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Effect of submerged macrophytes on the structure of lake ecosystem and trophic relationships among fishes

Ph.D. Thesis

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Annotation

This Ph.D. Thesis is focused on the effect of submerged vegetation on the structure of lake ecosystem and trophic relationships among fishes. Succession of submerged vegetation is a dynamic process and the vegetation community may change dramatically even during a year. Many environmental factors have impact on the succession of submerged vegetation and consequently the presence of the vegetation has substantial impact on the aquatic ecosystem. Nutrient level (mainly nitrogen and phosphorus) plays a key role in the succession of freshwater ecosystems. Further, herbivory significantly influences species composition and biomass of macrophytes and macroalgae. Herbivory of fish is a foraging strategy that is dependent on temperature. Foraging preferences of fish may suppress the palatable species. The rate of herbivory affects plant growth patterns and thus inpalatable species may be advantaged in the competition among plants. In aquatic ecosystems, herbivory is often performed by omnivorous species. The diet preferences of omnivorous fish differ among aquatic ecosystems and submerged vegetation can shape their trophic niches substantially.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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List of papers and author´s contribution

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I participated in the field sampling, provided the data processing and statistical analyses, and I wrote the manuscript with the help of co-authors.

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I participated in the field sampling, provided the data processing and statistical analyses, and I wrote the manuscript with the help of co-authors.

Declaration of originality

The co-authors fully acknowledge that Ivana Vejříková made a major contribution to all manuscripts presented. She substantially contributed to data collenting, data processing, statistical analyses as well as to writing of the manuscripts. All papers contain original results. All co-authors consent to the publication of individual papers in the dissertation of Ivana Vejříková and four authors listed below hereby support this statement with their signature.

RNDr. Jiří Peterka, Ph.D.   RNDr. Ing. Lukáš Vejřík, Ph.D.

doc. RNDr. Martin Čech, Ph.D.   RNDr. Marek Šmejkal, Ph.D.

Content

Introduction

The role and position of submerged vegetation in the ecosystem

Submerged vegetation plays very important role in aquatic ecosystems and influences many characteristics. Submerged vegetation is mainly formed by macrophytes (vascular plants living in the aquatic ecosystem), and also by macroalgae (aquatic algae that can be seen with the naked eye). Henceforth, submerged vegetation will be referred to as macrophytes in the text for simplicity, unless otherwise written. Macrophytes have direct impact on the nutrient cycles that affects physicochemical properties of water (Hutchinson, 1975). Within certain nutrient limits, macrophytes are able to maintain clearwater state via numbers of feedback mechanisms despite increased nutrient supply (Jeppesen et al., 1998). In addition, macrophytes may affect other abiotic variables such as light, temperature, and oxygen concentration (Carpenter and Lodge, 1986; Lillie and Budd, 1992).

Macrophytes also influence biotic variables, as they affect trophic structure and trophic relationships in both direct and indirect ways. They provide additional habitat and resources for other trophic levels of the food web (Carpenter and Lodge, 1986; Lillie and Budd, 1992). They affect processes on a wide range of spatial and temporal scales (Diehl, 1988; Diehl, 1992). In terms of fish, macrophytes function as a resource of food, as a refuge from predation and as a spawning substrate (Persson, 1993; Osenberg et al., 1994; Persson and Eklov, 1995; Paper I; II). Macrophytes offer a physical structure that affects both competitive and predatory relationships between different species and sizes of fish (Winfield, 1986; Persson, 1991). Habitats formed by macrophytes may positively affect the survival of fish fry by lowering the predation efficiency of piscivores (Savino and Stein, 1982; Persson and Eklov, 1995). The decreased predation efficiency in complex habitats may be caused by both decreased encounter rate between predator and prey and decreased capture success of the predator when the prey is encountered (Anderson, 1984; Main, 1987; Christensen and Persson, 1993). Macrophytes also increase the diversity of habitats and resources for macroinvertebrates that subsequently become to be an abundant food source for fish and may positively affect their growth (Diehl, 1992; Persson, 1993). Through these various pathways, submerged macrophytes have a positive effect on the other components of the aquatic ecosystems (Jeppesen et al., 1998). However, fish have

reverse effects on macrophytes directly via their consumption and indirectly via other trophic components or abiotic routes such as sediment feeding that induces turbidity. Therefore, fish also influence habitat structure by affecting the macrophytes (Jeppesen et al., 1998).

Macrophytes are also able to affect niche variation of fish individuals thanks to the increasing habitat complexity (Schluter et al., 2001; Svanbäck and Persson, 2004; Losos, 2010). Generalist fish populations commonly consist of specialized individuals. But the potential for and hence degree of individual niche variation may be markedly determined by habitat complexity (Diehl, 1992; Persson, 1993; Schriver et al., 1995). The occurrence of macrophytes provides offered littoral food resources, predation shelter, and thus new niches that may be occupied. It increases individual variation of generalist species, that enables interspecific niche segregation as it was shown on the example of rudd (*Scardinius erythrophthalmus*), roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*; Paper III), but also intraspecific niche segregation, as it was proved on the example of ruffe (*Gymnocephalus cernua*; Paper IV). Therefore, succession of macrophytes in the freshwater ecosystems essentially influences the overall structure of complex food webs in the system (Schindler and Scheuerell, 2002; Kratina et al., 2012; Paper V).

Succession of macrophytes

Succession of primary producers in a freshwater ecosystem is often connected with changes of a nutrient level (Portielje and Roijackers, 1995). It affects both the abundance and composition of macrophytes (Wetzel, 1975). With increasing nutrient level, the depth limit of macrophytes decreases (Chambers and Kalff, 1985), but at the same time the biomass per unit of area may increase (Wetzel, 1975). However, at some point of the high nutrient level, macrophytes most often disappear due to light limitation caused by increasing amount of floating phytoplankton (Gliwicz and Rybak,1976; Venugopal and Winfield, 1993; Moss et al., 1997). Therefore, from the perspective of macrophytes, the most productive state is in a slightly eutrophic system, where density of macrophyte biomass is high, but the area covered is not yet severely reduced (Jeppesen et al., 1998). Simultaneously with the changes in density and distribution of macrophytes, species

composition also changes. In northern temperate lakes, the succession often directs from characeans to elodeids in lakes with hard water and from isoetids to elodeids in lakes with soft water (Jeppesen et al., 1998). Isoetids are small rosette aquatic plants that make usually low biomass on the contrary characeans often form dense beds with a high areal biomass. Elodeids may also appear in high densities, but the biomass per unit volume is often smaller than that of characeans (Diehl, 1988). Characeae is part of macrophyte community during the initial stage in Central Europe (Portielje and Roijackers, 1995; Paper V). After the initial stage, Characeae usually disappear and are replaced by several species of macrophytes, such as *Potamogeton* and *Myriophyllum*. The transition is correlated with increasing nutrient level and with the colonization rate of new diaspores (Krahulec et al., 1987; Portielje and Roijackers, 1995). The most common ways of seed, oospores and vegetative propagules dispersion is by water, followed by wind and animals (Boedeltje et al., 2003, Soons et al., 2008; Xie et al., 2010). With increasing nutrient level, submerged macrophytes are often replaced by floating or emergent macrophytes (Krahulec et al., 1987; Portielje and Roijackers, 1995) or by periphytic and filamentous algae and subsequently, phytoplankton prevails. The phytoplankton bloom is the most common problem connected with eutrophication (Phillips et al., 1978; Jeppesen et al., 1998; Ibelings et al., 2007). The transition is caused by the competition for light, carbon and space that is necessary for the survival of primary producers. Therefore, the production zone moves towards or even above the water surface (Den Hartog and Segal, 1964; Carignan and Kalff, 1980; Portielje and Roijackers, 1995) due to light interception and availability of atmospheric carbon.

However, filamentous algae may sometimes prevail also with decreasing nutrient level. For instance, biomass of *Vaucheria* sp. positively correlates with decreasing level of nitrates that seem to be key factor for submerged vegetation in terms of nutrients (Paper V).

Apart from chemical processes such as nutrient flow and physical processes such as light intensity (Den Hartog and Segal, 1964; Carignan and Kalff, 1980; James et al., 1995), biological processes play also a key role in the succession of macrophytes. The most important biological processes are competition among species, that can be accompanied by allelopathy with an inhibitive effect (Gross, 2003; Hilt and Gross, 2008), and

herbivory, i.e. direct consumption of macrophytes by herbivorous or omnivorous species. Effect of herbivory poses macrophytes in front of a difficult trade-off whether to invest to a fast growth or to chemical defences against consumption (Bakker et al., 2016).

Herbivory

Specialization of herbivory in aquatic ecosystems was for many decades considered to be a sporadic phenomenon with irrelevant impacts on the water ecosystems (Hutchinson, 1975; 1981) and the predominant view was that freshwater and marine macrophytes did not participate in the food web. They were considered to be primarily part of the detritivorous pathway (Bakker et al., 2016). The impact of microfauna (i.e., crustaceans, snails and aquatic insects) on algal periphyton was the most frequently studied topic in terms of potential impact of herbivores (Jeppesen et al., 1998). Contrastly to these thoughts, studies from last decade prove that herbivory has important impact on the aquatic ecosystem (Bakker et al., 2016). The estimated amount of plant biomass removed by herbivores in freshwater and marine ecosystems is on average 40–48%, that is five to ten times more than the amount reported for terrestrial ecosystems (Turcotte et al., 2014; Wood et al., 2016). The reasons, why herbivory in aquatic ecosystems is so important, can be the variation in plant productivity, nutritional quality, resistance and tolerance to grazing and very probably by the lower C:N stoichiometry found in submerged plants (Cebrian et al., 2009; Bakker et al., 2016).

Primary producers in aquatic ecosystems have to invest their energy either into to the fast growth to succeed in competition, or into the chemical defences against herbivores such as primary producers in the terrestrial ecosystems (Herms and Mattson, 1992). However, these mechanisms have been described relatively recently in the aquatic ecosystems (Dorenbosch and Bakker, 2011; Kapuscinski et al., 2014). The chemical defence in the aquatic ecosystem is provided by secondary metabolites, such as alkaloids, glucosinolates and polyphenolics (Sotka et al., 2009). The consumption rate of macrophytes with chemical defence (e.g., *Myriophyllum* sp.) is much lower than those of palatable macrophytes (e.g., *Potamogeton pectinatus*) and macroalgae (e.g. *Chara* sp.) (Gross et al., 1996; Choi et al., 2002; Lake et al., 2002; Marko et al., 2008).

It has been discussed, whether herbivory in aquatic ecosystems may really affect species composition and biomass of macrophytes in a long-term perspective even when the macrophyte biomass has fast turn-over (Bakker et al., 2016). However, several studies have already proved the fact that both species composition and biomass of macrophytes may be influenced by grazing of herbivorous fish, waterfowl or even crayfish, depending on the locality (Van Donk and Otte, 1996; Matsuzaki et al., 2009; Moore et al., 2010; Paper II). For instance, in Lake Zwemlust (Netherlands), the macrophytes were significantly utilized by coots (*Fulica atra*) and rudd that altered the dominance of *Elodea nutallii* to codominance by *Ceratophyllum demersum* and *Potamogeton berchtholdii* (Van Donk and Otte, 1996). In Milada Lake, the marked effect of herbivory was proved by the unique underwater experiment under natural condition. *Potamogeton pectinatus* with no chemical defence against herbivory, but apparently competitively successful species, prevailed in the experimental plots where access of herbivores was prevented, whereas this species was suppressed in the open plots with access of herbivores (Paper II).

The proportion of fish species consuming plant material (at least partly) increases with decreasing latitude and, therefore, with increasing species richness in both marine (Floeter et al., 2005; González-Bergonzoni et al., 2012) and freshwater ecosystems (Wooton and Oemke, 1992; Ibáñez et al., 2009; Jeppesen et al., 2010). This globally coherent pattern in the relative and absolute richness of omnivorous fish species in all aquatic ecosystems along a latitudinal gradient was described by González-Bergonzoni et al. (2012). According to this study, both absolute and relative omnivorous richness increased towards lower latitudes more steeply than the gradient of total fish species richness. More theories discuss the higher relative richness of omnivorous fish species in tropical than in temperate regions (Wooton and Oemke, 1992; Pringle and Hamazaki, 1998; Ibáñez et al., 2009; Moss, 2010), namely i) the large-scale biogeographic and evolutionary theory stating that time has been too short for fish to develop a herbivorous (or omnivorous) strategy in temperate regions, or to disperse from areas where herbivory first occurred (Mead, 1970), ii) the change in the macroalgae assemblage characteristics, with higher nutritional quality and greater digestibility (Gaines and Lubchenco, 1982; Ferreira et al., 2004), iii) low biomass of primary producers during winter in the cold and temperate zone (Arrington et al., 2002), and iv) various temperature-dependent digestive constraints (Floeter et al., 2005; Behrens and Lafferty, 2007). The latter is called the

'digestive constraint' hypothesis and says that assimilation of plant matter becomes more difficult at low temperatures (Clements et al., 2009). The same constraint is known also in herbivorous reptiles characterized by digestive fermentation in the internal gut system where endosymbionts largely depend on higher temperatures to gain digestive efficiency (Espinoza et al., 2004). Overall, the intake of plant matter by herbivorous fish seems to be positively correlated with the temperature (Prejs, 1984; Behrens and Lafferty, 2007; Kapuscinski et al., 2012; Guinan et al., 2015). The threshold temperature seems to be around 16°C (Paper I). Below this temperature, plant matter was ignored by rudd, the European native fish species with most developed herbivory. In contrast, the plant matter was preferred against animal prey above temperature of 20°C and the proportion of plant-based diet increased with increasing temperature.

Fish species have two methods how to digest barely degradable high molecular weight polysaccharide material: i) chemical processing by acid hydrolysis in the stomach (e.g., Acanthuridae, Kyphosidae, Pomacentridae), or ii) mechanical processing by breakdown of plant matter in the muscular stomach (e.g., Acanthuridae, Sparidae) or by pharyngeal teeth (e.g., Cichlidae, Cyprinidae, Scaridae) (Prejs and Blaszczyk, 1977; Niederholzer and Hofer, 1979; Gerkings, 1994). The subsequent digestive process is dependent on symbiotic microorganisms and their cellulolytic activity (Niederholzer and Hofer, 1979; Kapuscinski et al., 2012). Saha et al. (2006) isolated microorganisms capable of cellulolytic digestion called *Bacillus circulans* and *B. megaterium* from grass carp (*Ctenopharingodon idella*) and tilapia (*Oreochromis mossambica*). A characterization of the isolated microorganisms revealed that they could grow in a wide range of pH levels (5–11) and temperatures (15–42°C). Thus, the lower temperature limit of 15°C is very close to the results of the experiment, where rudd consumed no plant matter in temperature of 16°C. In this temperature, the activity of microorganisms is probably so low that it is inefficient for rudd to utilize plant matter (Paper I).

In our conditions of the central Europe, fishes with the most developed herbivory are rudd, common nase (*Chondrostoma nasus*) and introduced grass carp (Dorenbosch and Bakker, 2011; 2012; Kapuscinski et al., 2012; Zapletal et al., 2019). Further, herbivory may be developed in other cyprinids such as roach, or carp (*Cyprinus carpio*) (Prejs and Jackowska, 1978; Prejs, 1984; Paper III). Plant matter may be surprisingly found also in

the diet of predatory fish, such as European catfish (*Silurus glanis*) (Copp et al., 2009; Vejřík et al., 2017), or perch (Zapletal et al., 2016). However, accidental ingestion of the plant matter during suction of benthic prey is a more probable explanation for the presence of macrophytes in catfish stomachs than intentional feeding on it (Vejřík et al., 2017).

The consumption of living macrophytes has been discussed so far. However, macrophytes may become a food source also after senescence and death. They become available as food source to shredders and deposit feeders (Kornijów et al., 1995). For example, many herbivores benefit from the additional substrate area provided by submerged macrophytes to periphytic algae (Jeppesen et al., 1998; Kornijów et al., 1995).

Summary of results, general conclusions and perspectives

As it was mentioned herbivory of fish is dependent on temperature, because fish rely on the production of enzymes by symbiotic microorganisms that are active at temperature higher than ca. 16°C. This finding is associated with a serious issue that was observed in a marine ecosystem. Biomass of kelp forests in temperate (mainly in south latitude) is decreasing due to increasing numbers of herbivorous consumers (Taylor and Schiel, 2010) that is apparently connected with climate change (Bennett et al., 2015), specifically with a migration of herbivorous fish from tropics poleward. Due to increasing water temperature, tropical fish can digest plant matter even in temperate climate where kelp forests occur (Vergés et al., 2007; 2009; 2014; 2016). However, there is no realistic solution that would stop this problem. The spread of herbivorous species in freshwater ecosystems has likely not been studied to date. Nevertheless, we can assume these changes are occurring (Paper I).

We have demonstrated in the underwater experiment that both herbivory and competition have strong impact on shaping of the macrophyte community composition. The results of our study may be applied in revitalisation or conservation projects conducted in aquatic ecosystems because the interventions may increase biodiversity and promote some endangered macrophyte species. However, this approach cannot be applied in all localities as not all species would benefit from similar interventions (Paper II).

Beside mentioned biological factors, also chemical and physical factors have essential effect on the succession of macrophytes. Especially, nutrient level fundamentally drives the succession. Although increase of nutrient level mostly occurs, nutrient level decrease was observed during the succession in our study site. For instance, transition between dominant groups of macroalgae (from Characeae to *Vaucheria* sp.) was described. It demonstrates that commonly neglected macroalgae may essentially contribute to the composition of submerged vegetation. Number of studies focused on the ecology of macroalgae as primary producers is limited (Schagerl & Kerschbaumer, 2009). Hence, future ecological studies focused on role of macroalgae (not only vascular plants) on the aquatic ecosystems are desirable (Paper V).

The presence of macrophytes increases habitat complexity. It brings various advantages such as new source of food, a refuge from predators or a spawning substrate. In addition, macrophytes can affect individual niche variation among generalist fishes with different ontogenetic trajectories, and hence the overall food-web structures in lake ecosystems. We revealed the impact of macrophytes on the interspecific niche variation of rudd, roach and perch (Paper III). Further, macrophytes can apparently affect also intraspecific niche segregation. We described the case of ruffe population consisting of shallow- and deep-water dwelling individuals (Paper IV). Although, clear impact of macrophytes on inter- and intraspecific niche variations were described, there is still space for future ecological studies. For instance, seasonal (temporal) variations caused by differing prey availability and environmental conditions could be revealed thanks to telemetry in conjunction with trophic studies.

Image attachments from the field sampling and data processing
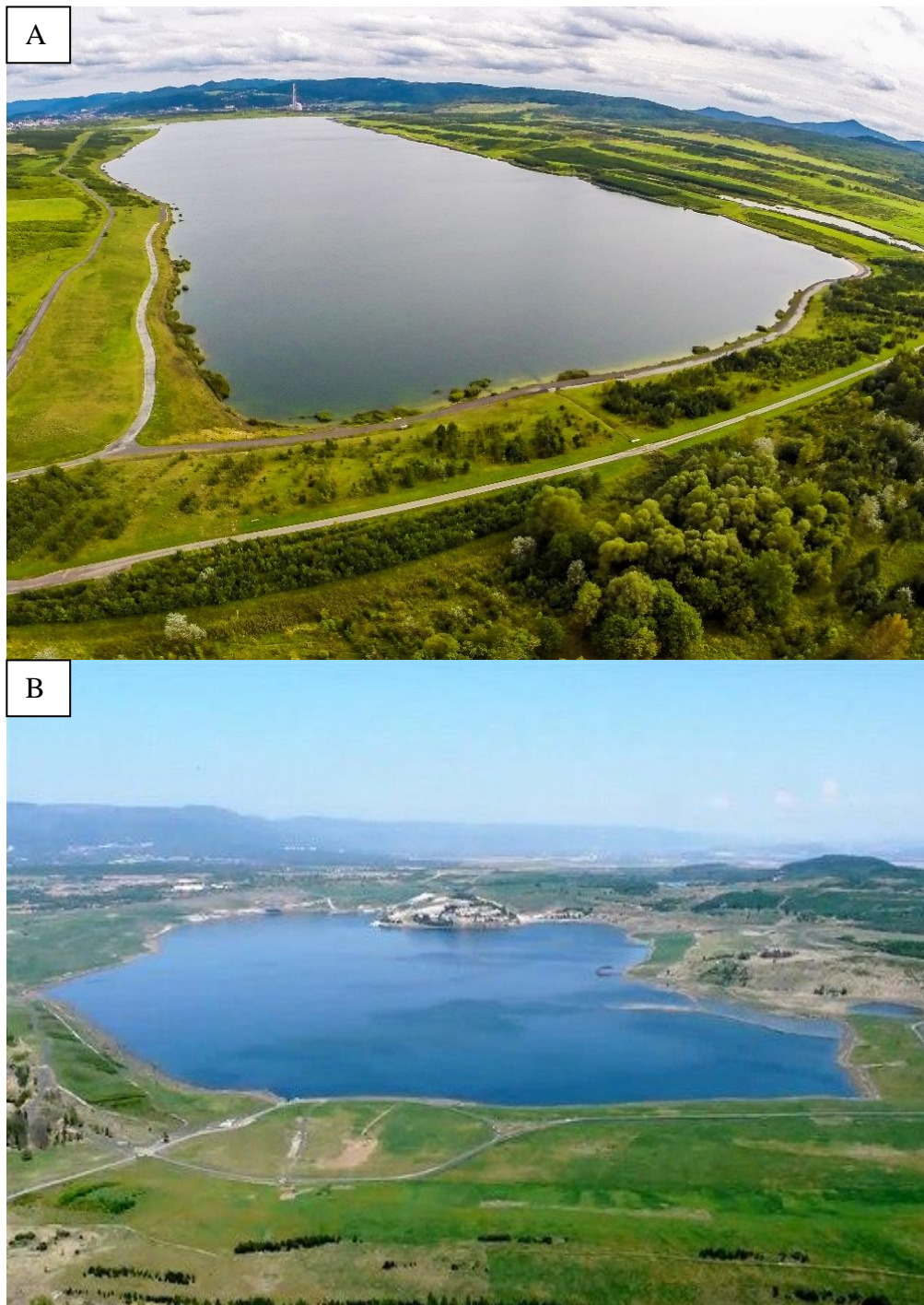
Figure 1. Two post-mining lakes that are the key sites in presented studies, A) The oligo-to mesotrophic Milada Lake (250 ha, 36×10^6 m^3, max. depth 25 m), and B) the oligotrophic Most Lake (311 ha, 70×10^6 m^3, max. depth 75 m).

Figure2. Monitoring of the vegetation cover and writing notes right in the water.

Figure 3. *Myriophyllum spicatum* and *Potamogeton pectinatus* in Milada Lake.

Figure 4. *Chara globularis* in Milada Lake.

Figure 5. Cover of *Vaucheria* sp. in Milada Lake.

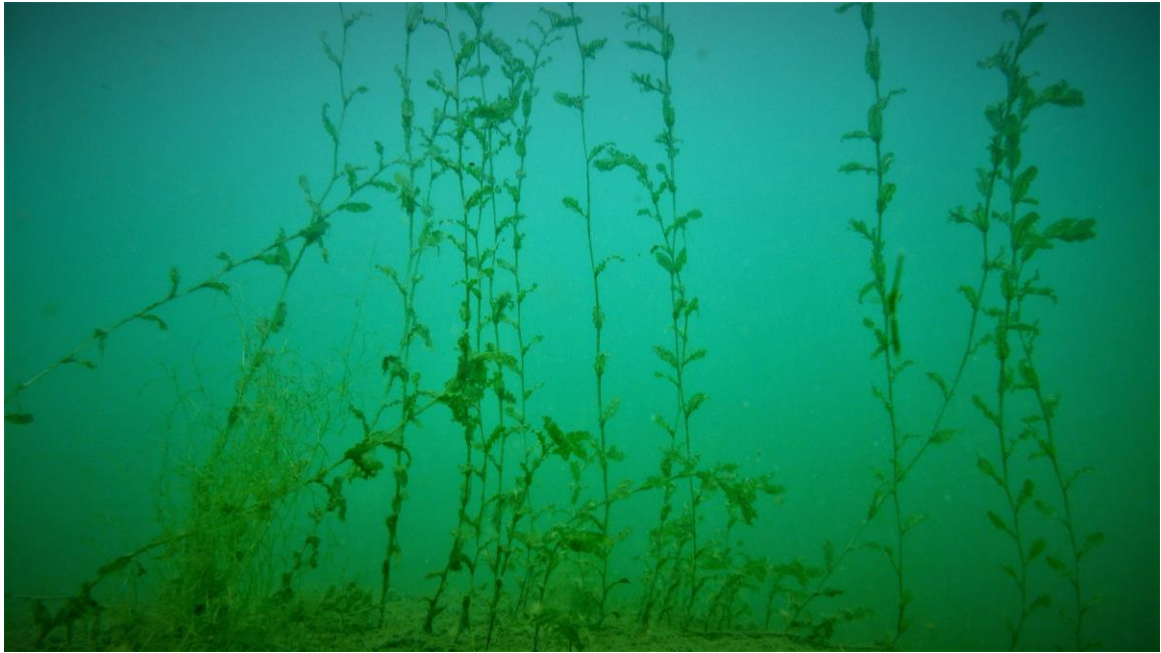**Figure 6**. *Potamogeton crispus* in Milada Lake.

Figure 7. Macrophytes provide a refuge for small fish in Milada Lake.

Figure 8. Monitoring of the cage experiment in Milada Lake

Figure 9. Perch (*Perca fluviatilis*) and rudd (*Scardinius erythrophthalmus*) from Milada Lake.

Figure 10. Preparation of samples for SIA provided in University of Jyväskylä, Finland.
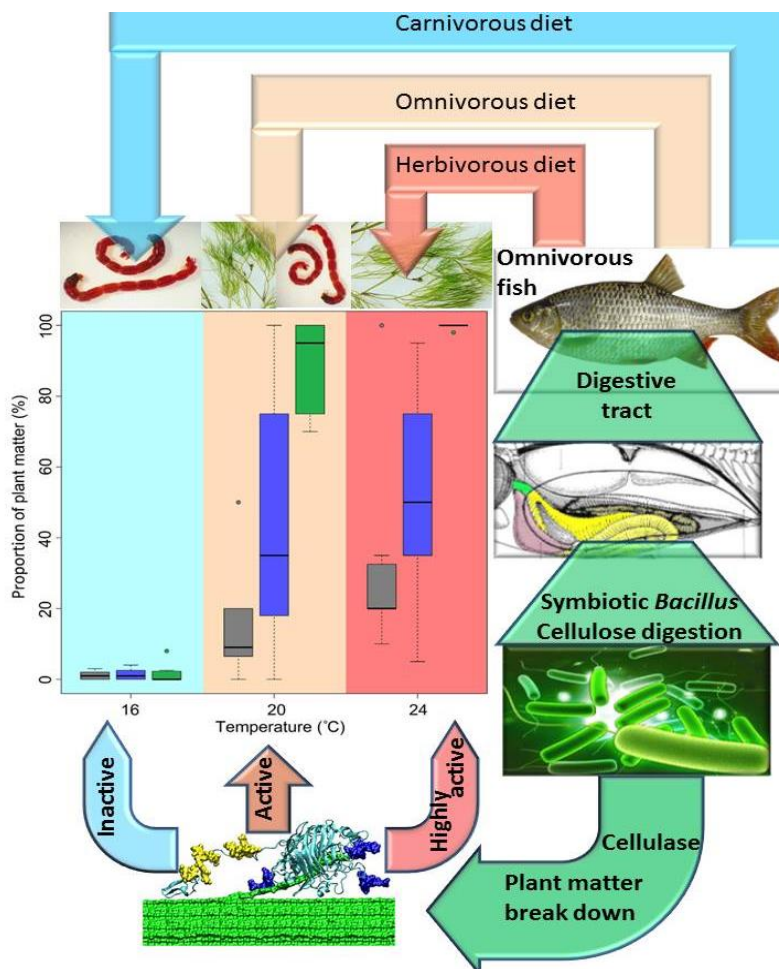

Figure 11. Scheme illustrating temperature mediated pattern of diet preferences of omnivorous fish. Low temperature inactivates enzyme cellulase responsible for cellulose digestion. In the mesocosm experiment (paper I), animal vs. plant diet was offered in three different ratios 1:1 (grey), 1:10 (blue), 1:400 (green) under three different temperatures (16, 20 a 24 °C).

