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Rotifer functionality as a potentially useful indicator of lake browning

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ABSTRACT

Lake browning is considered a severe water quality problem in lake ecosystems, but it has received considerably less attention in water protection than eutrophication. Current metrics used in lake surveillance monitoring programmes, including the European Union's Water Framework Directive, do not reflect browning sufficiently. The aims of the study were to explore the potential role of the functionality of rotifers as browning indicators and to improve understanding of the environmental parameters driving the functionality and diversity of rotifers. Seasonal data on rotifer communities and water quality from seven lakes with differing water colour and trophic conditions were analysed. The feeding guilds of rotifers enabled differentiation between lakes in terms of their ecological conditions, and, in particular, eutrophic and brown-water lakes were clearly distinguished from other lakes. The guild ratio of rotifers was positively affected by water colour, but inversely related to total phosphorus concentration. Our results suggest that zooplankton functionality provides a potential tool to assess ecosystem dynamics, particularly when assessing lake browning. Thus, our results suggest that application of the guild ratio of rotifers is a promising method to estimate the general browning status of lakes and may complement the metrics used in Water Framework Directive.

1. Introduction

Lake browning is a serious environmental problem in temperate and boreal regions (e.g. Creed et al., 2018; Sepp et al., 2018). Browning is mainly caused by increased export of coloured dissolved organic carbon (DOC) and iron (Fe) from catchment soils to water bodies (Kritzberg et al., 2020; Estlander et al., 2021). Several mechanisms behind browning have been suggested, including, for example, warming, increased precipitation and greening associated with climate change, changes in land use especially in peatland-dominated catchment areas, and decreased atmospheric sulphate deposition (Evans et al., 2005; Erlandsson et al., 2008; Weyhenmeyer and Karlsson, 2009). Since multiple stressors act simultaneously, their individual effects on lake browning is challenging to determine. For instance, in boreal regions, climate warming increases terrestrial organic matter (i.e. greening), the potential source of organic carbon to watersheds (Finstad et al., 2016). Additionally, reduced atmospheric sulphate deposition enhances the solubility and mobility of organic matter from the soil to the aquatic environment (Monteith et al., 2007). Alterations in light, temperature and availability of nutrients due to the browning have a profound effect on a lake's physico-chemical properties (Eloranta, 1999; Knoll et al., 2018; Horppila et al., 2023) and diverse effects on aquatic organisms. In addition to ecosystems, browning affects the domestic use of water, as well as ecosystem services linked to recreational values (Lavonen et al., 2013; Van Dorst et al., 2019).

Although browning is considered a severe water quality problem in lake ecosystems, it has received considerably less attention in water protection than eutrophication (Horppila et al., 2024). This also applies to monitoring of the ecological state of waters according to the Water Framework Directive (WFD) of the European Union, which is the main policy instrument for water protection in the EU. In WFD, biological quality elements, such as phytoplankton, macrophytes, phytobenthos, macroalgae, macroinvertebrates and fish, are the basis for the ecological status assessment of surface waters. The assessment compares the monitoring results of these biological parameters to reference conditions, which would be expected in conditions of minimal anthropogenic impact. The current biological indicators in WFD are mostly targeted at eutrophication and are not suitable for detecting the effects of browning (Sepp et al., 2018; Albrecht et al., 2022). Thus, further research is needed to develop new methods for assessing the ecological status of

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freshwater ecosystems in terms of browning. The changes in the lake's primary production due to browning provide a good example of how the capacity of WFD classification to reflect browning is limited. According to the status classes in WFD, the reduction of phytoplankton biomass indicates in all lake types that the lake is shifting towards a better ecological state, i.e. closer to the natural state (e.g. Poikāne et al., 2010). However, several studies suggest that the effect of browning on primary production is not always positive (Seekell et al., 2015; Solomon et al., 2015; Kelly et al., 2018; Bergström and Karlsson, 2019). Increasing DOC concentration can initially increase the lake's productivity by supplying additional energy and nutrients to phytoplankton (Klug, 2002), but when a certain DOC concentration is exceeded, the productivity of the ecosystem turns to decline due to the light extinction (Ask et al., 2009). Thus, it follows that primary production has an unimodal relationship with DOC concentration (Seekell et al., 2015; Solomon et al., 2015).

