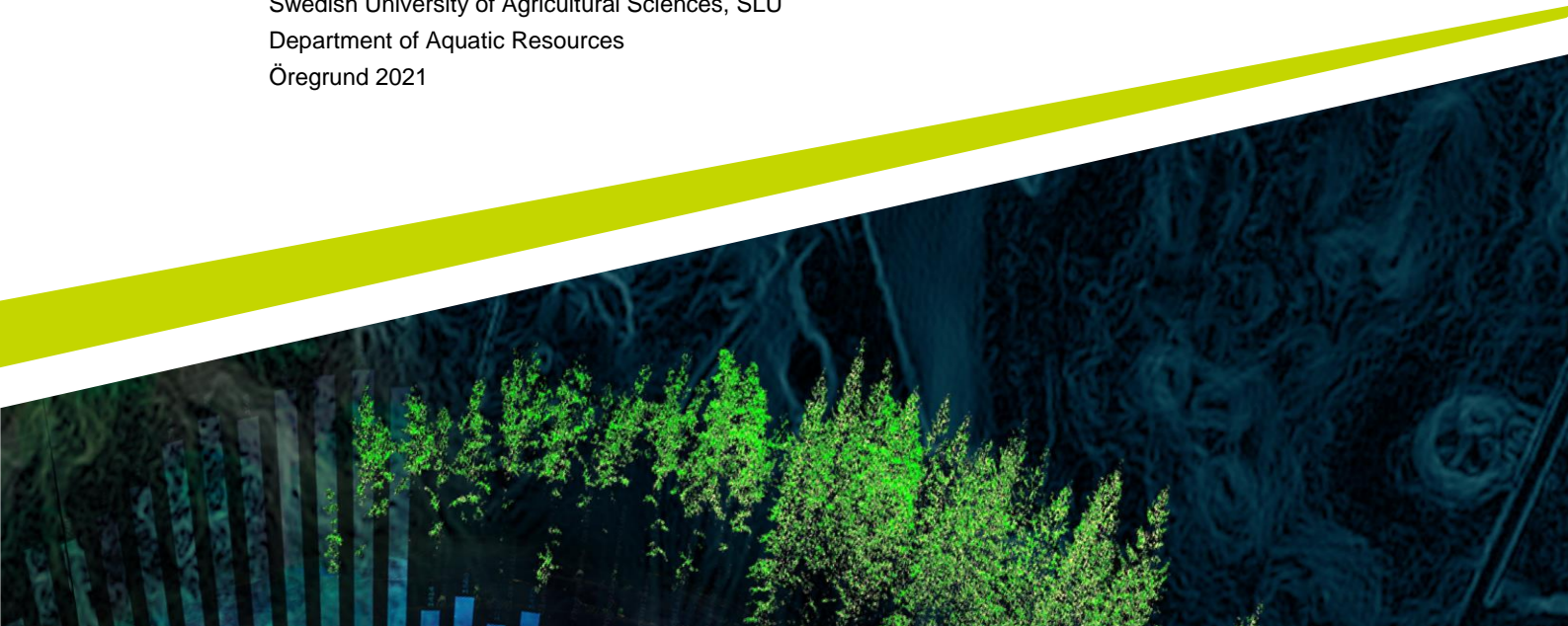# The effects of eutrophication and browning on prey availability and performance of the three-spined stickleback (*Gasterosteus aculeatus*)

*Påverkan av övergödning och brunifiering på bytestillgång och storspiggens (Gasterosteus aculeatus) kroppstillväxt*

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Olivia Bell

| | |
|---|---|
| **Supervisor:** | **Magnus Huss, SLU, Department of Aquatic Resources** |
| **Assistant supervisor:** | Aurélie Garnier, SLU, Department of Aquatic Resources |
| **Examiner:** | Josefin Sundin, SLU, Department of Aquatic Resources |

**Swedish University of Agricultural Sciences**

Faculty of Natural Resources and Agricultural Sciences

Department of Aquatic Resources

Institute of Coastal Research

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# Abstract

Shallow coastal areas often have high productivity and diversity, in part due to the high availability of light and nutrients. At the same time, they are exposed to multiple environmental pressures, such as browning and eutrophication. Browning is mainly caused by runoff bringing coloured dissolved organic matter (CDOM), reducing light availability in waters, whereas eutrophication is caused by high nutrient loading, leading to eutrophication symptoms such as algal blooms. Existing variation and further change in light and nutrients of coastal areas could have large implications for aquatic food webs, including fish. For instance, reduced light might alter food availability and reduce foraging abilities. Increased nutrient supply might, depending on the extent, instead increase food availability. In this study, I ask how browning and nutrient-enrichment, alone and in combination, affect benthic and pelagic invertebrate communities and how those changes, in turn, affect the performance of the three-spined stickleback (*Gasterosteus aculeatus*). The three-spined stickleback is not only a common model organism but also an increasingly common and important mesopredator in the Baltic Sea. Here, I performed a mesocosm experiment, including benthic and pelagic habitats and invertebrate prey, together with young-of-the-year three-spined stickleback as predators. In doing so, I examined the extent to which browning and nutrient-enrichment influenced the density, biomass and composition of stickleback prey, in addition to diet choice, body growth and condition of the stickleback. I found that prey availability increased with nutrient-enrichment but not with browning. The reason for the increase with added nutrients is most likely due to increased primary production, as evident in the much higher chlorophyll *a* concentrations. Accordingly, stickleback body growth was positively affected by nutrient-enrichment, probably because of increased food availability. In contrast, stickleback body growth and condition were negatively affected by browning, most likely due to negative effects of reduced visibility on feeding rates. Interestingly, nutrient-enrichment counteracted the negative effects of browning when combined. My findings add novel understandings about the potential for both eutrophication and browning to affect coastal food webs and fish body growth in the Baltic Sea.

*Keywords:* Baltic Sea, benthic habitats, body growth, climate change, coastal areas, diet, fish, stickleback, zooplankton

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# 1. Introduction

Shallow coastal waters are among the most productive and diverse ecosystems on earth (Ray 1991; Raffaelli et al. 2003; Waycott et al. 2009; Cesbron et al. 2019). Their high productivity is in part due to the high supply of light and nutrients to benthic habitats, enabling the growth of habitat-forming primary producers, such as aquatic plants, macrophytes, and benthic microalgae (Lefébure et al. 2013; Jäger & Diehl 2014; Ask et al. 2016; Östman et al. 2016; Cesbron et al. 2019; Kritzberg et al. 2020). Consequently, higher trophic level species, such as fish, thrive in these environments because of shelter and an abundance of benthic and pelagic prey (Raffaelli et al. 2003; Bergström et al. 2015; Kritzer et al. 2016; Östman et al. 2016). However, anthropogenic activities, e.g. causing variation in terrestrial runoff, can dramatically alter light and nutrient availability with large impacts on coastal food webs (Evans et al. 2006; Andersson et al. 2015; Mustaffa et al. 2020).