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Research articles

Paper I

Distribution of herbivorous fish is frozen by low temperature

SCIENTIFIC REP⚙RTS

Distribution of Herbivorous Fish Is Frozen by Low Temperature

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The number of herbivores in populations of ectothermic vertebrates decreases with increasing latitude. At higher latitudes, fish consuming plant matter are exclusively omnivorous. We assess whether omnivorous fish readily shift to herbivory or whether animal prey is typically preferred. We address temperature as the key factor causing their absence at higher latitudes and discuss the potential poleward dispersion caused by climate changes. A controlled experiment illustrates that rudd (*Scardinius erythrophthalmus*) readily utilize plant matter at water temperatures above 20 °C and avoid its consumption below 20 °C. Field data support these results, showing that plant matter dominates rudd diets during the summer and is absent during the spring. Utilizing cellulose requires the enzyme cellulase, which is produced by microorganisms growing at temperatures of 15–42 °C. Water temperatures at higher latitudes do not reach 15 °C year-round; at our latitude of 50°N~150 days/year. Hence, the species richness of omnivorous fish decreases dramatically above 55° latitude. Our results provide support for the hypothesis that strict herbivorous specialists have developed only in the tropics. Temperatures below 15 °C, even for a short time period, inactivate cellulase and cause diet limitations for omnivorous fish. However, we may expect increases in herbivory at higher latitudes caused by climate change.

A specialization of herbivory in aquatic ecosystems was previously considered a sporadic phenomenon with irrelevant impacts on the water communities[1,2]. However, recent studies indicate that herbivory in aquatic ecosystems has been overlooked by scientists in comparison to terrestrial ecosystems[3–6], and the impact of herbivory by both vertebrates and invertebrates on aquatic ecosystems is substantial[7–9]. Fishes have the following two methods of digesting barely degradable high molecular weight polysaccharide material: i) predominantly chemical processing by acid hydrolysis in the stomach (e.g., Acanthuridae, Kyphosidae, Pomacentridae) or ii) mechanical processing by breakdown of plant matter using a muscular stomach (e.g., Acanthuridae, Sparidae) or pharyngeal teeth (e.g., Cichlidae, Cyprinidae, Scaridae)[10–12].

A specialization in herbivory is often developed by fish and other ectothermic aquatic and terrestrial vertebrates at lower latitudes[13,14]. In contrast, ectothermic vertebrates (mainly fish) at higher latitudes that use plant matter are omnivores, consuming both plant and animal sources in their diets. At these latitudes, either a herbivorous specialization has not developed or evidence of this type of specialization is lacking in the fossil record[14–16]. The quantity of plant matter in the diet of omnivores and the number of ectothermic species capable of consuming plant matter rapidly decrease poleward[16–19], which can potentially be explained by a number of theories as follows: (i) short-term evolution and the inability of fish to migrate along latitudinal gradients[20], (ii) availability of a readily palatable plant diet at lower latitudes[21], (iii) absence of plant diet at higher latitudes during the winter[22], and (iv) constraints in the digestion of plant matter at low temperatures[14,18,23]. The last theory is supported by observations that marine herbivorous fish have spread to higher latitudes due to global warming[24,25].

Of the European native fish species found in lentic water, herbivory is most developed in rudd (*Scardinius erythrophthalmus*). The proportion of plant-based diet consumed (mainly macrophytes) is markedly higher for rudd than for other species, e.g., roach (*Rutilus rutilus*), even when a nutrient-rich diet is available[26]. Recent

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		Depth 0–3 m					Depth 0–20 m		
	Macrophyte cover.(%)		Depth 0–3 m Mean biomass of potential sources (g m^{-2})				Depth 0–20 m Mean density (ind. L^{-1})		
	Palatable macroph.	Unpalatable macroph.	Palatable macroph.	Unpalatable macroph.	Benthic invert.	*Dreissena*	Copepods	Cladocerans (*Daphnia*)	
								small	large
Milada	71	20	2122	598	5.4	167	23.5	10.1	2.7
Most	0.9	0.8	27	24	4.2	242	0.3	26.8	0.3

Table 1. Summary of the assessment of potential diet for rudd in Milada and Most Lakes. We assessed mean coverage and mean biomass of palatable and unpalatable macrophytes on the bottom in the depth of 0–3 m, and biomass of benthic invertebrates in the same depth. Further, we assessed mean density of zooplankton in the water column in the depth of 0–20 m.

studies examining macrophyte characteristics and the causes of selective herbivory in rudd assert that the greatest factors are C:N ratio and phenolic concentration, both of which are negatively correlated with herbivory rates. Lesser importance is assigned to dry matter content and the concentration of total soluble proteins[15,27,28]. The shared evolution of rudd and macrophytes also seems to be a relevant factor[28].

Although rudd are shown to prefer an animal diet in experimental conditions (aquarium, mesocosm)[7,15], natural observations indicate a preference for a plant-dominated diet in the wild[29,30]. The percentage of plant matter in rudd diet usually increases with size and age[31]. However, the key environmental factor driving these preferences is still unknown. According to previous findings, temperature seems to play an important role, as it is positively correlated with the intake of plant matter by omnivorous fish[14,26,30,32]. This trend has also been observed in other fish species[33]. It is often a consequence of changes in other environmental factors, such as plant availability[22] and the increased cellulolytic activity mediated by symbiotic microorganisms[10,30].

This study focuses on the food preferences of omnivorous fish using rudd as a model organism in two oligotrophic lakes exhibiting differences in both macrophyte occurrence and animal food availability. Our principal conclusions were drawn from mesocosm experiments testing rudd preferences for animal prey versus plant matter in the presence of different concentrations of food and under a range of temperature conditions (16, 20, 24 °C). The primary aim of the study was to determine if omnivorous fish readily shift to herbivory, or whether animal prey is always preferred. These results will enhance our understanding of the key factors causing the absence of ectothermic herbivores at higher latitudes. Additionally, recent climate change models predict rapid warming, particularly at higher latitudes. The results of this study can provide important insights into how many herbivorous fish species may disperse to new areas as water temperatures increase and fish species gain advantages in trophic competition by utilizing resources that are not available to other aquatic animals, including native species.

Results

An assessment of potential diet for rudd in studied lakes.
In both lakes, macrophytes and macroalgae (*Chara* and *Vaucheria*, henceforth referred to as macrophyte coverage for simplicity) occurred down to a depth of 12 m, but the abundance in the two locations differed noticeably. In Milada Lake in September, dense coverage by submerged macrophytes reached 91% at the primary rudd habitat depth of 0–3 m, with a mean wet mass of 2,720 g m^{-2} (Table 1). In May, the coverage was lower (60.1%), but macrophytes were still present with a mean mass of 1,275 g m^{-2}. The species occurring at this depth were as follows: *Potamogeton pectinatus* (September: 40%; May: 30%), *Myriophyllum spicatum* (20%; 12%), *Chara* sp. (19%; 11%), *Vaucheria* (11.5%; 7%), *Potamogeton trichoides* (0.2%; 0%), *Sparganium emersum* (0.15%; 0%), *Myriophyllum verticillatum* (0.05%; 0%), *Potamogeton crispus* (0.05%; 0.1%) and *Elodea canadensis* (0.04%; 0%). In Most Lake, there was only a sparse macrophyte coverage of 1.6% and 0.1% at a depth of 0–3 m, and a mean mass of only 51 g m^{-2} and 11 g m^{-2} in September and May, respectively. The species occurring at this depth were *Myriophylum spicatum* (September: 0.8%; May: 0.03%), *Potamogeton pectinatus* (0.3%; 0.04%), *Chara* sp. (0.2%; 0.03%), *Sparganium emersum* (0.2%; 0%) and *Potamogeton crispus* (0.1%; 0%). The shorelines of both lakes comprise stones covered by periphyton layers of similar densities, but an accurate biomass measurement was not taken.

Invertebrates living in the fine sediment, on the surface of the stones and macrophytes had a mean biomass 5.4 g m^{-2} and 4.2 g m^{-2} in Milada and Most Lakes, respectively. In both lakes, the following genera occurred in order of descending biomass: waterlouse (*Asellus aquaticus*), dragon fly larvae (Odonata), chironomid larvae (*Chironomus* spp.) and caddisfly larvae (Trichoptera). Additionally, zebra mussels (*Dreissena polymorpha*) occurred at high densities in both studied lakes and were the only potential rudd food source that was more abundant in Most Lake than in Milada Lake (Table 1). However, this food source was not found in rudd stomachs in either of the studied lakes (Table 2).

The mean density of zooplankton at 0–20 m depth was 36.3 ind. L^{-1} and 27.4 ind. L^{-1} in Milada and Most Lakes, respectively. Copepods (Copepoda) and large *Daphnia* were more abundant in Milada Lake, while small *Daphnia* were more abundant in Most Lake (Table 1).

Diet results revealed by Gut Content Analysis (GCA) and Stable Isotope Analysis (SIA).
GCA revealed that plant matter dominated the diet of rudd in September 2013 and 2014 (surface water temperature: 19.1–21 °C) in both lakes. For rudd older than one year in Milada Lake, 92.5% of the food consumed was plant matter in the form of macrophytes, and the rest was animal prey (Table 2). In Most Lake, plant matter also

Date	Lake	N	Proportion of given diet category in gut content, mean ± SD (%)						
			Macroph.	Periphyton	Detritus	Benthos	Zoopl.	*Dreissena*	A. insect
September	Milada	80	92.5 ± 22	0	0	7.5 ± 22	0	0	0
2013 and 14	Most	80	0	68 ± 34	25 ± 31	7 ± 13	0	0	0
May 2015	Milada	25	0	0	0	0	0	0	100
	Most	25	0	0	0	42 ± 41	13 ± 20	0	45 ± 40

Table 2. Mean proportion of diet categories (% ± standard deviation) in Milada and Most Lakes during September and May according to gut content analysis. The size range of rudd was 98–430 mm (TL; >1 year old). N = number of dissected individuals, Zoopl. = zooplankton and A. insect = aerial stage of aquatic insect.
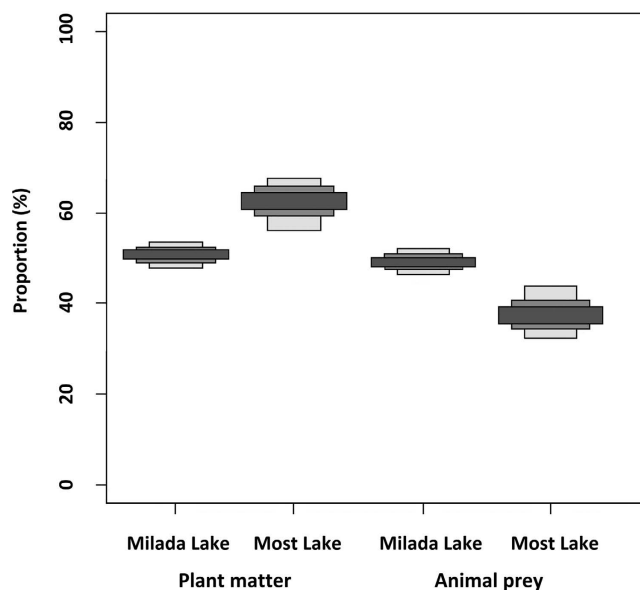

Figure 1. Probability proportion of plant matter and animal prey in assimilated diet of rudd in Milada and Most Lakes according to stable isotope analysis. Plant matter is presented by the categories macrophytes (Milada Lake), periphyton and detritus (both Most Lake). Animal prey is presented by the categories zooplankton, benthos, and zebra mussel (*Dreissena polymorpha*). The credibility intervals are 95, 75 and 25%. (see Supplementary Fig. S3 for SIA Biplots).

dominated but consisted of periphyton (68%) and detritus (25%). Animal prey accounted for 7% of the gut contents. In September in Milada Lake, juvenile rudd were strict herbivores consuming macrophytes, whereas in Most Lake they were strict zooplanktivores. In contrast to the warmer month of September, in May 2015 (surface water temperature: 13.1–14.2 °C), only animal prey was found in rudd digestive tracts. In Milada Lake, diet consisted solely of the aerial stage of aquatic insects. In Most Lake, it consisted of the aerial stage of aquatic insects, benthic invertebrates and zooplankton (Table 2).

The results of SIA indicated a lower proportion of plant matter in rudd diet in September than was suggested by GCA (Table 2 and Fig. 1). The SIAR stable isotope mixing model showed 51% and 62% plant matter consumed by rudd in Milada and Most Lakes, respectively. In contrast, SIA showed a higher percentage of animal prey consumption (Milada: 49%, Most: 38%) than the GCA (7.5%, 7%) (Table 2 and Fig. 1). No clear trend was observed between fish size and $\delta^{15}N$ or fish size and the proportion of plant matter in gut contents (Supplementary Figs S1 and S2). Additionally, there were no statistically significant differences in $\delta^{13}C$ and $\delta^{15}N$ between sampling years 2013 and 2014 (see Supplementary Table S1, and Fig. S3 for SIA biplots).

Results of feeding experiments. Under experimental conditions reflecting the natural conditions in Milada Lake (an animal prey to plant matter ratio of 1:400), plant matter dominated rudd diets at 24 °C (99.8%) and 20 °C (88.8%). In contrast, plant matter constituted an average of only 1.6% of gut contents at 16 °C (Fig. 2 and Table 3). The percentage of plant matter at 24 °C and 20 °C decreased with changing diet ratios. In the presence of a ratio of animal prey to plant matter of 1:10, plant matter comprised 52.5% and 44.5% of the gut contents, respectively. At 16 °C and a ratio of 1:10, the proportion of plant matter remained very low (1.4%). The final experiment used a ratio of animal prey to plant matter of 1:1, which differed the most from natural conditions of all the treatments. Under these conditions, the percentages of plant matter at 24 °C and 20 °C decreased to 31.9% and 15.1%, respectively. At 16 °C and a 1:1 ratio, the proportion of plant matter was only 1.1% (Table 3 and Fig. 2).

Both temperature and the ratio of available food sources significantly affected the diet preferences of rudd. The impact of temperature was greater ($F_{2,15} = 10.35$, $p < 0.01$) than the impact of diet ratio ($F_{2,15} = 2.67$, $p < 0.10$). The
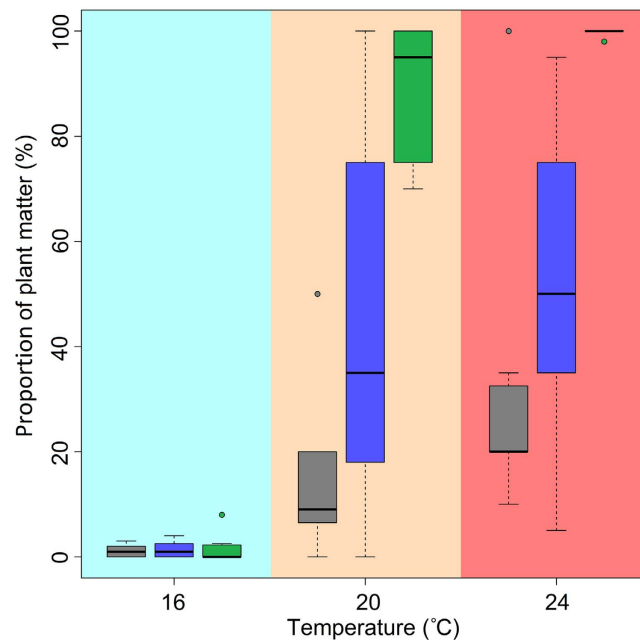
Figure 2. Proportion of plant matter in gut content of experimental rudd in given temperatures and three different diet ratios. The diet ratios of animal prey vs. plant matter were 1:1 (grey), 1:10 (blue) and 1:400 (green). Box and whiskers plots: upper and lower quartiles (boxes), median values (line inside the boxes), maximum and minimum values (whiskers), and outliers (circles) are shown.

T, Time	Repetition no.	Proportion of plant matter in given diet ratio (%)				
		1:400	1:10	1:1	0:1	1:0
13 °C, 168 h	1	—	—	—	100 ± 0	—
13 °C, 168 h	2	—	—	—	100 ± 0	—
16 °C, 24 h	1	2 ± 4	1.3 ± 1.5	1.3 ± 1.5	Empty	—
16 °C, 24 h	2	1.1 ± 1.3	1.5 ± 1.9	1 ± 1.2	Empty	—
20 °C, 24 h	1	87.5 ± 2	42.5 ± 41.9	14 ± 6.9	—	—
20 °C, 24 h	2	90 ± 14.1	46.5 ± 36.5	16.3 ± 22.8	—	—
24 °C, 24 h	1	100 ± 0	36.3 ± 21.4	23.8 ± 7.5	—	0 ± 0
24 °C, 24 h	2	99.5 ± 1	68.8 ± 27.8	40 ± 40.8	—	0 ± 0

Table 3. Mean proportion of plant matter in gut content of experimental rudd (% ± standard deviation) for each experiment in given diet ratios (animal prey vs. plant matter) and temperatures. "Empty" denotes situations when fish were found with no gut contents, whereas 0% of plant matter indicates presence of animal prey.

proportion of plant matter in rudd diet decreased with decreasing temperature. The interaction of temperature and diet ratio also had a statistically significant impact ($F_{8,8} = 31.11$, $p < 0.001$).

At 16 °C, all studied rudd exhibited very similar feeding behaviour regardless of diet ratio, and the percentage of plant matter consumed was consistently very low (an amount greater than 4% was found in only one individual). At 20 °C, food consumption was more variable, and we found individuals with digestive tract contents ranging from 100% plant matter to 100% animal prey. At 24 °C, the variability was lower than at 20 °C, with the highest variability found when using a 1:10 diet ratio (Table 3 and Fig. 2).

In the additional experiment with only animal prey available as a food source at 24 °C, all eight individuals had digestive tracts full of animal prey (100%). In contrast, in the trial with only plant matter available as a food source and a temperature of 16 °C, the digestive tracts of all ten individuals were entirely empty (even when using the spare individuals, see Methods). In the final additional experiment with only plant matter as a food source at 13 °C for 168 h (*i.e.,* assuming major starvation), all eight individuals utilized plant matter (100%; Table 3) and the specimens' entire digestive tracts were evenly filled with the plant material.

A slight difference in mass was observed before and after experiments for all rudd individuals. In most cases (except for the two trials discussed below), the slight mass increases were caused by the filling of the digestive tract. There was virtually no difference observed after subtracting the mass of gut contents. In the experiments with both animal prey and plant matter, the mean difference ± SD was 0.35 g ± 0.24 and was statistically insignificant for all experiments ($p > 0.1$). In the case of experiments with only plant matter available at 16 °C, a slight decrease in mass was observed (mean ± SD: 0.06 g ± 0.05) but it was also statistically insignificant ($F_{1,10} = 1.13$, $p > 0.1$). A greater decrease in mass was observed in the experiment with only plant matter at 13 °C. In this trial,

the mean decrease was 0.75 g ± 0.32, which corresponds to a mean mass decrease of 2.7% of total mass and was statistically significant ($F_{1,8} = 5.4$, $p = 0.02$).

Discussion

Based on the gut contents of rudd in both lakes in September (surface water temperature: 19.1–21 °C), rudd show a tendency towards herbivory during summer when a sufficient amount of macrophyte plant material is available. The results for Milada Lake demonstrate that this is also true for juvenile rudd, which contradicts the work by Nurminen *et al.*[34], who observed that rudd did not utilize a plant diet during their first year. However, if the only plant matter available is periphyton and/or detritus juvenile rudd ignore it and utilize zooplankton, as shown by the results for Most Lake. In contrast, older rudd consume periphyton and detritus extensively in the absence of macrophytes. When a sufficient number of macrophytes are present, periphyton and detritus are ignored; this was demonstrated by the results for Milada Lake.

Gut content analyses conducted in May (surface water temperature: 13.1–14.2 °C) show that the rudd completely disregarded plant matter even though macrophytes and periphyton were present. The observed tendency towards switching between food sources is consistent with other studies on rudd. Based on our results, temperatures approximately 20 °C seem to be a crucial threshold for transitioning to herbivory[26,30,31]. This trend is not only valid in our chosen model organism but can also be observed in other species. For example, a study focused on a rudderfish (*Girella nigricans*) showed that they exhibited the highest RNA:DNA ratio (the most intensive growth in a short-term period) when they utilized plant matter at temperatures above 22 °C. Whereas at 17 °C, a low RNA:DNA ratio indicated that fish were experiencing stress while utilizing plant matter[14]. According to the SIA conducted for rudd in September, the percentage of plant matter consumption was lower than that shown by GCA. This difference is likely caused by isotope turnover in fish tissue[35]. The tissue of rudd still contained an isotopic signal from food assimilated during colder periods, when animal prey was preferred. Nevertheless, we cannot exclude the possibility that the isotopic signal was enriched by the assimilation of bacteria responsible for cellulose digestion, rather than by the plants themselves[36].

The experimental portion of our study provided support for the hypotheses that both temperature and availability of food play important roles in the food preferences of rudd. Rudd were almost entirely herbivorous in conditions similar to those of Milada Lake, represented by trials with the highest ratio of plant matter to animal prey and temperatures of 20 °C and 24 °C. Decreasing plant matter and increasing animal prey led to a decrease in observed herbivory. This trend was more apparent at 20 °C than at 24 °C. Thus, plant matter seems to be more readily utilized in warm water when it is readily available.

The critical point for transition to plant utilization was observed at 16 °C. Rudd essentially consumed only animal prey at this temperature in all three experimental concentrations of animal prey and plant matter. Interestingly, in the experiment at 16 °C which provided only plant matter, the rudd preferred to consume no food rather than to consume only plant matter. It should be emphasized that this occurred after a period of starvation lasting four days. Such a strategy has been observed among carnivorous fish but is not common for omnivorous or herbivorous species[22].

These findings indicate that low temperature is the key factor driving the elimination of plant matter from the diet of rudd. This effect does not only apply to fish but is also observed in invertebrate omnivores such as copepods[37] and snails (*Lymnaea stagnalis*)[38]. Preference for plant matter decreases rapidly when the temperature falls below 20 °C. Plant matter is completely ignored when the temperature falls to 16 °C, which likely explains why Dorenbosch and Bakker[9] noted low preference for plant matter among rudd and grass carp (*Ctenopharyngodon idella*). The strong preference for animal prey observed by Dorenbosch and Bakker[15] was likely influenced by the low temperature in their experiment (18 °C), and by the ratio of available animal prey to plant matter (2.9:1). These results are consistent with our findings that rudd in both lakes utilized only animal prey in the spring, when the surface water temperature ranged from 13.1 to 14.2 °C. Piscivory was even observed in rudd during the spring, in low temperatures in the Niagara River[26].

In extreme cases, fish are likely capable of gaining a minimal amount of energy from plant sources even in cold water, as shown in when rudd started to utilize plant matter at 13 °C after 168 h of starvation in one of our experiments. This suggests that fish are able to ingest plant matter at low temperatures, but this behaviour is not sustainable in the long-term. The mean decrease in mass of rudd individuals in our experiment was 2.7%, supporting the theory of Behrens and Lafferty[14] that fish begin to experience stress at temperatures below 17 °C when consuming plant matter. However, exceptions were discovered in Antarctica, where extreme herbivory is found in four species of notothenioid fishes (Notothenioidei), which utilize algae[19], and references therein.

To better understand the observed trends related to temperature, it is necessary to understand how fish digest plant matter. Fish lack specific organs for plant matter digestion, such as the specialized stomachs found in herbivorous mammals and some birds[10,39]. Therefore, to use cellulose as a nutrient source they require the enzyme cellulase. B-1,4 glycosidic bonds must be cleaved to release glucose units[40]. For this process, fish depend on the production of enzymes by symbiotic microorganisms living in their digestive tracts[10,11]. The microorganisms enter the digestive tract via consumed food, primarily through detritus[10,31,41]. The cellulase levels in the digestive tracts of rudd are typically highest in late summer and lowest in early spring[10]. This is known from the work by Saha *et al.*[42], who isolated a series of *Bacillus circulans* and *B. megaterium* from grass carp and tilapia (*Oreochromis mossambica*). A characterization of the isolated microorganisms revealed that they could grow in a wide range of pH levels (5–11) and temperatures (15–42 °C). Similarly, a wide temperature range was found to be suitable for microorganisms with cellulase isolated from the cyprinid *Labeo rohita*[43]. Therefore, the cellulase produced by microorganisms capable of living in the digestive tracts of ectotherms can function at lower temperatures in comparison to other water organisms, where optimum temperatures for cleaving complex carbohydrates range from 30–60 °C[44–46]. Although the lower limit for cellulose functioning in microorganisms is 15 °C, herbivory by fish still seems to be unbeneficial from 16–19 °C, (based on our observation[26,30,32]), probably due to low cellulase

activity. Therefore, the precise temperature at which plant diet starts to be ignored by fish will vary by species and individual.

However, a temperature of approximately 15 °C is still relatively high for an aquatic ecosystem. At our study site (Milada Lake: 50.4°N), the surface water temperature is warmer than 15 °C for only 149 days (41%) each year on average (Palivový kombinát Ústí s.p., unpubl. data). The period of effective cellulose digestion is dramatically shortened with increasing latitude. This is likely the reason that species richness in omnivorous fish decreases dramatically above 55°, and why they are absent above 60°[16]. This trend is stronger in freshwater than in marine ecosystems, probably due to ocean currents that influence water temperature[16]. Warm currents can increase the temperature at higher latitudes, and the opposite can occur at lower latitudes. In contrast to marine conditions, in freshwater the temperature is more strictly correlated with latitude. The trend of decreases in the number of species utilizing plant matter at higher latitudes is also stronger for fish than for terrestrial ectotherms, such as reptiles[16,47]. This is likely due to differences in their habitats. In cold climates, reptiles have solved the problem of plant digestion by reducing their size. A small body can be more quickly warmed by air, especially with a warmed under layer that enables cellulase activity. This strategy is not viable in water ecosystems, which have a high specific thermal capacity.

Although these trends in fish herbivory are slightly less apparent in marine than in freshwater ecosystems, the poleward movement of herbivorous species in marine ecosystems due to climate change has already had an apparent negative impact. For example, losses of kelp forests caused by increases in herbivorous consumers have been observed[24,25,48,49]. The spread of herbivorous species in freshwater ecosystems has likely not been extensively studied to date. Nevertheless, we can assume that these changes are occurring. The impact of non-native fish species on macrophytes is evident as observed in studies regarding the introduction of rudd in New Zealand and North America[27,28]. We can also presume that fish popular for use in aquaculture, such as *Tilapia* spp. or grass carp[9,50], are likely to spread by human activity and will have negative impacts on macrophytes.

In conclusion, temperature is the cause of fish developing strict herbivorous specializations only in tropical regions, except for a few extreme cases as mentioned above. At higher latitudes, omnivorous fish only utilize plant matter[13,14], which is restricted by temperatures lower than 15 °C. Herbivorous fish need a regular intake of food; therefore an annual decrease in water temperature below 15 °C, even for a short time period, prevents fish speciation and herbivorous specialization at higher latitudes. Climate change is expected to have stronger impacts on water temperatures at higher latitudes, allowing the dispersion of herbivorous feeding behaviours by fish. This can benefit many omnivorous fish species and greatly shape the future of fish communities in lakes of higher latitude regions.

Methods

Study site.
The study was conducted in two newly created opencast mine lakes, Milada Lake (50°39′N, 13°58′E) and Most Lake (50°32′N, 13°32′E), in the Czech Republic (Fig. 3). The oligotrophic to mesotrophic Milada Lake has a mean summer total phosphorus (TP) in the surface layer of $<10\,\mu g\,L^{-1}$, and is located 80 km northwest of Prague (Fig. 3b). It has an area of 250 ha, a volume of 0.036 km³ and a maximum depth of 25 m. Aquatic restoration started in 2001 and was finished in 2011. Several species of macrophytes and algae are present at high levels of biomass to a depth of 12 m[51]. The biomass of rudd was 3.58 kg ha⁻¹ in 2013, and 2.50 kg ha⁻¹ in 2014. Other fishes present are perch (*Perca fluviatilis*), roach, ruffe (*Gymnocephalus cernua*), pike (*Esox lucius*), European catfish (*Silurus glanis*), tench (*Tinca tinca*) and pikeperch (*Sander lucioperca*). The oligotrophic Most Lake (TP $< 5\,\mu g\,L^{-1}$) is 75 km northwest of Prague. It has an area of 310 ha, a volume of 0.07 km³ and a maximum depth of 75 m. Aquatic restoration started in 2008 and was finished in 2014. Macrophytes were not abundant in 2014. The biomass of rudd was 1.9 kg ha⁻¹ in 2013 and 0.6 kg ha⁻¹ in 2014. Other fishes present are perch, roach, ruffe, pike, European catfish, tench and maraena whitefish (*Coregonus maraena*).