Zooplankton constitute an important link in lake food webs between the primary producers and other consumers such as fish (Ask et al., 2009; Estlander et al., 2010) and can be used as an indicator of ecological conditions in lakes (Jeppesen et al., 2011). In particular, rotifers are the most interesting group of zooplankton because they respond faster to environmental changes than crustacean zooplankton and appear to be sensitive indicators of changes in water quality (Gannon and Stemberger, 1978; Leech et al., 2018). Yet, the effects of environmental stressors on rotifer community composition are difficult to interpret, as the species composition and abundance show usually considerable seasonal fluctuations. In addition, they are continuously controlled by number of abiotic and biotic factors, and the strength of these factors vary among, and even within lakes (Pinel-Alloul et al., 1995). Despite the complexity of the taxonomic approach, referring to single species, distinct or rare species of rotifers, it can serve as a sensitive tool for measuring environmental gradients (Kuczyńska-Kippen et al., 2024). However, the complex species composition data can be simplified by classifying species into functional groups that assist to evaluate the effects of the environmental factors on community changes.

Rotifers exploit diverse food resources, and their feeding behaviour is associated with a range of behavioural, morphological and life-history traits (Gilbert and Bogdan, 2019; Gilbert, 2022). According to Pourriot (1977), rotifer communities can be roughly classified into two categories based on their predominant feeding mode: raptorial and microphagous species. Raptorial species catch or select single food items, whereas microphagous species are filter feeders that simultaneously collect multiple food items. This difference in food acquisition, the trait-based guild ratio, enables zooplankton community assessment through resource use. Despite the shortcomings of this rough classification (Gilbert, 2022), simplifying the multispecies rotifer communities facilitates the detection of complex environmental responses in rotifer communities. The guild ratio has been successfully used, for example, to express the trophic level of lakes (Špoljar et al., 2011) and has been applied in rotifer community ecology to estimate the contribution of species to ecosystem functioning (Virro et al., 2009; Obertegger et al., 2011; Lokko et al., 2017).

Rotifers have been shown to be potentially useful indicators of ecosystem functioning, biological diversity and community structure, and overall they have a key role in the functioning of ecosystems (Hatton et al., 2015). Here, we investigated the taxonomic diversity and the guild ratio of rotifer community in seven lakes with differing water colour and trophic conditions. Considering that the monitoring programmes under the WFD require new insights in terms of browning, our objective was to study whether the feeding guilds of rotifers could be a novel way to study browning effects on lake ecosystems. As increasing water colour seems to benefit predatory rotifers and decrease the abundance of herbivorous zooplankton taxa (Estlander and Horppila, 2023), we expected that increasing water colour would favour raptorial rotifer taxa. This would also be reflected in the predominant feeding mode of rotifers. We also hypothesized that the diversity of rotifers decreases along increasing water colour as shown in earlier studies (Karpowicz and Ejsmont-Karabin, 2018; Estlander and Horppila, 2023). From comparisons across time, we aimed to distinguish between seasonal variability and browning-induced changes in the rotifer community of the lakes.

2. Material and methods

2.1. Study lakes

The study was conducted in seven small forest lakes (2.2–13.8 ha) in Evo district (61 °13N, 25 °12E, Fig. 1), in southern Finland representing varying levels of water colour (14–340 mg Pt l^{-1}; corresponds to Secchi depths 450–60 cm) and trophic conditions (total phosphorus 9–77 μg l^{-1}) (Estlander et al., 2009, 2021).

The lakes were sampled fortnightly from June to September (7–8 sampling dates). Four lakes (Hokajärvi, Haarajärvi, Haukijärvi and Majajärvi) were sampled in 2006 and three lakes (Valkea Mustajärvi, Kyynäröjärvi and Käkilammi) in 2022. On each sampling date, three replicate water quality samples for physical and chemical analyses were taken with a Limnos tube sampler (volume 2.8 l) from the pelagic zone (top 0–3 m layer) in connection with zooplankton sampling. Secchi disc depth was measured, and vertical profiles of water pH, temperature and dissolved oxygen were determined *in situ* with a YSI 6820 CTD sonde (YSI Incorporated, Yellow Springs, OH, United States). From all the water samples, water colour and DOC concentration were analysed in the laboratory. DOC concentration was measured with a Shimadzu TOC 5000 A analyser, following the standard SFS-EN 1484 (Finnish Standards Association SFS, 1997). Water colour was measured spectrophotometrically as the absorbance of light at 410 nm wavelength (standard SFS-EN ISO 7887) (Finnish Standards Association SFS, 2011). In addition, total P concentration was analysed with a flow injection analyser (Gallery Plus, Lachat QuikChem) and chlorophyll-*a* concentration was analysed with a Hitachi F-4000 fluorescence spectrophotometer with excitation and emission wavelengths of 435 and 671 nm, respectively, after filtration on Whatman GF/C filters and extraction with ethanol.

