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Does browning-induced light limitation reduce fish body growth through shifts in prey composition or reduced foraging rates?

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Abstract

1. Browning of waters, coupled to climate change and land use changes, can strongly affect aquatic ecosystems. Browning-induced light limitation may have negative effects on aquatic consumers via shifts in resource composition and availability and by negatively affecting foraging of consumers relying on vision. However, the extent to which light limitation caused by browning affects fish via either of these two pathways is largely unknown.
2. Here we specifically test if fish growth responses to browning in a pelagic food web are best explained by changes in resource availability and composition due to light limitation, or by reduced foraging rates due to decreased visual conditions.
3. To address this question, we set up a mesocosm experiment to study growth responses of two different fish species to browning and conducted an aquaria experiment to study species-specific fish foraging responses to browning. Furthermore, we used a space-for-time approach to analyse fish body length-at-age across >40 lakes with a large gradient in lake water colour to validate experimental findings on species-specific fish growth responses.
4. With browning, we found an increase in chlorophyll *a* concentrations, shifts in zooplankton community composition, and a decrease in perch (*Perca fluviatilis*) but not roach (*Rutilus rutilus*) body growth. We conclude that fish growth responses are most likely to be linked to the observed shift in prey (zooplankton) composition. In contrast, we found limited evidence for reduced perch, but not roach, foraging rates in response to browning. This suggests that light limitation led to lower body growth of perch in brown waters mainly through shifts in resource composition and availability, perhaps in combination with decreased visibility. Finally, with the lake study we confirmed that perch but not roach body growth and length-at-age are negatively affected by brown waters in the wild.
5. In conclusion, using a combination of experimental and observational data, we show that browning of lakes is likely to (continue to) result in reductions in fish body growth of perch, but not roach, as a consequence of shifts in prey availability and composition, and perhaps reduced foraging.

KEYWORDS
climate change, pelagic food web, perch, roach, water colour

1 | INTRODUCTION

Global climate change, de-acidification, and changes in land use are leading to increased concentrations of dissolved organic carbon (DOC) and iron in temperate and boreal aquatic systems, leading to *browning* of waters and decreased light availability (Creed et al., 2018; Larsen, Andersen, & Hessen, 2011; Roulet & Moore, 2006; Weyhenmeyer, Müller, Norman, & Tranvik, 2016). Next to reduced light availability, this browning is often accompanied by increased nutrient concentrations (Creed et al., 2018; Findlay, 2003). Browning can have negative effects on many components of lake ecosystems, ranging across trophic levels, from reducing primary production and algal biomass (Ask et al., 2009; Vasconcelos et al., 2016), zooplankton production (Kelly, Solomon, Weidel, & Jones, 2014) and benthic invertebrate biomass (Vasconcelos et al., 2018), to fish growth and production (van Dorst et al., 2019; Karlsson et al., 2015). However, browning effects are rarely studied across multiple trophic levels and interacting species (but see Hansson et al., 2012; Vasconcelos et al., 2018). In addition, most studies to date have focused on the total effect of browner waters, instead of studying the effects of light limitation, increased DOC, or increased nutrient concentrations separately. Decreased light availability can affect aquatic systems in many ways, e.g. by reduced visibility (Davies-Colley & Vant, 1987; Morris et al., 1995), lower primary production (Ask et al., 2009; Seekell et al., 2015), and less heat penetration down the water column, which may lead to increased thermal stratification (Solomon et al., 2015). Nonetheless, there is limited knowledge on the relative importance of these consequences of light limitation through browning for fish body growth and production.

Decreased light availability can reduce the amount of basal production and biomass available to higher trophic levels by limiting photosynthesis (Ask et al., 2009; Jones, Solomon, & Weidel, 2012; Seekell et al., 2015; Vasconcelos et al., 2016). Whereas browning generally has a negative effect on benthic primary production, effects on pelagic primary production range from negative (Jansson, Bergström, Blomqvist, & Drakare, 2000), to neutral (Ask, Karlsson, & Jansson, 2012) and even positive (chlorophyll *a* [chl *a*]) (Kelly et al., 2016). The latter may partly be explained by increased nutrient concentrations with browning, which, up to a certain threshold, can compensate for reduced light availability, causing a hump-shaped relationship between DOC and whole-lake primary production (Kelly, Solomon, Zwart, & Jones, 2018; Seekell et al., 2015). Changes in primary production with browning may, in turn, change the biomass and alter the composition of secondary consumer communities (i.e. zoobenthic and zooplankton invertebrates) through bottom-up processes. Altered invertebrate prey communities due to browning could ultimately affect the amount of biomass available for predators such as fish (i.e. a bottom-up response), thereby potentially affecting their growth and productivity.

Most fish species are visual foragers and a decreased visibility caused by brown waters can strongly reduce foraging ability, as shown for some benthivorous and piscivorous fish (Jönsson, Ranåker, Nilsson, Brönmark, & Grant, 2013; Ranåker, Jönsson, Nilsson, & Brönmark, 2012). Considering that fish species have different modes of feeding (e.g. using vision or other senses) and feed on different prey items (e.g. benthic or pelagic prey), previous studies have shown that browning may affect foraging rates of different fish species in distinctive ways (Jönsson, Ranåker, Nilsson, & Brönmark, 2012; Weidel et al., 2017). For example, responses to browner waters for fish feeding on zooplankton seem to vary between fish species (Jönsson et al., 2012; Weidel et al., 2017). However, the extent to which reduced feeding rates with lower visibility contribute to observed patterns of decreased fish biomass production with browning in many temperate lakes (van Dorst et al., 2019; Karlsson et al., 2015) is unknown. Next to changes in feeding rates, lower visibility could also change prey selection, for example by reducing visual prey selectivity in some fish species (Estlander et al., 2010). The fact that feeding of fish species is affected differently by light limitation (Estlander et al., 2010; Jönsson et al., 2012; Weidel et al., 2017), begs the question of whether they also vary in their growth responses to browning, which would suggest that fish community composition may determine how fish community production is affected by browning.

The extent to which light limitation specifically caused by browning affects fish indirectly via changes in the prey community or directly by worsened conditions for visual feeding is still largely unknown. Here, we test the relative importance of changes in resource availability and composition due to light limitation, and reduced foraging rates caused by decreased visual conditions for fish growth responses to browning in a pelagic food web. Our main question was tested with a mesocosm experiment set up to study fish growth responses to browning in a pelagic food web. We also set up an aquarium experiment to study fish foraging responses to worsened visual conditions caused by browning. Finally, to test whether our experimental results hold also in the wild, we analysed fish body length-at-age data collected across a large gradient in lake water colour. We further generalise our results by asking if observed responses to browning vary depending on fish species identity.

2 | METHODS

2.1 | Species studied

The two fish species used in this study are Eurasian perch (*Perca fluviatilis*) and common roach (*Rutilus rutilus*), two common and often co-occurring fish species in northern European lakes and coastal waters.

Perch changes resource use over its lifetime, first feeding on zooplankton, switching to zoobenthos, and finally feeding on other fish (Eklöv & Persson, 1995; Hjelm, Persson, & Christensen, 2000). Roach can feed on zooplankton, algae, and zoobenthos, but do not exhibit strong ontogenetic diet shifts (Horppila, 1994; Persson, 1983). Roach are more efficient zooplankton feeders than perch (Byström & García-Berthou, 1999). While roach are efficient zooplankton feeders even in low light conditions (Bohl, 1979), percids, like *P. fluviatilis*, are vision-oriented selective predators and are therefore more dependent on their vision when feeding on zooplankton (Helfman, 1979).

2.2 | Mesocosm experiment

2.2.1 | Experimental setup

To study fish growth responses to light limitation in a pelagic food web, we performed a mesocosm experiment in 18 open tanks (3 m diameter × 1 m water depth) that were located outside from 10 August to 10 September 2017. The tanks were filled with *ca*. 7,000 L of filtered water (using a filter with 400-µm mesh size) from the adjacent lake Mälaren (59°33′N 17°87′E) on 9 August. We inoculated all tanks with similar amounts of zooplankton from a pooled sample collected from nearby ponds using a 70-µm mesh net on 10 August.

To separate fish growth responses caused by changes in resource availability and composition due to light limitation (shifts in prey community), and reduced foraging rates due to decreased visual conditions, we assigned three browning treatments and two fish species treatments using a factorial design (Figure 1). Browning treatments consisted of a clear control treatment (CL), a brown–early treatment (BE) aimed to create and test effects of browning on the prey community through decreased light availability before fish are present in the system, and to test the combined effects of (potentially) altered prey communities and a possible decrease in fish species-specific foraging rates caused by lower visibility after fish addition, and finally we had a brown–late treatment (BL) aimed to solely test the effect of potentially reduced foraging rates due to lower visibility on fish species-specific growth responses (Figure 1). To mimic browning of waters, we used 1,600 ml of Sera Blackwater Aquatan water conditioner (Sera GmbH, Heinsberg, Germany; hereafter blackwater) per mesocosm, which browns the water and reduces light availability (Figure S1) without changing pH. We analysed blackwater samples and found that it contains low amounts of total organic carbon (TOC) and nutrients, and increased concentrations of TOC by 3.8 mg/L (±0.096 *SD*), total phosphorus by 2.89 µg/L (±0.89 *SD*), and total nitrogen by 180.17 µg/L (±39.1 *SD*) at the start of the browning. However, the natural lake water used to fill the tanks contained much higher nutrient and carbon levels (TOC: 8.05 mg/L, total phosphorous: 18.25 µg/L, total nitrogen: 420.5 µg/L). In the first half of the experiment, no fish were present and only BE treatment mesocosms were browned (Figure 1). This allowed the zooplankton populations to establish without predators present and us to study whether browning-induced light limitation affected phytoplankton biomass (measured as chl *a*) and zooplankton biomass and composition. On day

