic zone of the lake (except in summer with the cyanobacteria *Dolichospermum*), whereas filamentous Zygnematales and difficult-to-eat algae were predominant in the wetland habitats where this large daphnia was present. The near-absence of filamentous algae may also be due to roach and Rudd, which are abundant in the lake and able to feed on these algae, see (Brož 2011; rybarstvi.eu). The higher presence of submerged plants may also explain the problem at site 2, as according to the results it negatively affects the diversity of phytoplankton, most noticeably green algae. Heteša et al. (2012) describe this as the result of a repressive effect due to the absorption of important biogens. A few algal representatives, however, seemed to prefer sites with more submerged plants, specifically those with a dense cover of *Utricularia*. We are talking about many *Dismiss*, which were not the most abundant here in terms of diversity, but in abundance, and other algae found exclusively here, elsewhere only in smaller numbers (some of them are also among the interesting representatives). In most cases this is the result of ecological linkage, see (Hall & McCourt 2017).



Since we categorized floating and submerged plants together, the negative effect of fish on the cover of these macrophytes that Pliska (2022) found was not statistically significant. However, at sites with fish, truly submerged plants were almost absent. The author mentions that these macrophytes are absent in habitats with a low abundance of large zooplankton species where vegetation turbidity or water bloom develops, thus suppressing macrophytes due to shading and increasing chlorophyll concentration. Corresponding with us, he also talks about the loss of emersion vegetation. He describes that the lack of vegetation negatively affects the zooplankton community by the loss of microhabitats normally helping it to resist increased predation pressure from fish. The negative trend of fish occupancy on diversity size was shown in NMDS analysis and in non-parametric correlation on zooplankton. Linear models were not very tight and, in addition, not significant according to ANOVA. The inclination of interesting species to sites with more fish may be explained by their claims to higher trophy. Sukop (2007) reported that the mass abundance of smaller waterfleas, cycloids, or rotifers associated with low water transparency indicates a strong predation pressure by the fish stock. Since we categorized floating and submerged plants together, the negative effect of fish on the cover of these macrophytes that Pliska (2022) found was not statistically significant. However, at sites with fish, truly submerged plants were almost absent. The author mentions that these macrophytes are absent in habitats with a low abundance of large zooplankton species where vegetation turbidity or water bloom develops, thus suppressing macrophytes due to shading and increasing chlorophyll concentration. Corresponding with us, he also talks about the loss of emersion vegetation. He describes that the lack of vegetation negatively affects the zooplankton community by the loss of microhabitats normally helping it to resist increased predation pressure from fish. The negative trend of fish occupancy on diversity size was shown in NMDS analysis and in non-parametric correlation on zooplankton. Linear models (Fig. 11) were not very tight and, in addition, not significant according to ANOVA. The inclination of interesting species to sites with more fish may be explained by their claims to higher trophy. Sukop (2007) reported that the mass abundance of smaller waterfleas, cycloids, or rotifers associated with low water transparency indicates a strong predation pressure by the fish stock. In our study, such a situation was partially observed only in the lake and pond. We cannot completely claim about mass occurrences of small zooplankton representatives and significantly low transparency, so we estimate the fish biomass at the mentioned sites from the data of Čejnová (2016) in the interval of 400-500kg.ha<sup>-1</sup> (max. 600). According to Spurný et al. (2015), this corresponds to an average mass of 460 kg.ha<sup>-1</sup> in pond environments in Czechia. According to Baxa (2018), we could thus categorize it at the very limit of low stocks, rather at the beginning of medium-high values. This could also be the reason why the trend with decreasing zooplankton diversity towards increased occupancy did not come out well in the linear regression. Musil (2016) mentions that even the same fish biomass, but differing in numbers and average size, have different impacts on zooplankton. The lower transparency of the large lake was also indirectly related to fish, which apparently correlated with the higher presence of chlorococcal algae, euglenids and cyanobacteria. Hazuková (2016), Heteša et al. (2014), Sukop (2007) and Kosík (2007) come up with the same results. Namely, the aforementioned fish predation transforms the zooplankton community into small species incapable to filter algae, which leads to vegetation turbidity, see (Třešňáková 2016). The inorganic sludge flowing out from waste pond also contributed to the low values of the pond (not only according to the community, there is also a lot of org. substances in the pond). Also, the higher O<sub>2</sub> deviations were obviously related to the mass of algae (O<sub>2</sub> saturation decreased with lower transparency), which will be caused by their death or thus increased bottom respiration and possible exposure to the risk of undesirable O<sub>2</sub> deficiencies, see (Janda et al. 1996). Also, the highest measured pH values (9) in spring could correspond to significant photosynthetic activity of algae and cyanobacteria, according to Hummelová (2016), but we did not find more of them in this period, as confirmed by the high transparency. Such results are also mentioned by other authors, e.g. (Hnátek 2016), and even so they attribute the high pH values mainly to phytoplankton. Its prolonged high levels can reduce zooplankton and algal diversity and promote cyanobacteria, see (Hudec 2010; Řihová Ambrožová 2007).