Light availability in shallow coastal waters is influenced by the amount of coloured dissolved organic matter (CDOM). Increased concentrations of CDOM, leading to browning of waters, is mainly caused by land-use change (e.g. in forestry methods), climate change (e.g. increased precipitation/runoff), and reduced acid deposition (Evans et al. 2006; de Wit et al. 2016; Kritzberg et al. 2020). Browning can influence both the productivity and composition of aquatic food webs (Solomon et al. 2015; Kritzberg et al. 2020; van Dorst et al. 2020). For instance, browning can reduce benthic primary production, which commonly initiates a lower overall food web productivity, including lower fish biomass production (Ask et al. 2009; Benoît et al. 2016; Leech et al. 2020). Some fish might also be negatively affected by browning through impaired visual conditions, reducing foraging rates (Ranåker et al. 2014; Weidel et al. 2017; Leech et al. 2020; van Dorst et al. 2020). For example, van Dorst et al. (2020) found that reduced visibility contributed to perch (*Perca fluviatilis*), but not roach (*Rutilus rutilus*), having lower body growth in brown relative to clear waters. Most studies on the causes and impacts of browning are from freshwater ecosystems (Flöder et al. 2006; Ask et al. 2009; Karlsson et al. 2009; Solomon et al. 2015; Kritzberg et al. 2020). However, increased input of CDOM has also been observed in coastal ecosystems (Wikner & Andersson 2012; Herrmann et al. 2015; Svedäng et al. 2018). The lack of knowledge concerning the impacts of browning on coastal ecosystems is problematic and is especially striking for organisms at higher trophic levels, such as fish. We specifically need this

knowledge given that climate change and an associated increase in precipitation may lead to increased runoff and further browning.

In contrast to browning, nutrient-enrichment often promotes primary productivity (Schindler 1977; Burkholder & Glibert 2013). At high enough concentrations, nutrients (i.e. nitrogen and phosphorous) cause eutrophication, triggering excess algal growth and the consequent shading of benthic primary producers (Meyercordt & Meyer-Reil 1999; Flöder et al. 2006; Smith & Schindler 2009; Heiskanen et al. 2019). In addition, nutrient-enrichment can trigger blooms of cyanobacteria and benthic filamentous algae, reducing opportunities for growth of habitat-forming macroalgae and submerged aquatic plants (Berger et al. 2003; Isæus et al. 2004; Wikner & Andersson 2012; Burkholder & Glibert 2013). Early stages of eutrophication have been found to be beneficial for fish due to increased prey availability (more zooplankton prey due to increased biomass of phytoplankton; Burkholder & Glibert 2013). Severe eutrophication, however, can result in blooms of inedible algae (Smith & Schindler 2009) and eventually hypoxia, reducing benthic secondary production (Steckbauer et al. 2011) and fish egg survival (Nordheim et al. 2020). Despite extensive research on eutrophication in coastal ecosystems, we know little about the combined effects of eutrophication and browning, especially concerning fish performance.

Although responses to increased inputs of CDOM and nutrients in aquatic ecosystems have been studied extensively as independent variables (Anderson et al. 2002; Evans et al. 2006; Burkholder & Glibert 2013; Kritzberg et al. 2020), they are, in many ecosystems, occurring simultaneously (Taipale et al. 2018; Bergström & Karlsson 2019). In the Baltic Sea region, future climate change is predicted to increase precipitation and runoff, bringing more CDOM and nutrients to lakes and coastal areas, resulting in either darker and more eutrophic waters, or slowing down the rate of recovery from eutrophication (Meyercordt & Meyer-Reil 1999; Faithfull et al. 2011; Wikner & Andersson 2012; Andersson et al. 2015; Blenckner et al. 2015; Andersen et al. 2017). An increase in runoff causing browning and nutrient-enrichment might, therefore, through bottom-up processes, result in altered fish production (Karlsson et al. 2009; Burkholder & Glibert 2013; Benoît et al. 2016). As intensified land use and climate warming are threatening coastal ecosystems in general, and the Baltic Sea's coasts specifically (Andersson et al. 2015), it is becoming increasingly important to understand and disentangle the combined impact of browning and eutrophication on coastal food webs.

Previous findings indicate that browning might reduce body growth and foraging rates of some fishes due to impaired visual conditions or reduced production of prey (Karlsson et al. 2009; Ranåker et al. 2012b; van Dorst et al. 2020). Conversely, increased nutrient supply might have a positive impact on the performance of mesopredatory fish, due to an increased amount of prey (secondary consumers; Burkholder & Glibert 2013). However, very eutrophic waters tend to have high

biomasses of inedible, or low quality, algae (Smith & Schindler 2009), which might impair fish body growth. Despite studies on eutrophication and browning alone, we do not know how fish are affected by the combination of these pressures. Three potential outcomes could be that the pressures behave *antagonistically*, whereby one pressure counteracts the effect of the other, *additively*, where the outcome results in the sum of the two pressures individually, or *synergistically*, whereby one of the pressures is strengthened by the other (Piggott et al. 2015).

Here, I ask how browning and nutrient-enrichment in shallow coastal ecosystems affect benthic and pelagic invertebrate communities, and how those changes, in addition to changes in visual conditions, affect mesopredatory fish. To answer this, I performed a mesocosm experiment, with each mesocosm including both benthic and pelagic habitats, with prey organisms from the Baltic Sea archipelago, using the three-spined stickleback (*Gasterosteus aculeatus*, hereafter referred to as stickleback) as focal species. In doing so, I examined the extent to which browning and nutrient-enrichment influence prey biomass and composition, in addition to diet choice and growth of sticklebacks.

# 2. Material and methods

## 2.1. Study site and species

This study was conducted from August until September 2020 in Forsmark, Sweden (60°24′12″N 18°10′0″E), next to a coastal area of the Baltic Sea. The Baltic Sea is a brackish inland sea surrounded by nine countries with a drainage area four times its surface area. With approximately 85 million people surrounding it, the Baltic Sea is threatened not only by climate change, but also by overfishing, runoff, pollution, and eutrophication (Andersson et al. 2015). In many areas of the Baltic Sea, seasonal eutrophication episodes have increased since the 1950s following the increased use of fertilizers, concurrent with expansions in agriculture and meat production (Österblom et al. 2007; Andersen et al. 2017; Heiskanen et al. 2019). At the same time, there have been substantial changes to the Baltic Sea fish community, the most notable one being the collapse of the eastern Baltic Sea cod (*Gadus morhua*) population in the early 1990s (Casini et al. 2008; Eero et al. 2015). In addition, there has been a shift from predator to prey fish dominance in some coastal areas, with observations suggesting that sticklebacks have increased substantially (Ljunggren et al. 2010; Eklöf et al. 2020).