2.2. Rotifer sampling and analyses

Three replicate zooplankton samples were taken with a Limnos tube sampler (volume 2.8 l) from the pelagial, at the deepest part of each lake. Tube sampler hauls were conducted at 1-metre intervals from the surface to the bottom and assembled into one integrated sample. Samples were filtered through a 50 μm mesh net and preserved in 4 % formaldehyde. Rotifers were identified to the lowest possible taxonomic level (genus or species) using the taxonomic keys of (Ruttner-Kolisko, 1972; Koste and Voigt, 1978; Pontin, 1978), counted, their length and width measured under an inverted microscope. From each species, 30 individuals were measured to estimate the carbon biomass using species-specific carbon regressions (Bottrell et al., 1976; Ruttner-Kolisko, 1977). Zooplankton species diversity was quantified using Simpson's diversity index (D),

$$D = 1 - \sum_{i=1}^{s} p_i^2$$

where s is species richness and p_i is the proportion of species i.

The value of D ranges from 0 to 1, and the greater the value, the greater the sample diversity.

To calculate the guild ratio of rotifers, we classified the rotifers according to Obertegger et al. (2011); thus, genera *Ascomorpha*, *Asplanchna*, *Collotheca*, *Gastropus*, *Ploesoma*, *Polyarthra*, *Synchaeta* and *Trichocerca* were designated as raptorial, and genera *Brachionus*, *Conochilus*, *Euchlanis*, *Filinia*, *Kellicottia*, *Keratella*, *Lecane*, *Notholca* and *Trichotria* were designated as microphagous. The guild ratio was calculated as,

Fig. 1. Locations of the studied lakes (n = 7) in southern Finland.

$$GR = \frac{(bm_{rap} - bm_{mic})}{bm_{tot}}$$

where bm_{rap} is the biomass of raptorial genera, bm_{mic} is the biomass of microphagous genera and bm_{tot} is the total biomass of rotifers. Values range from −1 to +1, with values < 0 indicating microphagous dominance and values > 0 indicating raptorial dominance.

2.3. Statistical analyses

Between-lake differences in surface water temperature, water colour, DOC, chlorophyll *a* and pH were compared using repeated-measures analysis of variance (ANOVA) with time (month) of sampling (4 levels; fixed), lake (7 levels; fixed). In addition, the same analysis was also used to compare the differences in the rotifer biomass, Simpson's diversity index and guild ratio between lakes. The between-lake pairwise comparisons were performed using Bonferroni t-tests. A stepwise multiple regression analysis with forward selection (α = 0.05) of predictors (water colour, total P, pH and lake surface area; Table 1) was used to explore the most predictive environmental factors affecting the biomass, diversity and guild ratio of rotifers. Collinearity between predictor variables were assessed using both correlation matrices and estimates of variance inflation factors and variables that had highly significant coefficients (>0.60 in Spearman's correlations) were excluded from multiple regression analysis. Standardized regression coefficients were used to conclude the relative importance of environmental variables for explaining variations in guild ratio, diversity and biomass of rotifers. Before the analyses, the datasets were checked for normality and, if necessary, log-transformed.

Table 1
Main water quality results from studied lakes. Values represent mean values with standard error of mean from June to September.

Lake	Surface area (ha)	Colour (mg Pt l^{-1})	DOC (mg l^{-1})	Chlorophyll *a* (μg l^{-1})	Total P (μg l^{-1})	pH
Valkea Mustajärvi	13	25 ± 6	6 ± 0.1	5 ± 0.3	10 ± 0.8	6.5 ± 0.03
Hokajärvi	8	59 ± 2	11 ± 0.1	3 ± 0.1	7 ± 0.6	6.9 ± 0.15
Haarajärvi	14	84 ± 3	13 ± 0.3	6 ± 0.2	11 ± 1.2	7.0 ± 0.08
Kyynäröjärvi	25	253 ± 12	26 ± 0.5	31 ± 1.4	65 ± 2.4	6.5 ± 0.03
Haukijärvi	2	344 ± 5	14 ± 0.4	6 ± 0.9	10 ± 0.9	6.5 ± 0.18
Majajärvi	3	344 ± 5	20 ± 0.4	15 ± 0.7	30 ± 3.3	6.3 ± 0.11
Käkilammi	10	348 ± 12	34 ± 0.8	11 ± 0.5	25 ± 2.5	6.3 ±0.08

3. Results

3.1. Water quality

All the study lakes were stratified from the beginning of June to the end of August and the epilimnion thickness varied from 0.6 to 3.7 m. The epilimnion was shallower in the dark-water lakes (Käkilammi, Haukijärvi and Majajärvi) and deepest in Valkea Mustajärvi. Correspondingly, the Secchi disk depth was the lowest (60 ± 5 cm) in Käkilammi and the highest (450 ± 10 cm) in Valkea Mustajärvi. In June, surface water temperature (0–1 m) varied from 18 to 19°C, the highest temperatures (21°C) were observed in July and the lowest temperatures were measured (12–13°C) in September. The average monthly surface water temperatures differed significantly in June and September between lakes ($F_{18,42} = 4.29$; $p = 0.04$), but the difference was less than 1.5°C.