No single reservoir appears to be significantly more ideal for maximising diversity as a whole or of faunistically interesting species, as they obviously have different needs. We can reliably speak of almost all aquatic sites as habitats suitable for conservation, not only in terms of the NCV index. The highest species richness of plankton was found in the littoral of the large lake and in wetland 5. This also applies to the number of interesting species. However, the other habitats do not lag behind. Loc. W2 hosted the fewest interesting species, but we still find great value in it, because the alga *Botryococcus terribilis*, which is very rare in Czechia, was found very frequently. No single reservoir appears to be significantly more ideal for maximising diversity as a whole or of faunistically interesting species, as they obviously have different needs. We can reliably speak of almost all aquatic sites as habitats suitable for conservation, not only in terms of the NCV index. The highest species richness of plankton was found in the littoral of the large lake and in wetland 5. This also applies to the number of interesting species. However, the other habitats do not lag behind. Loc. W2 hosted the fewest interesting species, but we still find great value in it, because the alga *Botryococcus terribilis*, which is very rare in Czechia, was found very frequently. Among all the interesting species in the sandpit predominate the ones, that are scattered in the Czech Republic. In the limnetic zone of the lake (pond) we observed a community approaching monotonous pond ecosystems, therefore there were many representatives common for eutrophic waters. Interesting representatives were also found here in not very small numbers. Some of them are known that their presence in more nutrient-rich places is the result of their ecological preference. This raises the question of whether an apparently lake with increased trophy and many fish should be considered a habitat suitable for conservation. The occurrence of these representatives could be linked to eutrophic conditions caused by ions other than phosphorus and nitrogen. The question is therefore whether, as the number of fish increases and these two nutrients increase in the reservoir, the interesting species will not disappear and be completely replaced by common ones, as described by Hnátek (2016). In terms of insects and spiders, the most species-rich site is W5, where we also find the most species in different categories of threat or with distinct habitat requirements. Two critically endangered species (*Dolomedes plantarius* and *Erotettix cyane*) are found here, among others, tied to nutrient-poor, mainly lowland wetlands. This demonstrates that W5 represents an excellent substitute for mesotrophic wetlands (Sychra & Malenovský 2015, Řezáč & Rothová 2020). However, ordination plots and Jaccard index values suggest that different species, often at various levels of threat, prefer different habitats, probably based on environmental characteristics other than those considered in our work. We observe the fewest redlisted species (2) at Loc. W1 and W3, but even here we find convincing reasons for protection and species with distinct habitat requirements. Nevertheless, it is almost certain that in the case of aquatic insects, the presence of more interesting taxa is negatively affected by the fish stock in particular, whose presence has far-reaching consequences. We can therefore say that by limiting fish occupancy we will achieve a very desirable community status at all sites.

For the ripicolous arthropods, we find several trends with changing values of the measured factors. Due to the lack of literature on this issue, it is difficult to compare our results and it would be advisable to devote more scientific effort (!) to issues related to the ecology of freshwater bank communities in the future. There are quite a few works dealing with the ecological value of shorelines. For example, Paetzold et al. (2008), Kleinwächter et al. (2003) investigated the effect of stream regulation and modification (alignment, riverbed reinforcement, flow alteration, damming) on riparian arthropod communities, concluding that homogenization of the riverbed affects them significantly negatively. Thus, it stands to reason that sand-gravel pits may be suitable refugia for species inhabiting such habitats (see Řehouňková et al. 2012). However, studies examining microhabitat preferences of individual groups are desperately lacking, as well as available information.