Water temperature and dissolved oxygen were measured using a calibrated YSI 556 MPS probe (YSI Incorporated - Yellow Springs, Ohio, USA) in both lakes, three times each during sampling in September 2014 and May 2015.

Collection of plant and invertebrate samples.
To obtain a qualitative assessment of macrophytes, two SCUBA divers visually assessed their occurrence at ten transects. Transects were marked from the shore to a depth of 12 m in both lakes in September 2013, September 2014 and May 2015 (Fig. 3b), using measuring tapes. The coverage of each macrophyte species, the uncovered bottom area, the percentage composition of each species, and the percentage of uncovered bottom area were measured at 1 m depth intervals. The results of duplicate measurements were averaged for a more accurate assessment. To obtain a quantitative assessment of macrophytes, SCUBA divers staked a quadrangle (50 × 50 cm) at six locations at a depth of 0–3 m (Fig. 3b) with 100% macrophyte coverage. All macrophytes and macroalgae were removed from the area, put into a sack, raised to the boat and weighed after being allowed to drain for 10 minutes. *Potamogeton* spp., *Chara* sp., *Vaucheria* and *Elodea canadensis* were determined to be potentially palatable to rudd, according to past studies[9,15,30] and our observations. *Myriophyllum* sp. was classified as an unpalatable species and was not included as a potential food source for rudd. The mean coverage of palatable species per 1 m² at a depth of 0–3 m (where most rudd were found by SCUBA divers and in gillnet catches) was estimated using both quantitative and qualitative information. Samples of palatable macrophytes were collected and frozen for stable isotope analysis. To obtain information about periphyton, the stony shoreline was assessed visually from the boat using polarized glasses.

Sampling was conducted for benthic invertebrates (animal prey), at a depth of 0–3 m in both lakes in September 2014. SCUBA divers sampled six locations evenly distributed along the lake; these were identical to the locations for quantitative assessment of macrophytes (Fig. 3b). At each location a plastic corer (length 50 cm, diameter 8 cm) was used to collect two samples of invertebrates living in the fine sediment in three depth zones (0.5, 1.5 and 3 m). All six subsamples were then combined to give one composite sample per locality. For
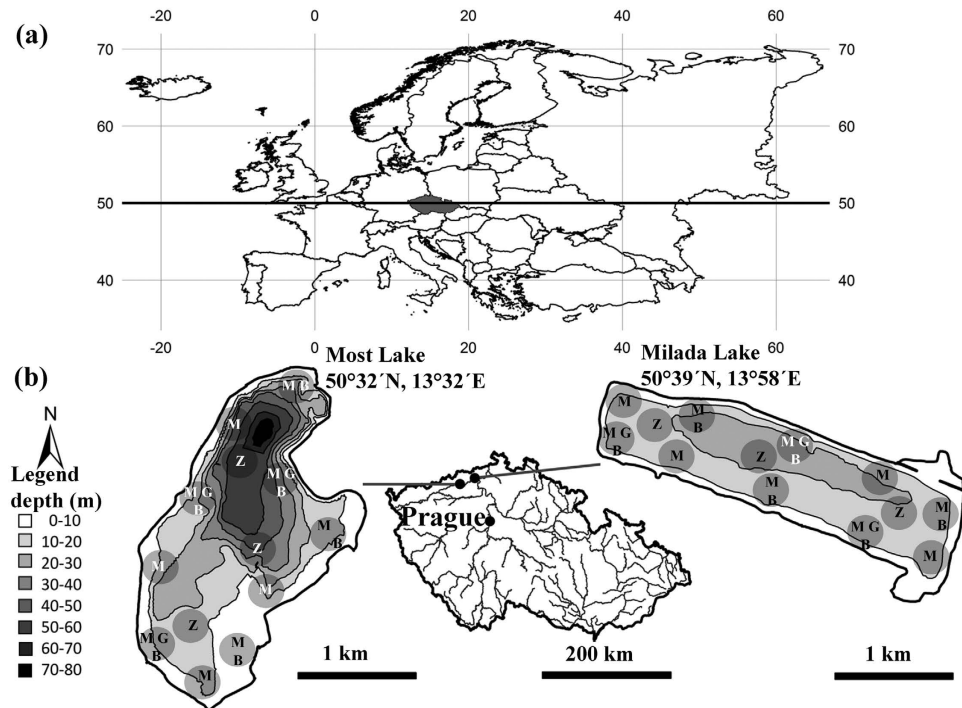
Figure 3. A map showing (**a**) the location of the Czech Republic within Europe (latitude of 50° north as bold line) and (**b**) the location of the two study sites, Milada and Most Lakes, in the Czech Republic. A detailed view of the bathymetric maps shows contour lines with relevant depths and sampling localities: B = benthic invertebrates sampling; G = gillnets sampling; M = macrophytes sampling; Z = zooplankton sampling. The figure was generated by the software ArcMap, version 10.2.2[59].

invertebrates living on the stones, a quadrangle (50 × 50 cm) was staked out, and all macroscopic invertebrates (most of which were waterlouse or zebra mussel specimens) were collected using forceps. Next, all upper stones were raised from the staked area to the boat to collect any remaining individuals. Two subsamples were obtained and combined to give one sample per location. The bottom cover of fine sediment and stones was measured at each location to determine the percent cover of each type of bottom material. In Milada Lake, invertebrates living on macrophytes were also collected from the 50 × 50 cm staked area. All macrophytes were collected, put into a sack, raised to the boat and rinsed, and all invertebrates were collected using forceps. Two subsamples were obtained and combined to give one sample per location. The previously mentioned two (benthic invertebrates from fine sediment and stones; Most Lake) or three (benthic invertebrates from fine sediment, stones and macrophytes; Milada Lake) samples were combined from each location. All invertebrates were counted, weighed and preserved in 4% formaldehyde for subsequent identification to the species level in the laboratory. To estimate mean biomass of invertebrates per 1 m^2 of the lake bottom, the mean biomass of invertebrates living in fine sediment and on stones and macrophytes was recalculated proportionally. Subsamples of benthic invertebrates occurring in the environment were collected and frozen for stable isotope analysis.

In both lakes, zooplankton was sampled in triplicate with a plankton net (diameter: 24 cm, mesh size: 100 μm) at three locations by a vertical haul at 0–20 m in September 2014 (Fig. 3). The samples were fixed with 4% formaldehyde. In the laboratory, the organisms were identified to order or genus and counted under the microscope (Olympus CX40, 100 magnifications) using a Sedgewick-Rafter chamber. *Daphnia*, the taxa most vulnerable to fish predation, were divided into the following two groups: small (<700 μm carapace length) and large (≥700 μm). The reported density of zooplankton is the mean of all nine samples. Zooplankton samples were collected and frozen for stable isotope analysis in March, May, July, September and November 2014.

Fish sampling. All animal handling (including fish sampling, GCA, SIA and the mesocosm experiment) was performed according to the guidelines of and with permission from the Experimental Animal Welfare Commission at the Ministry of Agriculture of the Czech Republic (Ref. No. CZ 01679). The Experimental Animal Welfare Commission approved all experimental protocols.

In both lakes, fish were sampled for GCA and SIA using benthic multi-mesh gillnets following the European standard[52]. Gillnets were set overnight; they were placed in the water 2 h before sunset and lifted 2 h after sunrise. Sampling was conducted three times each at depths of 0–3, 3–6 and 6–9 m at three locations. Altogether, 72 gillnets were set in both lakes and both in 2013 and 2014. All captured rudd were anaesthetised using a lethal dose of tricaine methanesulfonate (MS–222, Sigma Aldrich Co.). Rudd for the experiment were also sampled by electrofishing (600 W-pulsed DC current) in Milada Lake in September 2015.

Diet ratio	Mass ratio (g)	Duration (h)	Date of the experiment in 2015			
			T = 13 °C	T = 6 °C	T = 20 °C	T = 24 °C
1:400*	4.2:1,666	24	—	Sept. 14	Sept. 14	Sept. 14
1:10	152:1,518	24	—	Sept. 15	Sept. 15	Sept. 15
1:1	835:835	24	—	Sept. 16	Sept. 16	Sept. 16
1:0	1,670:0	24	—	—	—	Sept. 17
0:1	0:1,670	24	—	Sept. 17	—	—
0:1	0:1,670	168	Sept. 18–24	—	—	—

Table 4. Summary of conducted experiments, the diet ratios used (animal prey vs. plant matter) and mass ratios counted, temperatures, duration and dates of the experiments. The experiments were conducted always in two repetitions. Note that the total usable diet mass was ca. 1,670 g per tank in all experiments. * real lake diet ratio, Sept. means September.

In September 2013 and 2014, 80 rudd older than one year and 30 juveniles were used for GCA. In May 2015, 25 individuals older than one year were used; in all cases, the samples were from both lakes. The analysis was conducted on the same day as sampling. The mean sizes of the adult rudd used for GCA were 281.7 mm ± 92.4 (SD) and 191.3 mm ± 38.9 for Milada and Most Lakes, respectively. The mean mass measurements were 100.8 g ± .56.5 and 515.9 g ± .477.4, respectively. The mean sizes of the juvenile rudd were 65 mm ± 2.6 and 63.8 mm ± 2.4, and the mean mass measurements were 1.8 g ± 0.4 and 1.2 g ± 0.5 for Milada and Most Lakes, respectively. Fish scales were collected and used to age the fish. After collection, 76 rudd from Milada Lake and 56 rudd from Most Lake in September 2013 and 2014 were frozen to preserve them for SIA. The mean sizes of the rudd used for SIA were 271.4 mm ± 98.6 and 184.8 mm ± 47.2 for Milada and Most, respectively, and the mean mass measurements were 476.3 g ± 467.9 and 94.6 g ± 66 for Milada and Most, respectively.

GCA. For rudd obtained from the experiment and the lakes, gut contents were identified under a dissecting microscope and the percent composition of their diet by volume was visually estimated. The diet of experimental rudd was categorized into two groups based on what they were offered during the experiment (plant matter or animal prey). The diet of rudd obtained from the lakes was categorized into seven functional groups (macrophytes, periphyton, detritus, benthos, zooplankton, *Dreissena* and the aerial stage of aquatic insects). For the experimental individuals, the gut contents (wet mass) were also weighed (Kern CKE 3600–2, accuracy = 0.01 g) to determine wet mass.

SIA of fish and potential food sources. Dorsal muscle tissue was dissected from individual fish from both lakes and cleaned of scales and skin. All samples (fish and diet samples) were washed with distilled water. The samples were dried at 60 °C for 48 h and ground into a homogenous powder using a ball-mill Retsch MM 200 (Retsch GmbH, Haan, Germany). Small subsamples (0.520–0.770 mg) were weighed in tin cups for the analysis of δ^{13}C and δ^{15}N. The isotope analyses were performed using a Thermo Finnigan DELTA^{plus} Advantage continuous flow stable isotope-ratio mass spectrometer, connected to a Carlo Erba Flash EA1112 elemental analyser (Thermo Electron Corporation, Waltham, MA, USA) at the University of Jyväskylä, Finland. Measurements of δ^{13}C and δ^{15}N were reported in per mil (‰) notation, using internal standards with a known relationship to the international standards of Vienna Pee Dee belemnite (for carbon) and atmospheric N_2 (for nitrogen). Sample analyses also yielded the elemental composition of carbon and nitrogen (by mass). Because the C:N ratios were consistently lower than 3.5, isotope values were not lipid corrected[53].

Feeding experiments. From September 14 to 24, 2015, 12 different experiments were conducted and duplicated on the shore of Milada Lake, using different diet ratios and water temperatures. Each experiment lasted 24 h, except for one that lasted 168 h (Table 4). Fibreglass tanks were placed under a tent pavilion to prevent contact with rain and sunlight, and were filled with water from Milada Lake filtered through a sieve (mesh size 170 μm) (tank diameter = 1 m, surface of tank bottom = 0.785 m², total height of the tank = 0.8 m, height of water column = 0.64 m and total water volume in the tank = 502 L). During the experiments, water temperatures were measured at 4 h intervals, and the temperature was maintained with a maximum deviation of ± 0.6 °C. Oxygen volume was also measured at the same time intervals (ranging from 11.7 to 6.8 mg L^{-1} with the highest and lowest values at 13 °C and 24 °C, respectively). Water was heated or cooled to the required temperature using aquarium heaters (Eheim Jäger 300 W) and freezer packs containing refrigerant gel.

Diet ratios for the experiments were selected according to the occurrence of palatable plants and animal prey in the lake (see above and Table 1) estimated to the tank surface area. The estimated available ratio (by wet mass) of animal prey to plant matter in Milada Lake was 1:395.2, presented as 1:400 for simplification. Then, diet masses corresponding with each of the required ratios (1:10, 1:1, 1:0, 0:1) were calculated for each experiment (Table 4). *Potamogeton pectinatus* from Milada Lake and *Chironomus* sp. from cultivation were placed in the required amounts on the tank bottom. Both types of diet have been shown to be readily utilized by rudd. Prior to placement in the tank, macrophytes were rinsed properly to remove invertebrates, and the required mass was weighed after being allowed to drain for 10 minutes. Live *Chironomus* sp. were placed in shallow plastic buckets (25 × 25x 10 cm) to prevent them from hiding.

Three days prior to the experiment, the rudd were caught from Milada Lake by electrofishing. The rudd were starved for one day in cage nets in the lake, and further acclimatized for two more days at the selected

temperatures in extra tanks without food. The rudd individuals were measured, weighed and placed into the experimental tanks 4–6 h after the appropriate diet was added to the tanks. Five rudd individuals were used for each experiment (120 fish in total). After the experiment, the rudd were anaesthetised using a lethal dose of MS–222, weighed, and dissected for gut contents, which were also weighed to acquire a measurement of wet mass. Four individuals were analysed and one was kept as a spare for cases where individuals were empty. The mean size of rudd used for gut content analysis in the feeding experiments was 137.3 mm ± 17.6 (SD), and the mean mass was 29.4 g ± 9.3 (SD).

Statistical analysis.   The effects of temperature and diet ratio on rudd dietary preferences in our experiments were tested using a general linear model and a split-plot design. The observed percentage of plant diet of each fish was tested as nested in the tank variable, which was set as a random factor. The arcsine square root transformation was used to improve non-normal distribution of percentage data. One-way ANOVA was used to test for differences in fish mass before and after the experiment. Dependency between fish size and $\delta 15\,N$, and between fish size and proportion of plant matter in gut content was tested by a linear regression analysis. A nonparametric Kruskal–Wallis test (Statistica 12; Stat-Soft Inc., Tulsa, OK) was used to test for differences in $\delta^{13}C$ and $\delta^{15}N$ between years 2013 and 2014. The freeware package SIAR 4.0 for linear mixing models was used to determine the ratio of potential food sources to assimilated diet in both lakes[54,55]. Means ± SD of $\delta^{13}C$ and $\delta^{15}N$ from samples were entered into the model. Concentration dependence was included in the form of proportions of C and N in food sources[56] because these concentrations differ markedly between plant and animal samples (*cf.* Dataset). For carbon, we used a trophic fractionation factor of 0.4[57]. For nitrogen, we used 3.4 and 4.8 for animal prey and plant matter, respectively[57,58].

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Author Contributions

I.V., L.V., M.Č., M.V., Z.S., S.H.T.C., M.Š., P.B. and J.F. participated in the field work. I.V., L.V. and J.P. designed the study. I.V., J.S. and M.K. did the stable isotope analyses and SIAR model. I.V., L.V. and P.B. conducted the statistical analysis. I.V. and L.V. wrote the manuscript. All authors contributed substantial comments during manuscript preparation. The authors declare no conflict of interest.

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Paper II

Impact of herbivory and competition on lake ecosystem structure:

underwater experimental manipulation

SCIENTIFIC REP{O}RTS

Impact of herbivory and competition on lake ecosystem structure: underwater experimental manipulation

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Two basic ecological relationships, herbivory and competition, distinctively influence terrestrial ecosystem characteristics, such as plant cover, species richness and species composition. We conducted a cage experiment under natural conditions in an aquatic ecosystem to test the impacts of two treatments combined in a factorial manner: (i) a pulse treatment – removal of dominant competitors among primary producers (macroalgae *Chara* sp. and *Vaucheria* sp.), and (ii) a press treatment – preventing herbivore (fish, crayfish) access to caged plots. The plots were sampled once before the treatments were established and four more times within two years. Both treatments had a significantly positive impact on macrophyte cover and species richness and changed the macrophyte species composition. The effect of the macroalgae removal was immediate with the highest species richness occurrence during the first post-treatment monitoring, but the positive effect vanished with time. In contrast, preventing herbivore access had a gradual but long-lasting effect and reached a more steady-state over time. Two of the most common species showed contrasting responses, the palatable *Potamogeton pectinatus* was most supported by caging, while the distasteful *Myriophyllum spicatum* preferred open plots. Our findings may be applicable during the revitalisation of aquatic ecosystems that aims to increase macrophyte biodiversity.

Among plants, herbivory and competition play a key role in the formation of the plant community[1–3]. Herbivorous microfauna, mainly insects, induce the evolution of chemical defences in plants[4], and coevolution has led to a relatively high number of foraging specialisations. Nevertheless, plant communities are also shaped by macrofaunal herbivores[2,5] that are usually less specialised and activate both the mechanical and the chemical defences of plants. Though the effect of herbivory on terrestrial plant communities has been well studied, less attention has been focused on the impact of herbivory on communities of aquatic – particularly freshwater – macrophytes. Frequently studied factors that affect plant communities include water chemistry, sediment composition and hydro-morphological parameters[6–9]. In terms of herbivory, the impact of microfauna (i.e., crustaceans, snails and aquatic insects) on algal periphyton has been the most frequently studied topic considered thus far[10]. Nevertheless, recent studies from marine ecosystems have revealed the significant impact of herbivorous fish on macroalgae and seagrasses[11–13]. The wide retreat of kelp forests in cold areas was initiated by the climate-mediated dispersal of herbivorous fish to these localities[14–16]. An increase in temperature greater than the critical degree for cellulase-activation introduced new niches for herbivorous fish species[17]. The distribution of herbivorous freshwater fish has also recently changed; however, this change was driven by man-mediated dispersion, in addition to the natural species responses to climate changes[18–21].

Fish and crayfish may reduce the biomass and biodiversity of macrophytes by direct consumption as well as by indirect changes in the environment[22]. The rate of herbivory affects plant growth patterns and influences the trade-off between active growth and defence[1]. Plants face a trade-off between investments into the fast growth

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	DF	Macrophyte cover		Species richness	
		F	p	F	p
Removal	1	7.47	**0.01284**	4.07	0.05731
Caged	1	5.88	**0.02492**	0.68	0.41826
Removal*Caged	1	0.39	0.53730	0.01	0.92943
Error	20				
Time	4	40.12	**$<10^{-6}$**	32.69	**$<10^{-6}$**
Time*Removal	4	11.42	**$<10^{-6}$**	7.37	**0.00004**
Time*Caged	4	4.52	**0.00243**	3.06	**0.02110**
Time*Rem*Caged	4	1.19	0.32310	1.16	0.33281
Error	80				

Table 1. Result of repeated measures ANOVA for macrophyte cover and species richness in time. (Time is the repeated measure factor). DF – degrees of freedom, F – value of F statistics, p – p values. Significant effects ($p < 0.05$) are shown in bold.

that is necessary for success in terms of competition and into the activation of chemical defences against herbivores. This has long been well-documented in terrestrial ecosystems; however, similar findings from aquatic ecosystems have occurred relatively recently[20,23]. Observations and experiments have shown that the consumption rate of macrophytes that have a chemical defence (e.g., *Myriophyllum* sp.), is much lower than those of palatable macrophytes (e.g., *Potamogeton pectinatus*) and macroalgae (e.g., *Chara* sp.)[18,24–26]. The secondary metabolites produced by macrophytes (i.e., alkaloids, glucosinolates and polyphenolics) have an apparent impact on the feeding preferences of aquatic herbivores[27]. Nevertheless, this impact is not as well established as that observed in terrestrial plants[27,28]. In some terrestrial ecosystems, insects play an important role in herbivory, and herbivory is performed mainly by specialists[29]. However, other terrestrial ecosystems are also strongly shaped by vertebrate grazing, which is not very selective[2,5]. In aquatic ecosystems, herbivory is often performed by omnivorous species. Thus, the efficiency of macrophyte chemical defences may be lower than that of terrestrial plants[30,31]. The long-term monitoring of rudd (*Scardinius erythrophthalmus*) provided evidence about its effect on the formation of the macrophyte community[32]. A study on the plant-herbivore interactions using rudd and North American macrophytes in experimental conditions presented similar results[14]. However, another study observed no significant impact of rudd on macrophyte biomass when compared to grass carp (*Ctenopharyngodon idella*)[33].

Disturbances that cause the partial or total removal of plant cover initiate succession[34,35] and can result in an increase in species diversity[36]. The entire successional dynamic results from well-known trade-offs and constraints[37,38], with the competition-colonisation trade-off[36,39] being one of the most important. In terrestrial ecosystems, ruderal species dominate during the initial succession stages due to their high fecundity and ability to spread. In contrast, the best competitors are disadvantaged and cannot reach reproductive maturity due to the recurrence of disturbances; additionally, their diaspore production and dispersibility are usually low. The best competitors dominate in ecosystems where disturbances have been absent for a long time[40,41]. Though these patterns have been well-documented in terrestrial systems, it is not clear how these processes operate in aquatic ecosystems that are often insular and, thus, affected by dispersal limitations[42,43].

It is difficult to determine the real impact of herbivory and competition on macrophyte communities in lakes. Field observations may demonstrate changes in biodiversity[44,45] as well as in feeding preferences of herbivores[32,46]. However, without manipulation, it is impossible to disentangle the effect of individual factors.

We aimed to directly test the effect of competition among aquatic macrophytes and the effect of herbivory by manipulating both in the lake ecosystem. We utilised a cage experiment as a promising compromise to study the direct impacts of two ecological characteristics on macrophytes under natural conditions[19]. Competition was controlled by a "pulse treatment" (i.e., a treatment conducted only at the beginning[47]) including removal of the macroalgae. In addition, herbivory was controlled by a "press treatment" (i.e., a long-lasting treatment[48]) including no herbivore access during the experiment. We aimed to test the following hypotheses: (i) the decrease in competition caused by a pulse treatment will positively affect the macrophyte cover and species richness and will change the macrophyte species composition. Further, (ii) the decrease in herbivory caused by caging will also positively affect the macrophyte cover and species richness and will change the species composition. Finally, (iii) the effects of the two factors will be independent of each other.

Results

Regardless of the treatment, all plots underwent pronounced successional dynamics that was the same as the rest of the vegetation in the lake. This was marked by the significant effect of time in all the statistical analyses. Nevertheless, the successional dynamics always changed due to the effect of the experimental treatments.

Macrophyte cover. Hereafter, macrophyte cover refers to the cover of aquatic plants other than the manipulated dominant species of primary producers (i.e., macroalgae *Chara* sp. and *Vaucheria* sp.). Both treatments, i.e., macroalgae removal and prevention of herbivore access, had a significant positive effect on the macrophyte cover that changed over time; this included the significant main effects of both treatments and that of their interaction over time (Table 1). The increase in cover differed markedly in each treatment. Macroalgae removal resulted in an immediate significant increase in macrophyte cover during the first period (time 1; increase from 0% to 15%), followed by a gradual decrease to 1% over time (Fig. 1 and Table 2). The removal was successful, and the cover
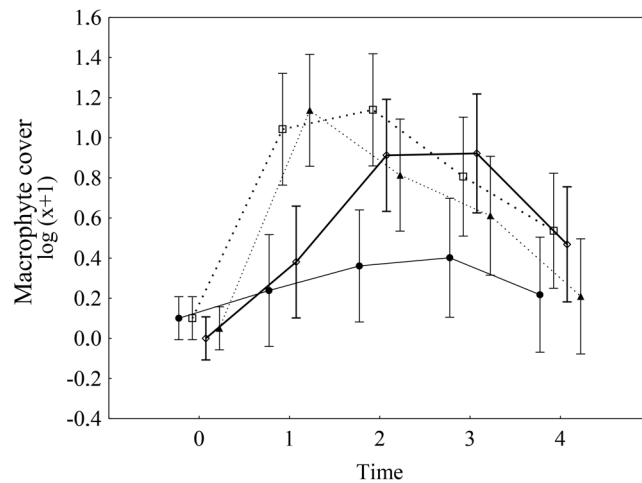
Figure 1. Impacts of two treatments (i.e., prevention of herbivore access and macroalgae removal) on macrophyte cover over time. Solid line = non-removal open plots (control), bold solid line = non-removal caged plots, dashed line = removal open plots, bold dashed line = removal caged plots. Data were log(x + 1)-transformed. The error bars are the 95% confidence intervals. The results of repeated measures ANOVA are in Table 1.

	Macrophyte cover		Species richness		Species composition	
	F	p	F	p	pseudo-F	p
Removal 0	0.23	0.640	0.23	0.640	0.2	0.852
Caged 0	0.23	0.640	0.23	0.640	0.2	0.845
Removal 1	**34.43**	**0.000**	**9.35**	**0.006**	**13.8**	**0.000**
Caged 1	0.03	0.859	0.02	0.898	1.0	0.391
Removal 2	**6.52**	**0.019**	**10.94**	**0.003**	**3.8**	**0.021**
Caged 2	**10.85**	**0.003**	3.26	0.085	**13.6**	**0.000**
Removal 3	0.11	0.747	1.48	0.238	0.3	0.859
Caged 3	**6.24**	**0.021**	0.20	0.659	**14.2**	**0.000**
Removal 4	0.05	0.828	1.83	0.191	<0.1	0.949
Caged 4	**4.62**	**0.043**	**7.73**	**0.011**	**15.7**	**0.000**

Table 2. Results of main effect ANOVAs (F statistics, DF = 1, 21 in all cases and corresponding p values) for the macrophyte cover and species richness in individual sampling dates, and test of significance of the effects on species composition in the RDA, pseudo-F values and significance obtained from the Monte Carlo permutation tests. Significant effects ($p < 0.05$) are shown in bold.

of dominant macroalgae decreased, on average, from 100% to 3% after the treatment; however, the cover gradually increased to 78% in time 4 (Fig. 2). Preventing the access of herbivores resulted in a slow gradual increase, from 0% to 11%, in macrophyte cover in time 3. The cover subsequently decreased to an average of 3% in time 4 (Fig. 1). The interaction of the two treatments was not statistically significant (neither itself, nor in the interaction with time); thus, the impacts of the two treatments on the macrophyte cover was additive on the log scale (Table 1). This might be interpreted as the two treatments having independent effects. In plots subjected to both caging and removal, the macrophyte cover increased in time 1, as it did in plots with only macroalgae removal treatment. In addition, a subsequent gradual decrease in macrophyte cover was observed, similar to the decrease seen in the cage-only plots (Fig. 1).

In the control plots, the changes were the least evident, and the macrophyte cover was the lowest (Fig. 1, Table 2). The explained variability in ANOVA showed that, macroalgae removal initially had a larger effect than did the prevention of herbivore access (Fig. 3a); however, the effects of macroalgae removal quickly faded. The decrease in the effect was apparently caused by the return of macroalgae to the experimental plots (Fig. 2). The prevention of herbivore access reached its maximum effect in time 2, and then the effect slowly decreased but remained significant until the end of the experiment. In contrast, the highest effect of the macroalgae removal was measured in time 1 and then decreased quickly (Fig. 3a).