Water colour varied considerably between the lakes (Table 1), being the lowest in Valkea Mustajärvi (25 ± 6 mg Pt l^{-1}) and the highest in Käkilammi (348 ± 12 mg Pt l^{-1}). Accordingly, the water colour differed significantly ($F_{18,42} = 21.66$; $p < 0.01$) between all other lakes, except between dark-water lakes Majajärvi and Haukijärvi (Table 1). Also, in the DOC concentrations we found a significant difference between lakes ($F_{18,42} = 10.27$; $p < 0.01$). It varied from 6 to 34 mg l^{-1}, being lowest in Valkea Mustajärvi and highest in Käkilammi, thus following the trend of water colour in lakes (Table 1).

The total P and chlorophyll-*a* concentrations showed a large variation between the lakes (Table 1), being the lowest in Hokajärvi (Total P: 7 ± 0.6 μg l^{-1}; Chlorophyll *a*: 3 ± 0.1 μg l^{-1}) and the highest in Kyynäröjärvi (Total P: 65 ± 2.4 μg l^{-1}; Chlorophyll *a*: 31 ± 1.4 μg l^{-1}). The relatively high total P and chlorophyll-*a* concentrations in the Lake Kyynäröjärvi distinguished it from the other lakes, and total P and chlorophyll-*a* concentrations showed relatively small within-lake variation overall (coefficient of variation ~ 20 %) during the summer. The average pH values in water column varied from 6.3 to 7.0, but showed no significant variation within or between lakes (Table 1). Spearman's correlations for water quality parameters indicated strong positive relationship between water colour and DOC concentration ($r = 0.86$, $p < 0.01$) and total P and chlorophyll *a* ($r = 0.90$, $p < 0.01$). However, water colour correlated weakly with total P ($r = 0.39$, $p < 0.05$) and with pH (*r*

= −0.43, $p < 0.05$), thus water colour, total P, pH and lake surface area were chosen as independent variables in the regression model. Variance inflation factors for water colour, total P, pH and lake surface areas also indicated minor collinearity (VIF < 2) among the independent variables.

3.2. Rotifer communities

We identified 38 rotifer taxa from the studied lakes (Appendix 1). The most abundant genera overall in the low-coloured lakes (Valkea Mustajärvi, Hokajärvi and Haarajärvi) were *Conochilus* sp. and *Kellicottia* sp., in the dark-water lakes (Haukijärvi, Majajärvi and Käkilammi) *Keratella* sp., *Polyarthra* sp. and *Asplanchna* sp. and in Kyynäröjärvi *Kellicottia* sp., *Filinia* sp. and *Conochilus* sp. In all study lakes, *Ascomorpha* sp., *Asplanchna* sp., *Conochilus* sp., *Gastropus* sp., *Kellicottia* sp., *Keratella* sp., *Polyarthra* sp., *Synchaeta* sp., and *Trichocerca* sp. were found throughout the study period, but their abundance showed relatively large within- and between-lake variation (Appendix 1).

The repeated-measures ANOVA revealed that rotifer diversity was significantly lower in the dark-water lakes (Haukijärvi, Majajärvi and Käkilammi) throughout the sampling season from June to September (Fig. 2; Table 2).

Simpson's diversity also showed a generally decreasing trend from June to September (Fig. 2) with the exception of Valkea Mustajärvi and Kyynäröjärvi, where the diversity displayed small variation between months (range 0.12). Rotifer diversity was positively affected by lake surface area (Table 3), which explained 39 % of the variance, while diversity was inversely related to water colour which explained an additional 14 % of the variance (Table 3).

The rotifer biomass varied significantly between lakes (Table 2), being the highest (2–53 μg C l^{-1}) in Kyynäröjärvi throughout the study period (Fig. 3). The biomass of rotifers was the lowest in Valkea Mustajärvi (1 μg C l^{-1}) and varied between 1 and 4 μg C l^{-1}. In other lakes, the biomass was almost similar (Hokajärvi average 3.6 ± 0.2 μg C l^{-1}; Haarajärvi average 3.3 ± 0.2 μg C l^{-1}; Haukijärvi average 3.0 ± 0.2 μg C l^{-1}; Majajärvi average 3.1 ± 0.2 μg C l^{-1}; Käkilammi average 3.0 ± 0.8 μg C l^{-1}) throughout the sampling season (Fig. 3). In the regression model, total P, pH and water colour accounted for 46 % of the variance in rotifer biomass (Table 3). The standardized coefficient for pH (0.54) was higher than those for total P (0.41) and water colour (0.43) indicating that pH was the most important factor.