   The stickleback is an abundant and generalist mesopredator that occurs across the northern hemisphere and is often used as a model organism in ecological and evolutionary research (Schluter & McPhail 1992; Gibson 2005; Moran et al. 2010; Lavin & McPhail 2011; Olsson et al. 2019). In the Baltic Sea, they spend most of their adult life in the open sea, but use shallow coastal areas for spawning and as a nursery (Bergström et al. 2015; Olsson et al. 2019). Being both generalist and specialist feeders (Schluter & McPhail 1992), sticklebacks feed on a range of prey items, including zooplankton and zoobenthos (Gill & Hart 1994; Jakobsen et al. 2003; Olsson et al. 2019), as well as fish eggs and larvae (Ljunggren et al. 2010; Byström et al. 2015; Nilsson et al. 2019). In the Baltic Sea, the stickleback population has increased substantially in many areas over the past few decades (Bergström et al. 2015; Olsson et al. 2019). The cause of this increase is not entirely known, although it has been suggested to be due in part to their ability to quickly

adapt to environmental change, such as eutrophication (Jakobsen et al. 2004; Candolin 2009), and because of predatory release (Eriksson et al. 2011).

## 2.2. Experimental design

In order to assess whether, and if so, why, eutrophication and browning alone and in combination might affect body growth, condition, and diet choice of sticklebacks, I performed an experiment with young-of-the-year (YOY) sticklebacks and prey organisms using 12 outdoor mesocosms (tanks). Mesocosms were free-standing, 0.68 m diameter x 1.11 m deep cylindrical soft plastic tanks filled with 350 litres of seawater from the adjacent waters of Forsmark, thus containing the same natural phytoplanktonic community. The experiment consisted of two phases; first, there was a three weeks' phase where I induced eutrophication and browning without fish present to allow for the pelagic and benthic communities of algae and invertebrates to establish and respond to the treatments without top-predators present (Figure 1). Secondly, I added fish for a two weeks' growth phase (Figure 1).

I implemented a 2x2 full factorial design with four treatments: browning only ($n$ =3), nutrient-enrichment only ($n = 3$), a combination of browning and nutrient-enrichment ($n = 3$), and a control exhibiting no browning or nutrient-enrichment ($n = 3$; Figure 1). To simulate browning, I added 80 ml of Sera Blackwater Aquatan water conditioner (Sera GmbH, Heinsberg, Germany) to each of the 6 mesocosms on day one. 8 ml of Sera Blackwater Aquatan was added to each mesocosm in the middle of the experiment to top up what had been removed with sampling. Sera Blackwater Aquatan has been found to mimic brown waters without considerably increasing concentrations of total organic carbon and nutrients (van Dorst et al. 2020). To induce eutrophication symptoms, similar to what has been observed in the Baltic Sea (see HELCOM 2018), 108 µmol nitrogen $L^{-1}$ (i.e. 1500 µg N $L^{-1}$, with $NH_4NO_3$) and 6.8 µmol phosphorus $L^{-1}$ (i.e. 211 µg P $L^{-1}$, with $KH_2PO_4$) were added in total to each of the 6 mesocosms (of which 3 were browned). The nutrients were added as a large pulse on day one (60 µmol N $L^{-1}$ and 3.8 µmol P $L^{-1}$) and then eight times as small pulses until fish addition (6 µmol N $L^{-1}$ and 0.38 µmol P $L^{-1}$ each pulse) together with water to replace the sampled water (sampling is described below).

Zooplankton were collected from the adjacent sea from the shoreline and by boat using both 20-µm and 70-µm mesh nets. The collected zooplankton were pooled together and equal amounts of 0.020 µg $L^{-1}$ (± 0.003 SE) were placed in each mesocosm. To create a natural benthic environment, I filled the mesocosms with a 4 cm layer of sand covered by a 3 cm layer of coastal sediment collected in the nearby area, which included naturally occurring microalgae and benthic invertebrates.

I collected YOY sticklebacks using hand nets from Forsmark's shallow coastal shoreline at ca 1 m depth. Once caught, I transferred the fish to the nearby experimental site where I measured their length and placed them into separate containers. The fish were left in the containers for several hours to minimize and standardize any impact of handling. Four healthy-looking individuals of similar size (means $\pm$ SE standard length $1.90 \pm 0.05$ cm) were then placed into each mesocosm, rendering a total of 48 fish used in the experiment.
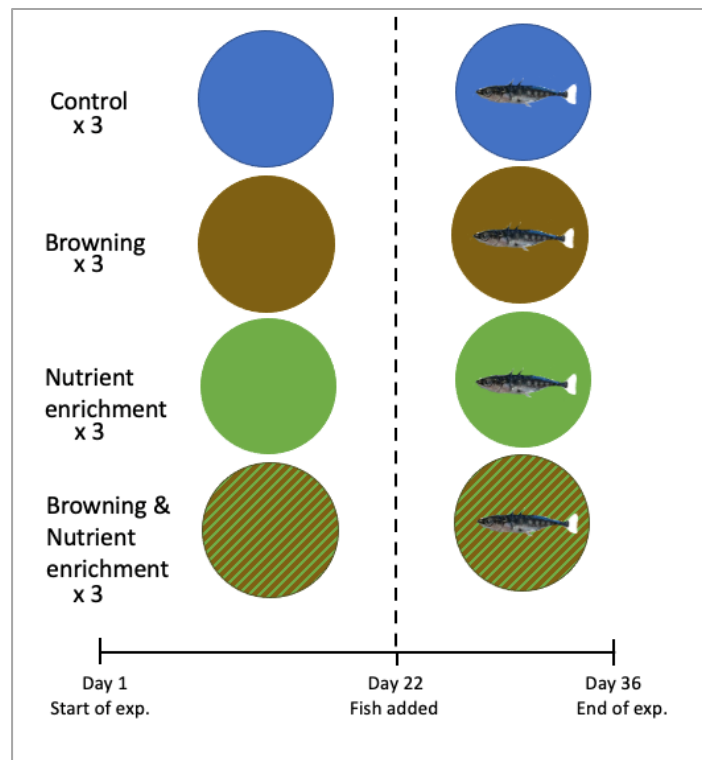


*Figure 1. Mesocosm experimental set-up with three mesocosms for each treatment (total n = 12) and four fish in each (total n = 48). Before fish were added on day 22 there was an initiation phase to allow for the communities of algae and invertebrates to establish and respond to browning and nutrient-enrichment. Sampling was done on day 1, 19, 29, and 36.*

## 2.3. Sampling protocol

At the end of the experiment, 44 out of 48 fish were found and caught with a net, euthanised in a benzocaine solution, and immediately stored at -20 ∘C. Two fish in one browned mesocosm, one fish in one nutrient-enriched mesocosm, and one fish in one browned and nutrient-enriched combination mesocosm were found, or assumed, dead. I sampled chlorophyll *a* (chl *a*) and zooplankton on day 1, 19 (before fish addition), 29, and 36 (after fish addition). Chl *a* and zooplankton were collected using a 600 ml tube sampler from three different positions of each mesocosm at 0.4 m depth and then combined as one sample (for a total of ~2000

ml). Each sample was filtered through a 70-μm mesh net and zooplankton were preserved in Lugol's solution. Then, I filtered 500 ml of the water through a 47-mm G/F microfiber filter, which I stored in aluminium foil at -20 ∘C until processed. The chl *a* content was extracted with 10ml of 96% ethanol and the fluorescence was measured with a spectrofluorometer. Extraction and measurements were done in darkness to avoid alteration of the chl *a*. Chl *a* (μg L$^{-1}$) was estimated using the following calibration regression

$$Chl\ a = 0{,}1182 \times fluorescence + 1{,}2201$$

Additionally, the multiple dilutions involved were taken into account (500ml of water filtered, extraction with 10 ml, and dilution(s) to fit the calibration range).