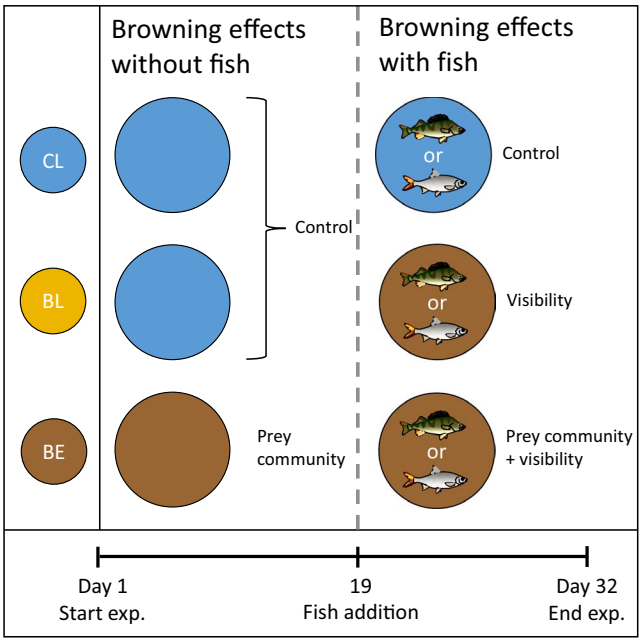

FIGURE 1 Set-up of the mesocosm experiment. Before adding fish, we had a clear control treatment (CL and BL) and a brown–early treatment (BE) to create and test effects of browning on the prey community through decreased light availability. From day 19, when fish were introduced we had a clear control treatment (CL), a brown–late treatment (BL) to test the effect of reduced visibility on fish species-specific growth responses, and a brown–early treatment (BE) to test the combined effect of (potentially) altered prey communities and reduced visibility on fish species-specific growth responses

19 of the experiment, BL treatment mesocosms were browned and fish were added to all treatments and mesocosms, allowing us to study fish growth responses. Comparison of the BE and the BL treatment allowed us to determine if alterations in resource availability and composition, or decreased fish foraging rates due to decreased visual conditions, affected fish growth the most, and if growth responses varied depending on fish species identity (i.e. roach or perch). This rendered 6 treatments during the second half of the experiment that all were replicated three times (three browning treatments × two fish species treatments × three replicates = 18 mesocosms; Figure 1).

2.2.2 | Experimental fish

Fertilised perch and roach eggs were collected in Lake Mälaren on 25–26 May 2017 and transferred to two nearby ponds (22.5 × 6 m, maximum depth 1.5 m). The eggs hatched in the beginning of June and the fish lived in these ponds and fed on natural invertebrate prey communities until the start of the experiment.

On 28 August we took out fish from the ponds using a seine net. We selected fish of similar size for each species, of which we preserved a subsample for size estimates (perch length 50.6 ± 4.1 mm and weight 1.29 ± 0.39 g, roach length 45.2 ± 2.7 mm and weight 0.79 ± 0.14 g, means ± 1 *SD*). To control for the size difference between perch and

roach in their effects on zooplankton, we added either 6 perch or 10 roach to the mesocosms. This was specifically done to achieve similar energy requirements (i.e. metabolic mass) of fish between mesocosms regardless of fish species present, such that potential variation in top-down influence of fish on lower trophic levels would only link to fish species identity and not to different energy requirements (see supplementary methods). We acclimatised the fish in containers with filtered lake water for a few hours before introducing them to the experimental mesocosms. The experiments in this study were conducted in accordance with national guidelines for animal care and the procedures employed were reviewed and approved by the regional ethical review board in Uppsala, Sweden (Dnr 5.8.18-03449/2017).

2.2.3 | Sampling

The mesocosms were sampled every 9 days before fish were added and every 6–7 days after fish addition. At each sampling occasion, water temperature was measured at the surface and at 0.5 meter depth (Figure S1). Photosynthetically active radiation (PAR) was measured at the surface, at 0.5, and 0.8 m depth with a LI-250A light meter with a LI-193SA spherical underwater quantum sensor (LI–COR Biosciences–Biotechnology, Lincoln, NE, USA, Table S2). From these PAR measurements, the light attenuation coefficient (k_z/m) was calculated as: $k_z = \ln\left(\frac{PAR_0}{PAR_z}\right)/z$. Where PAR_0 is the PAR at the surface, and PAR_z is the PAR at depth z (m). Blackwater addition increased the light attenuation coefficients 4–5 fold, from 0.678 m^{-1} ± 0.035 (mean ± *SE*) in the CL treatment, to 3.49 m^{-1} ± 0.066 and 3.74 m^{-1} ± 0.103 in the BE and BL treatment, respectively (Figure S2). These are all within the range of naturally occurring light attenuation coefficients in the study region (Karlsson et al., 2015). Water samples for chl *a* analyses were taken at 0.5-m depth with a 2-L water sampler. Chlorophyll *a* was used as a proxy for phytoplankton biomass. From each water sample, 500 ml water was filtered through a 47-mm diameter glass microfibre filter (Whatman™), after which the filter was frozen until analysis. The samples were analysed by extraction with acetone and using a spectrophotometer (full method description, https://www.sis.se/api/document/preview/5605/, in Swedish). Zooplankton samples were taken with a zooplankton net with a mesh size of 70 μm and preserved in Lugol's solution. The net was hauled from the bottom to the surface of the mesocosms (1 m, net diameter 25 cm, corresponding to a sampled volume of 49 L). Using a stereo microscope, cladocerans were determined to genus level, while copepods were identified as either cyclopoid, calanoid, or nauplii. For each taxa/group, up to 15 individuals were length measured to the nearest 0.01 mm (all if fewer). Zooplankton lengths were converted to population biomass (μg) with taxa-specific length-weight conversions (Bottrell et al., 1976; Dumont, Van de Velde, & Dumont, 1975). We also calculated biomass proportion of each taxa/group of the total zooplankton biomass.

At the end of the experiment, all fish were removed from the mesocosms with a seine net, euthanised in a benzocaine solution, blotted dry, and measured and weighed to the nearest mm and 0.01 g.

2.3 | Capture rate experiment

We performed a foraging experiment in aquaria (38.5 cm l × 19.5 cm w × 24.5 cm h), filled with 15 L of filtered lake water. We measured capture rates on *Daphnia longispina* (0.7 ± 0.1 mm, mean ± *SD*) of perch and roach of similar size as used in the mesocosm experiment (mean length ± 1SD, perch: 44.5 ± 2.7 mm, roach: 44.2 ± 2.4 mm). The experiment was conducted at three different light conditions: clear, intermediate (medium brown), and dark brown water; and two temperatures (19 and 25°C). Different levels of browning were simulated by adding Sera Blackwater Aquatan water conditioner (0, 2 and 8 ml to each aquarium, respectively). We measured perch and roach capture rate (no. of prey eaten in 1 min) of *D. longispina* after inoculating each aquarium with a density of four *D. longispina* per litre (total of 60 *D. longispina* per aquarium). We had between three and five replicates for each treatment. For a more detailed method description of the capture rate experiment, see supplementary methods.

2.4 | Lake data

Lake fish data were obtained from the Swedish National Register of Survey test-fishing (National Register of Survey test-fishing - NORS, 2016). We selected lakes that had length-at-age data for perch and/or roach for a minimum of five fish per selected age (1 and 5 year olds) for the time period 2006 to 2015. Lakes larger than 5 km^2 were excluded to limit variation in lake size. For all lakes included in our analyses we also have environmental data (e.g. absorbance of filtered water at 420 nm, temperature, and turbidity) sampled in July and/or August for at least 4 years during the same time period (Miljödata MVM database, https://miljodata.slu.se/mvm/Default.aspx, on 05-12-2016), and lake morphology data (area, mean depth; see Table S1). These selection criteria gave us a dataset of 49 small to intermediate sized lakes (area: 0.04–4.89 km^2) distributed all over Sweden, of which 43 contained perch and 40 roach (see Table S1). Lake water colour is reported as absorbance at 420 nm in samples taken at 0.5 m depth, where high absorbance is a proxy for brown water (Kirk, 1994). Absorbance was measured using filtered (0.45 μm filter) water in a 5-cm cuvette and converted to the Napierian absorption coefficient (a_{420}) as recommended by Hu, Muller-Karger, and Zepp (2002) (hereafter we use *absorbance* to refer to the Napierian absorption coefficient, see supplements). Mean absorbance measured across lakes during July and August was 6.36 m^{-1} (0.7–22.2 m^{-1}).

All fish were sampled using multi-mesh gillnets in the benthic and pelagic zones according to a standardised test-fishing method (Appelberg et al., 1995). For detailed information on the fish sampling method see van Dorst et al. (2019). All captured fish were identified to species and their total individual length was measured to the nearest mm. In order to obtain individual age estimates of perch and roach, random sub-samples were collected in proportion to the size distribution of the total catch. Fish that were sub-sampled for age determination were measured to the nearest millimetre and weighed to the nearest gram. Otoliths of perch and roach were used for age determination (Le Cren, 1947; Linløkken, Kleiven, & Matzow, 1991). We calculated mean

length-at-age (mm) at catch of age 1 and 5 perch and roach. Length-at-age 1 represents growth during the first year and length-at-age 5 is a result of the growth during the first 5 years of life. We included both length-at-age 1 and 5 in order to see if possible species-specific responses to browning hold over ontogeny.

2.5 | Statistics

To study if fish growth responses to browning were mostly caused by changes in resource availability and composition due to light limitation, or by reduced foraging rates due to decreased visual conditions we first test how browning influenced the lower trophic levels in our pelagic food web. We analysed treatment differences in chl *a* concentration and total zooplankton biomass over time with mixed-design analyses of variance models (mixed ANOVA, equivalent to a split-plot ANOVA) using the package *afex* in R (Singmann, Bolker, Westfall, & Aust, 2018). Response variables were ln-transformed before analyses. Analyses were performed separately on data collected before (day 1, 10 and 19) and after fish addition (day 19, 26 and 32). Before adding fish we only had two browning treatments: a clear control (CL and BL, analysed as one as BL was browned first when adding fish) or brown treatment (BE; Figure 1). Thus, for this first part of the experiment, the response variables were analysed with a two-way mixed ANOVA with browning as the between mesocosm variable and date as a within mesocosm (random) variable (formula: ln(response variable) ~ browning treatment × date). After adding fish we had three browning treatments (CL, BL, and BE, as BL was browned when adding the fish) and two fish species treatments (perch and roach; Figure 1). For this second part of the experiment, response variables were analysed with a three-way mixed ANOVA with browning and fish species treatments as between mesocosm variables and date as the within mesocosm (random) variable (formula: ln(response variable) ~ browning treatment × fish species × date). When the assumption of sphericity in the mixed ANOVA was not met, Greenhouse–Geisser sphericity corrected statistics are shown. If we found significant main or interactive effects we performed follow-up pairwise tests with Bonferroni adjustments using the *lsmeans* package in R (referred to as pairwise comparison in results) (Lenth, 2016).