Species richness. As with macrophyte cover, species richness was positively affected by both treatments (i.e., both had a statistically significant interaction with time), and their interaction was not significant, neither by itself, nor in its interaction with time (Table 1, Fig. 4). In addition, similar to macrophyte cover, the
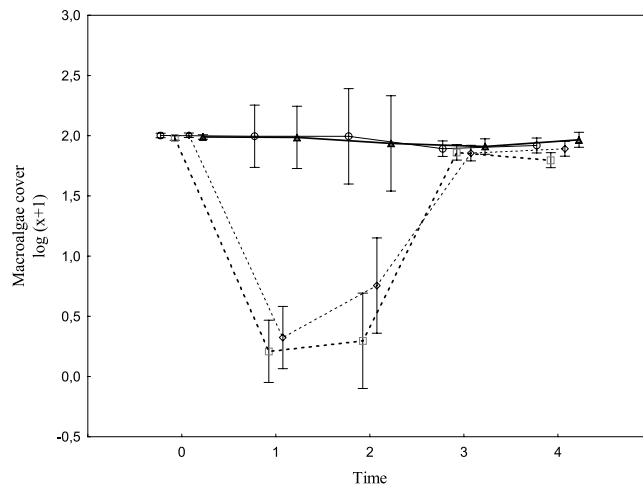
Figure 2. Macroalgae cover in the experimental plots over time. Solid line = non-removal open plots (control), bold solid line = non-removal caged plots, dashed line = removal open plots, bold dashed line = removal caged plots. The error bars are the 95% confidence intervals.

effect of removal was very pronounced at the beginning of the experiment, but this effect disappeared in the last two observations; in contrast, the effect of caging was rather negligible at the beginning of the experiment but appeared as significant in the last observation (Fig. 3b). In the case of macroalgae removal, species richness increased from one species in time 0 to five species in time 1 (each species was present in at least one of six plots for each treatment), and species richness subsequently decreased to one species per plot in time 4. The prevention of herbivore access resulted in a gradual increase in species richness, followed by a more steady-state (Fig. 4). The interaction of the two effects was not significant (Table 1). The lowest species richness was generally observed in the control plots (Fig. 4).

Species composition. In total, seven macrophyte species and two macroalgae species were observed in the experimental plots. The species composition showed pronounced dynamics, i.e., both common successional dynamics and differential responses to the treatments. The first PRC (principal response curves) axis (Fig. 5) was determined by the two most common species and their differential responses to caging; in contrast, *P. pectinatus* was strongly supported by the exclusion of herbivores, and *Myriophyllum spicatum* preferred the open plots. The PRC diagram (highly significant, pseudoF = 52.3, *p* = 0.0002, Fig. 5) clearly demonstrated the responses to experimental manipulations. While the response to macroalgae removal was immediate and pronounced, the effects vanished over time, as can be expected for a "pulse type" treatment; however, the effects of caging were gradual but rather long-lasting, corresponding to the "press type" treatment. This also corresponds to the amount of explained variability in individual RDA for the individual times (Fig. 3c, Table 2).

At the beginning of the experiment (i.e., time 1), the community composition was determined mainly by macroalgae removal (Fig. 5: the PRC curves in time 1 differ for removal, and in the RDA results for time 1, Supplementary Fig. S1, the first axis was determined by removal); additionally, most species responded positively to removal (with the exception of *Potamogeton crispus*). At time 2, caging was more important (the first RDA axis for times 2–4 were determined by caging). However, in time 2, both the effects were significant, and we can see a predominance of most of the species, i.e., *M. spicatum*, *Myriophyllum verticillatum*, *Elodea canadensis* and *P. crispus* predominated in the plots where macroalgae were removed. Interestingly, *P. pectinatus* responded positively to caging beginning in time 2, whereas the predominance of *M. spicatum* in the open plots started only in time 3. Only two species of macrophytes occurred in plots with no treatment: *M. spicatum* and *P. pectinatus*. Three species occurred in plots with prevention of herbivore access: *M. spicatum*, *P. pectinatus* and *P. crispus*. These three species, plus *M. verticillatum*, *E. canadensis* and *Batrachium trichophyllum*, occurred in plots where macroalgae were removed. In plots with both treatments, all mentioned species except *E. canadensis* were observed, and additionally, *Potamogeton pusillus* appeared.

Discussion

Our study demonstrated how competition and herbivory affected the structure of the aquatic ecosystem in terms of affecting the macrophyte cover, species richness and composition of macrophytes. The results supported our hypothesis that the pulse treatment (i.e., macroalgae removal) would result in a marked increase in the macrophyte cover and macrophyte species richness in the experimental plots; however, the initial effects quickly diminished and finally disappeared. The increase was apparent mainly during the first time period (i.e., time 1). Subsequently, the macrophyte cover decreased because macrophytes were gradually replaced by macroalgae. The species richness also started to decrease in time 1. A similar trend was observed during the succession stages of a drying lake in France, where the greatest species richness was observed during the first year[42]. A lake, like an island, is a semi-isolated ecosystem with a limited possibility of species dispersal from the outside[49]. Thus, a few competitively successful species may colonise the disturbed habitat after a short time, resulting in highest species richness occurrence during the initial succession stage[50]; however, species richness subsequently decreases due
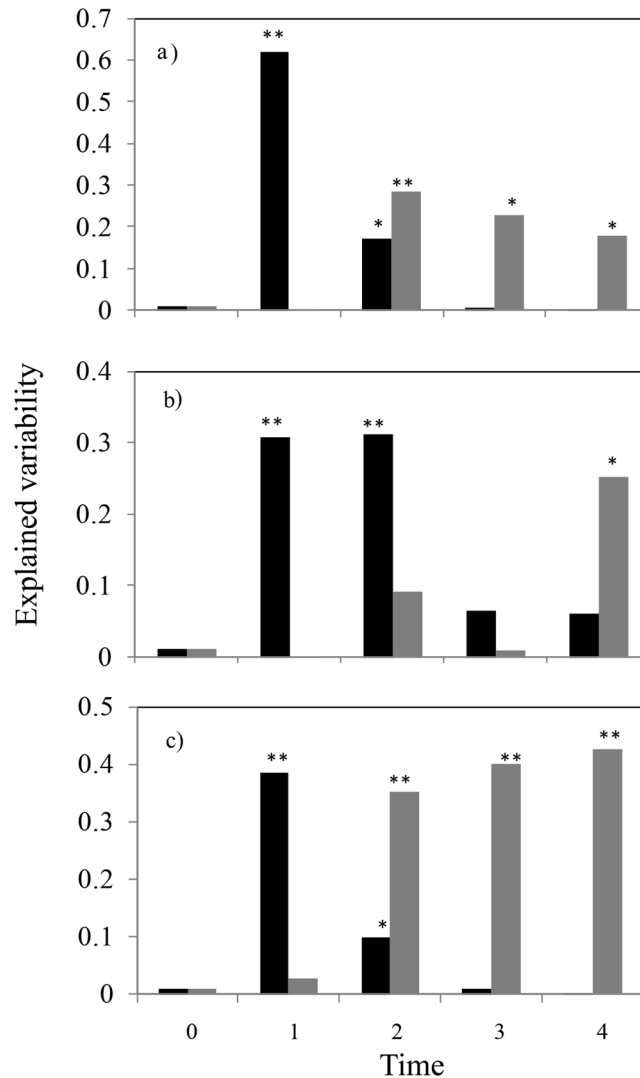
Figure 3. Proportion of total variability explained by two treatments, i.e. by macroalgae removal (black columns) and by prevention of herbivore access (grey columns) as main effects in ANOVA of (**a**) macrophyte cover and (**b**) species richness, and (**c**) in RDA of quantitative species composition calculated separately for each time interval (0–4). One and two asterisks show the significance of individual effects ($p < 0.05$ and $p < 0.01$, respectively, obtained from corresponding F-tests of the main effects in ANOVA in (**a**,**b**), and by Monte Carlo permutation test in (**c**). Detailed results of individual ANOVAs are in Table 2, and individual ordination diagrams are in Supplementary Fig. S1.

to competitive exclusion. Macrophyte species that occurred only in plots after the pulse treatment – macroalgae removal (i.e., after the elimination of competition) included *M. verticillatum*, *E. canadensis* and *B. trichophyllum*. These three species were scarce, and each of them accounted for less than 1% of the total vegetative cover in the lake[17]. In addition, *M. verticillatum* is included on the IUNC red list and is classified as vulnerable (A2c). Although *E. canadensis* is an invasive species and is spreading worldwide[51], the conditions in Milada Lake, such as trophy, water chemistry, sediment composition and hydro-morphological parameters[6–8], are probably sub-optimal for its growth. The next species, *B. trichophyllum*, was observed in Milada Lake for the first time. Its occurrence demonstrated the apparent impact of disturbances on the community of primary producers in the lake. In terms of the condition of our study site, all the species mentioned above represent species that have the ability to quickly colonise and have low competitive ability[36]. Considering the isolation of Milada Lake, we may exclude hydrochory (i.e., water dispersal through ditch networks) in terms of colonisation by the new species *B. trichophyllum*. We assumed that waterfowl may have had an important impact on the dispersal of macrophytes to our locality, but we have no clear evidence to support our theory. Nevertheless, this hypothesis has been generally accepted[52–54]. Specifically, internal dispersal, which refers to the eating and excretion of seeds by waterfowl, has a more important role than does external dispersal, which refers to seeds that have attached to the body surface, a process that has been overestimated in the past[55]. However, we admit that the species could have already been in the lake, but if so, it was present only at very low densities that were not recorded during our regular monitoring.
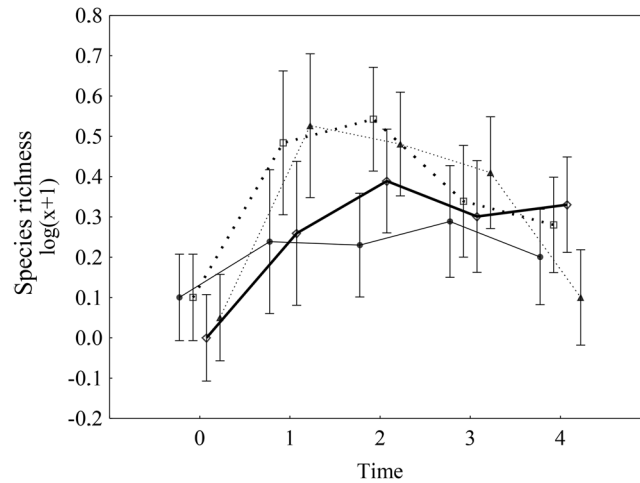
Figure 4. Impacts of two treatments (i.e., prevention of herbivore access and macroalgae removal) on macrophyte species richness over time. Solid line = non-removal open plots (control), bold solid line = non-removal caged plots, dashed line = removal open plots, bold dashed line = removal caged plots. Data were log(x + 1)-transformed. The error bars are the 95% confidence intervals. The results of repeated measures ANOVA are in Table 1.
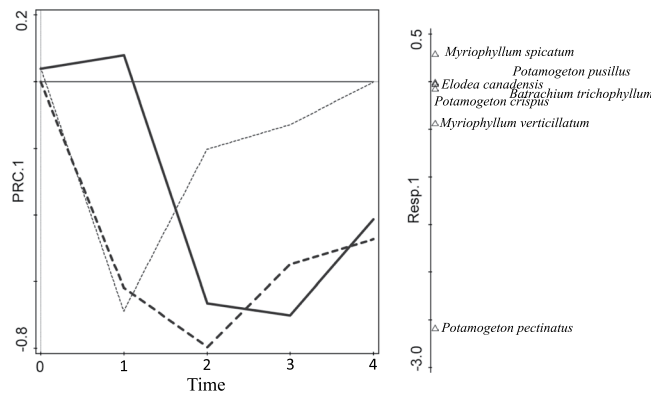

Figure 5. Response of the community to experimental manipulations visualised using principal response curves (PRC). The horizontal axis (time) corresponds to individual observations, with 0 being the baseline (just before the treatments were imposed; the variation in the baseline provides the size of the expected variation in plots under the same treatment). The x-axis (light solid line) corresponds to the reference group (we deliberately selected the non-removal open as the control), bold solid line = non-removal caged plots, dashed line = removal open plots and bold dashed line = removal caged plots. The Resp. 1 axis on the right shows the species score on the first PRC axis and is aimed to help interpret the response (e.g., negative values of the PRC.1 correspond to prevalence of *Potamogeton pectinatus*).

Our hypothesis that the decrease in herbivory caused by caging will positively affect both macrophyte cover and species richness was confirmed. The effect appeared much later and was particularly pronounced in terms of species composition, which increased with time. The results support the latest findings that herbivory in aquatic ecosystems is noticeable. Until the 1990s, herbivory on aquatic vascular plants was considered relatively unimportant; however, new findings show that 48% of plant biomass may be removed due to herbivory. This is, in fact, a pressure that is five to ten times greater than that reported in terrestrial ecosystems[56]. According to Wood *et al.*[57], changes in plant abundances were reported at relatively low herbivore densities, suggesting that, in aquatic systems, greater herbivore densities overwhelm plant compensatory growth responses. The more intensive utilisation of plant biomass in aquatic ecosystems is probably due to low C: N ratio (i.e., the high content of nitrogen) in aquatic ecosystems[56]. We focused on the level of impact of herbivory on the macrophyte cover and species richness. Our complex monitoring of fish populations at the study site showed that the abundance of potential herbivorous fish able to affect the macrophyte community was 9–15 kg ha^{-1} (J. Peterka *et al.*, unpubl. data). Rudd has the greatest impact in the studied lake[17,58]. Thus, rudd poses the most herbivorous pressure on the macrophytes in this experiment. Other potential herbivores include roach (*Rutilus rutilus*)[58] and spiny-cheek crayfish (*Orconectes limosus*; based on our observations), which are abundant in the lake but present a lower impact. We may exclude the impact of herbivorous waterfowl[52], particularly the mute swan (*Cygnus olor*) and common coot

(*Fulica atra*), because the experiment occurred at the depth of 3–4 m[59]. In addition, a recent meta-analysis by Wood *et al.*[57]. referred to substantial between-taxa differences in the effects of herbivores on the abundance of freshwater and marine macrophytes. Fish have large impacts on macrophytes, while insects and birds have relatively low impacts on macrophytes. The reason for these differences may be the mobility and habitat preferences of each of these groups. Fully aquatic species, which permanently live underwater, have been shown to produce the greatest impacts on aquatic plants and are often considered to be ecosystem engineers[60]. Two species from our herbivores were invasive species in certain parts of the world, e.g., the spiny-cheek crayfish in Europe[61] and rudd in North America and New Zealand[18,20]. Therefore, it is advisable to have knowledge about their structuring role in an aquatic ecosystem.

The decrease of herbivory caused by caging also affected the species composition. This result is in accordance with the theory that herbivory has an important impact on the succession of macrophytes[56]. At our study site, the highest expansion was recorded for *P. pectinatus*, while the lowest cover was recorded for *M. spicatum*. These two species were the most common macrophytes in the experimental area as well as in the entire lake. The results clearly showed a preference for *P. pectinatus* by herbivores, but *M. spicatum* was ignored; furthermore, *M. spicatum* predominated in the open plots. The predominance of *P. pectinatus* in the caged plots appeared earlier than did the predominance of *M. spicatum* in the open plots. This suggested that *M. spicatum*, as a competitively weak species, was resistant to herbivory and took advantage of the open plots that suppressed the other species, particularly *P. pectinatus*. This result is in agreement with previous studies that showed that *P. pectinatus* was readily utilised by rudd[17,20,23]. The distaste for *M. spicatum* was probably caused by their high phenolic concentration, which is used as a chemical defence against herbivory[23]. The low number of *M. spicatum* in closed plots was probably due to its low competitiveness compared to *P. pectinatus* and other macroalgae that are more successful when herbivory is absent. This result demonstrates a typical trade-off, i.e., species with a chemical defence against herbivory are bad competitors and vice versa[1]. Like *P. pectinatus*, *P. crispus* was also positively affected by the "prevention of herbivore access" treatment but in considerably lower abundances. This result is in accordance with the claim that *Potamogeton* species generally represent a genus preferred by herbivores[17,20,32], probably due to their low chemical defences[23]. *P. crispus* tries to defend itself with tough and partially spine-like leaves, but it seems that this type of defence is not very effective.

We found no significant interaction between the two treatments; thus, we can conclude that the effects of herbivory and competition were independent in our case (as no interaction means additivity on the log scale, for both species richness and macrophyte cover; additionally, the caging means the same percentage increase in both variables in the control and removal plots, and removal means the same percentage increase in the caged and open plots). Nevertheless, a positive effect of both treatments together was recorded for *P. pusillus* and *B. trichophyllum*. Both species were rare in the experiment as well as in the entire study lake. This indicated favoured utilisation by herbivores (results for similar species in[20,32]) and highlighted their low competitiveness due to the suboptimal conditions in the lake, which was mainly represented by low nutrient availability[6,7,10]. However, due to the scarcity of these two species, they had very small effect on the results of the statistical tests.

We have clearly demonstrated that both herbivory and competition were strong ecological forces that shaped the macrophyte community composition. The results of our study may also be applied in revitalisation or conservation projects conducted in aquatic ecosystems. The regular disturbance of a lake bottom in a restricted area covered by macrophytes may lead to an increase in the biodiversity and preservation of rare species in the community, either by the colonisation of the uncovered bottom or by strengthening the diaspore production in the system[42,62]. A similar effect has been observed in various terrestrial ecosystems[63]. In contrast, human-induced disturbances of seagrasses in marine ecosystems may have a negative impact on sensitive species[64]. Thus, the controlled disturbances should be evaluated individually. Our results implied that disturbances such as those studied in our experiment will increase the biodiversity in an aquatic ecosystem. However, this approach cannot be applied without precautionary measures, as not all species would benefit from similar interventions. The treatment should be mainly performed in a limited area and should be continued after the pilot data have been evaluated. Though it is well-known that the species composition and quantity of the fish community affect the water quality[65], we have newly demonstrated that vertebrate herbivory can decrease the quantity and species richness of aquatic macrophyte communities. We have also demonstrated the effect of macroalgae competition on macrophytes. Thus, management that decreases these effects might lead to the increase in biodiversity and may even promote some endangered macrophytes species.

Methods

Study site.　The study was conducted in the newly created, opencast mine Milada Lake (50°39′N, 13°58′E; Fig. 6a) in the Czech Republic (Fig. 6b). Milada is oligo- to meso-trophic with mean summer total phosphorus in surface layer (TP) $<10\,\mu g\,L^{-1}$. The lake has an area of 250 ha, a volume of 0.036 km³ and a maximum depth of 25 m. Aquatic restoration started in 2001 (when the water level was 122 m.a.s.l.), and the final water level (i.e., 145.7 m.a.s.l.) was obtained in 2011. Several species of macrophytes and macroalgae are present at high biomasses to a depth of 12 m[17].

Experimental design and sampling.　The experiment was conducted in the southwest part of Milada Lake (N: 50°39′09.4″, E: 13°56′11.9″) at a depth of 3–4 m (i.e., the depth with the highest macrophyte biodiversity; I. Vejříková, unpubl. data) and started in May 2014. Experimental plots were delineated by cages made of stainless steel with a width of 2 × 2 m and a height of 1.6 m. The optimal sizes were chosen based on the sizes of potential herbivores[66] and the accuracy of cover estimates that generally decreases with the size of the plot. Two treatments were applied: (i) the removal of macroalgae *Chara* sp. and *Vaucheria* sp. (i.e., the pulse treatment), and (ii) the prevention of herbivore access (i.e., the press treatment) by using closed cages made of a stainless steel net with mesh sizes of 20 mm (the controls were open cages). A full factorial design formed four combinations when either the
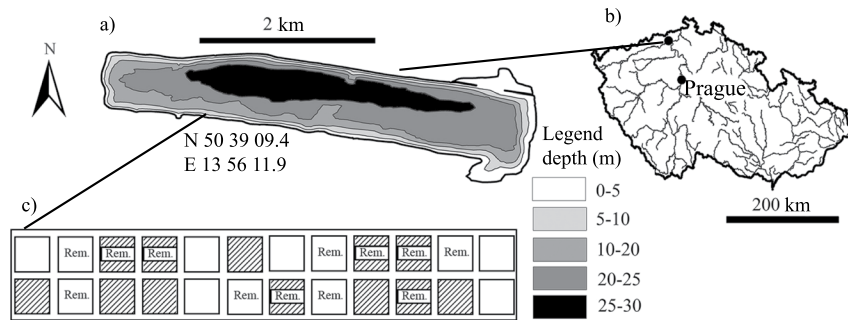
Figure 6. Study site. (**a**) Bathymetric map of Milada Lake, with the location of the experiment. (**b**) Map of the Czech Republic, showing location of the lake. Both maps were generated by the software ArcMap, version 10.2.2[69]. (**c**) Schematic design of the experiment: 24 experimental plots with different treatments. White and patterned squares represent open and closed cages, respectively. *Rem* stands for the macroalgae removal.

first treatment, the second treatment, both treatments, or no treatment was applied. Each combination was replicated six times, and the plots were completely randomised (with restrictions to prevent the clumping of identical treatments in space; Fig. 6c). We also attempted a third treatment, i.e., transplanting two bunches of *M. spicatum* and two bunches of *P. crispus* to the plots, but the plants did not establish (i.e., transplants were seen dead in time 1); thus, this third treatment was disregarded. The potential herbivores were rudd, roach and spiny-cheek crayfish. The abundance and biomass of rudd was 7 ind. ha^{-1} and 3 kg ha^{-1}, respectively, in 2014, and 91 ind. ha^{-1} and 7 kg ha^{-1}, respectively, in 2015. The abundance and biomass of roach was 149 ind. ha^{-1} and 12 kg ha^{-1}, respectively, in 2014, and 14 ind. ha^{-1} and 2 kg ha^{-1}, respectively, in 2015 (J. Peterka *et al.*, unpubl. data). Both fish species were concentrated in the littoral section of the lake, which also has high macrophyte occurrence (based on our observations). The plots were initially sampled before the experimental treatment and then four times after the treatment, i.e., two samples in July and September in both 2014 and 2015, respectively. The cover of all aquatic plants and algae was visually estimated by two independent SCUBA divers in each plot. The plot was visually divided into four 1 × 1-m subunits, and the cover was estimated in given subunits and averaged by each diver; finally, the mean cover obtained from both divers was used as the final value. The estimation of the macrophyte cover varied between the divers by 1.8% (from 0 to 8%). The water transparency varied from 3 to 6 m during the experiment. To prevent potential shading, the microalgae and sediments were regularly removed by a small broom from the cages, particularly from the lid of the closed cages.

Statistical analysis. The macrophyte community was characterised by species richness, macrophyte cover (univariate response variables), and species composition. The univariate variables (i.e., species richness, macrophyte cover and macroalgae cover) were analysed using repeated measures ANOVA. The data were log(x + 1)-transformed before analysis to improve normality and homoscedasticity. Because the effect of both treatments changed over time, we conducted separate analyses (i.e., separate two-way ANOVAs) for each individual observation. We also presented the amount of explained variability by each of the main effects at each time point (the amount of explained variability was calculated as SS_{effect}/SS_{total} for each treatment). Because it was a manipulative experiment, the explained variability can be considered a measure of strength of the effect of manipulation, and the statistical significance is proof of causality. The univariate analyses were conducted using Statistica 12 (StatSoft Inc.).

The species composition was analysed in the framework of constrained ordinations. Because the data were in the form of repeated observations, we used PRC[67,68]. Because the PRC (highly significant) demonstrated that the effects of the two treatments changed considerably over time (similar to the univariate analyses), we used simple analyses of the effects of the two treatments (i.e., removal and caging) from individual observation times. In this case, variation partitioning[68] was used to quantify the independent effects of the two factors and to test for their significance. Because the two factors were orthogonal, the overlap of their two effects was, by definition, zero; thus, we were only interested in the size of the two effects. This procedure provided a multivariate equivalent of the ANOVA results.

The multivariate analyses were conducted using CANOCO5, and the significance of the effects was obtained using Monte Carlo permutation tests (in PRC, with a corresponding hierarchical permutation scheme), with 4999 permutations.

Data availability. The dataset analysed during the current study is available from the corresponding author on reasonable request.

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Acknowledgements

Author Contributions

I.V., M.Č. and J.P. designed the study. I.V., L.V., L.K., Z.S. and J.P. did the field work. I.V., L.V. and J.L. did the statistical analyses. I.V., L.V. and J.L. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Paper III

Macrophytes shape trophic niche variation among generalist fishes

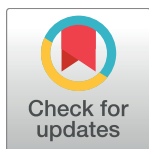# Macrophytes shape trophic niche variation among generalist fishes

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Abstract

Generalist species commonly have a fundamental role in ecosystems as they can integrate spatially distinct habitats and food-web compartments, as well as control the composition, abundance and behavior of organisms at different trophic levels. Generalist populations typically consist of specialized individuals, but the potential for and hence degree of individual niche variation can be largely determined by habitat complexity. We compared individual niche variation within three generalist fishes between two comparable lakes in the Czech Republic differing in macrophyte cover, i.e. macrophyte-rich Milada and macrophyte-poor Most. We tested the hypothesis that large individual niche variation among generalist fishes is facilitated by the presence of macrophytes, which provides niches and predation shelter for fish and their prey items. Based on results from stable nitrogen (δ^{15}N) and carbon (δ^{13}C) isotopic mixing models, perch (*Perca fluviatilis* L.) and rudd (*Scardinius erythrophthalmus* (L.)) showed larger individual variation (i.e., variance) in trophic position in Milada as compared to Most, whereas no significant between-lake differences were observed for roach (*Rutilus rutilus* (L.)). Contrary to our hypothesis, all the three species showed significantly lower individual variation in the relative reliance on littoral food resources in Milada than in Most. Rudd relied significantly more whereas perch and roach relied less on littoral food resources in Milada than in Most, likely due to prevalent herbivory by rudd and prevalent zooplanktivory by perch and roach in the macrophyte-rich Milada as compared to macrophyte-poor Most. Our study demonstrates how the succession of macrophyte vegetation, via its effects on the physical and biological complexity of the littoral zone and on the availability of small prey fish and zooplankton, can strongly influence individual niche variation among generalist fishes with different ontogenetic trajectories, and hence the overall food-web structures in lake ecosystems.

Introduction

Generalist species that feed on multiple trophic levels (*cf.* [1]) commonly have a fundamental role in ecosystems. Generalists can, for instance, regulate the abundance, composition and niche use of organisms at lower and higher trophic levels and also integrate spatially distinct habitats and food-web compartments [2], thereby affecting the structure and stability of food webs [3,4]. Generalist populations with wide trophic niche typically consist of highly specialized individuals that only use a subset of the entire population niche [5]. Such individual specialization can, in turn, have complex population-, community- and ecosystem-level effects, including speciation processes, intra- and inter-specific interactions as well as nutrient cycles and energy flow ([6–8] and references therein). However, the occurrence and degree of individual niche variation, and hence potential for speciation and habitat coupling, within generalist populations is commonly controlled by niche availability and ecological opportunities [9–11].

In lakes, the niche availability and ecological opportunities are strongly controlled by the presence and extent of macrophyte vegetation, both through the direct effects on habitat complexity and the overall ecosystem productivity and also indirectly through reduced predatory effects (*e.g.* [12,13] and references therein). Besides affecting the abundance, size and community composition of zooplankton, benthic macroinvertebrates and small fish [12,14,15], macrophytes can also directly contribute to the diet of generalist fishes [13,16]. The effects of macrophyte vegetation on individual niche variation among generalist fishes can largely be shaped by the species' foraging strategy and ontogenetic trajectories. However, there is limited empirical evidence of how macrophyte vegetation affects individual niche variation and hence the overall population-level niche width of generalist fishes. Here, we use a novel opportunity to compare how different succession stages (*i.e.*, abundance) of macrophyte beds influence trophic niche width of three generalist fishes with contrasting foraging strategies. Perch (*Perca fluviatilis* L.), roach (*Rutilus rutilus* (L.)) and rudd (*Scardinius erythrophthalmus* (L.)) are all generalist and widely spread fishes [17], the former two species commonly dominating the fish communities in European lakes [18–20]. However, the three species show fundamental differences in their foraging strategies. Perch commonly undergo ontogenetic dietary shift from zooplankton to benthic invertebrates and finally to piscivory [21,22], whereas roach and particularly rudd become increasingly herbivorous with increasing size [17,23,24]. Hence, the species can be expected to show markedly different responses to the presence or absence of macrophytes, both in terms of the trophic position occupied by the populations and individuals within, as well as in terms of their use of littoral and pelagic food resources.