To evaluate the biomass of benthic macro-invertebrates in the mesocosms, I took benthic core samples (6.6 cm diameter) on day 19 (before fish addition) and day 36 (after fish addition). I sampled three different positions of each mesocosm and then combined the samples and filtered them through a net with 500 μm mesh size. I hand-picked all visible organisms and preserved them in ethanol.

I measured temperature and photosynthetically active radiation (PAR) (Appendix, Figure A1 and A2) on day 1, 19, 29, and 36. Temperature was measured at 0.4 m depth and PAR at 0.1, 0.4, and 0.65 m depth using a LI-250A light meter with a LI-193SA spherical underwater quantum sensor (LI-COR Biosciences-Biotechnology, Lincoln, NE, USA). The light attenuation coefficient (K$_z$ m$^{-1}$) was calculated from PAR measurements as

$$K_z = \ln\left(\frac{PAR_0}{PAR_z}\right) / z$$

Where PAR$_0$ is PAR at the surface and PAR$_z$ is PAR at depth z (m). Browning caused a higher light attenuation (Mixed ANOVA: $F_{(1,8)} = 185.01$, $p = <0.0001$), whereas the light attenuation in treatments with nutrient-enrichment exhibited only a trend of being higher than the control (Mixed ANOVA: $F_{(1,8)} = 5.01$, $p = 0.056$; Appendix, Figure A1). There was no interaction effect (Mixed ANOVA: $F_{(1,8)} = 0.06$, $p = 0.811$) or difference over time (Mixed ANOVA: $F_{(1,12)} = 1.88 = p = 0.199$; Appendix, Figure A1). Browning (GLMM: $t = 1.55$, $p = 0.121$) or Nutrient-enrichment (GLMM: $t = 1.51$, $p = 0.132$) did not affect temperature. However, there was a very minor but statistically significant interaction effect (GLMM: $t = -2.145$, $p = 0.032$) and temperature decreased over time in all treatments (GLMM $t = -9.82$, $p < 0.0001$; Appendix, Figure A2).

## 2.4. Laboratory analyses

In the laboratory, stickleback individuals were dabbed dry, measured and weighed to the nearest mm and 0.01 g. Fulton's condition factor (*K*, also referred to as body condition) was calculated using the formula:

$$K = \frac{100 \times weight\ (g)}{length\ (cm)^3}$$

Next, I removed stomachs from each individual to analyse the diet. Zooplankton and zoobenthos found in the water samples, benthic cores, and stomachs, were counted, identified, and measured to the nearest 0.01 mm using a stereomicroscope. I identified cladocerans and rotifers to genus level, whilst copepods were identified as either cyclopoid, calanoid, or nauplii. Benthic invertebrates, of which most were chironomid larvae, were identified to genus level. To be able to calculate zooplankton and zoobenthos biomasses for both invertebrates in the environment and diet samples, I measured the lengths of up to 15 individuals (all if fewer had been found in the sample in total) of each taxon/group per sample and converted lengths to dry mass using taxa-specific length-weight regressions (Dumont et al. 1975; Bottrell et al. 1976; Méthot et al. 2012).

The experiment was carried out in accordance with national guidelines for animal care and approved by the regional ethical review board in Uppsala, Sweden (5.2.18-4771/17).

## 2.5. Statistical analyses

Mesocosms exhibiting 50% or less of initial numbers of fish at the end of the experiment were excluded from the analyses, which meant that one of the browned-only mesocosms was excluded. All statistical analyses were executed in the statistical software *R* (R Core Team 2019). Normality was assessed using Shapiro-Wilks tests. Before the experiment, there was no significant difference in fish length between treatments (Generalized linear mixed-effects model: Browning (B) $z = 0.42$, $p = 0.676$, Nutrient-enrichment (N) $z = 0.279$, $p = 0.780$, B:N $z = -0328$, $p = 0.743$). I analysed treatment effects (browning and nutrient-enrichment) on fish length, weight, and body condition (i.e. response variables) using a two-way mixed-design analysis of variance (mixed ANOVA) using the *afex* package (Singmann et al. 2021) for normally distributed data. When the data was non-normally distributed, I instead used a generalized linear mixed-effects model (GLMM) with a gamma distribution and inverse link function using the *lme4* package (Bates et al. 2020). I treated mesocosms as random variables, rending the following statistical model:

response variable ~ browning * nutrient-enrichment + mesocosm.

I also analysed treatment effects over time on Chl *a* concentration, the number of chironomid larvae and zooplankton biomass (total, copepod and rotifer) using either a three-way mixed ANOVA or a GLMM as:

response variable ~ browning * nutrient-enrichment + (day(mesocosm)).

As there were few, if any, cladocerans left after fish addition, I only analysed them on day 19 with the same formula as above but removing 'day'. When there

was a significant main or interactive effect, I performed follow-up pairwise comparison tests with Tukey adjustments using the *emmeans* package (Lenth et al. 2021).

To statistically test if there were differences in community and diet composition between treatments for zooplankton, I performed a permutational multivariate analysis of variance (PERMANOVA) using the *adonis* function in the *vegan* package (Oksanen et al. 2020), with 999 permutations and low stress scores (<0.2). Taxa/group were square-root transformed to reduce the weight of highly abundant species. The PERMANOVA was based on distance matrices of zooplankton taxa/group biomasses and diet taxa/group biomasses and counts using the Bray-Curtis dissimilarity index. Zooplankton community composition was analysed both over time, including day as a third independent variable, and separately on day 19 (before fish addition), and day 29 and 36 (after fish addition). When there was a significant main or interactive effect, I performed follow-up pairwise comparisons using the *pairwise adonis* function. To visualise the results of the PERMANOVA, I used non-metric multidimensional scaling (NMDS) plots using the *metaMDS* function in the *vegan* package.