Because zooplankton taxa-specific biomass data did not adhere to assumptions of a mixed ANOVA because of too many zeroes, we studied biomass of three common zooplankton taxa/groups and zooplankton community composition on one date before fish addition (the final date, day 19, allowing for enough time for zooplankton to respond to browning and also representing the starting values of prey available for fish) and one date with fish present (the middle date, day 26, as most zooplankton were depleted on the final date). We analysed treatment differences in biomass of *Bosmina* sp. and copepods for these two dates, and *Daphnia* sp. on day 19 (as they were almost completely consumed by day 26) with analyses of variance models (ANOVA). Response variables were ln-transformed before analyses. We statistically tested for differences in zooplankton community composition among treatments for these two dates with permutational multivariate analysis of variance (PERMANOVA (Anderson, 2001)), using the *adonis* function in the *vegan* package, with 999 permutations. The PERMANOVA was based on distance matrices of zooplankton taxa/group biomasses using the Bray–Curtis dissimilarity index (which can handle zero-skewed community composition data) (Clarke & Warwick, 2001). To assess the extent of unequal variance in our data sets (to which PERMANOVA is sensitive, (Anderson & Walsh, 2013)), PERMANOVAs were followed by *betadisper* tests, a multivariate analogue of Levene's test for homogeneity of variances. These were not significant, suggesting no treatment effects on variance. To visualise differences in community composition we graphed biomass proportions for each zooplankton taxa/group to the total zooplankton biomass. In addition we used non-metric multidimensional scaling (NMDS) plots, again based on distance matrices of zooplankton taxa/group biomasses using the Bray–Curtis dissimilarity index (Clarke & Warwick, 2001). The NMDS were performed with the *metaMDS* function in R's *vegan* package (Oksanen et al., 2019). Each ordination ran for 100 iterations, or until the lowest stress score was found. Stress scores were sufficiently low (<0.2) in all runs, such that data could be interpreted in two dimensions.

To test for treatment effects on growth responses (measured as increase in weight from the start to the end of the experiment) of perch and roach in the mesocosm experiment we carried out two one-way ANOVA's (ln(perch growth) ~ browning treatment, and ln(roach growth) ~ browning treatment). We assessed survival rate with a two-way ANOVA (survival rate ~ browning treatment × fish species). Tukey-HSD post hoc tests were performed when the ANOVA was significant.

We analysed effects of browning on fish foraging rates, measured in the aquarium experiment, with two two-way ANOVA models, one per fish species with browning (3 levels) and temperature (two levels) as explanatory variables (capture rate = browning treatment × temperature). Interactions were not significant for either model and therefore removed. Temperature is present in the study to generalise the results, but is not a variable we specifically want to test for in this study.

For the lake study, we analysed the influence of water colour (absorbance) on ln-transformed length-at-age 1 and 5 of both perch and roach with linear regression models. We ran an ANCOVA for each species, with water colour (absorbance) as an independent measuring variable and age (1 and 5) as a nominal variable, to study if the effect of water colour on length-at-age differed between ages. In addition, we repeated the linear regression analyses including multiple environmental covariates likely to affect fish body growth (see supplementary methods and results).

All analyses were based on significance levels $p < 0.05$ (two-sided tests) and were done in R 3.4.2 (R Core Team, 2017).

3 | RESULTS

3.1 | Mesocosm experiment

3.1.1 | Browning effects before fish addition

Prior to fish addition, browning initially had a slight positive effect on chl *a* concentrations (Table 1, Figure 2). After this initial increase there

TABLE 1 Mixed ANOVA models on the effects of browning (CL = clear/BE = brown–early/BL = brown–late), fish species identity (perch/roach), and time (day 1, 10, 19, 26, 32) on chlorophyll a concentration and total zooplankton biomass, before and after fish addition

Explanatory variables	Chlorophyll *a* concentration	Zooplankton community biomass
Before fish addition		
Browning (CL/BE)	$F_{(1,16)} = 25.70^{***}$	$F_{(1,16)} = 0.28$
Time	$F_{(2,32)} = 37.85^{***}$	$F_{(1.10,17.65)} = 70.45^{***}$
Browning × Time	$F_{(2,32)} = 6.95^{**}$	$F_{(1.10,17.65)} = 0.83$
With fish		
Browning (CL/BE/BL)	$F_{(2,11)} = 45.76^{***}$	$F_{(2,12)} = 2.48$
Fish species	$F_{(1,11)} = 4.77$	$F_{(1,12)} = 1.92$
Browning × Fish species	$F_{(2,11)} = 0.29$	$F_{(2,12)} = 0.39$
Time	$F_{(2,22)} = 31.71^{***}$	$F_{(2,24)} = 25.89^{***}$
Browning × Time	$F_{(4,22)} = 10.38^{***}$	$F_{(4,24)} = 2.55^{+}$
Fish species × Time	$F_{(2,22)} = 1.99$	$F_{(2,24)} = 3.06^{+}$
Browning × Fish species × Time	$F_{(4,22)} = 0.47$	$F_{(4,24)} = 1.62$

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{+}p < 0.1$.

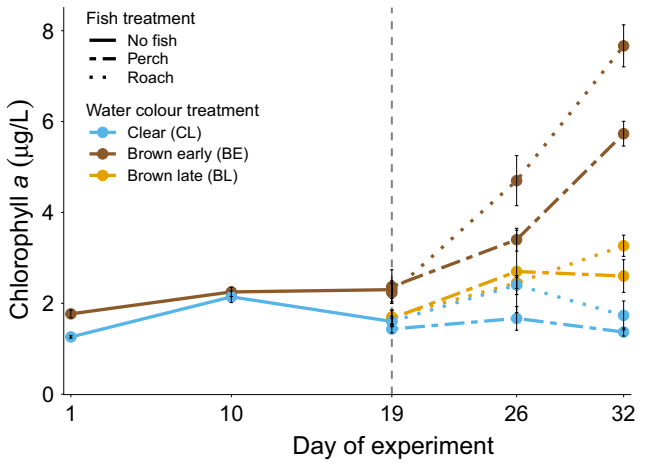

FIGURE 2 Chlorophyll *a* concentrations (means ± *SE*) over time in different browning treatments, before and after addition of perch or roach on day 19 of the mesocosm experiment (vertical grey dashed line)

was a slight decrease in chl *a* concentrations before fish addition in the clear, but not the brown, treatment (Figure 2, Clear treatment: pairwise comparison dates: $p < 0.05$ (Figure 2). Consequently, chl *a* concentrations were higher in the brown than in the clear treatment on the final day before fish addition (Figure 2, pairwise comparison: $p < 0.0001$).

Whereas we found an overall increase in zooplankton biomass over time, there was no effect of browning on total zooplankton biomass (Figure 3a, Table 1) prior to fish addition. However, zooplankton community composition differed between clear and brown mesocosms at the time when fish were introduced (Day 19; Figure 4a and Figure S4a, PERMANOVA = $F_{(1,16)} = 2.26$, $p = 0.043$). This seems mainly caused by a higher biomass proportion (Figure 4a) and absolute biomass of *Daphnia* sp. in brown compared to clear water mesocosms on this date (Figure 3b, biomass ANOVA: $F_{(1,16)} = 6.19$, $p = 0.024$). There was also a lower biomass proportion of *Bosmina* sp. in brown than in clear water mesocosms (Figure 4a, for community composition on all dates see Figure S3). However, there were no differences between treatments in absolute biomass of *Bosmina* sp. (Figure 3c, ANOVA: $F_{(1,16)} = 2.29$, $p = 0.1499$) or copepods (Figure 3d, ANOVA: $F_{(1,16)} = 0.0244$, $p = 0.8779$) on day 19.

3.1.2 | Browning effects with fish

Fish responses

Perch body growth was negatively affected by early browning (ANOVA: $F_{2,6} = 7.941$, $p = 0.0206$), being lower in the BE (0.35 ± 0.039 g, mean ± *SE*) than in the CL (0.62 ± 0.068, Tukey-HSD: $p = 0.027$; Figure 5) and the BL (0.60 ± 0.075, Tukey-HSD: $p = 0.037$) treatments. Perch body growth in the BL treatment, however, did not differ from the CL treatment (Tukey-HSD: $p = 0.966$). In contrast, there were no effects of water colour treatment on roach body growth (ANOVA: $F_{2,6} = 0.417$, $p = 0.677$; Figure 5). Roach had a lower mean survival (94%) than perch (100%; ANOVA: $F_{1,14} = 4.73$, $p = 0.047$), irrespective of browning.

Chlorophyll a

After fish addition, browning further increased chl *a* concentrations (Table 1), with chl *a* concentrations being lowest in the CL and highest in the BE treatment, and BL in between (Figure 2, pairwise comparisons: $p < 0.05$). In both brown treatments (BE, BL), chl *a* concentrations increased throughout this second part of the experiment (Figure 2, Table 1), with treatment differences being largest at the end of the experiment (pairwise comparisons between browning treatments on the final date: $p \leq 0.001$). Fish species identity had no effects on chl *a* levels (Table 1).