Among Coleoptera order, we compared two families (Carabidae, Staphylinidae) whose representatives have a close association with riparian habitats (Hůrka 1996, Smetana 1958). It seems that these beetles are most limited by the vegetation cover on the banks. Greater vegetation cover caused by succession progression causing shading and vertical habitat fragmentation. Such an environment can degrade visual habitat conditions, which is likely to be a limiting factor for predators, visually oriented shore beetles (and other insect predators). It is also apparent that more advanced succession limits the occurrence of particularly threatened and ecologically specialised species. The slope of the shoreline probably also has some influence on the communities. Too steep slope banks prevents beetles from moving easily on the surface and also makes it difficult for individuals to access the water. For Heteroptera in general, we do not observe any distinct or significant relationships with different bank parameters. However, if we consider only strictly ripicolous and predatory species (similar to the selected beetles), such as *Saldula* spp. we observe a similar trend to the beetles, showing an affinity for sites with low cover.

In terms of habitat preferences based on substrate characteristics, burrowing white-winged insects are the most studied (Srba et al. 2012). In our study, we do not treat the white-winged in the statistical analysis due to the small number of sampled sites. For quantitative collections suitable for intercomparison, standard colour plates were not used in the original methodology (Vácha & Zeman 2018), but only collection in ground traps and entomological nets for a standardized period of time. In our work, these methods were only effective at sites with lower vegetation; at other sites, netting was difficult to manipulate and burrowing insects were quite rare. Although there is an objectively noticeable significant decline in the diversity of the white-winged warblers at the study sites (especially at T3 and T4), we cannot attribute this change to successional development alone. Ideally, the colour plate method combined with net collection is the method of choice for collecting waders (Roulston et al. 2007).

Invertebrates of terrestrial sites have a significant positive correlation with increasing grain size fraction ratios >1 mm to <1 mm. Habitats with larger grain size fractions generally more closely resemble gravel banks of oxbow rivers, where many of these species (e.g. *Bembidion modestum*, *B. femoratum*, *B. stephensii*) occur naturally. Species that inhabit sandy banks in the wild (*Cicindela arenaria*, *Chaenius vestitus*, etc.) have an affinity for sites with a smaller grain size fraction - but of the sites we examined, all similar sites were at least somewhat overgrown with vegetation and did not provide the most suitable refuge for such species. This was shown, among other things, by a correlation which shows that succession has a negative effect on bioindicator-suitable species of gastropods. We do not find any significant trends for spiders, but most species (especially the web species) seem to have some affinity for wetter habitats. For other groups (Auchenorrhyncha, Heteroptera), their apparent (not significant) attachment to the environment seems to be more related to their dependence on the food plant and their well-developed migratory abilities.

For spiders, we do not see trends as strong. We also observe that we are not detecting the presence of as many shoreline specialist spiders as we would expect to find on the sandpit (Řezáč 2022). For many web spiders, the presence of vegetation is crucial for the construction of trapping webs, but for the most part, unlike open habitat species, they are not listed on the Red List of Threatened Species (Řezáč et al. 2015). Given that most of the habitats in the sandpit were already in an advanced successional stage at the time of the survey and that habitats of the initial stages were very fragmented and sporadic, we question whether spider species of conservation concern (e.g., *Arctosa* spp., *Prinerigone vagans*, etc., as well as some beetles or Heteropterans) need sites with a larger area and the necessary habitat integrity for their occurrence.

Penetrability did not significantly affect the communities of any surveyed group. Rather, its values are of importance for burrowing organisms (Heneberg 2010), which were not present at riparian sites. Probably because of this, penetrability was not one of the important environmental variables shaping the zoocenoses of arthropods on shorelines. Similarly, neither the effect of shoreline ruggedness nor grain size appeared to be crucial. It is clear that both variables influence the communities significantly, which is already based on the ecology of individual species (Hůrka 1996, Buchar & Růžička 2002, Boukal et al. 2017), but more comprehensive research is needed to confirm this theory, looking at more diverse sites to avoid misinterpretation based on random unconsidered environmental factors.

## Restoration reaccommodation

As can be seen from the results, it is not advisable to plant fish in the created water bodies. An exception would be the introduction of a predator in optimal proportion to undesirable herbivorous or omnivorous fish. In our reservoirs, the theoretical goal is to achieve a balanced fish stock with an optimal weight ratio of predatory and non-predatory fish species of 1:3-7 (Spurný et al., 2015). In practice, it turns out that the use of predatory fish can be complicated on reservoirs of several tens of hectares or more (Adámek et al., 2010). The maximum amount of fish should stick to the seasonal limit of 350 kg.ha<sup>-1</sup> average biomass see (Pecha, 2021). However, lower sets would be more appropriate, Adámek et al. (2010) states that a threshold of 100 kg. ha<sup>-1</sup> is the condition for top-down (briefly called steady state). Thus, these measures should ultimately aim to support the development of large zooplankton, littoral fauna and benthos. This should lead to an increase in water clarity, which is positively correlated with biodiversity. Water with higher transparency will not only promote the growth of submerged plants, which are the habitat for many species of aquatic invertebrates, but also attracts a wide range of organisms, including vertebrates such as amphibians and birds (Pecha 2021). After all, such a condition is also highly desirable in terms of aesthetic appeal.