# 3.  Results

## 3.1.  Fish body growth and condition

During the two weeks growth period, stickleback in the control grew on average by 22% in body length, with nutrient-enrichment both with and without browning by 28% and 30% respectively, and with browning alone 17%. Stickleback body condition was negatively affected by browning but was not affected by nutrient-enrichment, irrespective of water colour (Figure 2a; Tables 1 and 2). Nutrient-enrichment increased stickleback body length and weight, while there was a trend of decreased stickleback body size with browning (Figures 2b-c; Tables 1 and 2). Thus, nutrient-enrichment and browning exhibited a tendency for an antagonistic effect on body size and condition, with the negative effect of browning being counteracted by the positive effect of nutrient-enrichment (Figure 2; Table 2).
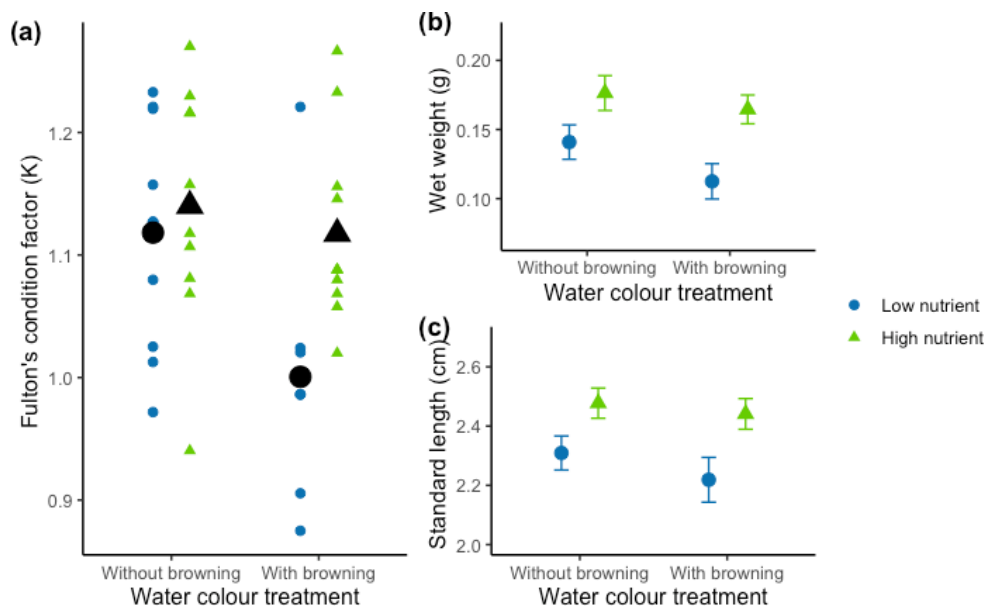


*Figure 2. (a) Fulton's condition factor (K), (b) wet weight (g), and (c) standard length (cm) of stickleback at the end of the experiment with or without browning and nutrient-enrichment. Mean Fulton's condition factor is displayed in black and each coloured point indicates individual fish (displayed instead of SE due to non-normal data distribution), whereas weight and length values are means ($\pm$ SE).*

Table 1. Generalized linear mixed model (GLMM) for Fulton's condition factor and Mixed ANOVAs for wet weight and standard length. B = Browning, N = Nutrient enrichment. (p < 0.0001 "***", p < 0.001 "**", p < 0.05 "*", p < 0.1 "·")

| Statistical test | Variable | Parameter | t/F-value | p-value |
|---|---|---|---|---|
| GLMM | *Fulton* | | | |
| | | B | -2.54 | 0.0110 * |
| | | N | 0.94 | 0.3470 |
| | | B:N | 1.22 | 0.2240 |
| Mixed ANOVA | *Weight* | | | |
| | | B | $4.99_{(1,7)}$ | 0.0606 · |
| | | N | $22.25_{(1,7)}$ | 0.0022 ** |
| | | B:N | $0.55_{(1,7)}$ | 0.4807 |
| Mixed ANOVA | *Length* | | | |
| | | B | $4.08_{(1,7)}$ | 0.0830 · |
| | | N | $37.02_{(1,7)}$ | 0.0005 *** |
| | | B:N | $0.61_{(1,7)}$ | 0.4606 |

Table 2. Pairwise comparison post hoc test for Fulton's condition factor, wet weight, and standard length. B = browning, N = nutrient-enrichment, BN = browning combined with nutrient-enrichment. Values are p-values (p < 0 "***", p < 0.001 "**", p < 0.05 "*", p < 0.1 "·").

| Fulton | | Control | B | N | BN |
|---|---|---|---|---|---|
| | Control | | 0.0534 · | 0.7827 | 0.9998 |
| | B | | | 0.0040 * | 0.0644 · |
| | N | | | | 0.7366 |
| | BN | | | | |
| Weight | | Control | B | N | BN |
| | Control | | 0.2738 | 0.0769 · | 0.3231 |
| | B | | | 0.0096 * | 0.0318 * |
| | N | | | | 0.6914 |
| | BN | | | | |
| Length | | Control | B | N | BN |
| | Control | | 0.3165 | 0.0216 * | 0.0707 · |
| | B | | | 0.0041 * | 0.0102 * |
| | N | | | | 0.7905 |
| | BN | | | | |