Zooplankton responses

Total zooplankton biomass decreased over time after fish addition but (similar to before fish addition) there was no effect of browning

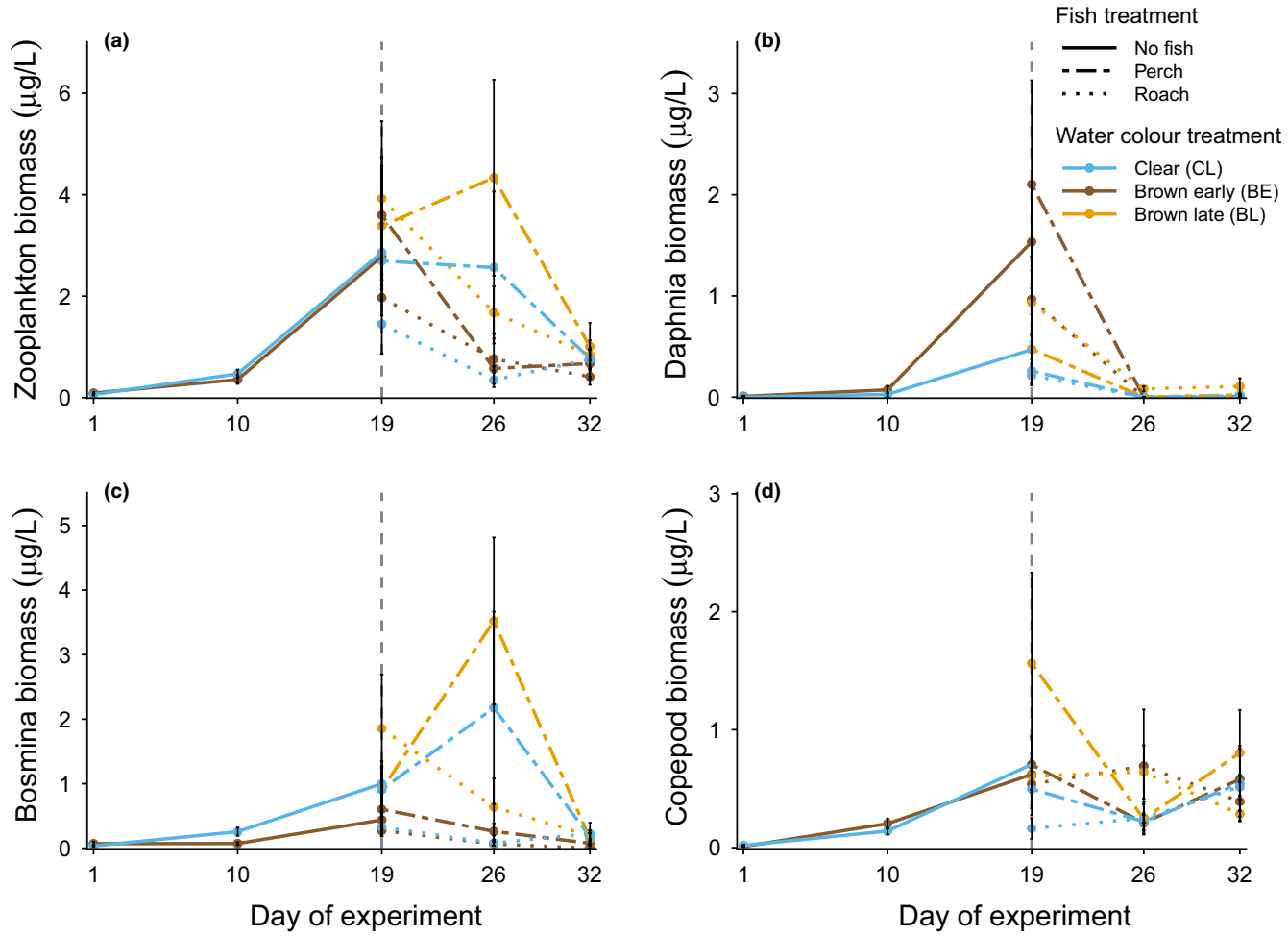

FIGURE 3 (a) Total zooplankton biomass, (b) *Daphnia* sp. biomass, (c) *Bosmina* sp. Biomass, and (d) copepod biomass for different browning treatments over time, before and after addition of perch or roach on day 19 of the mesocosm experiment (vertical grey dashed line). All values are means ± *SE*

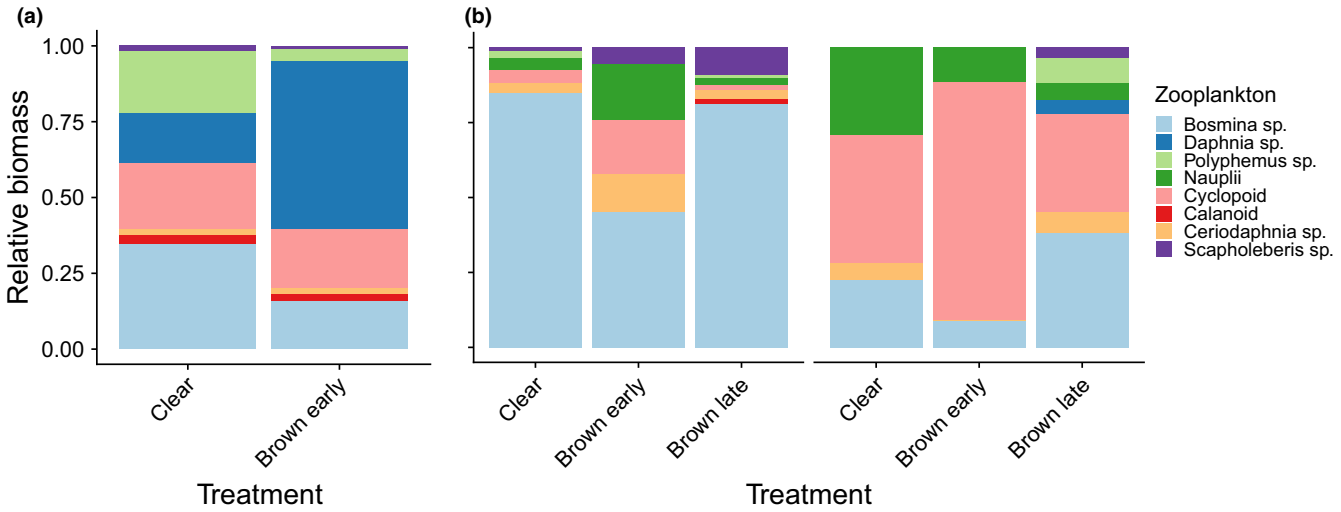

FIGURE 4 Zooplankton community composition as relative biomasses of all groups in the different water colour and fish species treatments on (a) day 19 and (b) day 26 of the mesocosm experiment

(Figure 3a, Table 1). However, similar to before fish addition, there was an effect of browning on zooplankton community composition when fish were present (Figure 4b and Figure S4b, PERMANOVA: $F_{(1,12)}$ = 2.13, p = 0.047). The difference in community composition on this date seems mainly to be caused by a higher biomass proportion of cyclopods and lower biomass proportion of *Bosmina* sp. in

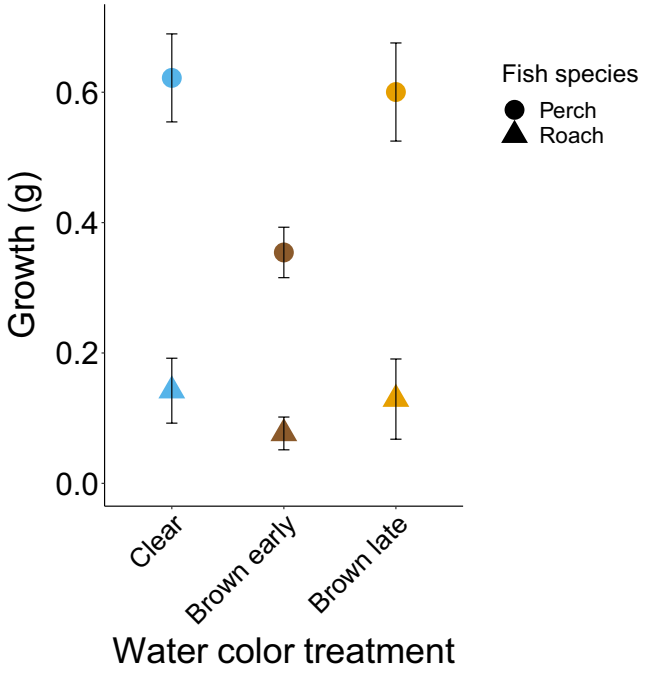

FIGURE 5 Means ± *SE* of body growth (g) of perch and roach in different browning treatments in the mesocosm experiment [Colour figure can be viewed at wileyonlinelibrary.com]

the BE compared to BL and CL treatments (Figure 4b). There was also a lower absolute *Bosmina* sp. biomass in the BE compared to the BL treatment on day 26 of the experiment (Tukey-HSD: BL-BE, p = 0.024, Figure 3c). *Daphnia* sp. almost completely disappeared from all treatments when fish were present (Figure 3b). Also, fish species identity had an effect on zooplankton community composition (Figure 4b and Figure S4b, PERMANOVA: $F_{(1,16)}$ = 3.81, p = 0.008, for community composition on all dates see Figure S3), with mesocosms with perch being dominated by *Bosmina* sp. while mesocosms with roach were dominated by copepods.

3.2 | Capture rate experiments

There was a tendency of a negative effect of brown water colour on capture rate of *D. longispina* by perch (ANOVA: $F_{2,22}$ = 3.4156, p = 0.051, Figure 6a). In contrast, capture rate of *D. longispina* by roach was not affected by water colour (ANOVA: $F_{2,21}$ = 1.9256, p = 0.17, Figure 6b). Temperature had no effect on capture rate of *D. longispina* by either species (perch: ANOVA: $F_{1,22}$ = 2.531, p = 0.13, roach: ANOVA: $F_{1,21}$ = 2.2777, p = 0.15, Figure 6).

3.3 | Lake data

There were negative relationships between absorbance and length-at-age for both 1- and 5-year-old perch (age 1: R^2 = 0.1, $F_{(1,41)}$ = 4.41, p = 0.042, age 5: R^2 = 0.31, $F_{(1,41)}$ = 18.25, p = 0.00011), but not for roach (age 1: R^2 = 0.00041, $F_{(1,38)}$ = 0.015, p = 0.902, age 5: R^2 = 0.083, $F_{(1,41)}$ = 3.456, p = 0.071, Figure 7). The negative effect of high absorbance (i.e. brown waters) on length-at-age was stronger in old than in young perch (ANCOVA: absorbance*age: $F_{(3,82)}$ = 4.04, p = 0.0476, Figure 7). Results were similar when including environmental covariates likely to influence fish growth (Table S3).

4 | DISCUSSION

Browning of waters can strongly impact aquatic ecosystems (van Dorst et al., 2019; Karlsson et al., 2015; Kelly et al., 2014; Vasconcelos et al., 2016). However, it is unknown if browning-induced light limitation affects food webs, and especially predators, mainly through changes in resource availability and composition or through direct effects on consumer feeding capacities. Here we experimentally show that browning leads to species-specific growth responses in fish, and that these probably come about through shifts in the zooplankton (prey) community, possibly in combination with reduced foraging rates. In the brown early (BE), but not the brown late (BL) treatment, we found a strong increase in chl *a* concentrations, shifts in zooplankton community composition, and a decrease in perch but not roach body growth. The latter is likely to be linked to observed shifts in prey (zooplankton) composition with browning. In addition, we found some evidence for reduced capture rates in perch, but not roach, in response to decreased visual conditions. However, reductions in foraging were not large enough on their own to cause reduced perch growth in the mesocosm experiment, as they only did so when combined with prey compositional changes (in the BE treatment). In a comparative analysis of fish growth in over 40 lakes, we confirmed that perch but not roach growth is negatively affected by brown waters in nature.