3.3. Guild ratio

As with diversity, the dark-water lakes differed significantly from the other lakes in terms of the guild ratio (Fig. 4; Table 2). The guild ratios were constantly > 0 from June to September in Haukijärvi, Majajärvi and Käkilammi, suggesting raptorial dominance (Fig. 4). In contrast, in Kyynäröjärvi microphagous rotifers dominated during the entire research period from June to September (Fig. 4).

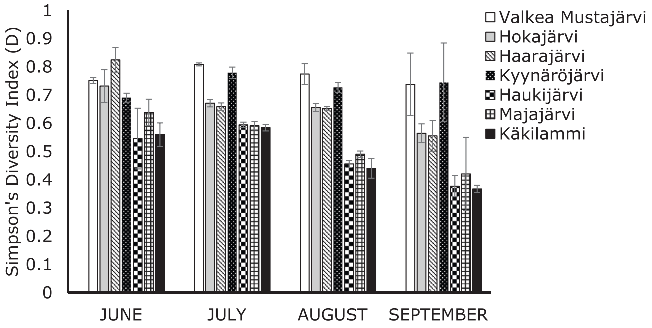

Fig. 2. The monthly average diversities (Simpson's Diversity Index D) in the studied lakes with 95 % confidence intervals. Lakes are in order of increasing water colour from left to right.

Table 2
Repeated-measures analysis of variance for diversity, biomass and guild ratio of rotifers in the study lakes from June to September.

		df, df error	MS	*F*	*p*
Diversity	Month	3,42	0.089	64.17	<0.001
	Lake	6,14	0.160	108.42	<0.001
	Month x Lake	18	0.009	6.82	<0.001
Biomass	Month	3,42	0.453	129.58	<0.001
	Lake	6,14	1.209	285.18	<0.001
	Month x Lake	18	0.313	89.69	<0.001
Guild ratio	Month	3,42	0.206	8.34	0.001
	Lake	6,14	1.426	61.82	<0.001
	Month x Lake	18	0.106	4.29	<0.001

Table 3
Results of stepwise multiple regression analyses for the guild ratio (GR), diversity (D) and biomass of rotifers.

Model	Variable	Coefficient	Standardized coefficient	R^2	*F*	*P*
GR (n=28)	Intercept	−0.225				
	Water colour	0.003	0.74	0.41	17.89	< 0.001
	Tot-P	−1.859	−0.76	0.65	16.76	< 0.001
	pH	−0.757	−0.54	0.79	18.13	< 0.001
D (n=28)	Intercept	0.649				
	Lake area	0.071	0.46	0.39	16.32	< 0.001
	Water colour	−0.552	−0.41	0.52	7.24	< 0.001
Biomass (n=28)	Intercept	−6.107				
	Tot-P	0.008	0.41	0.18	5.70	0.025
	pH	0.877	0.54	0.33	5.45	0.028
	Water colour	1.819	0.43	0.46	5.62	0.026

Also, in Valkea Mustajärvi, Hokajärvi and Haarajärvi microphagous feeding dominated, although the guild ratio values were close to zero (Valkea Mustajärvi average −0.05 ± 0.06; Hokajärvi average −0.17 ± 0.07; Haarajärvi average −0.08 ± 0.05) (Fig. 4). In general, the guild ratios showed relatively small variation within lakes, but significant variation was observed in Hokajärvi, Haukijärvi and Kyynäröjärvi (Fig. 4; Table 2). In Kyynäröjärvi and Haukijärvi, the guild ratio was the highest in August and in Hokajärvi in September (Fig. 4). In Valkea Mustajärvi, Majajärvi and Käkilammi, the guild ratio showed no significant variation between months. The guild ratio of rotifers was explained by water colour, total P and pH, accounting for 79 % of the total variance (Table 3). The water colour accounted for 41 %, total P 24 % and pH only 14 % of the total variance. However, the guild ratio responded differently to these environmental variables. The guild ratio was positively affected by water colour, but inversely related to total P and pH (Table 3), suggesting that increasing water colour favours raptorial rotifer taxa, while increasing total P concentration promotes microphagous rotifers.