The competitive coexistence of perch and roach is one of the most extensively studied examples of intra- and inter-specific interactions among generalist fishes. A general succession from dominance of perch to roach with increasing productivity has been documented for lakes of different trophic states [18,25,26], as well as within reservoirs with pronounced longitudinal gradients [27,28]. Roach are efficient zooplanktivores, but may also feed on macroinvertebrates and non-animal food. Perch are ontogenetic carnivores and start their lives by feeding on zooplankton and later shift to macroinvertebrates to finally become piscivorous [21]. Both species mutually influence each other by way of asymmetrical competitive and predator-prey interactions. Roach are more efficient zooplanktivores in open water and thus force young-of-the-year perch to ingest benthic macroinvertebrates earlier in the season if zooplankton resources are reduced [29,30]. This leads to increased intra-specific competition between perch age classes and thereby to reduced growth and potential to a piscivorous niche shift [30]. In some lakes, perch and roach show marked niche segregation with perch feeding mainly on benthos among macrophyte vegetation and roach feeding on zooplankton in the pelagic areas [29]. However, habitat complexity can fundamentally affect intra- and inter-specific

interactions among perch and roach. Structured habitats offer high biomasses of macroinvertebrates [31,32] and thereby facilitate competitive dominance of the more efficient benthivorous perch [29,33]. Moreover, perch is also more efficient zooplanktivore in structured habitats as compared to roach [34]. Consequently, the presence of structurally complex habitats, such as littoral macrophytes, generally reduces the bottlenecks for ontogenetic niche shifts and increases the growth of perch, even if perch and roach are forced to coexist in these sheltered habitats due to the presence of piscivorous predators [32,35]. Although the perch-roach interactions are well studied, there is limited empirical evidence of how macrophyte vegetation can influence trophic niche width of generalist fishes, including also rudd.

We compared isotopic niche widths of generalist perch, roach and rudd between two deep, post-mining lakes in northern Czech Republic: Lake Milada with abundant macrophyte vegetation and Lake Most where macrophytes are practically absent. In Milada, macrophytes evidently increase the structural complexity of the littoral zone and hence provide niches and shelter against fish predation for various invertebrate taxa [13]. This high physical and biological complexity may hence support a more complex food-web structure in Milada as compared to the macrophyte-poor Most. However, the three generalist fishes likely utilize different trophic niches and may also respond differently to between-lake differences in niche availability. Therefore, we raised three main hypotheses: i) Firstly, due to their different foraging strategies and ontogenetic trajectories, we expected significant niche segregation between the three generalist species, with perch occupying the highest trophic position due to piscivory and rudd occupying the lowest trophic position and relying most heavily on littoral food resources due to herbivory. ii) Secondly, we expected that the abundant macrophyte vegetation in Milada would facilitate higher trophic variability within the generalist fish populations than in the macrophyte-poor Most, as individuals can utilize a wider selection of niches (*i.e.*, prey items and habitats) in the former ecosystem. iii) Thirdly, we expected perch to be particularly responsive to higher niche availability since, unlike more invertivorous and herbivorous roach and rudd, perch can shift to piscivory and utilize small prey fish, which in turn are expected to be more abundant in the macrophyte-rich Milada.

Materials and methods

Study lakes

The study was conducted in two newly created comparable post-mining lakes in northern Czech Republic, Milada and Most (Fig 1), with a permission of the owner of the study sites, Palivový kombinát Ústí, státní podnik. The lakes originate from flooding of restored brown coal opencast mines. Aquatic restoration of Milada was performed in years 2001–2011 and nowadays several species of macrophytes and macroalgae are present in high biomasses to a depth of 12 m (average vegetation cover of > 65% at 0–12 m depth, consisting mainly of *Potamogeton* sp., *Myriophyllum* sp. and *Chara* sp.). Restoration of Most was performed in years 2008–2014, but no abundant macrophyte vegetation has been developed (average vegetation cover of < 1% at 0–6 m depth) (see [36] for more details). In years 2013–2014, the three study species composed in abundance 94–96% and 64–65% of the total catches in Milada and Most, respectively. The relative abundances of the species showed no significant between-lake differences (Wilcoxon matched pairs test: p = 0.9, Z_6 = 0.104; S1 Table), with perch and roach being consistently the most abundant and rudd being the least abundant species. Other fishes present in both lakes are ruffe (*Gymnocephalus cernua* (L.)), pike (*Esox lucius* L.), European catfish (*Silurus glanis* L.) and tench (*Tinca tinca* (L.)), whereas pikeperch (*Sander lucioperca* (L.)), asp (*Leuciscus aspius* (L.)) and Prussian carp (*Carassius gibelio* (Bloch)) are only found in Milada and maraena whitefish (*Coregonus maraena* (Bloch)) are only found in Most.
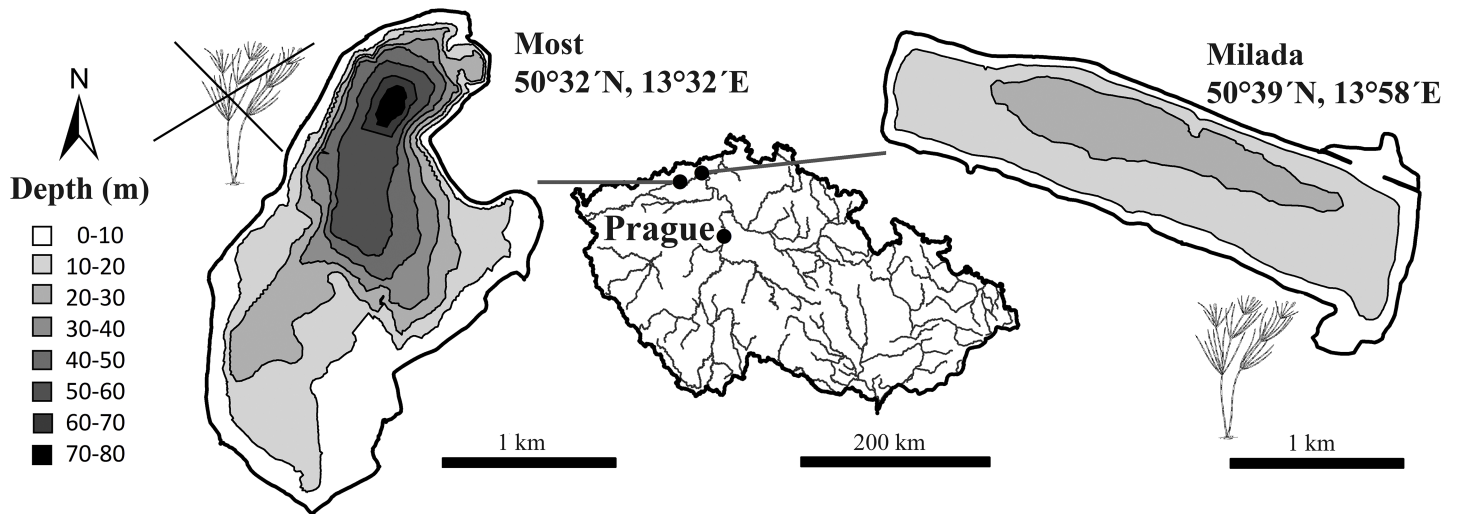
Fig 1. A map showing the location and bathymetric maps of macrophyte-rich Milada Lake and macrophyte-poor Most Lake in northern Czech Republic.

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Fish sampling

Animal treatment was performed in accordance with the guidelines and permission from the Experimental Animal Welfare Commission under the Ministry of Agriculture of the Czech Republic (Ref. No. CZ 01679). The work was approved by the Ethics Committee of the Czech Academy of Sciences. All sampling procedures and experimental manipulations were approved by the Czech Academy of Sciences. The field study did not involve endangered or protected species.

Fish for stable isotope analysis (SIA) were sampled in September 2013 and 2014 by benthic (height 1.5 m, length 30 m) and pelagic (height 3 m, length 30 m) multi-mesh gillnets (12 mesh sizes ranging from 5 to 55 mm knot-to-knot, [37]) in both lakes. The gillnets were set overnight (2 h before sunset and lifted 2 h after sunrise) at depths of 0–3, 3–6, 6–9 and 9–12 m at two benthic and one pelagic localities in three repetitions for both types of gillnets in both lakes and years. Altogether 36 gillnets (24 benthic and 12 pelagic) were set in each lake and year, summing to a total of 144 gillnet nights and 8,640 m^2 gillnet area. All captured fish were immediately anaesthetized by a lethal dose of tricainemethanesulfonate (MS–222, Sigma Aldrich Co.), identified, measured, weighed and dissected for gut content analysis. Muscle tissues from randomly chosen perch, roach and rudd individuals were stored frozen at –20°C prior to final preparation for SIA.

Sampling of food sources for SIA

Littoral and pelagic basal resources (macrophytes and macroalgae,particulate organic matter, POM) and invertebrates (benthic macroinvertebrates, zooplankton) were sampled for SIA to study the overall food-web structure in both study lakes and to estimate the trophic position and the relative reliance of generalist fishes on littoral *versus* pelagic food resources using two-source isotopic mixing models (*cf.* [38,39]). Macrophytes, macroalgae, POM and benthic macroinvertebrates were collected in September 2013 and 2014, whereas zooplankton was sampled in September 2013 and in March, May, July, September and November 2014. Macrophytes, macroalgae and benthic macroinvertebrates were collected by two SCUBA divers, using plastic corer in case of macroinvertebrates. Pelagic POM samples were obtained by filtering 20 L of surface water (collected from the uppermost 5 m) through a 50-µm sieve to prevent

contamination by animal matter prior to the filtration under pressure through a glass microfiber filter (GF/C 1.2 μm; Whatham, U.S.A.). Zooplankton was sampled with a 100-μm mesh plankton net by several vertical hauls throughout the uppermost 20 m of the water column. All littoral and pelagic food sources were sorted, cleaned of detritus and other unwanted material, identified to genus or family level and stored frozen at –20°C prior to final preparation for SIA. Only soft body tissue was dissected from molluscs and trichopterans with cases.

Stable isotope analyses

All frozen fish, invertebrate and basal resource SIA samples were dried at 60°C for 48 h and ground into a homogenous powder using a ball-mill Retsch MM 200 (Retsch GmbH, Haan, Germany). Small subsamples (0.520–0.770 mg) were weighed into tin cups for the analysis of $\delta^{15}N$ and $\delta^{13}C$. All SIA were conducted using a FlashEA 1112 elemental analyser coupled to a Finnigan DELTA[plus] Advantage mass spectrometer (Thermo Fisher Scientific Corporation, Waltham, MA, U.S.A.) at the University of Jyväskylä, Finland. Stable nitrogen and carbon ratios are expressed as delta values ($\delta^{15}N$ and $\delta^{13}C$, respectively) relative to the international standards for nitrogen (atmospheric nitrogen) and carbon (Vienna PeeDeeBelemnite). Analytical precision was always better than 0.20‰ for both isotopes, and was based on standard deviation of repeated analysis of working standards (pike white muscle tissue and birch leaves) inserted in each run after every five samples. As C:N ratios were consistently lower than 3.5 (*i.e.*, 96% of cases), obtained stable isotope values of fish were not lipid corrected [40].

In this study, SIA was applied to compare individual niche variation within the generalist fishes in macrophyte-rich Milada and macrophyte-poor Most. Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes are widely used to study lake food webs and to estimate the long-term diet of consumers [39] as well as niche width of consumer populations [41,42]. While $\delta^{15}N$ values indicate the trophic position of an organism in the food web, the $\delta^{13}C$ values reflect the long-term, assimilated carbon sources of consumers, such as the relative proportions of littoral (benthic) *versus* pelagic (planktonic) food for fish [39]. Hence, SIA provides a powerful tool to study food-web complexity (*e.g.*, food-chain length) and energy flow pathways in the ecosystems.

Primary producers and consumers can show marked differences in $\delta^{15}N$ and $\delta^{13}C$ values between different sites [43,44]. Therefore, the $\delta^{15}N$ and $\delta^{13}C$ values of higher consumers, such as fish, need to be standardized with the isotopic baselines prior to comparisons across ecosystems. Here, the fish $\delta^{15}N$ and $\delta^{13}C$ values were standardized across the study lakes by estimating the trophic position (*TP*) and the relative reliance on littoral *versus* pelagic carbon sources (hereafter littoral reliance, *LR*), respectively, using the two-source isotopic mixing models described in [38]. The mean $\delta^{15}N$ and $\delta^{13}C$ values of macrophytes (*i.e. Myriophyllum* sp., *Potamogeton* sp., *Elodea* sp.) together with macroalgae (*i.e. Chara* sp.) and of pelagic POM were used as the littoral and pelagic isotopic baselines, respectively. Long-lived primary consumers, such as algae-grazing snails and filtrate feeding zooplankton or clams, are commonly used for estimating isotopic baselines since they often show lower spatial and temporal variation in $\delta^{15}N$ and $\delta^{13}C$ as compared to primary produces [38]. However, in the present study, it is more justified to use macrophytes and macroalgae as the littoral baseline since some of the study species (*i.e.*, roach and particularly rudd) are facultative herbivores and thus can utilize these plant foods with the most elevated $\delta^{13}C$ values. For the *TP* and *LR* computations, the commonly used trophic fractionation factors of 3.4‰ for $\delta^{15}N$ and 0.4‰ for $\delta^{13}C$ were applied [38].

Statistical analyses

Depending on normality of the data, either parametric *t*-tests or non-parametric Mann-Whitney *U*-tests were used to test between-lake differences in standard length, trophic position

71

(*TP*) and littoral reliance (*LR*) of the generalist fishes. Analysis of variance (ANOVA) with Tukey's HSD (honest significant difference) pairwise comparisons were used to test differences in *TP* and *LR* between the three species (*i.e.*, inter-specific niche segregation within the lakes). Between-lake differences in the trophic niche width of the generalist species (measured as variance of *TP* and *LR*) were tested with Levene's test for equality of variances, which takes into account differences in sample sizes and is robust to non-normality. All statistical analyses were done in R 3.1.1 [45].

Results

Interspecific niche segregation

The three generalist fishes showed significant niche segregation in terms of their trophic position both in the macrophyte-rich Milada ($F_{2,423}$ = 200.1, $p < 0.001$) and in the macrophyte-poor Most ($F_{2,336}$ = 73.6, $p < 0.001$). In both lakes, perch occupied on average the highest trophic position whereas rudd occupied the lowest trophic position (Table 1; Fig 2; $p < 0.001$ for all pairwise comparisons). The species also showed significant differences in their long-term use of littoral *versus* pelagic food resources in Milada ($F_{2,423}$ = 525.8, $p < 0.001$), but not in Most ($F_{2,336}$ = 2.00, $p = 0.137$). In Milada, rudd relied the most whereas perch relied the least on littoral food resources (Table 1; Fig 2; $p < 0.001$ for all pairwise comparisons).

Between-lake differences in food webs and intraspecific niche variation

Perch collected for SIA were on average smaller (W = 10,167; n = 326; $p < 0.001$) whereas rudd were on average larger (W = 3,476; n = 134; $p < 0.001$) in Milada as compared to Most. No significant between-lake differences were observed in roach size (S2 Table).

All the three generalist fishes, but perch in particular, occupied a significantly higher trophic position in the macrophyte-rich Milada than in the macrophyte-poor Most (Table 1). Rudd relied significantly more whereas perch and roach relied less on littoral food resources in Milada than in Most (Table 1), likely due to prevalent herbivory by rudd and prevalent zooplanktivory by perch and rudd in Milada as compared to Most.

The isotopic niche widths estimated for the three generalist fishes differed significantly between the macrophyte-rich Milada and the macrophyte-poor Most. Perch and rudd showed significantly higher individual variation (*i.e.*, variance) in trophic position in Milada than in Most, whereas no between-lake differences were observed for roach (Table 2; Fig 2).

Table 1. Mean (SD) and range of trophic position (TP) and littoral reliance (LR) estimates of the generalist fish populations in macrophyte-rich Milada and macrophyte-poor Most.

	Milada			Most			
	n	Mean (SD)	Range	n	Mean (SD)	Range	Statistics
TP							
Perch	167	4.64 (0.48)	3.23–5.93	159	3.79 (0.33)	3.02–4.49	t = 18.93; df = 292.82; **$p < 0.001$**
Roach	183	4.23 (0.32)	3.15–5.14	122	3.65 (0.35)	2.54–4.62	t = 14.82; df = 244.19; **$p < 0.001$**
Rudd	76	3.54 (0.39)	2.65–4.20	58	3.18 (0.30)	2.37–4.05	W = 3,305; n = 134; **$p < 0.001$**
LR							
Perch	167	0.39 (0.07)	0.22–0.63	159	0.48 (0.17)	0.10–0.86	W = 8,209; n = 326; **$p < 0.001$**
Roach	183	0.42 (0.07)	0.28–0.79	122	0.53 (0.24)	0.04–0.97	W = 7,276; n = 305; **$p < 0.001$**
Rudd	76	0.70 (0.08)	0.47–0.86	58	0.49 (0.12)	0.18–0.69	W = 4,164; n = 134; **$p < 0.001$**

Sample sizes (n) and results from the statistical tests are shown, with significant between-lake differences ($p < 0.05$) marked in bold.
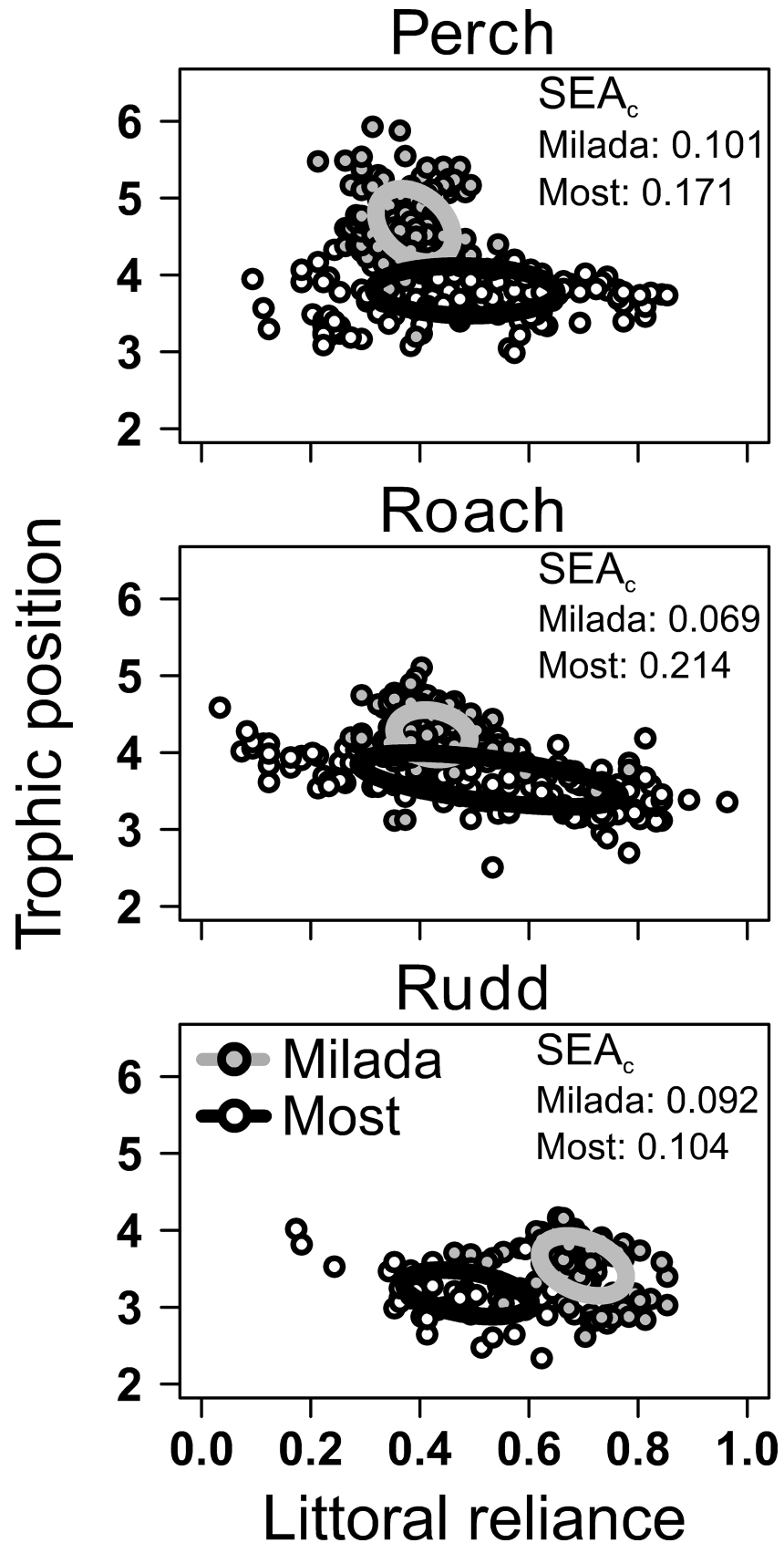
72

Fig 2. Trophic position versus relative littoral reliance of generalist perch, roach and rudd in the macrophyte-rich Milada (grey dots) and macrophyte-poor Most (white dots). The trophic position (*i.e.*, the average position relative to primary producers at which an organism feeds) and the littoral reliance (*i.e.*, the proportion of assimilated food obtained from the littoral habitat) are based on the isotopic values of fish muscle tissue and of the littoral and pelagic basal resources (see Materials and methods and [38] for details). The ellipses depict the core isotopic niches of the fish populations estimated as the sample-size corrected standard ellipse areas (SEAc; estimates shown in the figures; see [68] for details).

All the three species showed lower individual variation in the relative littoral reliance in macrophyte-rich Milada as compared to macrophyte-poor Most, with some individuals using mainly littoral benthic (*LR* > 0.5) and some mainly pelagic planktonic food resources (*LR* < 0.5) (Table 1; Fig 2). The variance tests were repeated after fixing the ranges in fish standard length between the lakes (*i.e.*, after excluding exceptionally small and large individuals) but this did not change the results.

Discussion

Our study demonstrates how habitat complexity, in terms of macrophyte vegetation, can influence the degree of individual niche variation within and resource competition between coexisting generalist species, but also how generalists with different feeding strategies can respond differently to differences in niche availability. As hypothesized, the three generalist species showed a higher intra-specific niche variation and a more distinct inter-specific niche segregation in the presence of abundant macrophyte vegetation. In essence, the generally higher trophic position of generalists suggests a more complex food-web structure (*i.e.*, a longer food chain) in the macrophyte-rich Milada as compared to the macrophyte-poor Most. Perch and rudd showed higher individual variation in trophic position in Milada than in Most, apparently due to high trophic positions of some specialized piscivorous perch and low trophic positions of some specialized herbivorous rudd. At the same time, roach with limited potential to piscivorous or herbivorous specialization showed minor response to between-lake differences in macrophyte vegetation. Contrary to trophic position, the generalists in Milada showed less individual variation in littoral *versus* pelagic resource use than the conspecifics in Most, possibly due to a general paucity of preferred food resources in the latter ecosystem. Overall, our study gives further insights into the fundamental role of macrophytes in shaping the structure and function of lake food webs via their effects on trophic niche variation among generalist fishes.

Table 2. Individual niche variation within the generalist fish populations in macrophyte-rich Milada and macrophyte-poor Most, measured as the variance in trophic position (TP) and littoral reliance (LR) estimates.

		Variance		
	n	Milada	Most	Levene's test
TP				
Perch	326	0.228	0.104	$F_{1,324}$ = 19.563; ***p* < 0.001**
Roach	305	0.104	0.122	$F_{1,303}$ = 152; *p* = 0.219
Rudd	134	0.152	0.092	$F_{1,132}$ = 4.377; ***p* = 0.038**
LR				
Perch	326	0.0050	0.0283	$F_{1,324}$ = 80.323; ***p* < 0.001**
Roach	305	0.0048	0.0576	$F_{1,303}$ = 102.450; ***p* < 0.001**
Rudd	134	0.0063	0.0137	$F_{1,132}$ = 13.892; ***p* < 0.001**

Results from the Levene's test of equality of variances are shown, with significant between-lake differences (*p* < 0.05) marked in bold.

74

The results from stable isotope analyses support our first hypothesis predicting significant long-term trophic niche segregation between the three generalist species. Perch, roach and rudd are all generalist species, but they show markedly different ontogenetic trajectories, with perch shifting diet from zooplankton to benthic macroinvertebrates and finally to fish [46,47] and rudd commonly shifting diet from zooplankton to benthic macroinvertebrates and finally to macrophytes [23,36]. Roach typically prefer zooplankton diet throughout ontogenesis, but may shift to benthic macroinvertebrates and plant matter if the zooplankton resources are scarce or absent [48]. The present study supports this conception of niche segregation between the three generalists as perch generally occupied the highest whereas rudd occupied the lowest trophic position. Herbivory is particularly common among rudd and hence this foraging strategy likely reduces resource competition with the more zooplanktivorous and benthivorous roach [16,49]. In the present study, roach and rudd showed significant differences in relative littoral reliance only in Milada. There, the abundant macrophyte vegetation apparently provide a profitable food resource for herbivorous rudd, but also a predation shelter for large-sized zooplankton, which was one of the main prey items for roach (S3 Table), as observed elsewhere [49,50]. Hence, our results suggest that macrophyte vegetation can reduce niche overlap, and hence interspecific resource competition, between coexisting generalist species [33,51,52]. The three generalist species likely compete for zooplankton resources at early life-stages, but the niche overlap apparently decreases with the individuals' age and size due to increased piscivory by perch [21,33] and herbivory by rudd [53]. At the same time, piscivory by large perch generates novel intra- and inter-specific interactions as these individuals can predate upon smaller conspecifics as well as upon early life stages of other, potentially competitive fish species [21,33,46,54].

Macrophyte vegetation increases the overall physical complexity of the lake littoral zone, but also affects competitive and predatory interactions between different species and ontogenetic stages of fish [18,33,55]. Moreover, macrophytes can have direct effects on the lake total production via both increased primary production and increased density of benthic macroinvertebrates [15], large-sized zooplankton [12] as well as juvenile fish [56], but also indirectly via reducing resuspension of nutrients and fine particles from the sediment [13,57,58]. The effects of macrophytes on physical and biological complexity of the littoral zone can ultimately shape the overall structure and function of lake food webs [15,59]. While most studies have focused on macrophyte effects on competitive and/or predator–prey interactions [13,15,33], there has been limited empirical evidence of how macrophytes affect individual specialization among generalist species. The present study hence gives novel insights into how macrophyte vegetation can influence trophic niche variation within, as well as inter-specific resource competition between, generalist fishes with different foraging strategies. In particular, we found elevated and more variable trophic position of generalists in Milada, suggesting increased food-web complexity under the presence of macrophytes. This perception is supported by previous studies indicating that food-chain length generally increases with increasing resource availability [60,61], and it also supports our second hypothesis predicting higher intraspecific niche variation in the macrophyte-rich Milada. Our results also demonstrate clear between-lake differences in use of littoral and pelagic food resources, with generalists showing markedly larger individual variation in the macrophyte-poor Most. This high individual variation in littoral and pelagic resource use of generalists might indicate increased intra-specific competition due to a general paucity of food, forcing individuals to specialized foraging. This argument is consistent with the theory of trophic adaptability [62] and supported by previous studies from estuarine fishes where a scarcity of preferred food resources has led to population-level niche expansion [63]. This could explain why for instance some roach in Most have apparently specialized to zooplanktivory ($LR < 0.5$) and some other individuals have specialized to

75

herbivory ($LR > 0.5$) instead of utilizing generalist diet consisting largely of preferred, large-sized benthic macroinvertebrates and terrestrial insects.

Our study demonstrates the fundamental role of habitat complexity (*i.e.*, the presence/absence of macrophyte vegetation) in shaping individual variation in and resource use of generalist fishes. Such individual- and population-level differences in trophic niches of generalists can have complex effects on the community composition, through top-down and bottom-up trophic cascades, as well as on the entire ecosystems through altered energy-flow pathways and habitat coupling [5,7,64,65]. For example, the partial population-level niche specialization by herbivorous rudd, zooplanktivorous roach and piscivorous perch suggests high predation pressure on lower trophic levels and also limited habitat coupling by the species in the macrophyte-rich Milada. However, besides habitat (spatial) complexity, the intra- and inter-specific interactions within lake food webs can be fundamentally shaped by seasonal (temporal) variation in prey availability and environmental conditions [66]. Moreover, the other coexisting fishes, including piscivorous pike, catfish, pikeperch and zooplanktivorous whitefish, may have unexplored impacts on the niche width and resource use of the studied generalist species. Hence, seasonal studies and more controlled mesocosm experiments could give valuable insights into how macrophytes shape individual niche variation as well as intra- and inter-specific interactions among generalist fishes. Telemetry studies, in conjunction with trophic studies, could also provide important information about how macrophyte vegetation affects habitat use and activity levels of different generalist species and their ontogenetic stages, as well as individuals with different personalities [67].