## 3.2. Diet

Benthic taxa, specifically chironomid larvae, dominated the diet of sticklebacks in terms of biomass (Figure 3a; Appendix Figure A3a), but pelagic zooplankton dominated in numbers, except in treatments with nutrient-enrichment where around half of the gut content was benthic taxa (Figure 3b; Appendix Figure A3b). There were large variations in the number of prey within treatments (Appendix Figure A3b). There were no treatment effects on diet composition in terms of biomass (Appendix Figure A4a; Table 3). When looking at count data, nutrient-enrichment led to more variation in taxonomic composition (Appendix Figure A4b, Table 3). *Keratella sp.* tended to dominate with browning-only, whereas, *Chydorus sp.*, adult copepods, nauplii, and chironomid larvae were all common with nutrient enrichment (Figure 3b).
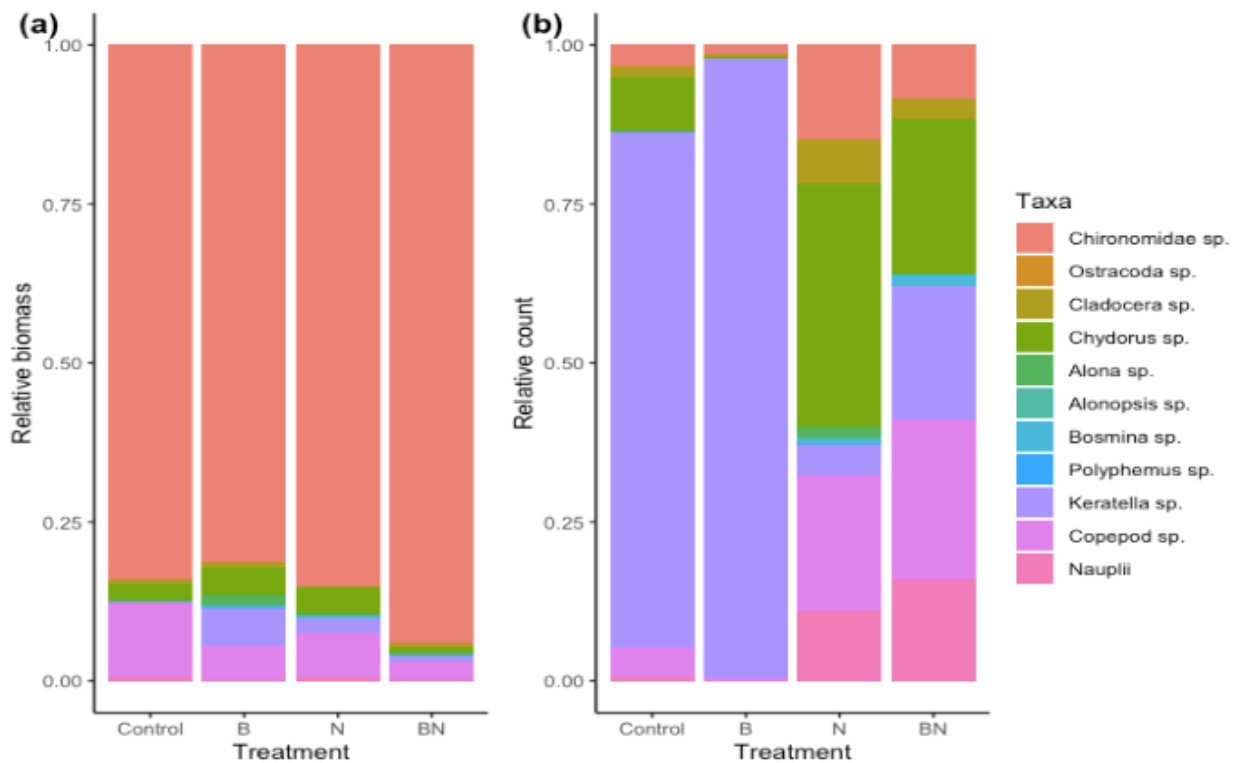


*Figure 3. Stickleback diet composition as (a) relative biomass and (b) relative numbers of different taxa (benthic taxa: Chironomidae sp., Ostracoda sp., cladocera sp., Chydorus sp., Alona sp., and Alonopsis sp.; pelagic taxa: Bosmina sp., Polyphemus sp., Keratella sp., copepod sp., and nauplii). for the control, browning alone ("B"), nutrient-enrichment ("N"), and browning and nutrient-enrichment combination ("BN").*

*Table 3. PERMANOVAs for diet composition based on biomass and number of prey items of different taxa/groups. B = browning, N = nutrient enrichment. (p < 0.0001 "***", p < 0.001 "**", p < 0.05 "*", p < 0.1 ".").*

|  | | *F-value* | R2 | *p-value* |
|---|---|---|---|---|
| *Biomass* | | | | |
| | B | $1.27_{(1)}$ | 0.03 | 0.250 |
| | N | $1.50_{(1)}$ | 0.04 | 0.169 |
| | B:N | $1.25_{(1)}$ | 0.04 | 0.296 |
| *Count* | | | | |
| | B | $1.83_{(1)}$ | 0.04 | 0.094 · |
| | N | $2.21_{(1)}$ | 0.05 | 0.040 * |
| | B:N | $0.85_{(1)}$ | 0.02 | 0.541 |

## 3.3. Invertebrates

The number of chironomid larvae was positively affected by nutrient-enrichment, although they decreased after fish addition in all treatments (Figure 4; Table 4). Before fish addition, on day 19, nutrient-enrichment had a positive effect on total zooplankton biomass (Figure 5a; GLMM: $t$ = -2.58, $p$ = 0.010) and an overall positive effect on copepod biomass (Figure 5b; Table 4). Total zooplankton, copepod, cladoceran, but not rotifer biomass, decreased after fish addition (Figure 5a-d; Table 4). There was no treatment or time effect on rotifer biomass, although there was a tendency towards lower biomass in the treatment with both browning and nutrient-enrichment (Figure 5d; Table 4).
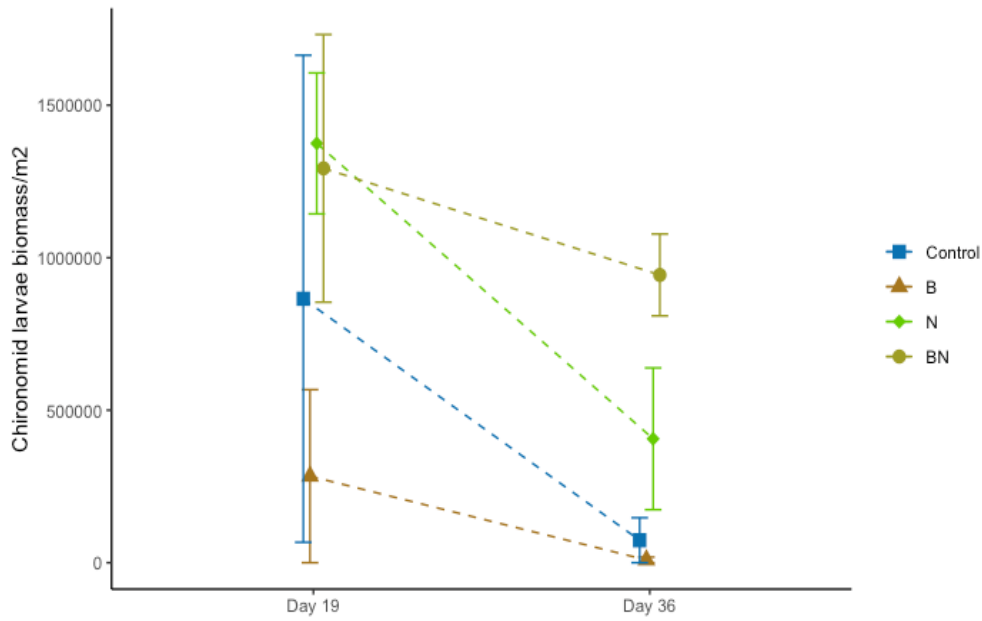
*Figure 4. Biomass of chironomid larvae before (day 19) and after (day 36) fish addition in the treatments without (Control) or with browning ("B"), nutrient enrichment ("N"), or the combination of browning and nutrient-enrichment ("BN"). All values are means (±SE).*
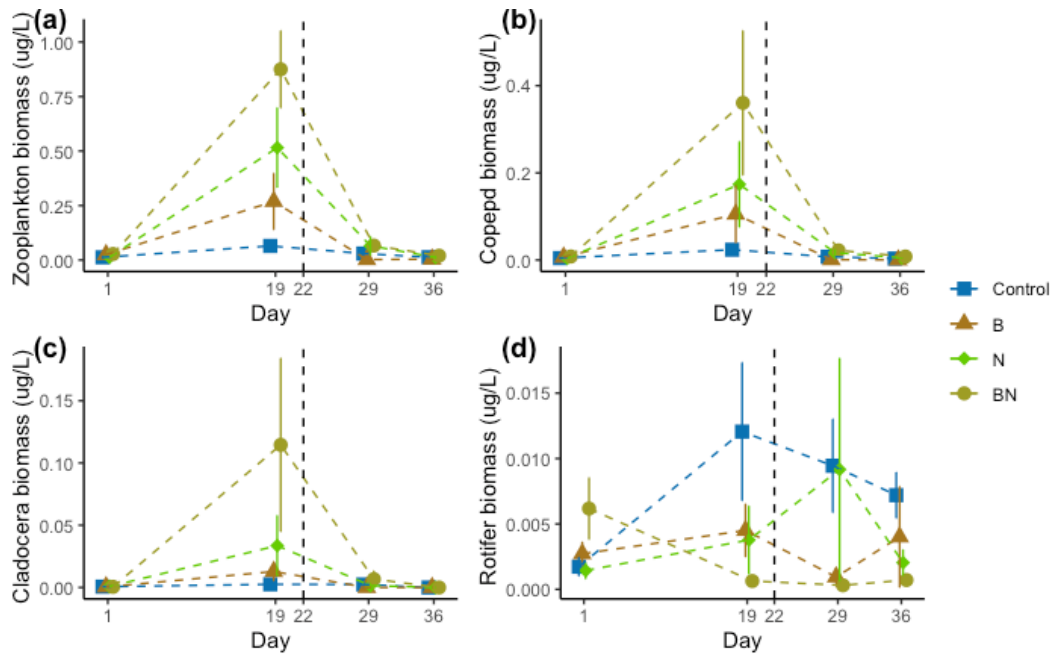