4. Discussion

Rotifers have been shown to be effective indicators of lake trophic status (Gannon and Stemberger, 1978; Mäemets, 1983; Ejsmont-Karabin, 2012). Based on our results, rotifers turned out to be a promising indicator also for estimating lake browning, since the feeding guild ratio performed well in distinguishing dark-water lakes from lakes with low colour or high nutrient concentration. Both water colour and total P concentration were significant environmental factors explaining the guild ratio variation, but the effects were opposite, thus suggesting

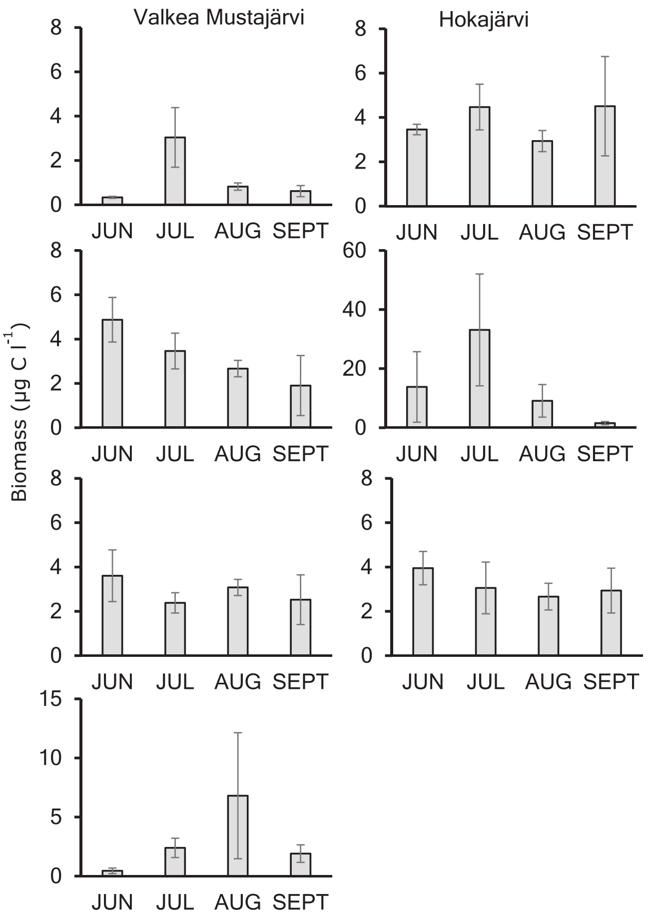

Fig. 3. Average monthly biomass of rotifers (µg C l^{-1}) in the studied lakes with 95 % confidence intervals. Lakes are in order of increasing water colour from left to right and from top to down.

that the guild ratio response to browning and eutrophication may differ. Our results displaying a declining pattern in rotifer diversity with increasing water colour are also in line with earlier studies (Urrutia-Cordero et al., 2017; Karpowicz and Ejsmont-Karabin, 2018; Estlander and Horppila, 2023) in which lake browning has been linked to reduced phytoplankton and zooplankton diversity. Diversity was also positively related to lake surface area, as observed in previous studies (e.g. Obertegger et al., 2010), likely due to increased habitat diversity as lake size increases. In addition, our results indicate that rotifer biomass reflects also lake productivity, although our model explained only 18 % of the variance in biomass due to the small number of lakes. Thus, our finding is also consistent with general zooplankton–lake productivity relations (e.g. Canfield and Jones, 1996).

Interestingly, the guild ratio in three of the dark-water lakes, Haukijärvi, Majajärvi and Käkilammi was clearly distinct from the other studied lakes and raptorial feeders dominated the community. Despite the relatively high water colour of Lake Kyynäröjärvi, it differs from other dark-water lakes due to its high concentrations of phosphorus (> 60 µg l^{-1}), chlorophyll-*a* (45 µg l^{-1}) and recurrent cyanobacterial blooms (Taipale et al., 2016; Estlander et al., 2021). Thus, it seems that eutrophic conditions favoured microphagous rotifers, such as *Kellicottia bostoniensis*, *Filinia longiseta*, *Conochilus unicornis* and *Keratella* spp. Gilbert (2022) suggested that genera *Filinia* and *Conochilus* are microphagous rotifers, the diets of which consist mainly of fine detritus / organic aggregates, picoplankton, and 2–10 µm nanoplankton, and *Keratella* and *Kellicottia* are polyphagous rotifers. The diet of polyphagous rotifers overlaps with the diet of microphagous rotifers but also includes slightly larger food particles (20–50 µm microplankton). For example, Pinel-Alloul et al. (1996) showed that < 20 mm algae components increase with lake trophy; thus, the dominance of microphagous rotifers in the Lake Kyynäröjärvi may suggest sufficient availability of pico- and nanoplankton. Yet, the relationship between phytoplankton and guild ratio remains to be clarified in future studies, since phytoplankton communities were not analysed in present study.