Supporting information

S1 Table. Relative abundances (%) of the generalist species caught with survey gillnets from macrophyte-rich Milada and macrophyte-poor Most in 2013 and 2014.
(PDF)

S2 Table. Means (SD) and ranges of standard length (mm) and wet mass (g) of perch, roach and rudd sampled from macrophyte-rich Milada and macrophyte-poor Most in 2013–2014.
(PDF)

S3 Table. Relative proportions of main prey items in the gut contents of perch, roach and rudd caught from macrophyte-rich Milada and macrophyte-poor Most in 2013–2014.
(PDF)

S4 Table. Data for PONE-D-16-38804.
(PDF)

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76

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79

Paper IV

Some like it deep: Intraspecific niche segregation in ruffe

(*Gymnocephalus cernua*)

ORIGINAL ARTICLE

WILEY | Freshwater Biology

Some like it deep: Intraspecific niche segregation in ruffe (*Gymnocephalus cernua*)

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Abstract

1. Generalist fishes commonly show intraspecific niche segregation along the littoral–pelagic resource axis in lakes. Recent studies have shown that the deep, cold and seemingly unproductive profundal zone can also offer underutilised resources and facilitate specialised individuals, and can contribute to lake food webs via methane-derived carbon pathways. Despite numerous examples from salmonid fish species, such intraspecific niche segregation along a littoral–profundal resource axis has not been reported in percids or other predominantly littoral benthivorous fishes.

2. Here, we describe a case of ruffe (*Gymnocephalus cernua*: Percidae) populations consisting of shallow- and deep-water dwelling individuals in two post-mining lakes in the northern Czech Republic. Results from stable isotope (δ^{15}N and δ^{13}C) mixing models indicated that littoral and profundal food resources dominated the long-term diets of ruffe individuals caught from shallow- and deep-water habitats, respectively. No similar niche segregation along the littoral–profundal gradient was observed in coexisting perch (*Perca fluviatilis*: Percidae) which used more pelagic food than the benthivorous ruffe.

3. The observed littoral–profundal niche segregation in ruffe was more pronounced in the macrophyte-rich and poorly oxygenated Lake Milada, where high habitat complexity and strong interspecific interactions in the littoral zone as well as the absence of competing fish species in the profundal zone likely promote narrow trophic niches of shallow- and deep-water dwelling ruffe

4. Our study provides novel evidence of littoral–profundal niche segregation in a predominantly benthivorous fish species. The results also show that intraspecific niche variation can be affected by lake ecosystem characteristics, in particular by the availability of and competition for littoral and profundal resources. Although more research is needed to confirm potential early divergence of shallow- and deep-water dwelling ruffe as well as cascading effects on lake ecosystem processes (e.g. nutrient cycling and competitive and predatory interactions), our study indicates that individual ruffe may show limited habitat and food-web coupling due to their restricted use of shallow or deep resources.

1 | INTRODUCTION

The deep, dark and cold profundal zone in lakes has traditionally been seen as an unproductive habitat acting as a sink rather than as a source of nutrients and energy (Wetzel, 2001), and hence has been presumed to be of minor importance for ecosystem structure and function. However, recent research has shown that this habitat can actually provide niches and thereby support polymorphism (i.e. speciation) particularly among salmonids such as *Coregonus* and *Salvelinus* fish species (e.g. Harrod, Mallela, & Kahilainen, 2010; Klemetsen, 2010; Muir, Hansen, Bronte, & Krueger, 2015). Moreover, recent stable isotope and microbial studies have shown that, alongside pelagic and littoral primary production, methane-oxidising bacteria occurring at oxic-anoxic interfaces can contribute substantially to food webs in some eutrophic and humic lakes (Belle et al., 2016; Jones & Grey, 2011). In such cases, pelagic zooplankton and profundal chironomid larvae typically act as the trophic link to fish (Ravinet, Syväranta, Jones, & Grey, 2010; Sanseverino, Bastviken, Sundh, Pickova, & Enrich-Prast, 2012) which, in turn, can reduce lake methane efflux via top-down control of zooplankton that graze on methanotrophic bacteria (Devlin, Saarenheimo, Syväranta, & Jones, 2015). Thus, consideration of profundal benthic habitats is a prerequisite for comprehensive limnological and ichthyological studies (cf. Vadeboncoeur, Vander Zanden, & Lodge, 2002), but the potential of generalist consumers to integrate the littoral and profundal habitats and food-web compartments (e.g. Ruokonen, Kiljunen, Karjalainen, & Hämäläinen, 2012; Schindler & Scheuerell, 2002; Vanni, 2002) remains understudied. Here, we provide empirical evidence of how intraspecific niche segregation to littoral and profundal trophic niches can reduce habitat coupling by a widespread and often abundant benthivorous fish species, the ruffe (*Gymnocephalus cernua*).

Like many small fishes with minor socio-economic value, ruffe has been the subject of relatively few limnological and ichthyological studies (Gutsch & Hoffman, 2016; Hölker & Thiel, 1998). However, there has been a growing concern about the potential impacts of invasive ruffe on native, commercially important fish species in the Great Lakes of North America as well as in central and northern Europe (Hayden, Harrod, Sonninen, & Kahilainen, 2015; Hayden et al., 2013; Leigh, 1998; Winfield, Rösch, Appelberg, Kinnerbäck, & Rask, 1998). Besides resource competition, ruffe has the potential to reduce the recruitment and abundance of native fishes via predation on eggs and under-yearlings of other fish (Adams & Tippett, 1991; Gutsch & Hoffman, 2016; Ogle, 1998). Due to its well-developed sensory physiology (e.g. good vision in low light and sensitive cephalic lateral-line system), ruffe is exceptionally well adapted to feed in the deep and dark profundal zone (Bergman, 1988; Ogle, 1998; Schleuter & Eckmann, 2006). The use by ruffe of profundal habitat

and food resources may reduce resource competition with coexisting percid and coregonid species (e.g. Hayden et al., 2013, 2015). Moreover, in some eutrophic lakes with anoxic profundal habitats, ruffe can feed largely on profundal chironomids which, in turn, gain most of their energy from consumption of methane-oxidising bacteria (Ravinet et al., 2010). Hence, unlike the more oxygen-sensitive salmonid species, ruffe may utilise the profundal resources in eutrophic lakes and thereby show intraspecific niche segregation, with individuals predominantly using either shallow or deep habitat and food resources. Such intraspecific niche segregation may reduce habitat coupling in lake ecosystems, as individual ruffe show only limited potential for transferring nutrients and energy across habitat boundaries, as observed on individuals of some other fish species (cf. Eloranta, Knudsen, & Amundsen, 2013; Quevedo, Svanbäck, & Eklöv, 2009). However, to the best of our knowledge, no previous studies have investigated the potential of benthivorous fishes for intraspecific niche segregation along a littoral–profundal resource axis, although some predominantly littoral species of sculpins and gobies do occasionally inhabit the lake profundal zone (Dickman, 1995; Ruzycki & Wurtsbaugh, 1999; Walsh, Dittman, & O'Gorman, 2007). Moreover, improved understanding of how lake community and ecosystem properties affect trophic ecology of ruffe will be necessary for future monitoring and evaluation of potential impacts of this highly invasive species on recipient ecosystems (e.g. Gutsch & Hoffman, 2016; Ogle, 1998).

Here, we used nitrogen (δ^{15}N) and carbon (δ^{13}C) stable isotope analyses (hereafter SIA) to investigate intraspecific niche (i.e. long-term dietary) segregation and potential habitat coupling by coexisting ruffe and perch (*Perca fluviatilis*) caught from different depth zones in two post-mining lakes, Lake Milada and Lake Most (hereafter Milada and Most), in the northern Czech Republic. We expected ruffe to show significant intraspecific niche segregation along a depth gradient in both lakes, with shallow- and deep-water dwelling individuals feeding predominantly on littoral and profundal benthic food resources, respectively. Minor niche overlap with ruffe and no corresponding intraspecific niche segregation along the littoral–profundal resource axis was expected in perch due to their inferior ability for efficient resource utilisation in deep, dark and cold environments (Bergman, 1987, 1988). Moreover, the two study lakes differ in their abiotic and biotic characteristics: Milada has an anoxic profundal zone as well as abundant macrophyte vegetation (Vejříková et al., 2016) and relatively dense populations of competitive and predatory fish species in the littoral zone, whereas Most has a well-oxygenated water column, dense ruffe population in the littoral zone and a profundal habitat dominated by maraena whitefish (*Coregonus maraena*: Salmonidae). Therefore, we expected ruffe to show more pronounced intraspecific niche segregation in Milada

where the absence of profundal competitors and the potential presence of methane-derived carbon pathways could offer underutilised resources for deep-water dwelling ruffe, while the high habitat complexity and strong interspecific interactions should facilitate a narrow trophic niche of shallow-dwelling ruffe. In essence, the expected intraspecific niche segregation along the littoral–profundal resource axis would indicate limited habitat and food-web coupling by ruffe individuals, with potential cascading impacts on lake ecosystem processes (cf. Quevedo et al., 2009 and references therein).

2 | METHODS

2.1 | Study lakes

The two study lakes, Milada and Most, are both medium-sized (surface area = 252 and 311 hectares, respectively), relatively deep (Z_{mean} = 16 and 22 m, Z_{max} = 25 and 75 m), oligotrophic (mean summer total phosphorus <10 and <5 μg/L) and dimictic post-mining lakes in the northern Czech Republic. The Secchi depth varies between 4–9 m and the summer thermocline depth between 6–13 m (Figure S1). The summer stratification commonly occurs between late May and early October, with typical surface and bottom water temperatures approaching 20°C and 5°C (Figure S1). The deeper Most has a well-oxygenated water column down to 50 m depth, whereas in Milada the profundal zone suffers from poor oxygen conditions below 15 m depth (Figure S1).

Roach (*Rutilus rutilus*) and rudd (*Scardinius erythrophthalmus*) are the most abundant cyprinid fish species coexisting with ruffe and perch in the lakes (Table S1). The other fishes inhabiting both lakes include pike (*Esox lucius*: Esocidae), European catfish (*Silurus glanis*: Siluridae) and tench (*Tinca tinca*: Cyprinidae), whereas maraena whitefish are only found in Most, and a few pikeperch (*Sander lucioperca*: Percidae), asp (*Leuciscus aspius*: Cyprinidae) and Prussian carp (*Carassius gibelio*: Cyprinidae) are found in Milada (Vejříková et al., 2016). In 2013–2014, ruffe was numerically the most abundant fish species caught from the deep (>9 m) benthic habitats in Milada whereas perch dominated the shallow littoral habitats (Table S1). In Most, maraena whitefish dominated the deepest benthic and pelagic habitats, whereas ruffe was the most abundant fish species caught from the shallow littoral habitats (Table S1).

2.2 | Fish sampling

Ruffe and perch were sampled in September 2013 and 2014 using benthic (height 1.5 m) and pelagic (height 3 m) multi-mesh Nordic gillnets (Appelberg et al., 1995). On each occasion, three sets of both benthic (bottom) and pelagic (suspended) gillnets were set overnight at different depth zones (i.e. at 0–3, 3–6, 6–9, 9–12, 12–18 and >20 m depth) to obtain representative samples and data for fish density in different habitats. At both lakes, the survey fishing was done at two benthic and one pelagic sampling stations, the latter being located at the deepest parts of the lakes (i.e. 20–25 m in Milada and 35–70 m in Most). However, no ruffe or perch were

caught below 18 m depth and the profundal-dwelling fish caught from depths of 9–12 and 12–18 m were pooled as a 9–18 m catch depth to increase sample size for statistical analyses. Species, sampling station and depth, standard length (mm), wet weight (g) and sex were recorded for each fish. In addition, sagittal otoliths, stomach and a small piece of dorsal muscle tissue were dissected from random subsamples of fish and stored for subsequent age, diet and stable isotope analyses, respectively.

2.3 | Sampling of food sources for SIA

Littoral (macrophytes and macroalgae) and pelagic (particulate organic matter, POM) basal food resources as well as littoral and profundal benthic macroinvertebrates and pelagic zooplankton were sampled for SIA to study the overall food-web structure in both study lakes (see Figure S2) and to estimate the long-term diets of ruffe and perch caught from different depth zones. Macrophytes, macroalgae, POM, and benthic macroinvertebrates were collected in September 2013 and 2014, whereas zooplankton was sampled in September 2013 and in March, May, July, September and November 2014. Two scuba divers collected macrophytes, macroalgae and benthic macroinvertebrates, using a plastic corer for the latter. Additional samples of profundal chironomid larvae were obtained from sediment that was attached to the sinker line of deep-water benthic gillnets. Pelagic POM was collected by filtering 20 L of surface water (taken from the uppermost 0–5 m) through a 50-μm sieve to remove animal matter and then under pressure through a glass microfiber filter (GF/C 1.2 μm; Whatham, U.S.A.) to obtain a bulk sample for SIA. Pelagic zooplankton, comprising mainly *Daphnia* sp. and Cyclopidae sp., was sampled with a 100-μm mesh plankton net by taking several vertical hauls throughout the uppermost 20 m of the water column. All littoral, profundal and pelagic food sources were sorted, cleaned of detritus and other unwanted material, identified to genus or family level and stored frozen at −20°C prior to final preparation for SIA. Only soft body tissue was dissected from molluscs and from trichopteran larvae with cases.

2.4 | Stable isotope analyses

All frozen fish, invertebrate and plant SIA samples were finally dried at 60°C for 48 hr and ground into a homogenous powder using a ball-mill Retsch MM 200 (Retsch GmbH, Haan, Germany). Small subsamples (0.520–0.770 mg) were weighed into tin cups for the analysis of $\delta^{13}C$ and $\delta^{15}N$. All SIA were conducted using a FlashEA 1112 elemental analyser coupled to a Finnigan DELTA^{plus} Advantage mass spectrometer (Thermo Fisher Scientific Corporation, Waltham, MA, U.S.A.) at the University of Jyväskylä, Finland. Analytical precision was ±0.20‰ for both $\delta^{15}N$ and $\delta^{13}C$, and was determined by repeated analysis of internal working standards [pike white muscle tissue and birch (*Betula pubescens*: Betulaceae) leaves] inserted in each run after every five samples. Fish stable isotope values were not lipid-corrected because the low C:N ratios (<3.5 in 98% of the

cases) indicated low lipid content of the ruffe and perch muscle tissue (Kiljunen et al., 2006).

Two-source isotopic mixing models were applied to estimate the trophic position (TP) and relative littoral reliance (LR) of ruffe and perch caught from different depths zones (Post, 2002). Besides the long-term dietary niche of fish, TP and LR are commonly used to study food-chain length and habitat coupling in lake ecosystems, respectively (Layman et al., 2012; Post, 2002). Finally, analysis of variance (ANOVA) with Tukey's honest significant difference pairwise comparisons were run in R 3.1.1 (R Core Team, 2014) to test differences in size, TP and LR of ruffe and perch caught from different depth zones (Table 1).

The SIAR isotopic mixing model (Parnell, Inger, Bearhop, & Jackson, 2010) was run in R 3.1.1 to evaluate the relative contributions of littoral, profundal and pelagic food sources to long-term diets of ruffe and perch caught from different depth zones, thus indicating intra- and interspecific niche segregation as well as habitat coupling by fish individuals. For the LR, TP and SIAR computations, the commonly used trophic fractionation factors of $3.4 \pm 1.0\permil$ for $\delta^{15}N$ and $0.4 \pm 1.3\permil$ for $\delta^{13}C$ were applied (Post, 2002). For LR and TP computations, the mean $\delta^{15}N$ and $\delta^{13}C$ values of macrophytes (*Myriophyllum* sp.: Haloragaceae; *Potamogeton* sp.: Potamogetonaceae; *Elodea* sp.: Hydrocharitaceae) and macroalgae (*Chara* sp.: Characeae) and of pelagic POM were used as the littoral and pelagic isotopic baselines, respectively (Figure S2).

3 | RESULTS

A total of 73 and 99 ruffe as well as 167 and 159 perch were sampled for SIA from Milada and Most, respectively. The standard length of ruffe ranged from 29 to 140 mm and the wet mass from 1 to 58 g. Perch length ranged from 45 to 375 mm and wet mass from 1 to 1,290 g. Ruffe were generally shorter than perch in both Milada ($t = 12.3$, $df = 236.9$, $p < .001$) and Most ($t = 12.0$, $df = 196.3$, $p < .001$). Ruffe caught from the deepest (9–18 m) areas tended to

TABLE 1 Results from ANOVA run to test differences in size (standard length, mm) as well as estimated trophic position (TP) and littoral reliance (LR) of ruffe and perch caught from different depth zones (0–3, 3–6, 6–9 and 9–18 m) in Lakes Milada and Most. Statistically significant differences ($p < 0.05$) are highlighted in bold

Species	Milada			Most		
Variable	F	df	p	F	df	p
Ruffe						
Size	3.27	3.95	**.025**	0.99	3.69	.401
TP	20.21	3.69	**<.001**	3.39	3.95	**.021**
LR	34.37	3.69	**<.001**	11.9	3.95	**<.001**
Perch						
Size	4.50	3.163	**.005**	1.21	3.146	.308
TP	1.44	3.163	.233	0.18	3.146	.907
LR	5.60	3.163	**.001**	14.57	3.146	**<.001**

be larger than those caught from the sublittoral (6–9 m) habitat in Most, but not in Milada (Table 1, Figure S3). In contrast, perch caught from the deepest areas tended to be smaller than conspecifics caught from the shallower areas in Milada (Table 1, Figure S3).

The $\delta^{15}N$ and $\delta^{13}C$ values indicated clear isotopic segregation between different trophic levels and between littoral and pelagic taxa, respectively (Figure S2). The most common littoral benthic macroinvertebrates sampled for SIA and found in ruffe stomachs (Table S2) included chironomid larvae (e.g. *Tanytarsus* sp., *Ablabesmyia* sp., *Procladius* sp., *Cricotopus* sp., *Polypedium* sp.), *Asellus aquaticus* (Asellidae) amphipods, *Lymnaea* sp. (Lymnaeidae) snails and odonate larvae, whereas the profundal benthos was dominated by *Chironomus* sp. and Orthocladiinae sp. chironomid larvae. The mean $\delta^{15}N$ value of ruffe was the highest of all coexisting fish species in Milada, and was equivalent to that of piscivorous pike in Most (Figure S2). The intermediate $\delta^{13}C$ values of ruffe and perch, as well as of most coexisting cyprinid and predatory fishes, suggested that the species used both littoral and pelagic carbon sources. In contrast, the exceptionally high $\delta^{13}C$ values of rudd in Milada indicated foraging on littoral benthos and plant material, while the low $\delta^{13}C$ values of maraena whitefish in Most indicated a predominantly zooplanktivorous diet (Figure S2).

Ruffe caught from the deepest habitats exhibited significantly higher estimated TP than conspecifics in the shallow-water areas, in both Milada and Most (Table 1, Figure 1). Moreover, ruffe caught
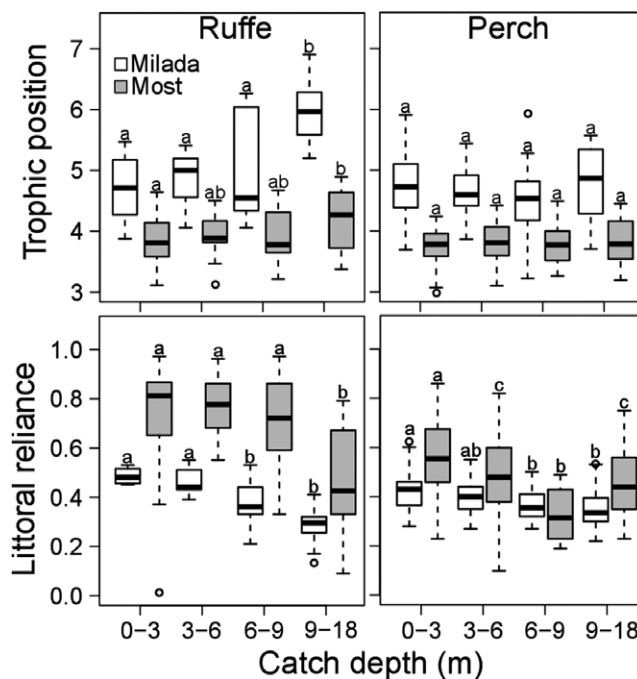

FIGURE 1 Boxplots of estimated trophic position and littoral reliance of ruffe and perch caught from different depth zones in Lakes Milada and Most. In the boxplots, lines indicate median, boxes show upper (75%) and lower (25%) quartiles and whiskers represent range (minimum and maximum values) with outliers indicated by circles. Statistically significant differences between groups (Tukey honest significant difference: $p < .05$) are shown with letters

from the deep habitats relied significantly less on littoral carbon sources LR than individuals caught from shallower habitats in Milada and in Most (Table 1, Figure 1). No significant differences in TP or LR were found in ruffe caught from the shallow depth zones (Figure 1). However, a few ruffe individuals in Most caught from the shallow- and deep-water habitats had exceptionally low and high LR estimates (Figure 1), respectively, suggesting that these fish had mainly been feeding in a different habitats from those in which they were caught.

In contrast to ruffe, no significant shifts in TP were observed in perch caught along the depth gradient in Milada or in Most (Table 1, Figure 1). However, the LR of perch decreased with increasing catch depth, in both Milada and Most (Table 1, Figure 1). Similar patterns in LR were observed in both lakes, except that perch caught from the deepest areas (9–18 m) in Most relied equally on littoral food resources as perch caught from 3 to 6 m depth (Figure 1). While very few perch were caught from the pelagic habitats in Milada (Table S1), no significant differences in TP ($t = -0.22$, $df = 8.81$, $p = .831$) or LR ($t = 1.48$, $df = 8.56$, $p = .175$) were observed between littoral- and pelagic-dwelling perch in Most.

The results from the SIAR isotopic mixing model demonstrated clear long-term dietary segregation between ruffe caught from different depth zones (Figure 2). In both lakes, ruffe caught from the shallow littoral areas relied mainly on littoral food resources, whereas the conspecifics caught from deep habitats relied substantially more on profundal food resources. This pattern was observed in both lakes, but was particularly evident in Milada (Figure S1, Table S1). The contribution of pelagic (zooplankton) food to ruffe diet was low in both lakes and at all depths. In contrast to ruffe, perch showed no clear trends in their use of profundal food resources but instead showed indications of a shift from a littoral diet towards a more pelagic diet with increasing depth (Figure 2). In general, the SIAR results indicated partial niche segregation between the two percids, with ruffe feeding solely on littoral and profundal benthic food and perch using also pelagic zooplankton resources.
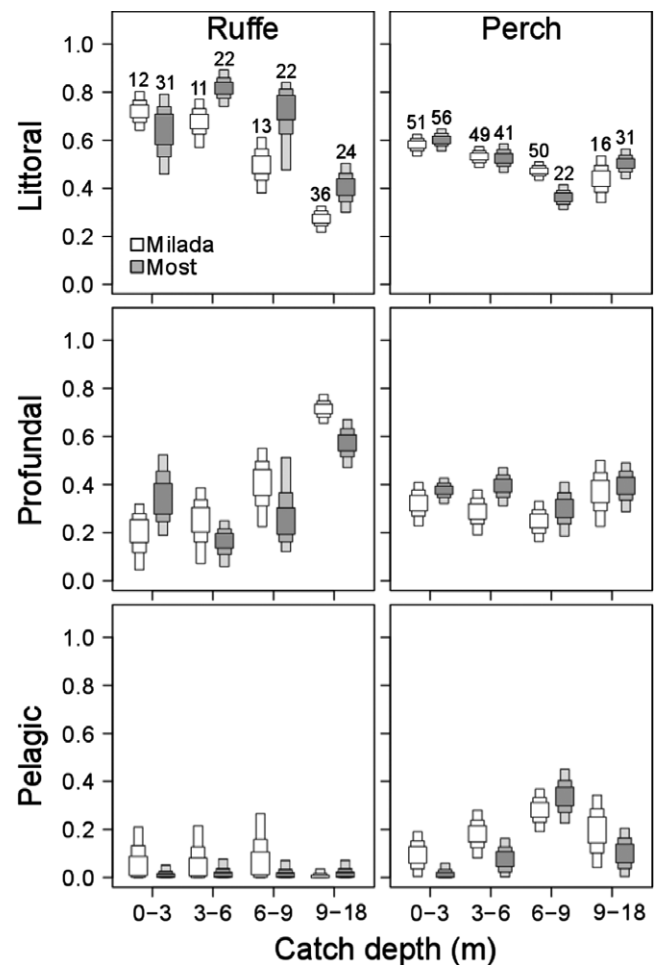

FIGURE 2 Relative proportions of littoral, profundal and pelagic food sources in the long-term diets of ruffe and perch caught from different depths in Lakes Milada and Most. The estimates are based on results from SIAR isotopic mixing model comparing the $\delta^{15}N$ and $\delta^{13}C$ of fish muscle tissue to those of putative littoral, profundal and pelagic invertebrate food sources. The boxes indicate 50%, 75% and 95% Bayesian credibility intervals for the estimated dietary proportions. The numbers of analysed fish are shown above the bars

4 | DISCUSSION

Our results demonstrate clear intraspecific niche segregation of ruffe along the littoral–profundal resource axis. To the best of our knowledge, this is the first evidence of littoral–profundal niche segregation in percids or predominantly benthivorous fishes. The coexisting perch showed only minor dietary overlap with ruffe and no evidence of littoral–profundal niche segregation, and instead indicated a shift from a littoral towards a more pelagic diet with increasing catch depth. Our results suggest that lake abiotic and biotic characteristic can affect the degree of intraspecific niche segregation in ruffe, as the long-term dietary segregation was more evident in Milada where abundant littoral macrophyte vegetation and coexisting species as well as the underutilised profundal resources likely promote narrow trophic niches of shallow- and deep-water dwelling ruffe. As discussed below, the observed littoral–profundal niche segregation in

ruffe may be affected by competitive and predatory interactions, and influence coupling of shallow- and deep-water habitats, with potential cascading effects on lake ecosystem processes.

4.1 | Intraspecific niche segregation and habitat coupling by ruffe

Most limnological and ichthyological studies have focused on pelagic and littoral processes, while the profundal zone has often been neglected due to its perceived lower importance for lake ecosystem structure and function (Wetzel, 2001). However, our study provides further evidence that this seemingly unproductive habitat can contribute to lake food webs through efficient utilisation of deep-water food resources by some fish. Our stable isotope data from ruffe demonstrate significant long-term dietary segregation between shallow- and deep-water dwelling individuals. Such intraspecific niche

segregation along the littoral–profundal gradient has been widely reported for salmonids (e.g. Harrod et al., 2010; Klemetsen, 2010; Muir et al., 2015) but, to the best of our knowledge, not previously reported for percids or predominantly benthivorous fishes, although some gobies and sculpins can inhabit lake profundal zones (Dickman, 1995; Ruzycki & Wurtsbaugh, 1999; Walsh et al., 2007). The pronounced differences in physical and biological properties of the different lake habitats are commonly considered as the main environmental drivers of niche specialisation within fish populations (e.g. Harrod et al., 2010; Klemetsen, 2010; Schluter, 1996). This is likely the ultimate reason for the observed intraspecific niche segregation between shallow- and deep-water dwelling ruffe in Milada and Most. Due to its physical and biological complexity, the littoral zone can provide various microhabitats for invertebrates and fish which encourages specialised adaptations for efficient resource utilisation (e.g. Šmejkal et al., 2014). In contrast, the deep-water dwelling individuals and species may benefit from reduced resource competition and predation pressure (e.g. Kahilainen, Lehtonen, & Könönen, 2003) and maintain a relatively large size and good condition through specialised foraging on profundal benthic food (Hayden et al., 2015), as observed on ruffe in Milada. Indeed, our results are consistent with previous studies indicating that the profundal zone can provide underutilised food resources for ruffe, which has a well-adapted mechanosensory system that helps efficient foraging in darkness and on profundal benthic food resources dominated by chironomids (Hayden et al., 2013, 2015; Hölker & Thiel, 1998).