*Figure 5. Zooplankton biomass over time for (a) total zooplankton, (b) copepods, (c) cladocerans, and (d) rotifers in treatments without (Control) or with browning ("B"), nutrient-enrichment ("N"), or the combination of browning and nutrient-enrichment ("BN"). The black vertical dashed line on day 22 indicates when fish were added. Note the different y-axis scales. All values are means (±SE).*

Table 4. Generalized linear mixed model (GLMM) for chlorophyll a for day 19 (before fish addition) and 36 (after fish addition) of the experiment and Mixed ANOVAs for day 19, 29, and 36 for total zooplankton, copepod, and rotifer biomass, but only day 19 for cladocerans due to zero individuals at later dates. Mixed ANOVA for day 19 and 36 for the biomass of chironomid larvae. B = browning, N = nutrient-enrichment. ($p < 0.0001$ "***", $p < 0.001$ "**", $p < 0.05$ "*", $p < 0.1$ "·").

| Variable | Statistical test | Parameter | *t/F-value* | *p-value* |
|---|---|---|---|---|
| *Chlorophyll a* | GLMM | | | |
| | *Day 19, 36* | B | -4.11 | <0.0001 *** |
| | | N | -9.68 | <0.0001 *** |
| | | B:N | 4.33 | <0.0001 *** |
| | | Day | -1.95 | 0.0513 · |
| *Total Zooplankton* | GLMM | | | |
| | *Day 19, 29, 36* | B | -0.04 | 0.9655 |
| | | N | 1.38 | 0.1682 |
| | | B:N | 0.73 | 0.4649 |
| | | Day | -8.55 | <0.0001 *** |
| *Copepod* | Mixed ANOVA | | | |
| | *Day 19, 29, 36* | B | $0.01_{(1,7)}$ | 0.9391 |
| | | N | $11.34_{(1,7)}$ | 0.0119 * |
| | | B:N | $0.93_{(1,7)}$ | 0.3666 |
| | | Day | $14.27_{(1.5,10.8)}$ | 0.0015 ** |
| *Cladocera* | Mixed ANOVA | *F* | | |
| | *Day 19* | B | $3.02_{(1,7)}$ | 0.1256 |
| | | N | $2.45_{(1,7)}$ | 0.1616 |
| | | B:N | $0.002_{(1,7)}$ | 0.9640 |
| *Rotifer* | Mixed ANOVA | *F* | | |
| | *Day 19, 29, 36* | B | $4.41_{(1,7)}$ | 0.0739 · |
| | | N | $4.11_{(1,7)}$ | 0.0823 · |
| | | B:N | $0.07_{(1,7)}$ | 0.8052 |
| | | Day | $2.06_{(1.3,8.8)}$ | 0.1868 |
| *Chironomid larvae* | Mixed ANOVA | *F* | | |
| | *Day 19, 36* | B | $0.58_{(1,4)}$ | 0.4902 |
| | | N | $18.17_{(1,4)}$ | 0.0130 * |
| | | B:N | $1.52_{(1,4)}$ | 0.2855 |
| | | Day | $13.76_{(1,4)}$ | 0.0207 * |

The zooplankton community composition changed over time in all treatments (PERMANOVA: $F_{(2)} = 11.29$, $P = 0.001$), from communities dominated by large copepods and cladocerans, to relatively more small zooplankton, such as rotifers and nauplii (Figure 6). Nutrient-enrichment had a significant effect on community composition on all dates (Table 4, Appendix Figure A5), most likely because there was a higher number of zooplankton taxa present with nutrient-enrichment compared to other treatments. At the end of the experiment, on day 36, the browning