Perch, but not roach, body growth was lower in mesocosms that were browned already at the start of the experiment (BE treatment) compared to fish growth in the clear water and the BL mesocosms, while the late browning treatment did not lead to lower growth. In addition, we found limited evidence for decreased capture rates on *D. longispinus* by perch (but not roach) in brown waters. This shows that the extent to which light limitation caused by browning affects capture rates on zooplankton can vary between fish species (see also Jönsson et al., 2012; and Weidel et al., 2017). The lack of a negative capture rate response of roach to light limitation confirms previous studies, where the absence of a response was assumed to be due to the short distance at which zooplankton prey are detected by fish (Jönsson et al., 2012). The results of the mesocosm experiment suggest that decreased visual feeding conditions due to browning on its own probably has a limited direct negative effect on perch or roach body growth, at least not in pelagic food webs and given the level of browning studied here. Rather, the negative effect on perch body growth with early browning is probably a consequence of factors other than decreased foraging capacity alone. The lack of a growth response in the BL treatment suggests that changes are the prey community in response to light limitation

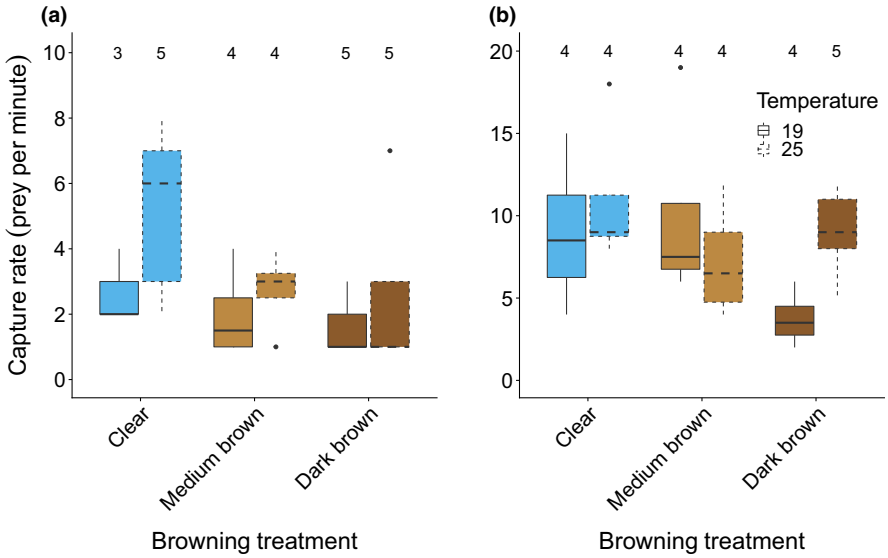

FIGURE 6 Number of *Daphnia* sp. eaten in the capture rate experiment during 1 min (starting after the first Daphnia was consumed) by (a) perch and (b) roach, shown with box and whisker plots, where the box represent the median, and 25th and 75th quantiles, and the whiskers represent the smallest observation greater than or equal to lower hinge – 1.5 × interquartile range and the largest observation less than or equal to upper hinge + 1.5 × interquartile range [Colour figure can be viewed at wileyonlinelibrary.com]

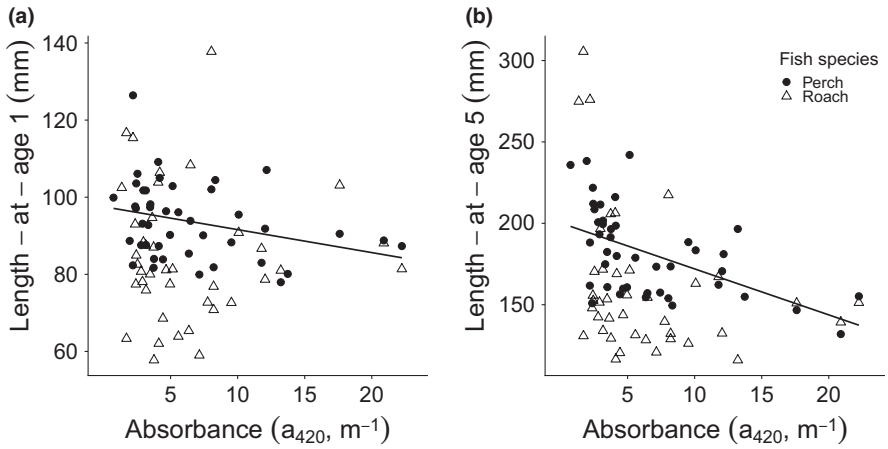

FIGURE 7 Length-at-age of (a) 1- and (b) 5-year-old perch and roach in Swedish lakes. Regression lines represent significant relationships between mean absorbance (higher absorbance means browner lakes) and perch length-at-age (1 or 5 years)

(which developed before fish addition), perhaps in combination with lower capture rates, led to slower body growth of perch in brown waters.

Before fish addition there was an increase in chl *a* (proxy for pelagic phytoplankton biomass) in the BE treatment, similar to what was found in a long-term lake study by Leach, Winslow, Hayes, and Rose (2019). However, higher chl *a* concentrations in brown waters, in the absence of increased nutrient concentrations, are probably caused by an increase in the amount of chl *a* per phytoplankton cell with decreased light conditions (Fennel & Boss, 2003; Geider, MacIntyre, & Kana, 1997) rather than an actual increase in phytoplankton biomass, as we see no subsequent increase in zooplankton biomass.

In contrast to chl *a*, total zooplankton biomass did not respond to browning before (or after) fish addition. However, zooplankton community composition did shift with browning. Before fish addition, there was a higher biomass and proportion of *Daphnia* sp. and a lower proportion of *Bosmina sp.* in brown than in clear waters. A study by Wissel, Boeing, and Ramcharan (2003) found a similar dominance of *Daphnia* sp. in brown compared to clear waters in the absence of fish. The observed changes in zooplankton community composition in our experiment before fish addition are likely to be either a consequence of potential changes in phytoplankton community composition or a direct effect of decreased light penetration. Previous studies have shown that zooplankton species prefer different phytoplankton (Mitra et al., 2014; Sommer & Sommer, 2006), but the extent to which there was a shift in phytoplankton composition in our study is unknown (phytoplankton was not sampled, only chl *a*). Browning may also change zooplankton community composition by blocking harmful ultraviolet (UV) radiation penetrating the water column (Williamson, Stemberger, Morris, Frost, & Paulsen, 1996), as zooplankton species are differently equipped to cope with UV radiation (Williamson, 1995). For example, *Daphnia* sp. has been shown to have a higher survival and reproduction rate when UV-B light is excluded (Zellmer, 1995, 1998), which is in concordance with the increased biomass and proportion of *Daphnia* sp. we found with browning.

After fish addition, we observed differences between treatments across all trophic levels. The observed increase in treatment differences of chl *a* concentrations could be the consequence of the

observed changes in zooplankton community composition when fish were added, given that different zooplankton taxa can vary in feeding efficiency and selectivity on different phytoplankton species (Mitra et al., 2014; Sommer & Sommer, 2006). However, we did not measure this in our experiment. Similar to before fish addition, total zooplankton biomass did not differ between treatments, but specific taxa and zooplankton community composition did. After fish were added, all *Daphnia* sp. (which were more abundant in the BE compared to clear treatment at the end of the no-fish part of the experiment) were soon eaten in all treatments. The rapid decline in *Daphnia* sp. biomass is expected as they are a desirable zooplankton prey for many fish species (Giles, Street, & Wright, 1990; Mills, Confer, & Ready, 1984). However, in addition, there was a lower biomass of *Bosmina* sp. in the BE compared to the BL and clear treatments. The fact that there was more of a difference in community composition between the BE and the BL treatment than between the BL and clear treatment, suggests that most of the difference in community composition between treatments after fish addition stems from differences that developed already before fish were added and that this was maintained when fish were present.

These differences in zooplankton communities may explain the decreased growth rate of perch in the BE treatment. After the fish had eaten their preferred *Daphnia* prey, there were fewer other cladoceran prey (*Bosmina* sp.) left to feed on in the BE treatment. The cause of the difference in growth response between the two fish species could be partly due to the fact that perch and roach differ in zooplankton prey preferences (in the absence of browning). Small roach seem to be more selective for cladocerans such as *Bosmina* sp. (Byström & García-Berthou, 1999; Hammer, 1985), while small perch are less selective in their prey choice (Byström & García-Berthou, 1999). This can explain the relatively lower biomass of *Bosmina* sp. in mesocosms with roach during the experiment compared to mesocosms with perch, irrespective of browning. In addition, at a given size, roach can sustain positive growth rates on less zooplankton than can perch (Byström & García-Berthou, 1999), and roach may therefore have been less affected than perch by any change in preferred zooplankton availability and community composition caused by browning. Furthermore, the capture rate experiment showed that perch but not roach zooplankton capture rate may be negatively affected by water colour. Accordingly, the combination of a different zooplankton community composition caused by browning before fish addition, species-specific feeding responses to water colour, species-specific differences in prey selection, and the higher feeding efficiency of roach probably led to a decrease in perch but not roach body growth in the BE treatment.

That perch but not roach grew slower in response to brown waters, as suggested by our experimental results, was confirmed in our analysis of fish growth in lakes. Both young and old perch had a lower length-at-age in brown compared to clear lakes, with the older perch having the most negative response. The latter is probably a result of that larger perch feed mainly on zoobenthos and fish (Amundsen et al., 2003), which both can be more negatively affected by browning than zooplankton (Vasconcelos et al., 2018), and because the feeding on zoobenthos and fish prey itself relies more on vision than does zooplankton feeding (Jönsson et al., 2012; Ranåker et al., 2012). Furthermore, larger fish need higher resource levels than small ones to sustain high growth rates (Byström & Andersson, 2005; Hjelm & Persson, 2001), which, in combination with a stronger decrease in prey levels for large perch, can explain the stronger negative effect on length-at-age of older individuals. As in the mesocosm experiment, roach body growth in lakes was not influenced by water colour. Thus, we conclude that fish growth responses to browning of waters are species specific (as also shown for growth of young fish over a DOC gradient in Benoît, Beisner, & Solomon, 2016).