Generalist consumers typically play a fundamental role in lake ecosystems as they can move nutrients and energy between habitats and integrate spatially distinct food-web compartments through highly mobile and flexible foraging strategies (Schindler & Scheuerell, 2002; Vander Zanden & Vadeboncoeur, 2002; Vanni, 2002). However, such habitat coupling may be limited if the consumer populations consist of highly specialised individuals, as is the case in some polymorphic fish populations (e.g. Harrod et al., 2010; Klemetsen, 2010; Quevedo et al., 2009). Our study provides strong evidence that individuals within ruffe populations can segregate into littoral and profundal trophic niches, remaining in and feeding in the respective habitat, and therefore offering limited coupling of the productive littoral and unproductive profundal habitats. However, a few ruffe individuals in Most caught from the shallowest and deepest habitats did show exceptionally low and high reliance on littoral food resources, respectively (Figure 1), suggesting that these fish had mainly fed in another habitat than that in which they were caught (cf. Ruokonen et al., 2012). This observation was supported by limited stomach contents data (see Table S2) indicating that a few ruffe individuals caught from the deep-water habitats in Most had actually recently eaten littoral prey items. Moreover, in both lakes, ruffe inhabiting the sublittoral and metalimnetic (6–9 m) areas evidently utilised and thereby coupled the shallow- and deep-water habitats and food-web compartments. Hence, the intraspecific niche segregation in ruffe is not absolute, with a few individuals exhibiting generalist utilisation of both littoral and profundal habitat and food resources. Due to the lack of genetic and morphological data, we

cannot say whether the shallow- and deep-water dwelling ruffe in Milada and Most show signs of early divergence to ecologically distinct morphs as observed in several postglacial fishes (Schluter, 1996). However, the fact that generalist foraging behaviour was exhibited by some individuals perhaps suggests that the observed intraspecific niche segregation reflects opportunistic behaviour by individuals rather than emergence of distinct morphs. Telemetry and video recording studies could reveal whether ruffe undergo diurnal or seasonal habitat shifts even if they predominantly feed on either shallow-water or deep-water food resources.

4.2 | Interspecific interactions and impact of lake characteristics

The littoral zone is commonly the most diverse and productive habitat, particularly in oligotrophic clear-water lakes (Vadeboncoeur et al., 2002; Wetzel, 2001). However, the littoral resources may become less accessible for fish if the littoral zone is inhabited by competitive or predatory species (Werner & Hall, 1979). Our results correspond to previous observations from lakes with relatively diverse fish communities, where ruffe seem to avoid resource competition and predation through frequent utilisation of the profundal habitat and food resources (Gutsch & Hoffman, 2016; Hayden et al., 2013; Hölker & Thiel, 1998). The elevated TP (estimated from $\delta^{15}N$ values) of ruffe in Milada and Most is probably mainly a consequence of substantial foraging on profundal chironomids with high $\delta^{15}N$ values (Figure S2), but may also partly reflect temporary foraging on fish eggs (Adams & Tippett, 1991; Gutsch & Hoffman, 2016; Ogle, 1998) or cannibalism on ruffe under-yearlings. No such prey were found from ruffe stomachs in Most (Table S2), but that was to be expected because all fish species in the lake, except whitefish, spawn in spring or early summer, long before our sampling of ruffe in August. However, substantial predation on eggs and/or under-yearlings in early season could elevate $\delta^{15}N$ values of ruffe muscle tissue which, followed by reduced feeding rate and slowed isotopic turnover of the muscle tissue in mid-summer when most benthic insect larvae have hatched, could lead to persistence of high $\delta^{15}N$ values through late summer. Further studies are needed to verify the occurrence of such feeding behaviour by ruffe and its possible impacts on lake community and ecosystem processes.

In contrast to ruffe, and as hypothesised, perch showed only minor intraspecific niche segregation along the littoral–profundal resource axis and only minor dietary overlap with ruffe due to their more prominent foraging on pelagic food. Hence, in conjunction with previous observations (Hayden et al., 2013; Hölker & Thiel, 1998; Schleuter & Eckmann, 2006), our study demonstrates clear differences in niche use and adaptive potential between the two percid species, with ruffe being clearly a mechano-sensory oriented benthivore and perch being a visually oriented predator that frequently feeds on zooplankton (Bergman & Greenberg, 1994). When compared to other common generalist percid and cyprinid species found in European temperate lakes, perch seems competitively dominant due to its exceptionally wide potential trophic niche, including

piscivory (Persson & Hansson, 1999). Moreover, perch commonly exhibit dietary and morphological adaptations along the littoral–pelagic resource axis, with littoral-dwelling individuals feeding mainly on large benthic macroinvertebrates and having a more robust body shape as compared to their predominantly zooplanktivorous, more slender pelagic-dwelling conspecifics (Bartels, Hirsch, Svanbäck, & Eklöv, 2012; Quevedo et al., 2009), although no such littoral–pelagic specialisation in perch were observed in our study lakes. However, perch, and particularly juveniles and spawning individuals, may still use the deep and often hypoxic profundal zone as a refuge from predation (Čech, Kratochvíl, Kubečka, Draštík, & Matěna, 2005; Čech et al., 2010; Vejřík et al., 2016).

Our survey fishing and stable isotope data indicate between-lake differences in ruffe niche use, which probably result from the contrasting biotic and abiotic characteristics of the study lakes. Compared to conspecifics in Most, ruffe in Milada seemed to be subjected to stronger interspecific interactions because abundant competitive percid and cyprinid species as well as predatory pike inhabited the littoral zone (Table S1). Overall, the ruffe population in Milada seemed to be forced towards a more profundal trophic niche as indicated by the relatively low δ^{13}C and high δ^{15}N values (Figure S1) as well as by the derived SIAR estimates. In contrast, maraena whitefish seemed to dominate in the deepest habitats in Most so that deep-water dwelling ruffe in that lake may be subjected to stronger interspecific resource competition. Although we lack data on profundal bottom substrata and prey resources, the oligotrophic and deep Most with its steep bathymetry (see Figure 3 in Vejříková et al., 2016) and cold profundal zone (Figure S1) may provide less profitable profundal resources for ruffe than the more productive Milada. This could partly explain the predominantly littoral niche of ruffe in Most and their less pronounced littoral–profundal niche segregation. In some highly eutrophic lakes with an anoxic profundal zone, ruffe find a suitable, under utilised niche by feeding on large profundal chironomids, which, in turn, can be fuelled by methane-derived carbon pathways (Jones & Grey, 2011; Ravinet et al., 2010). However, despite poor oxygen conditions below 15 m depth in Milada (Figure S1), the relatively high δ^{13}C and δ^{15}N values of profundal chironomids and other invertebrates observed in both study lakes suggest only a minor contribution of methane-derived carbon pathways to the lake food webs (cf. Grey, 2016; Jones & Grey, 2011).

4.3 | Conclusions and future research

Our results suggest that habitat availability and interspecific resource competition affect the ecological opportunity (cf. Losos, 2010) for intraspecific niche segregation and hence littoral–profundal habitat coupling by ruffe. Besides highlighting the largely neglected role of profundal trophic pathways in some lake ecosystems, ruffe is evidently a species well-adapted to feed in cold, dark, and low-oxygen conditions (Hölker & Thiel, 1998; Schleuter & Eckmann, 2006). This ability to exploit profundal resources and particularly the widely available chironomid prey, as well as tolerance of

variable abiotic conditions, seems to account for the wide natural distribution and successful invasion of ruffe to various lake ecosystems (cf. Faulks, Svanbäck, Ragnarsson-Stabo, Eklöv, & Östman, 2015; Gutsch & Hoffman, 2016; Hayden et al., 2015). Predicted browning of lakes in the northern hemisphere, resulting from climate change and increased loading of terrestrial organic matter (Larsen, Andersen, & Hessen, 2011), may further increase the relative availability of profundal food and habitat resources and hence encourage competitive dominance of ruffe, but also reduce intraspecific niche segregation as observed in perch (Bartels et al., 2012). Hence, future ecological studies of ruffe will be essential for understanding and predicting the impacts of this invasive species on native communities and lake ecosystems, including potential predatory impacts on methanotrophic organisms and thereby on lake methane emissions (cf. Devlin et al., 2015). Moreover, seasonal sampling and/or simultaneous analysis of another tissue with a shorter isotopic turnover rate, such as liver (Layman et al., 2012 and references therein), could indicate whether the high estimated TP of ruffe is temporally stable or reflects only a brief but substantial predation on eggs and/or under-yearlings in early growing season. Finally, genetic and morphological studies could confirm whether shallow- and deep-water dwelling ruffe can show signs of early divergence as observed in salmonids (e.g. Harrod et al., 2010; Klemetsen, 2010; Muir et al., 2015).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Paper V

Succession of submerged vegetation in a newly created lake with nutrient level decrease over a ten-year monitoring period

Succession of submerged vegetation in a newly created lake with nutrient level decrease during a ten-year monitoring period

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Running title: Factors influencing macrophyte succession

Keywords: Characeae, environmental factors, macroalgae, macrophytes, species composition, *Vaucheria*

Summary

1. Succession of submerged vegetation is a dynamic process that is affected by many characteristics and so it is commonly difficult to distinguish the main driving factors.

2. The succession from the early stages in a newly created lake was monitored for ten years by SCUBA divers. The main focus was on apparent changes in the percentage cover of primary producers (macrophytes and macroalgae). In total, data on 33 environmental factors (chemical, physical and biological) were collected and the importance of their impact was statistically analyzed. Besides macrophytes, we also monitored macroalgae that mainly occupied deep areas (down to 12 meters) and were neglected in literature in contrast to macrophytes.

3. Uniquely the trophic state of the study site decreased in contrast to most water bodies worldwide. Changes in the species composition of vegetation cover during the succession, including replacement of the dominant group of Characeae with *Vaucheria* sp. (both macroalgae), correlated mainly with a decrease in total nitrogen. Further, the biomass of herbivorous fish and also availability of uncovered lake bottom played important roles in the succession of macrophytes and macroalgae.

4. Even some changes in submerged vegetation during the succession of newly created lake could be expected in any case, the main shifts apparently correlated with studied factors. The main impact of nutrient level decrease was according to our expectations.

Introduction

Disturbances and particularly formation of new ecosystem bring partial or total removal of primary producers and initiate succession (Glenn-Lewin & Van der Maarel, 1992; Johnson and Miyanishi, 2007). The entire succession dynamic is based on well-known trade-offs and constraints (Connell & Slatyer, 1977; Tilman, 1990), the most important of which is the competition-colonisation trade-off (Levins & Culver, 1971, Wilson, 2011). Pioneer species dominate during the initial succession stages due to their high fecundity and ability to spread readily. In contrast, diaspore production and dispersibility of the best competitors are usually low. Moreover, recurrence of disturbances does not allow them to reach reproductive maturity. The best competitors dominate in stable ecosystems and set up a so-called climax community (Roxburgh et al., 2004; Cadotte, 2007; Marleau et al., 2011).

Primary producers have to invest their energy, either into to the fast growth that is necessary for success in competition, or into the activation of chemical defences against herbivores. These rules are well-known for terrestrial ecosystems (e.g. Herms & Mattson, 1992), however, similar processes in aquatic ecosystems have only been described relatively recently (Dorenbosch & Bakker, 2011; Kapuscinski et al., 2014).

In terms of submerged vegetation, the pioneer community of freshwater ecosystems in the temperate climate zone commonly includes Characeae (Portielje & Roijackers, 1995). After the pioneer stage, Characeae usually disappear and are replaced by several species of macrophytes. The rate of the transition positively correlates with nutrient level and with the colonization rate of new diaspores (Krahulec et al., 1987; Portielje & Roijackers, 1995). The most common means of dispersion of seeds, oospores and vegetative propagules of submerged macrophytes is by water, followed by wind and animals (Boedeltje et al., 2003, Soons et al., 2008; Xie et al., 2010). As in terrestrial ecosystems, the early succession stages in aquatic ecosystems are the most diverse in terms of number of species (Willby et al., 2001; Murphy, 2002). However, the macrophyte community is generally poorer in species in comparison to terrestrial ecosystems (Edvardsen & Okland, 2006).

When the nutrient level increases, submerged macrophytes are often replaced by floating or emergent macrophytes (Krahulec et al., 1987; Portielje & Roijackers, 1995) or by periphytic and filamentous algae. Consequently, this turns into phytoplankton blooms, the most common problem connected with eutrophication (Phillips et al., 1978; Jeppesen et al., 1992; Ibelings et al., 2007). In a eutrophic state, the survival of primary producers depends on their ability to compete for light, carbon and space. Therefore, the production zone

moves towards or even above the water surface (Den Hartog & Segal, 1964; Carignan & Kalff, 1980; Portielje & Roijackers, 1995) due to light interception and availability of atmospheric carbon. Enhanced primary production increases the pH level, which in turn enhances the CO_2 flux from the atmosphere (Portielje & Lijklema, 1995). In a low trophic state, the rooted macrophytes utilize nutrients from the sediment (Barko & Smart, 1980; Carignan & Kalff, 1980), however, they are capable of significant nutrient uptake from the water column (Phillips et al., 1978; Agami & Waisel, 1986). At an intermediate trophic state, nutrient utilization from the sediment and the water column can occur alternately. Thus, the importance of the root system decreases with increasing trophic state of the water (Portielje & Roijackers, 1995).

Light is definitely the key physical factor for primary producers, and absence of light due to turbidity or shading is the main limiting factor (Bakker et al., 2013). Therefore, high biomass of vegetation cover does not always mean high species richness, but only several species that were able to compete and survive the conditions (Hilt, 2006). In terms of chemical factors, nitrogen concentration (in the form of nitrate $N-NO_3$) is a major predictor of vegetation cover, more important than the concentration of phosphorus in the water (James et al., 2005; Lambert & Davy, 2011). With higher nitrate availability higher competition pressure occurs and leads to the mentioned prevalence of floating macrophyte species or phytoplankton algae that results in light limitation. A decrease in macrophyte species richness occurs commonly under the threshold concentration of 1–2 mg $N-NO_3$ L^{-1} in winter (James et al., 2005) or 0.6 mg $N-NO_3$ L^{-1} under experimental conditions (Barker et al., 2008). Nitrate can even have a toxic effect on Characeae (Lambert & Davy, 2011). The observed decrease in Characeae species richness was above the mean annual concentration threshold of ca. 2 mg $N-NO_3$ L^{-1} (Lambert & Davy, 2011). Changes in physical and chemical factors may also be induced by changes in biological factors. For instance, predation on zooplankton by planktivorous fish may increase turbidity, or feeding of benthic animals from the bottom intensifies sediment resuspension (Jeppesen et al., 1997; Gulati & van Donk, 2002).

Biomass and species composition of vegetation cover can also be influenced directly by grazing of herbivorous fish and waterfowl depending on the locality (Moore et al., 2010; Vejříková et al., 2018). In Lake Zwemlust in the Netherlands, the macrophyte vegetation was markedly utilized by coots (*Fulica atra*) and rudd (*Scardinius erythrophthalmus*) that altered the dominance of *Elodea nutallii* to codominance by *Ceratophyllum demersum* and

4

Potamogeton berchtholdii (Van Donk & Otte, 1996). Another biological factor affecting species richness is allelopathy, it has been documented in all groups of primary producers (cyanobacteria, micro- and macroalgae as well as angiosperms), and mostly has an inhibitive effect (Gross, 2003; Hilt & Gross, 2008). Further, field studies have proved that submerged vegetation is also dependent on many hydrological factors such as lake variables, including lake area, altitude, shoreline complexity, connectivity, conductivity, water quality and sediment composition (Bakker et al., 2013 and related studies).

The number of studies focusing on submerged vegetation is increasing (Bakker et al., 2013; Bakker et al., 2016), however, the presence of macroalgae (except Characeae) in aquatic ecosystems is under-examined despite the fact that they are common and visible macroscopically (Messyasz et al., 2018). Several species of macroalgae, e.g. genus *Vaucheria*, are able to survive in seasons and habitats that are fatal for macrophytes. Specifically, under conditions with low temperature and low light intensity such in the cold season or in habitats at greater depths (Schagerl & Kerschbaumer, 2009; Messyasz et al., 2018). In addition, the genus *Vaucheria* sp. has recently become a model organism in cell physiology, due to its large siphonous cell tubes suitable for micromanipulation experiments (Fischer-Arnold, 1963; Mine & Okuda, 2003). Nevertheless, a number of studies focused on the ecology of macroalgae as primary producers and provided under natural conditions is limited (Schagerl & Kerschbaumer, 2009).

The present study is focused on vegetation succession in a newly created lake with four abundant species of macrophytes, two species of macrophytes with rare presence, and two important species of macroalgae because of their dominance during monitoring. Monitoring of a wide spectrum of environmental factors (chemical, physical and biological) brings knowledge about their importance during succession and apparently reveals the triggers of species transition within succession. Based on literature, we supposed the largest impact to be that of nutrient level (e.g. Portielje & Roijackers, 1995; Lambert & Davy, 2011). Most studies conducted under natural conditions describe situations with trophic state increase, whereas nutrient level decrease was recorded in our study site. Further, we expected a significant impact from grazing by herbivores, which was observed in a manipulative experiment (Vejříková et al., 2018), and also of competition for light due to the possible fatal consequences of the shading effect (Stiers et al., 2011; Bakker et al., 2013).

Methods

Study site

The study was conducted in Milada Lake (50°39′N, 13°58′E), Czech Republic, that was newly created by the flooding of an abandoned open cast brown-coal mine (Fig. 1). It is an oligotrophic lake with mean summer total phosphorus (TP) in the surface layer of <10 µg L^{-1}. It has an area of 250 ha, a volume of 0.036 km^3 and a maximum depth of 25 m. Aquatic restoration of the coal mine started in 2001 and was finished in 2010 with the final water level of 146 m a.s.l.

The annual volume of water inflow varied during hydric revitalization (Fig. 2). 41% of the water volume between the years 2007 and 2009 was supplied by controlled inflow from Kateřina Reservoir. In 2010, only 10% came from Kateřina Reservoir and the remainder from the catchment basin. The highest water inflow from the catchment basin was in 2010, 60% more than in other years between 2007 and 2016 (Fig. 2; Rous, 2016).

Macrophytes and macroalgae were present to a depth of 12 m. Macrophytes consisted of *Myriophyllum spicatum*, *Potamogeton pectinatus*, *P. crispus*, *Elodea canadensis*, and two rare species *Myriophyllum verticillatum* and *P. pusillus*. The macroalgae present are Characeae (dominated by *Chara globularis* > 90%) and *Vaucheria* sp. (Vejříková et al., 2018).

The Fish community consisted of rudd (considered to be the main herbivorous species), roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), ruffe (*Gymnocephalus cernua*), pike (*Esox lucius*), European catfish (*Silurus glanis*), tench (*Tinca tinca*) and pikeperch (*Sander lucioperca*).

Monitoring of submerged vegetation

Monitoring of submerged vegetation was conducted annually in September 2007–2016. Monitoring started at a depth of 2 meters because a steep stone wall to a depth of 2 meters was created encircling the entire lake to prevent damage to the shoreline by waves. Below 2 meters the lake bottom is covered with sediment and has a gradual slope. To obtain a qualitative assessment of submerged vegetation, two SCUBA divers visually assessed the occurrence of the species present. Monitoring was conducted on ten transects evenly distributed along the lake (Fig. 1), however between 2007 and 2011 fewer number of transects were monitored in exceptional circumstances (not less than five evenly distributed along the lake). Submerged vegetation commonly did not outreach 12 m, only in a few

6

cases, macroalgae cover continued to a depth of 13.5 m. The percentage cover of each species, and the percentage of uncovered bottom area were recorded at 1-m depth intervals. The results of duplicate measurements were averaged for a more accurate assessment.

Monitoring of environmental factors

Between the years 2002–2015, chemical parameters were analyzed monthly from water sampled at 2-m intervals from three profiles marked by anchored buoys. From 2016, chemical parameters were analyzed only from the middle profile and three depths (water surface, middle, bottom; Fig. 1). All controlled inflows were concurrently monitored. Monitoring of 27 chemical parameters was conducted by the company Labe River Authority, state enterprise, OVHL Hradec Králové according to the Czech technical standards (BOD 5, pH, conductivity 25, ANC-4.5, BNC-8.3, N-NO$_2$, N-NH$_4$, N-NO$_3$, TN, N org. drain, TP, Cl, SO$_4$, DOM, POM, CHOD Cr, HCO$_3$, CO$_3$, TOC, CO$_2$ dis., Na, K, Fe, Mn, Ca, Mg, Chlorophyll a). The most important chemical parameters were determined as follows: Total nitrogen (TN) by continuous flow analysis and spectrophotometric method, bicarbonate (HCO$_3^-$) using acid neutralizing capacity and pH, Ca^{2+} by complexometric titration and TP by continuous flow spectrophotometry. TP, during most measurements, was under the detection limit (< 10 μg L^{-1}) and no trend of decrease/increase could be recorded, thus total phosphorus could not be included in the statistical analysis due to inapplicable information. To simplify graphical interpretation we present only representative years 2007, 2010, 2013 and 2016 (Fig. 5, Tab. 2).

The fish community (including herbivorous rudd used as an environmental factor) was sampled annually in September by standardized multi-mesh gillnets. For details see Vejříková et al. (2016).

Further, volumes of water inflows, volume percentage of new water, water level and percentage of uncovered bottom were used as environmental factors (see Study site and Monitoring of submerged vegetation for description).

Statistical analysis

Redundancy analysis (RDA) was used to test the effects of environmental factors on the percentage cover of each species within the monitored years. The environmental variables were tested by forward selection on centered response variables. Statistical significance

was analyzed using Monte Carlo permutation tests (number of permutations: 4999). RDA was carried out using CANOCO5 (Šmilauer & Lepš, 2014).

A chisquare test ($\chi 2$) was used to compare the percentage cover of macrophytes versus macroalgae in three depth profiles (2–4, 4–6 and 6–8 m) in four representative years (2007; 2010; 2013; 2016). A paired *t*-test was used to compare percentage cover of Characeae and *Vaucheria* sp. in the east versus west side of lake. The chisquare test ($\chi 2$) and *t* test were conducted using Statistica 13 (TIBCO Software Inc., 2017).

Results

<u>Changes in species composition of submerged vegetation during succession</u>

Three apparent changes in species composition of submerged vegetation were observed between years the 2007 and 2016 in Milada Lake. The first apparent change was observed in 2008 when a marked decrease in cover of *P. crispus* and *M. spicatum* was recorded. In 2007, 61.6 % of total cover was comprised of *P. crispus* and *M. spicatum* (Fig. 3). According to RDA (forward selection), these species were positively correlated with three environmental factors (Fig. 4). Out of 78.3% of total exploratory variables, 11.7%, 7.8% and 5% were explained by biomass of herbivorous fish, percentage of uncovered bottom and level of HCO_3^-, respectively. However, the impacts were statistically insignificant ($p \geq 0.08$) due to the high statistical significance of the level of TN that plays an important role in the following change (see below). Trends of these environmental factors are illustrated in Table 1. From 2008, macroalgae dominated in the lake, at first Characeae which were later replaced with *Vaucheria* sp. Macroalgae increased from 64.6% (2010) to the highest percentage cover of 91.4% in 2012 (Fig. 3).

The Second significant change was observed in 2011 when *Vaucheria* sp. appeared and its cover rapidly increased in the following years. *Vaucheria* sp. comprised 9.1% and 60.8% of total vegetation cover in 2011 and 2016, respectively. *Vaucheria* sp. dominated in deep parts (6–8, 8–10 m) where no macrophytes prospered, similar to Characeae. Percentage cover of Characeae gradually decreased from 72% in 2011 to 10.5% in 2016 with increasing cover of *Vaucheria* sp. The transition was highly correlated with the level of TN in the water. The level of TN started to decrease from 2010 when controlled flooding was stopped (Fig. 2). As revealed by RDA (forward selection) the decrease in TN was the key and highly significant factor affecting the succession of submerged vegetation in the lake

8

(F=6.6; p=0.0002). The level of TN explained 45.3% of total variability. A similar trend was recorded for the decrease in Ca^{2+} in water, however, only 8.4% of total variability was explained by this environmental factor (p=0.2; Fig. 4). See Table 1 for trends of the environmental factors including TN and Ca^{2+}.

The third apparent change occurred in 2013 when *P. pectinatus* was first recorded with cover 4.9%, the cover increased to 25.6% in 2015 (Fig. 3). In contrast to the increase of *P. pectinatus*, a decrease in *E. canadensis* (11.2% in 2009, 0% in 2014) and *M. spicatum* (19.1% in 2013, 6.1% in 2016) was recorded. The change was caused by competition among species caused by a decrease in TN, biomass of herbivorous fish and percentage of uncovered bottom. All mentioned changes led to the dominance of *Vaucheria* sp. (60.8%) in 2016, followed by *P. pectinatus* (22.6%).

<u>Depth and spatial distribution of species of submerged vegetation in the lake</u>

Percentage cover of uncovered bottom decreased and also submerged vegetation spread to deeper parts with time (Fig. 5). Maximum depth of occurrence of *Vaucheria* sp. and Characeae in 2016 was 13.5 and 12.7 m, respectively. Occurrence of macrophytes versus macroalgae varied with the depth gradient. Macroalgae dominated mainly at depth 6–8 m with the exception of 2007 when macroalgae dominated at 4–6 m depth. In contrast, macrophytes dominated mainly at 2–4 m. All mentioned dominances were statistically significant (Tab. 2). No species of macrophytes occurred deeper than 8 m.

Significant differences in the spatial distribution of both types of macroalgae were found. *Vaucheria* sp. was first recorded in 2011 in the west side of the lake, and its percentage cover was significantly higher in the west than in the east for all monitored years (t=3.55, df=5, p<0.05). In contrast, the presence of Characeae was lower in the west than in the east side (t=5.47, df=5, p<0.005; Fig. 6). Moreover, a decrease in Characeae with time was more apparent in the west side. This trend correlated with the difference in TN, because the level of TN was slightly (but significantly) lower in the west side with higher occurrence of *Vaucheria* sp. than in the east side (t=3.78, df=4, p<0.05; Fig. 6).

Discussion

Our study was conducted in a newly created lake and thus changes in the macrophyte community could be expected in any case. However, observed correlations, especially correlation with nutrient level decrease, apparently influenced the course of the succession.

9

Succession of primary producers in a freshwater ecosystem is often connected with an increase of trophic state (Portielje & Roijackers, 1995) that leads to the very common problem of eutrophication (Van Straten, 1986). In contrast, the trophic level decreased in Milada Lake. In the initial succession stage, the high influx of nutrients was caused by inflow from Kateřina Reservoir and by apparent degradation of terrestrial plants that were suddenly submerged (pers. observ.). Subsequently, nutrient level decreased due to accumulation in sediment and in aquatic organisms. Thus, the main changes in submerged vegetation were connected with a decrease in nutrient level, in contrast to most freshwater bodies.

High percentage cover of *P. crispus* and *M. spicatum* was already observed before monitoring in 2006 (Čech et al., 2009). During the first monitored year 2007, *P. crispus* and *M. spicatum* comprised 62% of total cover. However, rapid decrease was recorded in 2008 for both *P. crispus* (from 30% to 1%) and *M. spicatum* (from 31% to 11%) and is described in Čech et al. (2010). In contrast, percentage cover of Characeae increased from 34% to 80%. This transition was connected with a biomass decrease in herbivorous fish probably caused by stocking of predatory fish (Vejřík et al., 2017). Consequently, biomass of palatable *E. canadensis* (Van Donk & Otte, 1996) and Characeae (Lake et al., 2002) increased due to decreased pressure from herbivorous fish. *E. canadensis* and Characeae covered a substantial part of the uncovered bottom (Čech et al., 2011), and thus competition for light, carbon and the available space increased (Den Hartog & Segal, 1964; Carignan & Kalff, 1980). This is in accordance with the results of our underwater manipulation experiment. *M. spicatum* and *P. crispus* were positively correlated with a decrease in competition by the removal of macroalgae (Vejříková et al., 2018). High abundances of *M. spicatum* and *P. crispus* in the presence of high biomass of herbivorous fish in the initial stage of succession were apparently caused by the chemical and physical defence of both species. *M. spicatum* contains high concentrations of phenolic compounds (Dorenbosch & Bakker, 2011; Kapuscinski et al., 2014) and *P. crispus* tries to defend itself with tough and partially spine-like leaves (Vejříková et al., 2018). Other species from the lake lack similar defence mechanisms. The second reason for the decrease and subsequent vanishing of *P. crispus* is the decrease in nutrient levels given that this species prefers a high trophic state (Cao et al., 2018).