Based on the results of our research, we particularly recommend the creation of just such a mosaic of pools, wetlands and ponds as in our sandpit (see Fig. 1). The aim of this is thus to offer a wide range of measures of the different factors of conditions in the pools. Thus:

- pools with smaller and larger depths (up to 1.5 to 2 m deep - a rugged bottom forming different depth gradients is suitable).

- allowing the water areas to become overgrown with littoral and other macrophytes (but not allowing the area to become completely overgrown!).
- if the only water location in the sandpit is a lake, then we find it possible to create differently deep pools with different bottom profiles on the banks of the lake
- Continuous restoration of pools (ideally using heavy machinery) to create a gradient of pools of different stages of succession

For large lakes with excessively steep banks (these are created by one method of sand extraction by excavating a pit sideways), we should create a more gradual gradient (ideally  $<5^\circ$ ) to form a large shoreline zone and shallow water. It is necessary to take into account that a significant reduction in slope will occur at a considerable distance from the original shoreline. Ideally, some parts of the shallow waters should be re-introduced to form various pools. As regards the nature of such pools, we adhere to point 1. The pools should have variously indented banks with not too much steepness and a bank - this part should have a normal depth of up to 60 cm.

On terrestrial sites, we particularly recommend maintaining the regularity of anthropogenic disturbances, which have been shown to increase the diversity of endangered species at the site (Řehouňková et al. 2012). The ideal frequency of such interventions would be once every three years. Both areas of fine-grained sand and coarse gravel should be cared for in this way, as different species composition occurs on surfaces of different grain size. In addition to invertebrates, we can maintain both communities of endangered annual plants and vertebrates in this way. For future maintenance of the sandpit we recommend:

- regularly remove infestations of pioneer and invasive plant species (pine, reed, acacia, buttercup)
- disturbance of habitats with heavy machinery or hand tools, especially in wet areas
- restoration of the shorebird wall, which is now not serving its purpose (the current wall is damaged by erosion and partly overgrown with infestations), according to its current state - it is essential to maintain its near-zero plant cover
- involvement of local residents in the management of the sandpit

As part of the protection of endangered bird species, we urge increased care in handling material during the breeding season, particularly in riparian sites where carelessness could easily result in the unintentional killing of unhatched birds.

## Conclusions

No one aquatic site came out as the ideal one, but it can be argued that sites with richer littoral and the presence of various aquatic macrophytes had a greater diversity of species of interest in aggregate. We can also conclude that the uncontrolled presence of fish disrupts communities, thus we consider the size of the fish assemblage as a key factor altering the structure of the environment. At terrestrial sites, it is evident that succession affects the communities of almost all groups negatively and also changes the physical parameters of the environment. Initial stage habitats host the greatest diversity of threatened species.

You can see a virtual tour of our sandpit at this link: <https://flycamczech-vr.click/plana.html> and the documentary film <https://youtu.be/p90WZ9jiJhc>

## Acknowledgements (see Appendix 1)

## Resources (see Appendix 5)

**Project tags (select all appropriate):**

This will be use to classify your project in the project archive (that is also available online)

**Project focus:**

- Beyond quarry borders
- Biodiversity management
- Cooperation programmes
- Connecting with local communities
- Education and Raising awareness
- Invasive species
- Landscape management
- Pollination
- Rehabilitation & habitat research
- Scientific research
- Soil management
- Species research
- Student class project
- Urban ecology
- Water management

**Flora:**

- Trees & shrubs
- Ferns
- Flowering plants
- Fungi
- Mosses and liverworts

**Fauna:**

- Amphibians
- Birds
- Insects
- Fish
- Mammals
- Reptiles
- Other invertebrates
- Other insects
- Other species

**Habitat:**

- Artificial / cultivated land
- Cave
- Coastal
- Grassland
- Human settlement
- Open areas of rocky grounds
- Recreational areas
- Sandy and rocky habitat
- Screes
- Shrub & groves
- Soil
- Wander biotopes
- Water bodies (flowing, standing)
- Wetland
- Woodland

**Stakeholders:**

- Authorities
- Local community
- NGOs
- Schools
- Universities