These species-specific responses to browning can potentially shift the outcome of interspecific interactions such as competition. Furthermore, as reproduction rates generally increase with body-size, decreased growth rates caused by browning can lead to reduced population growth or biomass of certain species. Also, top predators, such as pike (*Esox lucius*) feeding on roach and perch, can probably be affected by species-specific growth responses of prey fish to browning, depending on species and size-preferences. Thus, not only may browning influence fish community composition, but we can expect different responses depending on the fish community present at the onset of browning, ranging from minor to major negative effects on community biomass and production.

In this study, we deliberately only looked at pelagic food web responses to browning due to shifted light conditions. However, in nature, browning is often accompanied with a significant concurrent increase in nutrients and DOC (Creed et al., 2018). Accordingly, previous experimental studies have often added humic substances to create browning treatments, leading not only to a decreased light availability but also a substantial increase in DOC and nutrient concentrations (e.g. Hansson et al., 2012; Vasconcelos et al., 2016; Vasconcelos et al., 2018). Whereas such a set-up creates a more realistic scenario, it makes it impossible to distinguish between effects of reduced light availability and increased nutrient/carbon input. Our experiment was designed to single out the effect of decreased light availability (with a much smaller addition of TOC and nutrients compared to the natural lake water used in all tanks), but still replicates impacts of browning across trophic levels similar to what is observed in nature (Ask et al., 2009; van Dorst et al., 2019; Karlsson et al., 2015) even without the addition of nutrients. It is, however, difficult to mechanistically explain lower trophic level responses in our experiment. For example, to elucidate the mechanisms underlying the browning-induced shifts in the zooplankton community, data on phytoplankton biomass and composition would be helpful, as would browning experiments on zooplankton capture rates of phytoplankton similar to the ones we did on fish. The capture rate experiment gives us some important insights on the possible mechanisms behind the effects of browning on fish, but to directly link the capture rate results to our mesocosm experiment is difficult. In our foraging experiment, we only used one zooplankton species, *Daphnia longispina*. *Daphnia* sp. was present in the mesocosm experiment and eaten rapidly by the fish in the experiment,

but was certainly not the only species present in that study. Ideally, we would have replicated the capture rate experiment with multiple zooplankton species, to study if the particular zooplankton species fed on influenced fish feeding responses in different water colours. Furthermore, light levels (PAR) differed between the mesocosm and capture rate experiment. However, the degree of light reduction between clear and brown treatments in both experiments is comparable.

Our findings increase our understanding of how browning affects pelagic food webs in temperate and boreal lakes. We show that browning can reduce fish growth—for some but not all fish species—through changes in resource availability and composition caused by light limitation, possibly in combination with a negative effect of decreased visibility on fish foraging rates. The species-specific responses we found in the experiments were reflected by the lower length-at-age of perch, but not roach, observed in brown water lakes in the large colour gradient of natural lakes. Lower body growth of some species is key to explain the lower fish biomass production in brown lakes (as reported by van Dorst et al., 2019), and suggests that we can expect different biomass production responses to browning depending on fish species present.

In conclusion, as temperate and boreal lakes get browner, we can expect shifts in zooplankton prey composition and fish foraging rates, and consequential species-specific reductions in fish body growth. These fish species-specific reductions in growth to browning will probably affect competitive and predator–prey interactions, and ultimately entire lake ecosystems.

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DATA AVAILABILITY STATEMENT

The data used for this manuscript are openly available on Zenodo at https://doi.org/10.5281/zenodo.3603049.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supplementary information

Does browning-induced light limitation reduce fish body growth through shifts in prey composition or reduced foraging rates?

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Supplementary methods

Energy requirements fish mesocosm experiment

In order to calculate energy requirements (*Em*, i.e. metabolic demand per unit time) of perch and roach of a certain size we used the function:

$$Em = \rho_1 w^{\rho_2}$$

Where w is the weight of an individual in gram and ρ_1 and ρ_2 are the metabolic scalar and allometric exponent, respectively, assumed to be 0.033 and 0.77 for both species (Persson et al. 1998, Claessen et al. 2000). Based on size measurements of a subsample of fish caught to be used in the experiment we calculated the number of roach individuals (45 mm, 0.89 g) that would result in the same total metabolic demand as six perch individuals (52 mm 1.60 g), rendering 10 roach. Based on later size measurements of a larger subsample of individuals, the average body weights were slightly lower than what was initially estimated based on the first smaller subset of individuals. The latter size estimate would have resulted in 9 rather than 10 roach being used to standardize metabolic mass.

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Aquarium experiment

The fish in the aquarium experiment had the same origin as the fish used in the mesocosm experiment. Perch and roach roe were collected in Lake Mälaren on 25 - 26 May 2017 and immediately transferred to two nearby ponds (1 for perch and 1 for roach, both being 22.5 x 6 meter with a maximum depth of 1.5 meter). The roe of both species hatched in the ponds in the beginning of June, where after the fish fed on the natural invertebrate prey communities in the ponds until the start of the aquarium experiment (16 August 2017). We collected fish from the ponds with a sein net the day prior to the start of the experiment. We selected perch and roach of similar sizes as used in the mesocosm experiment (mean length ± 1SD, perch: 44.5 ± 2.7 mm, roach: 44.2 ± 2.4 mm). Fish were starved and acclimatized in aquaria at experimental temperatures (19 or 25 ˚C) for 12-18 hours before the start of the experiment.

The capture rate experiment was carried out in aquaria (38.5 cm l *19.5 cm w *24.5 cm h), filled with 15 L (20 cm water depth) of filtered lake water. We covered the bottom, back and sides of the aquaria with dark blue plastic. A 20W halogen light bulb was hung 20 cm above each aquarium and a white fabric was placed in between the aquarium and the light bulb in order to create closer to natural

light conditions. No other lights were present in the room, and the light from the light bulb above the aquarium was blocked with black fabric so no light would come in directly through the front of the aquarium. The experiment was conducted at two temperatures (19 and 25˚C) and three different light conditions: clear, intermediate and dark brown water. Trials for each treatment combination were replicated 3-5 times. Different levels of browning were simulated by adding Sera Blackwater Aquatan water conditioner (Sera GmbH, Heinsberg, Germany). To create the medium brown and dark brown treatments, 2 ml and 8 ml blackwater respectively were added to the 15 litre aquaria. The photosynthetically active radiation (PAR), measured at the bottom of the aquarium, was 3.13 µmol s^{-1} m^{-1} ± 0.059 (mean ± SE) in the clear treatment, 2.26 µmol s^{-1} m^{-1} ± 0.08 in the intermediate browning treatment and 1.08 µmol s^{-1} m^{-1} ± 0.04 in the dark browning treatment.

At the start of each experimental trial, four *Daphnia longispina* (0.7 ± 0.1 mm, mean ± SD, occasionally another species of Daphnia or Ceriodaphnia may have been present) per liter (60 *Daphnia longispina* in total per aquarium) were introduced into the aquarium from above, and the water was lightly stirred to distribute zooplankton evenly in the aquarium. For treatments with perch, we introduced one perch into the aquarium after the addition of Daphnia. The trial started if the perch caught its first prey in the first 6 minutes. If a perch did not eat in the first 6 minutes after being introduced into the aquarium, the trial was stopped and neither the zooplankton nor fish were used for further trials (48 trials were started, of which 26 were successful). After the fish captured its first prey, we counted the number of zooplankton consumed (including this first one) for one minute. Whether a zooplankton was consumed or not was determined by visual observation. The zooplankton were visible to the naked eye, and the predation behaviour of the fish was clear.

As the roach were stressed when alone in an aquarium and took a long time to acclimate to the aquarium, a different set-up was used for the trials with roach. For treatments with roach, we instead added two roach to the aquarium 1.5 hour prior to the start of the trial. Daphnia were added as in trials with perch. If one of the roach individuals captured a prey within the first six minutes, we observed zooplankton feeding in the same way as for perch, i.e. for one minute and counted the number of zooplankton consumed (including this first one) (31 trials were started, of which 25 were successful). At the end of the experiment the fish (both perch and roach) were removed from the aquarium, euthanized in a benzocaine solution, and measured to the nearest mm.

Lake data

Absorbance, Napierian coefficient

We calculated the Napierian coefficient ($a_{420,}$ in m^{-1}) from the absorbance of filtered lake water (0.45 µm filter) at 420 nm in a 5cm cuvette ($AbsF_{420nm/5cm}$) as:

$$a_{420} = (AbsF_{420nm/5cm} * \ln(10))/\mathrm{OL} \qquad (1)$$

where a_{420} is the Napierian coefficient, $AbsF_{420nm/5cm}$ the measured absorbance of filtered water at 420 nm, and OL is the optical path-length (in m).

Multiple linear regression lake data

To study fish length-at-age, we, in addition to the simple linear regression with water colour (a_{420}), ran a multiple linear regression with multiple environmental covariates that may influence fish body growth and that were available for most lakes (excluding interactions between covariates). We selected mean summer temperature, total phosphorus, total nitrogen, turbidity (NTU), pH, and lake mean depth and area as covariates. For perch, 40 lakes were included in the analyses and for roach 35 (this is a few less than in the main analyses, due to missing data on covariates for some lakes). We ran a variance inflation factor (VIF) analyses to check for collinearity, and excluded one of the variables rendering a high VIF (>10, total phosphorus). See the outcome of the multiple linear regression in table S2.

Supplementary tables

Table S1 - Location, environmental conditions and physical characteristics of the 49 study lakes. Mean values of temperature (°C), absorbance (Napierian coefficient, a_{420}, m^{-1}), total phosphorus (P, µg/l), total nitrogen (N, µg/l), pH, and turbidity (NTU) are based on samples collected between 2006 and 2015. Lakes had either perch, roach or both species present.