The dominance of raptorial species in the dark-water lakes was associated with relatively high biomass of *Asplanchna priodonta*, *Ploesoma truncatum* and *Polyarthra* spp. Genera *Ploesoma* and *Asplanchna* are predatory rotifers and can eat protists and small rotifers as large as 500 µm (Gilbert, 2022 and references therein). In very humic lakes, bacterial productivity is often elevated (Lennon and Pfaff, 2005) if nutrients (phosphorus and nitrogen) are also available (Tulonen et al., 1992). Arndt (1993) suggested that rotifers are generally unable to suppress the microbial web through grazing because of their relatively low grazing rate compared with the growth rate of bacteria and protozoa. Here, the nutrient concentrations were relatively high in the Lakes Majajärvi and Käkilammi, which should favour bacterial production and, further, benefit protozoans through bacterial grazing, providing a stimulating effect on their consumers, raptorial rotifers. Thus, we postulate that the potentially high abundance of protozoans in the dark-water lakes contributes to raptorial feeding dominance, since according to Arndt (1993) rotifers should be efficient predators of protozoans. It is also possible that food resources for microphagous feeders were scarce in the dark-water lakes Haukijärvi, Majajärvi and Käkilammi, since, for example, Paczkowska et al. (2019) suggested a negative correlation between nanophytoplankton biomass and dissolved organic matter related variables.

Furthermore, Cronberg et al. (1988) showed that *Asplanchna* is capable of eating freshwater flagellated microalga *Gonyostomum semen* (Ehrenberg), the cell size of which has generally been considered too large for many filter-feeding zooplankton (Burns, 1968). *Gonyostomum* is a unicellular flagellate belonging to the class Raphidophyceae and is abundant in brown lakes with high DOC concentrations (Burns, 1968; Lepistö et al., 1994; Lebret et al., 2018). Challenges in filtering the samples with net, due to the slime ejected from the trichocysts of *G. semen* at cell disturbance, and relatively high chlorophyll concentration in Majajärvi and Käkilammi indicated that *Gonyostomum* was present in these lakes, demonstrated also in previous studies (Estlander et al., 2009). Consequently, the abundance of *Gonyostomum* should favour raptorial over microphagous feeders, especially in the dark-water lakes where resources are limited due to a thin epilimnetic layer. However, further research is needed to elucidate the roles of phytoplankton species and protozoans.

Generally, lake productivity reflects food resource availability and thus, zooplankton abundance is expected to increase with lake productivity (e.g. McCauley and Kalff, 1981). This also holds in our study for rotifers, as the rotifer biomass was positively related to total P and considerably higher in the eutrophic Kyynäröjärvi. Yet, the rotifer communities of the dark-water lakes differed notably from other lakes due to low diversity and moderate biomass, despite relatively high chlorophyll-*a* and nutrient concentrations. However, chlorophyll-*a* does not reflect phytoplankton biomass directly, since the chlorophyll content can vary between species and is also dependent on light, nutrients and temperature (Foy, 1987). Moreover, in addition to algae, rotifers have been shown to exploit other food resources, such as suspended detrital aggregates, bacteria, protozoa and other rotifers (Gilbert, 2022 and references therein). Thus, the chlorophyll-*a* concentration does not necessarily solely reflect food resource availability.

Haukijärvi, Majajärvi and Käkilammi were distinct lakes with very high water colour (> 300 mg Pt l^{-1}). In such lakes, primary production is strongly restricted due to effective light extinction in the water column (Arvola, 1984; Jones, 1992), and strong thermal stratification with oxygen depletion in the hypolimnion may limit potential habitats for zooplankton (Kelly et al., 2018). Habitat loss may in turn increase interspecific competition in zooplankton communities sharing limited