The second and most apparent change in appearance and the ready dominance of *Vaucheria* sp., and by contrast decrease in Characeae corresponded with a change in hydrological

level. Controlled inflow from Kateřina Reservoir was stopped in 2010. The water from Kateřina Reservoir was rich in nutrients and the nutrient level started to decrease rapidly from 2012. During the rapid decrease in nutrient level, the percentage cover of Characeae decreased from 65% to 11% in 2016. The same trend continued and no Characeae were found in the lake in 2018 (Vejřík, L. & Peterka, J. unpubl. data). In contrast, percentage cover of *Vaucheria* sp. increased from 11% in 2011 to 61% in 2016.

Although, Characeae commonly dominate in low trophic lakes (Portielje & Roijackers, 1995; Van den Berg et al., 1999) and also have strong allelopathic activity (Vermaat et al., 2000; Hilt & Gross, 2008), this group was replaced by *Vaucheria* sp. The transition happened probably due to a large decrease in level of $N-NO_3$ to 0.35 mg L^{-1} in 2016, when $N-NO_3$ decreased under critical limit due to accumulation of present $N-NO_3$ to other primary producers and low input of new nutrients from tributaries. Characeae are very sensitive to high concentrations of $N-NO_3$, however, they are not able to grow in water with too low concentrations. The optimal level for Characeae seems to be 0.5–2 mg L^{-1} (Lambert & Davy, 2011). *Vaucheria* sp., similar to Characeae (Van den Berg et al., 1999), has more intensive growth in winter than in summer (Ozimek, 2006). Therefore, $N-NO_3$ from dead macrophytes during the winter season is readily accumulated by *Vaucheria* sp. (Vejříková in prep.). *Vaucheria* is able to accumulate redundant nutrients in a nutrient sink (Simons, 1994) and thus it can grow in waters with very low nutrient levels (Schagerl & Kerschbaumer, 2009). The question that is unresolved is whether Characeae were not suppressed by allelopathy from *Vaucheria* sp. Unfortunately, allelopathic compounds have not been tested in our study and information about this species is still limited (Messyasz et al., 2018). However, the major impact of level of nitrogen on Characeae and *Vaucheria* sp. occurrence is also indicated by the spatial distribution of macroalgae. Higher occurrence of *Vaucheria* sp. was recorded in the west side of the lake with lower concentration of TN, in contrast to Characeae showing higher abundances in the east side of the lake.

Third apparent change during succession was the occurrence of *P. pectinatus* in 2013 and gradual increase in its percentage cover. Due to the decrease in nutrient level in the lake, *P. pectinatus* was placed in a better position than *E. canadensis,* which has higher nutrient requirements (Portielje & Roijackers, 1995). Moreover, *P. pectinatus* profited from the decrease in biomass of herbivores caused by predatory fish (Vejřík et al., 2017). *P. pectinatus* is a competitively successful species (Vejříková et al., 2018) that is, however, vulnerable to grazing (Hilt 2006; Vejříková et al., 2016) mainly due to no chemical defence

(Dorenbosch & Bakker, 2011). For instance, the biomass of *P. pectinatus* decreased by over 90% due to grazing, recorded by Hilt (2006). Further, preference of *P. pectinatus* by fish was observed in Matsalu Bay in Estonia (Hidding et al., 2010A), and in Lauwersmeer in The Netherlands (Hidding et al., 2010B). Low herbivorous pressure positions *P. pectinatus* as a more competitively successful species than *M. spicatum* that must invest energy in chemical defence (Vejříková et al., 2018).

The area of uncovered bottom decreased during succession. Therefore, competition among species increased with increasing shading effect of competitively successful species (Bakker et al., 2013). This is probably the reason why macroalgae smaller in size occurred in deeper parts where macrophytes do not meet the requirements for photosynthetically active radiation (Portielje & Roijackers, 1995). Macroalgae such as Characeae (Van den Berg et al., 1999; Van Nes et al., 2002; Bakker et al., 2010) and *Vaucheria* sp. (Schagerl & Kerschbaumer, 2009) have low requirements for temperature and photosynthetically active radiation. Thus, the fluctuation of biomasses is low during the year, they can survive in winter and also in deeper parts than macrophytes. Specifically, *Vaucheria* sp. has the typical ability to absorb blue light (Kataoka et al., 2001). This depth segregation of macrophytes and macroalgae has a positive impact on the structuring of the ecosystem that leads to an increase in biodiversity in the lake (Jeppesen et al., 1992).

Important impact of nutrients, herbivory and competition on succession of Milada macrophyte community was according to our expectations. However, only nutrient level was statistically significant, specifically, the level of total nitrogen that decreased during succession. The importance of nutrient level is in accordance with Barker et al. (2008) and Lambert and Davy (2011), which we proved for natural succession in a lake with nutrient level decrease. The other environmental factors, specifically herbivory and competition (presence of uncovered bottom), were statistically insignificant most probably due to the high significance of TN. Nevertheless, the impact of these factors is apparent and is in accordance with other studies, namely with Marklund et al. (2002) and Vejříková et al. (2018) in terms of herbivory and Stiers et al. (2011) and Bakker et al. (2013) in terms of competition.

Acknowledgments

Data availability

The dataset analyzed during the current study is available on reasonable request.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Author Contributions

I.V., L.V., M.Č., M.Ř. and J.P. participated in the field work. I.V., L.V. and J.P. designed the study. L.V. conducted the statistical analysis. I.V. and L.V. wrote the manuscript. All authors contributed substantial comments during manuscript preparation.

13

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20

Table 1. Five environmental factors (total nitrogen (TN), herbivorous fish, uncovered bottom, bicarbonate (HCO_3^-) and Ca^{2+}) with major impact on succession of submerged vegetation in Milada Lake according to RDA (forward selection). $N-NO_3$ comprised 87% of TN in 2007 with gradual decrease to 43% in 2016.

Year	TN	Herbivorous fish	Uncovered bottom	HCO_3^-	Ca^{2+}
	mg L^{-1}	kg 1000 m^{-2} of nets	%	mg L^{-1}	mg L^{-1}
2007	2.98	38.01	70.30	324.21	58.44
2008	2.34	6.91	46.47	309.05	56.85
2009	2.68	6.55	18.17	295.97	56.23
2010	2.50	8.85	22.08	303.71	55.26
2011	3.20	17.36	13.66	304.07	59.13
2012	2.29	25.86	5.23	296.50	50.92
2013	1.88	9.05	15.09	294.86	50.16
2014	1.44	8.26	8.89	300.98	51.96
2015	1.15	2.15	22.30	297.62	46.33
2016	1.11	1.07	5.11	297.62	72.57

Table 2. Statistical significance of the highest percentage cover of macrophytes and macroalgae at given depth profiles in 2007, 2010, 2013 and 2016.

Year	Dominating group	Depth (m)	$\chi 2$	p
2007	Macrophytes	2–4m	104	$p<0.001$
	Macroalgae	4–6m	61	$p<0.01$
2010	Macrophytes	2–4m	92	$p<0.001$
	Macroalgae	6–8m	23	$p<0.05$
2013	Macrophytes	2–4m	131	$p<0.001$
	Macroalgae	6–8m	13	$p<0.005$
2016	Macrophytes	2–4m	93	$p<0.001$
	Macroalgae	6–8m	10	$p<0.05$

22

Figure legends

Figure 1. Location of Milada Lake in the Czech Republic and bathymetric map of the lake with ten transects (numbers 1–10) of macrophyte monitoring, and three profiles (A, B, C) where water was sampled for subsequent chemical analyses.

Figure 2. Volume of water inflow from Kateřina Reservoir (black), deep wells (dark grey) and from the rest of the catchment basin (light grey) in 2007–2016. The white line shows surface water altitude and the black dashed line shows level of total nitrogen in the lake (TN, mg L^{-1}).

Figure 3. Species composition of submerged vegetation in Milada Lake throughout ten year monitoring period counted as weighted average from depths 2−10 m: Characeae (black)*, Vaucheria* sp. (grey), *Potamogeton crispus* (dash), *Myriophyllum spicatum* (white), *Elodea canadensis* (dots), *P. pectinatus* (bricks). Rare species *P. perfoliatus* and *M. verticillatum* (cover<1% in all years) were not included.

Figure 4. Redundancy analysis (RDA) of the percentage cover of six macrophytes and two macroalgae in Milada Lake in relation to level of total nitrogen (TN), level of calcium (Ca^{2+}), level of bicarbonate (HCO_3^-), biomass of herbivorous fish and percentage of uncovered bottom throughout years 2007–2016.

Figure 5. Decrease of uncovered bottom (in percentage, grey dash) and by contrast spreading of submerged vegetation to deeper parts (depths 2–4, 4–6, 6–8 and 8–10 m) with time in a) 2007, b) 2010, c) 2013, and d) 2016 is illustrated. Percentage cover of *Myriophyllum spicatum* (white), *Potamogeton crispus* (diagonal dash), *Elodea canadensis* (dots), Characeae (black)*, Vaucheria* sp. (grey), *Potamogeton pectinatus* (bricks) is shown.

Figure 6. Percentage cover of dominant macroalgae *Vaucheria* sp. (grey) and Characeae (black), and macrophytes (white) in 2011–2015 from depths 2–10 m on c) west and d) east side of the lake. Level of total nitrogen (TN, mg L^{-1}) by year is present.
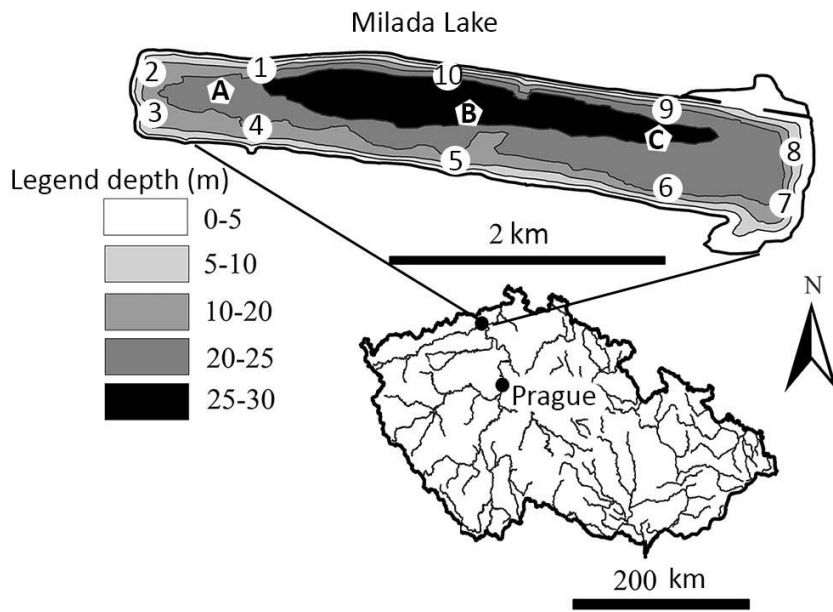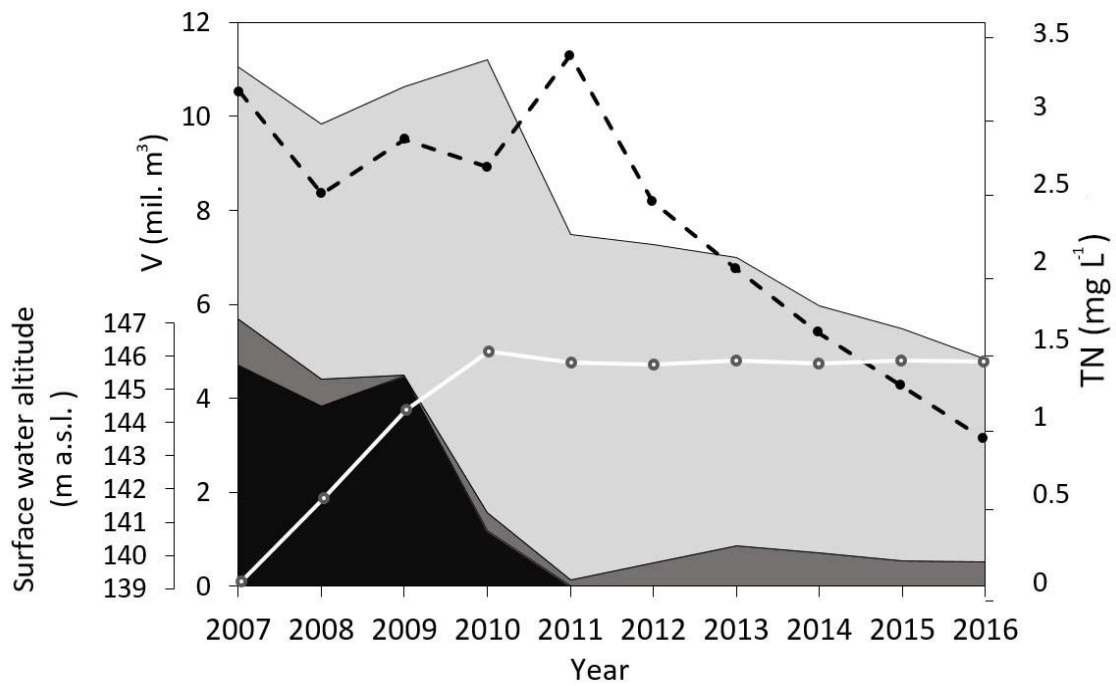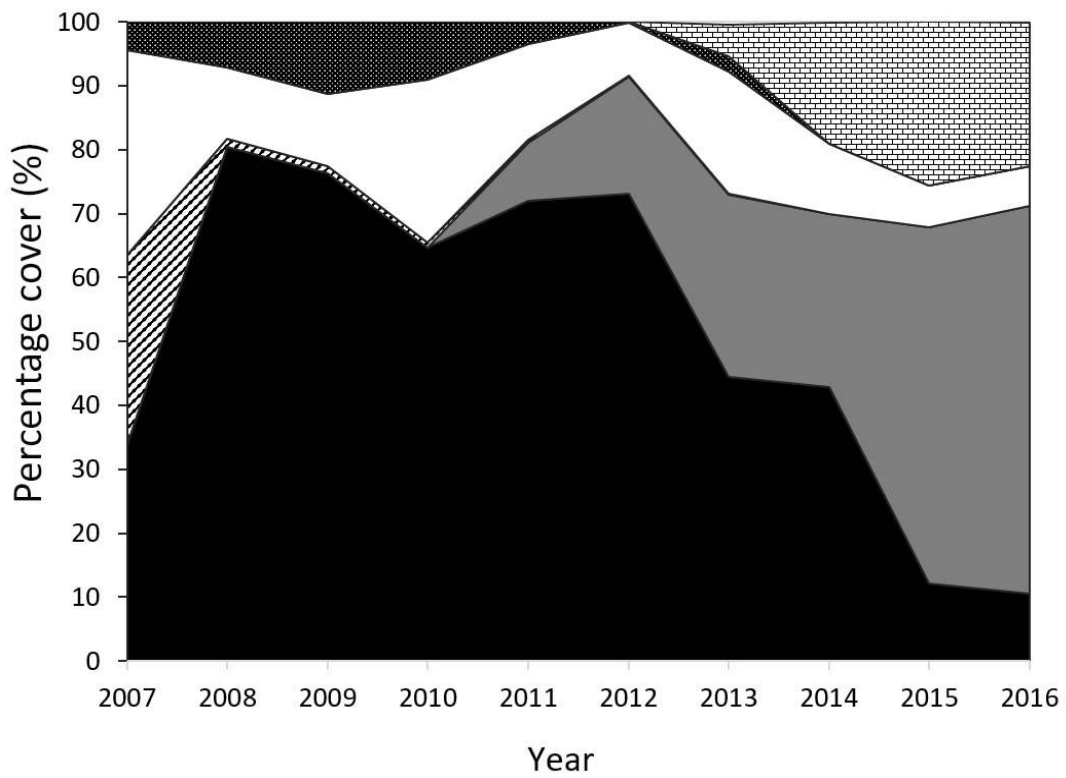
23

Figure 1.

Milada Lake

Legend depth (m)
- 0-5
- 5-10
- 10-20
- 20-25
- 25-30

2 km

N

Prague

200 km

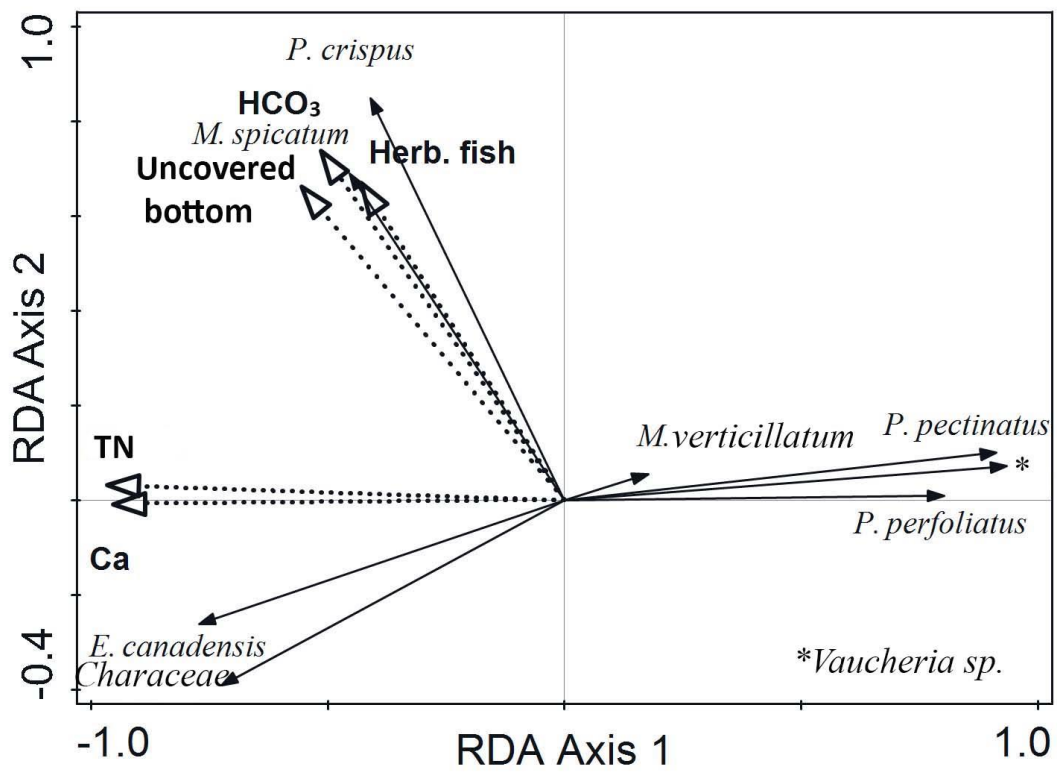**Figure 2.**

Figure 3.

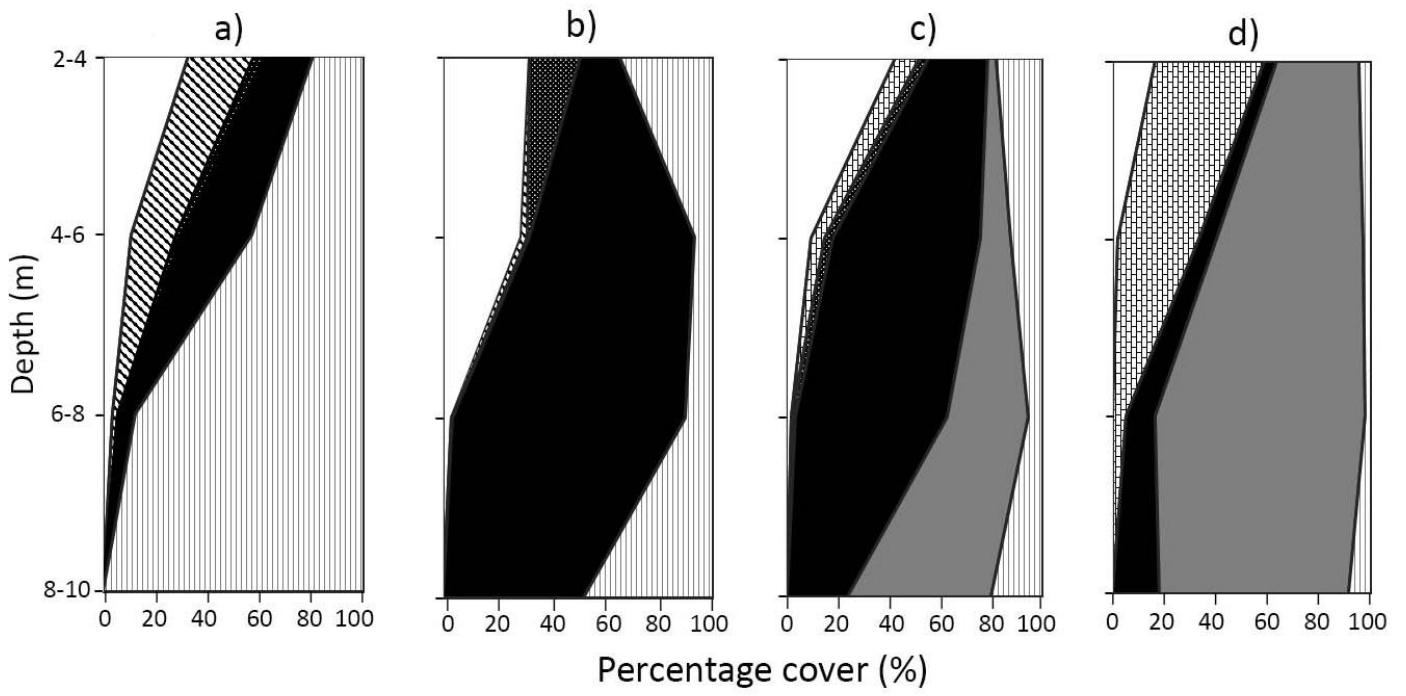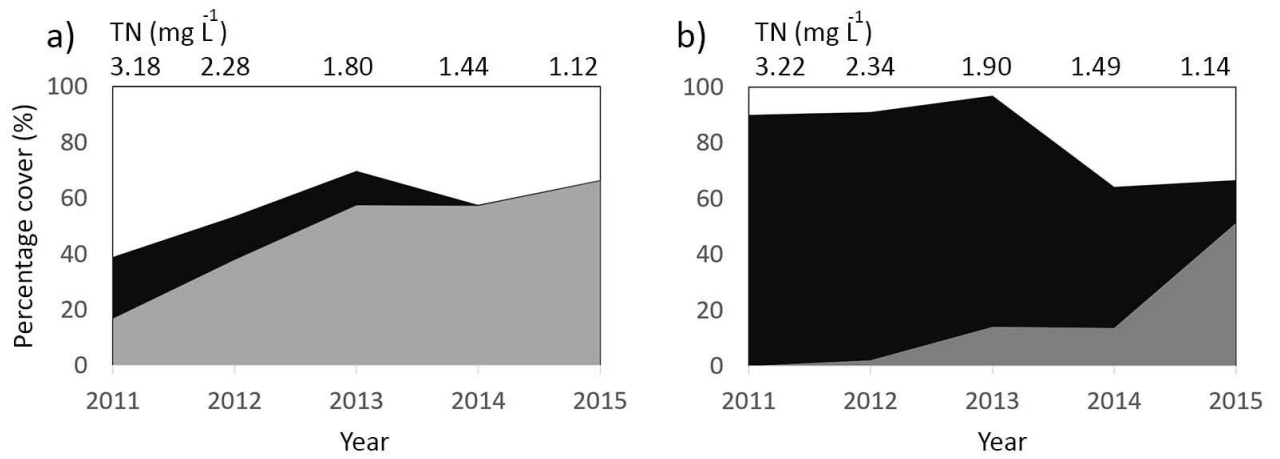
Figure 4.

Figure 5.

Figure 6.

List of co-authored research papers with IF not included in this Ph.D. Thesis

Vejřík, L., **Vejříková, I**., Kočvara, L., Blabolil, P., Peterka, J., Sajdlová, Z., Jůza, J., Šmejkal, M., Batroň, D., Kubečka, J., Čech, M. (*in press*) The pros and cons of the invasive freshwater apex predator, European catfish Silurus glanis, and powerful angling technique for its population control. *Journal of Environmental Management*.

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Oral and poster presentations at conferences

Vejříková I.: Impact of water temperature on fish herbivory, Fresh Blood for Freshwater, 23–26 April 2019, Tihany, Hungary.

Vejříková I.: Distribution of Herbivorous Fish Is Frozen by Low Temperature. Ekologie 2017 – 6th Conference of the Czech Society for Ecology, 25–27 September 2017, Praha, CR.

Matějíčková I., Vejřík L., Syväranta J., Kiljunen M., Čech M., Vašek M., Sajdlová Z., Chung S.H.T., Šmejkal M., Blabolil P., Frouzová J., Peterka J.: Is evolutionary trend to fish herbivory in higher latitude frozen by low temperature? Oral presentation. Species on the Move, 9-12 February 2016, Hobart, Tasmania.

Vejřík L., Matějíčková I., Frouzová J., Jůza T., Kubečka J., Čech M.: Hypoxic pelagic zone as a refuge for small fish in a freshwater ecosystem. Poster presentation. Species on the Move, 9-12 February 2016, Hobart, Tasmania.

Matějíčková I., Peterka J., Syväranta J., Vejřík L., Kočvara L.: Strong effect of macrophytes on fish trophic relationships, example of cyprinids in post-mining lakes. Poster presentation, Conference Ecology of Fish in Lakes and Reservoirs, 8–11 September 2014, ČB, Czech Republic, (1st place in Student poster competition).

Vejřík L., Čech M., Matějíčková I., Frouzová J., Jůza T., Kubečka J.: Hypoxic pelagial as a refuge for small fish in freshwater ecosystem. Poster presentation, Conference Ecology of Fish in Lakes and Reservoirs, 8–11 September 2014, České Budějovice, Czech Republic.

Matějíčková I.: Factors influencing succession of macrophytes in post-mining lakes revitalized using hydric way. Oral presentation, Wetland Seminair, 20–21. 2. 2014, Třeboň.

Matějíčková I., Gewa B., Manumbor M.: Comparing species richness of Geometrid moths between Riverine and Primary Forests in Wanang III. Poster presentation. Conference Ecosystem Services and Biodiversity under the Changing World, 17–21 March 2012, Ryukoku University, Japan.

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Education

2019	Final doctoral exam and rigorous state examination (RNDr.)
Since 2013	Doctoral study in Hydrobiology, Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 31
2010 – 2013	Master degree in Secondary School Teacher Education – Biology, Faculty of Science, University of South Bohemia in České Budějovice
2010 – 2013	Master degree in Ecosystem Biology (Hydrobiology), Faculty of Science, ČB
2007 – 2010	Bachelor degree in Environmental Science, Faculty of Science, ČB
1999 – 2007	The First Czech Grammar School in Karlovy Vary, Národní 25

Awards

Vojtěch Jarošík Award for excellent student publication in Ecology (Distribution of Herbivorous Fish Is Frozen by Low Temperature. Scientific Reports) awarded by ČSPE.

1st place in Student poster competition: Matějíčková I.: Strong effect of macrophytes on fish trophic relationships, example of cyprinids in post-mining lakes. Ecology of Fish in Lakes and Reservoirs, 8–11 September 2014, České Budějovice.

Working experience

Since 2019	Member of project QK1920011 Methodology of predatory fish quantification in drinking-water reservoirs to optimize the management of aquatic ecosystems
2016–2018	Maternity leave
2016	Student stay abroad at the University of Tasmania
2015	Month-long stay in laboratory of Aquatic Sciences, University of Jyväskylä, Finland, stable isotope analysis
2014–2017	Institute of Hydrobiology, BC CAS, member of project MacFish supported by Norwegian Financial Mechanism (7F4316). Job description: Quantitative and qualitative surveys of the fish stock of post-mining lakes, macrophytes monitoring, SIA and publication
2014, 2015	Member of ichthyological research of Brabantse Biebosch Reservoir, Netherland
2014	Member of ichthyological and parasitological research in Svalbard
2014	Month-long stay in laboratory of Aquatic Sciences, University of Jyväskylä, Finland
2013–2016	Institute of Hydrobiology, member of project CEKOPOT (CZ.1.07/2.3.00/20.0204)
2013	Stable Isotope Course, Freising, Germany
2013	Pedagogical Training at Grammar School in K.Vary and J. V. Jirsíka in ČB
Since 2012	Ichthyological research of lakes, reservoirs and other water bodies in CR
2011	Member of tropical research course in Papua-New Guinea
2009–2012	Practical training in chemical laboratory of Institute of Hydrobiology BC CAS, MikroChem LKT, Třeboň and Water and Wastewater Company in K. Vary

Effect of submerged macrophytes on the structure of lake ecosystem and trophic relationships among fishes