Lake	Latitude (WGS84)	Longitude (WGS84)	Absorbance (a_{420}, m^{-1})	Temperature (°C)	P (µg/l)	N (µg/l)	pH	Turbidity (NTU)	Mean depth (m)	Area (ha)	Perch and/or Roach
Havgårdssjön	55.4831	13.3578	1.36	19.38	79.38	1105.67	8.23	8.12	3.1	54	Roach
Krageholmssjön	55.5016	13.7446	1.70	20.36	125.41	1265.56	8.52	13.63	5	214	Roach
Blanksjön	56.2147	15.1802	4.08	21.61	7.07	532.92	7.20	0.85	4.9	19	Perch and Roach
Lillasjön	56.2253	15.1459	2.20	22.15	9.60	483.50	7.43	1.70	2	10	Perch and Roach
Bäen	56.2460	14.3775	8.34	18.64	15.98	489.22	5.94	1.38	3.4	58	Perch
Stora Ålagylet	56.2845	14.7052	8.21	21.59	9.50	557.75	6.99	0.81		4	Roach
Örsjön	56.2864	14.6851	4.19	21.35	9.80	444.89	6.44	1.15	3.5	18	Perch and Roach
Västra Hultasjön	56.3420	14.4464	1.71	20.75	6.14	370.42	7.56	0.80		8	Roach
Brunnsjön	56.5972	15.7281	22.24	20.28	12.39	769.28	5.73	1.26	5.3	10	Perch and Roach
Stora Skärsjön	56.6712	13.0658	2.36	20.06	8.06		7.05		3.9	32	Perch and Roach
Gyltigesjön	56.7532	13.1740	20.90	19.04	15.13	702.17	6.80		9.1	40	Perch and Roach
Fiolen	57.0920	14.5296	2.97	19.15	13.45	475.83	6.75	1.46	3.9	156	Perch and Roach
Gyslättasjön	57.1080	14.4835	8.04	19.38	14.45	460.83	6.79	2.43	2.8	32	Perch and Roach
Nässjön	57.1723	13.0674	6.48	19.93	16.00	384.25	6.92	2.08	2.7	52	Perch and Roach
Hagsjön	57.2644	13.6865	13.22	19.73	8.50	427.13	6.87	1.04	4.6	24	Perch and Roach
Stengårdshultasjön	57.5578	13.8020	10.08	19.02	7.29	427.00	6.92	0.75	7.1	489	Perch and Roach
Stora Härsjön	57.7095	12.3217	2.41	20.37	4.14	330.78	7.38	0.49	14.1	257	Perch and Roach
Allgjuttern	57.9479	16.0963	2.44	19.91	3.89	289.22	6.82	0.62	11.7	18	Perch and Roach
Fräcksjön	58.1482	12.1812	5.58	20.72	9.76	369.44	6.74	1.01	4.1	28	Perch and Roach
Granvattnet	58.2260	11.7707	3.63	18.76	24.42	525.38	6.59	3.15	1.6	18	Roach
Geten	58.5610	15.7217	17.61	20.24	20.88	638.56	6.36	1.76	3.6	20	Perch and Roach
Humsjön	58.6188	14.4809	3.33	18.37	10.38	374.44	6.84	1.98	4	21	Perch

Skärgölen	58.7631	16.2339	2.80	20.45	5.88	322.56	7.02	0.78	7	18	Perch and Roach
Långsjön	58.8346	14.7208	9.53	20.30	11.48	470.72	6.45	0.94	4.2	67	Perch and Roach
Björken	58.8562	17.3702	3.15	21.26	7.62	379.44	7.40	0.74	12.5	137	Perch and Roach
Rotehogstjärnen	58.8150	11.6124	11.78	19.23	13.58	435.83	5.71	1.27	3.6	16	Perch and Roach
Älgsjön	59.0949	16.3694	12.05	21.06	27.35	736.72	6.87	2.76	2.5	36	Perch and Roach
Stora Envättern	59.1149	17.3535	3.47	20.99	6.89	392.00	6.69	0.92	5.4	38	Perch and Roach
Stensjön	59.1745	18.3244	4.12	19.99	5.74	355.56	6.86	0.83	9.1	39	Perch and Roach
Långsjön	59.1903	18.2969	6.36	20.12	7.75	367.50	6.21	1.13	3.8	9	Perch and Roach
Bysjön	59.3024	12.3399	2.91	18.95	11.92	286.11	6.86	1.38	7.4	113	Perch and Roach
Tärnan	59.5570	18.3660	3.50	20.62	11.01	478.11	7.37	1.75	4.3	105	Perch
Ulvsjön	59.6098	12.2937	4.67	18.08	7.08	311.22	6.39	0.74	10	49	Perch and Roach
Sparren	59.6912	18.3128	3.17	21.19	35.33	942.13	8.37	4.22	6.6	288	Perch and Roach
Lien	59.8087	15.5288	5.15	19.26	4.00	236.17	6.96		7.8	149	Perch and Roach
Övre Skärsjön	59.8371	15.5503	7.43	19.16	5.84	340.89	5.91	0.58	6.1	169	Perch
Dagarn	59.8970	15.6867	2.53	20.09	5.32	306.78	7.00	0.78	5.1	172	Perch and Roach
Västra Skälsjön	59.9347	15.5529	0.74	19.28	3.32	176.39	7.14	0.48	6.6	43	Perch
Skifsen	60.0755	14.4094	8.21	18.09	11.00	368.56	6.15	1.18	2.6	32	Perch and Roach
Tryssjön	60.4406	15.0880	12.16	17.49	7.26	314.67	6.37	0.66	7.2	30	Perch
Rädsjön	60.8198	14.3184	1.95	17.62	6.83	215.39	6.84	0.59	8.8	58	Perch
Källsjön	61.6331	16.7354	13.73	18.94	9.08	405.22	6.70	0.90	7.1	24	Perch
Stensjön	61.6428	16.5753	4.96	19.23	5.59	234.50	6.50	0.81	4.3	59	Perch and Roach
Väster Rännöbodsjön	62.3301	16.9872	4.43	20.00	12.74	305.00	7.26	1.53	6.2	48	Perch and Roach
Storsjön	62.5444	17.6657	7.76	19.46	14.45	368.11	6.91	1.38	2.6	309	Roach
Degervattnet	63.8728	16.2293	3.76	18.91	5.46	248.89	7.17	0.61	5.1	158	Perch and Roach
Remmarsjön	63.8620	18.2726	7.14	18.31	8.61	254.17	6.60	0.82	5	140	Perch and Roach
Pahajärvi	66.7709	23.3529	2.19	16.40	11.87	262.89	7.02	1.66	3.9	132	Perch
Jutsajaure	67.0590	19.9436	3.73	15.41	7.67	230.89	6.86	1.32	2.3	113	Perch and Roach

Table S2 - Values of photosynthetically active radiation (PAR) at the surface, 0.5 meter depth and 0.8 meter depth for all sampling days and water colour treatments. PAR varies between dates due to difference in sun and cloud cover.

Day of experiment	Water colour treatment	PAR Surface	± SE	PAR -0.5	± SE	PAR -0.8	± SE
1	CL	532	± 37	344	± 24	265	± 22
1	BL	518	± 42	330	± 30	256	± 27
1	BE	462	± 28	63	± 4	21	± 2
10	CL	1548	± 15	1169	± 25	1003	± 16
10	BL	1552	± 30	1182	± 26	984	± 18
10	BE	1265	± 23	226	± 8	102	± 3
19	CL	929	± 242	726	± 189	573	± 151
19	BL	963	± 244	707	± 197	586	± 168
19	BE	760	± 167	154	± 36	76	± 19
20	BL	1050	± 170	148	± 17	63	± 7
26	CL	124	± 6	81	± 4	64	± 3
26	BL	101	± 6	14	± 1	6	± 0
26	BE	108	± 6	19	± 1	9	± 1
32	CL	877	± 212	667	± 172	570	± 150
32	BL	654	± 159	109	± 27	45	± 11
32	BE	747	± 159	152	± 35	73	± 17

Table S3 - Outcome of multiple linear regressions for length-at-age (LAA) 1 and 5 for perch and roach.

	Perch LAA 1 (adj. R^2= 0.194, F= 2.341*)			**Perch LAA 5** (adj. R^2= 0.559, F= 8.064***)		
	Estimate	t	p	Estimate	t	p
Intercept	4.356	11.923	<0.0001***	4.837	14.262	<0.0001***
Colour (a_{420})	-0.0142	-2.470	0.019*	-0.016	-3.001	0.0052**
Temperature	0.0211	1.202	0.238	-0.0354	-2.173	0.037*
Total nitrogen	0.000337	1.257	0.218	0.000778	3.126	0.00376**
Turbidity (NTU)	-0.0526	-1.087	0.285	-0.187	-4.161	0.00022***
pH	-0.0292	-0.567	0.574	0.161	3.358	0.00204**
Mean depth	-0.00286	-0.407	0.687	-0.000178	-0.027	0.978
Area	-0.00004	-0.224	0.824	-0.000188	-1.049	0.302
	Roach LAA 1 (adj. R^2= 0.047, F= 1.239)			**Roach LAA 5** (adj. R^2= 0.276, F= 2.852*)		
	Estimate	t	p	Estimate	t	p
Intercept	4.013	4.573	<0.0001***	5.272	5.696	<0.0001***
Colour (a_{420})	0.00273	-0.210	0.835	-0.0206	-1.498	0.146
Temperature	0.0238	0.667	0.510	0.000736	0.020	0.985
Total nitrogen	0.000134	0.320	0.751	0.000351	0.794	0.434
Turbidity (NTU)	0.0208	0.545	0.590	0.0243	0.604	0.551
pH	-0.0108	-0.090	0.929	-0.0361	-0.286	0.777
Mean depth	-0.0107	-0.837	0.410	-0.0117	-0.868	0.393
Area	-0.00015	-0.380	0.707	0.0000358	0.088	0.931

Supplementary figures

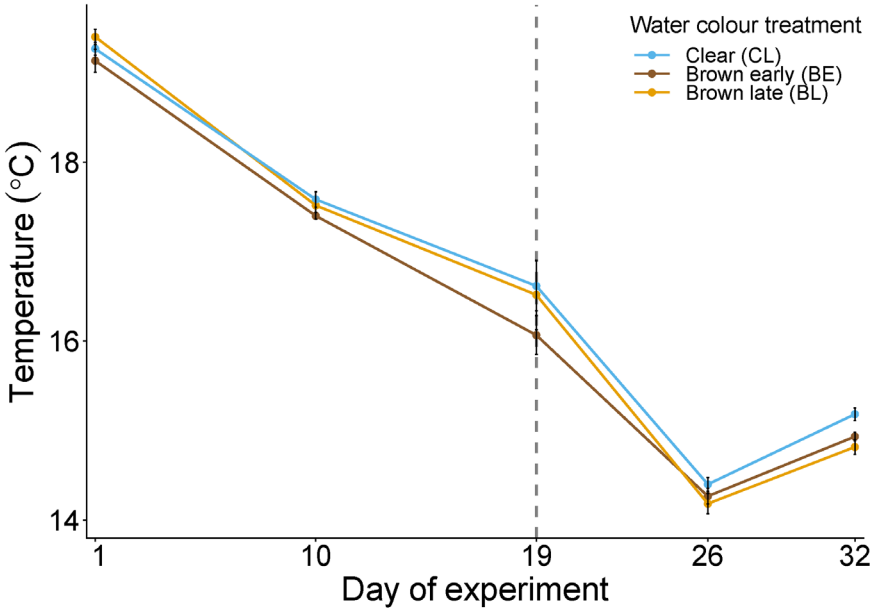

Figure S1 - Mesocosm temperatures in different treatments over the experimental period, measured at 0.5 m depth. On day 1 and 19 (vertical grey dashed line) we browned the BE and BL tanks, respectively. There were no significant differences in temperature between browning treatments over time (mixed ANOVA: Colour: p = 0.30, Date: p = <0.0001, Colour*Date: p = 0.29).