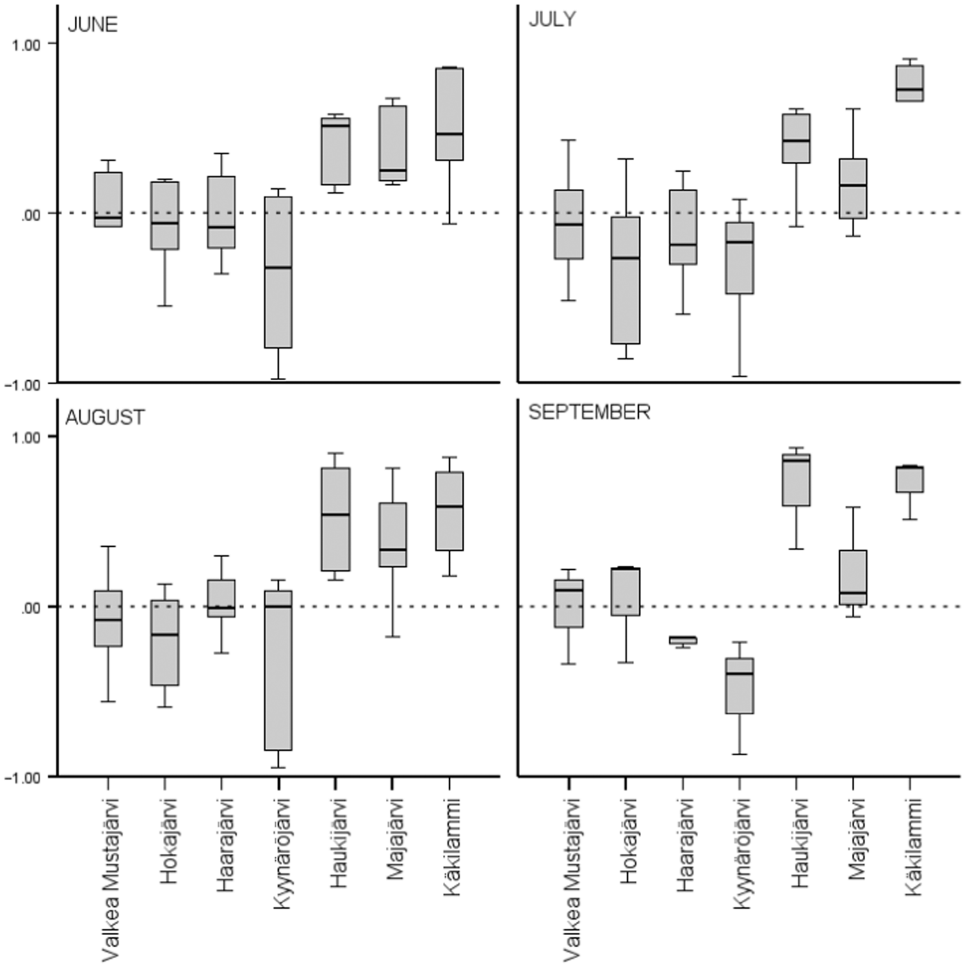

Fig. 4. Box plots of monthly guild ratios (GR) for the studied lakes. Values < 0 indicate microphagous dominance and values > 0 indicate raptorial dominance. The horizontal lines inside the box plots indicate the median, and the boundaries of the box plots indicate the 25th and 75th percentiles. Whiskers above and below indicate the minimum and maximum values. Lakes are in order of increasing water colour from left to right.

resources (Karpowicz and Ejsmont-Karabin, 2018) and, further, be reflected in diversity, which in the lakes with highest water colour was notably lower than in the Hokajärvi, Haarajärvi and Kyynäröjärvi. Several studies have also shown that the biomass of rotifers is also sensitive to predator abundance (e.g. Conde-Porcuna and Declerck, 1998; Diéguez and Gilbert, 2002). In our study, the predatory rotifer genera *Ploesoma* and *Asplanchna* were common in the dark-water lakes. These genera are able to consume other rotifer species so effectively that they shape the whole community (Gilbert, 2022 and references therein). We did not consider other predators (e.g. chaoborids or fish) in this study, but previous studies (Estlander et al., 2010) suggest that chaoborids are one of the main predators of rotifers in these lakes. In addition, chaoborid densities are higher in Majajärvi and Haukijärvi compared with Hokajärvi and Haarajärvi (Estlander et al., 2010), which may be reflected in the biomass and diversity of rotifer communities in the dark-water lakes, especially together with a high abundance of predatory rotifer species.

In addition to predation, rotifers may also suffer from competition with crustacean zooplankton (e.g. MacIsaac and Gilbert, 1989) and microphagous rotifers are more sensitive than raptorial feeders to competition with filter-feeding cladocerans (Obertegger and Manca, 2011). In the study lakes, the biomass of crustacean zooplankton is on average highest in Kyynäröjävi, Käkilammi and Majajärvi (Estlander et al., 2010; Estlander and Horppila, 2023). As microphagous feeders formed most of the rotifer biomass in Kyynäröjärvi, where also the cladoceran biomass is higher than in the dark-water lakes), we suggest that food competition was not the only explanation for the between-lake differences in the functional groups. However, since competitive interactions and predation were not studied here, these issues need further study and refinement in the future.

5. Conclusions

The results of this study emphasize the importance of understanding how the browning-driven changes may cascade in the lake food web. Lake browning can alter ecological functions, with potential consequences for the food-web structure of lakes. Our results suggest that application of the guild ratio of rotifers is a promising tool to estimate the general browning status of lakes in terms of ecosystem processes and the interrelationship between trophic levels. Yet, additional research is needed to confirm our results in other lakes and to examine and extend the applicability of the guild ratio. We also suggest that there is a need to develop monitoring programmes under the WFD to monitor the ecological consequences of lake browning. When considering the current metrics used in the WFD, the functionality of zooplankton may complement the metrics used in WFD.

CRediT authorship contribution statement

Leena Nurminen: Writing – review & editing. **Mikko Olin:** Writing – review & editing, Resources. **Salla Rajala:** Writing – review & editing, Investigation. **Jukka Horppila:** Writing – review & editing, Supervision, Resources. **Satu Estlander:** Writing – original draft, Validation, Supervision, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.limno.2024.126194.

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