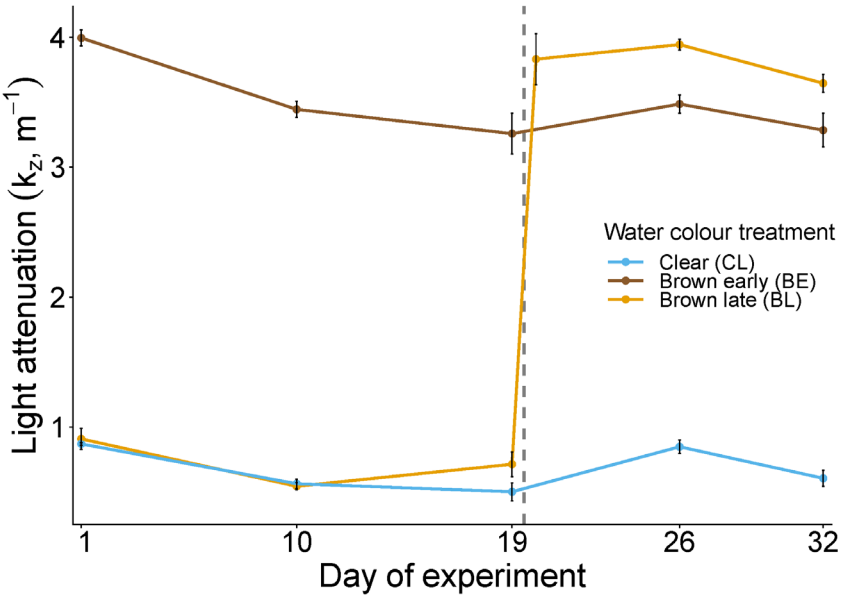

Figure S2 - Light attenuation (k_z, m^{-1}) in different treatments over the experimental period. On day 1 and 19 (vertical grey dashed line) we browned the BE and BL tanks, respectively.

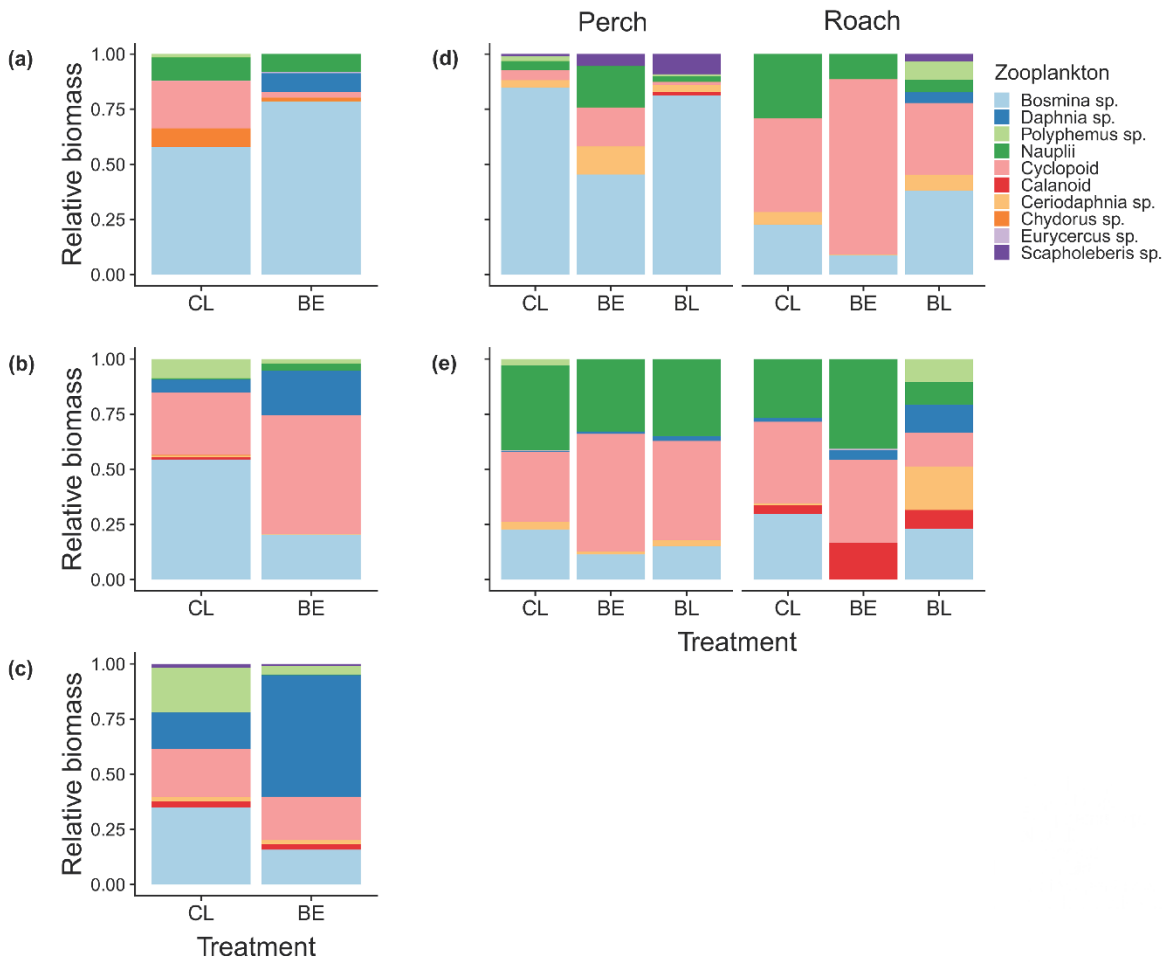

Figure S3 - Zooplankton composition is shown as relative biomasses before fish addition on day 1 (a), 10 (b), 19 (c) and after fish addition on day 26 (d) and 31 (e), in the different browning treatments (CL = clear, BE = brown early, and BL = brown late).

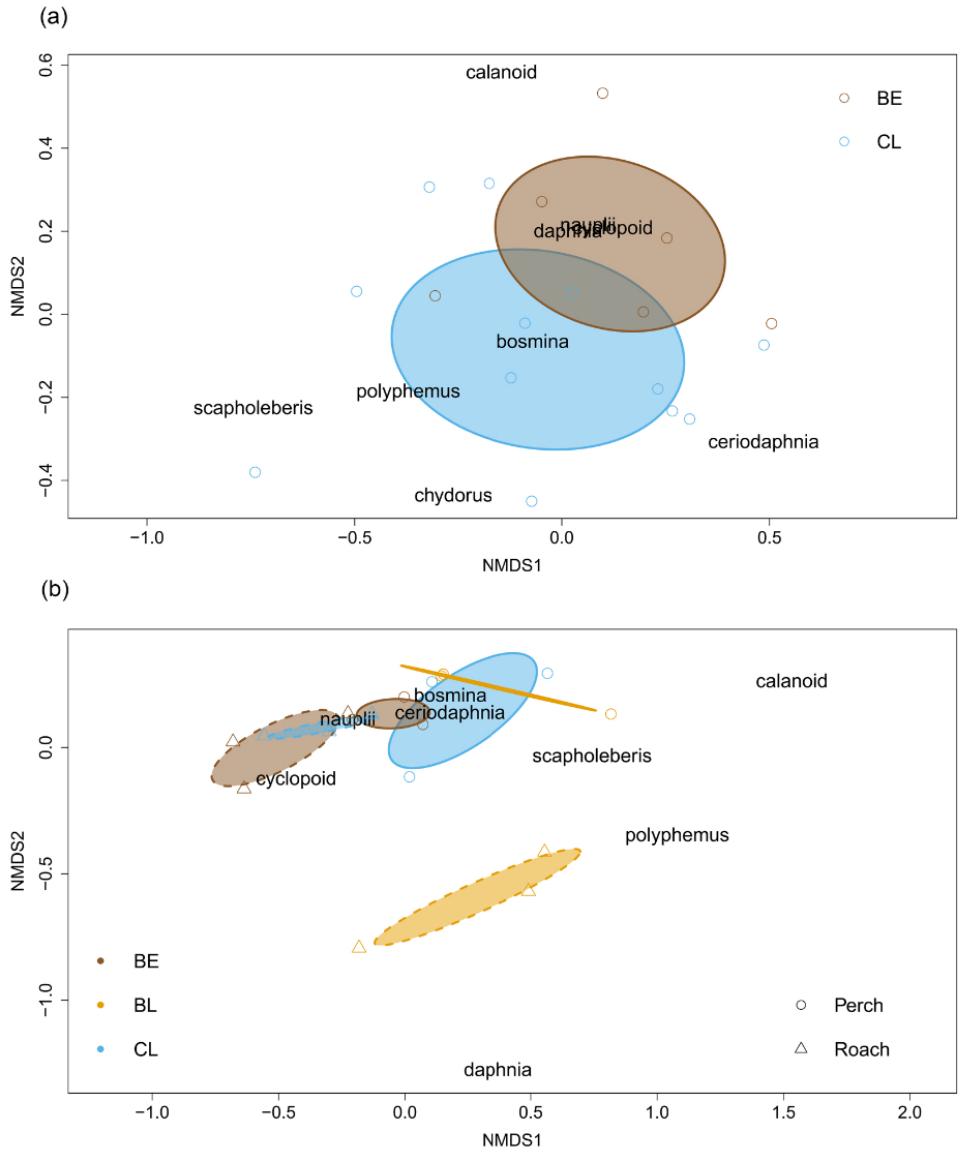

Figure S4 Zooplankton community composition in the different water colour and fish species treatments shown as non-metric multidimensional scaling (NMDS) plots on (a) day 19 and (b) 26 (stress values 0.18 and 0.11 respectively). Coloured areas are ellipse area of standard deviation per treatment, in figure (b) solid lines represent perch, and striped lines roach.