only treatment was dominated by small zooplankton, such as *Keratella sp.* and nauplii, whilst the nutrient-enriched treatments, exhibited relatively fewer *Keratella sp.* and more adult copepods and nauplii (Figure 6).
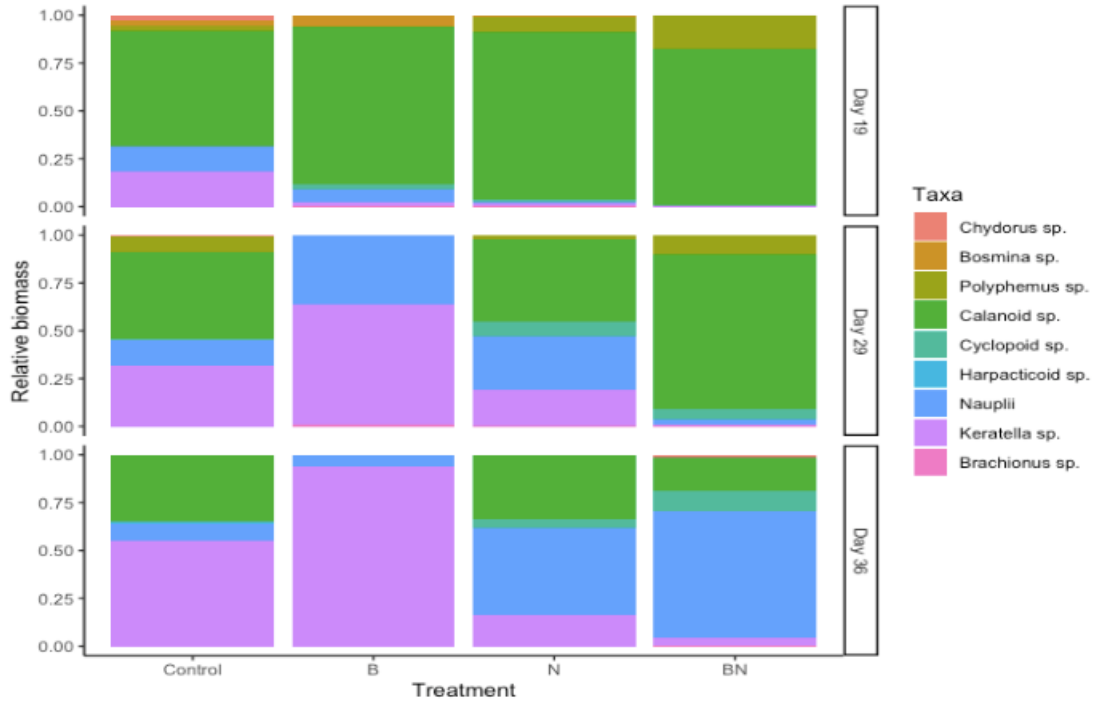


*Figure 6. Zooplankton community composition (relative biomass) on day 19, 29, and 36 for treatments without (Control) or with browning ("B"), nutrient-enrichment ("N"), or the combination of browning and nutrient-enrichment ("BN"). Fish were added on day 22.*

*Table 5. PERMANOVAs for zooplankton community composition on day 19, 29, and 36 of the experiment. Fish were added on day 22. B = browning, N = nutrient-enrichment (p < 0.0001 "***", p < 0.001 "**", p < 0.05 "*", p < 0.1 ".").*

|  | Parameter | *F-value* | R2 | *p-value* |
|---|---|---|---|---|
| *Day 19* | | | | |
| | B | 1.93$_{(1)}$ | 0.10 | 0.143 |
| | N | 7.18$_{(1)}$ | 0.38 | 0.003 ** |
| | B:N | 1.88$_{(1)}$ | 0.10 | 0.148 |
| *Day 29* | | | | |
| | B | 1.76$_{(1)}$ | 0.10 | 0.157 |
| | N | 5.05$_{(1)}$ | 0.29 | 0.017 * |
| | B:N | 3.76$_{(1)}$ | 0.21 | 0.027 * |
| *Day 36* | | | | |
| | B | 0.87$_{(1)}$ | 0.07 | 0.510 |
| | N | 4.04$_{(1)}$ | 0.32 | 0.015 * |
| | B:N | 0.67$_{(1)}$ | 0.05 | 0.626 |

## 3.4. Chlorophyll *a*

Nutrient-enrichment, with and without browning, had a substantial positive effect on chl *a* concentration (Figure 7; Tables 4 and 6), while browning alone had a marginally positive effect (Figure 7; Table 6). There was no effect of time on chl *a* concentration before (day 19) or after (day 36) fish addition in any of the treatments (Figure 7; Table 6).
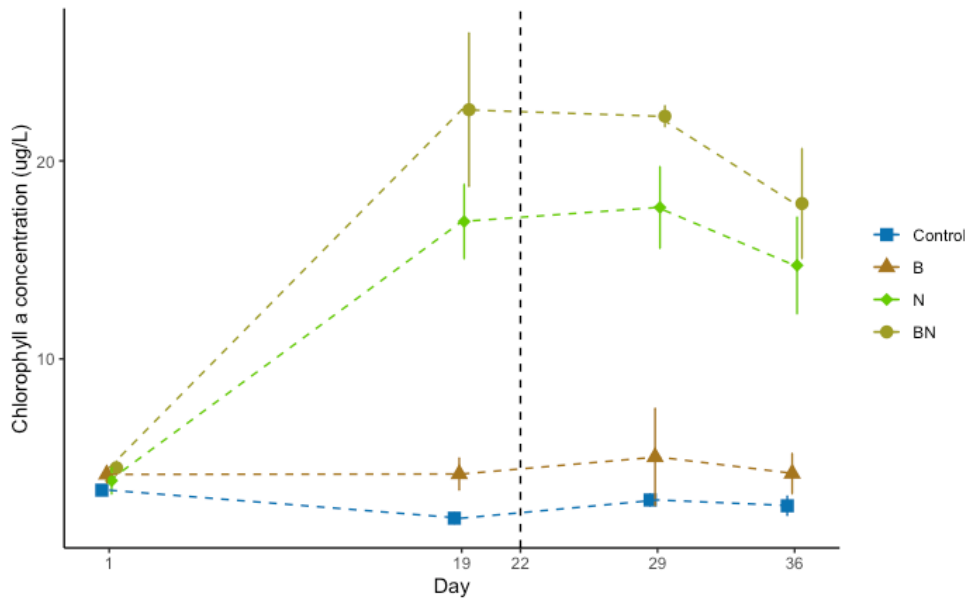


*Figure 3. Chlorophyll a concentrations over time in treatments without (Control) or with browning ("B"), nutrient-enrichment ("N"), or the combination of browning and nutrient-enrichment ("BN"). The black vertical dashed line on day 22 indicates when fish were added. All values are means (± SE).*

*Table 6. Pairwise comparison with Tukey´s post hoc test for chlorophyll a before (day 19) and after (day 36) fish addition. Ctrl = Control, B = browning, N = nutrient-enrichment, BN = browning combined with nutrient-enrichment. Values are p-values (p < 0.0001 "***", p < 0.001 "**", p < 0.05 "*", p < 0.1 "·"). Values in bold are p-values for each treatment when comparing values before and after fish addition, indicating no differences.*

|  | B 19 | N 19 | BN 19 | Ctrl 36 | B 36 | N 36 | BN 36 |
|---|---|---|---|---|---|---|---|
| Ctrl 19 | 0.0010 * | <0.0001 * | <0.0001 * | **0.5171** | 0.0004 * | <0.0001 ** | <0.0001 ** |
| B 19 |  | <0.0001 * | 0.0267 * | 0.2630 | **0.5171** | <0.0001 ** | 0.0025 * |
| N 19 |  |  | 0.5159 | <0.0001 * | 0.0566 · | **0.5171** | 0.9999 |
| BN 19 |  |  |  | <0.0001 * | 0.8034 | 0.1156 | **0.5171** |
| Ctrl 36 |  |  |  |  | 0.0010 * | <0.0001 ** | <0.0001 ** |
| B 36 |  |  |  |  |  | <0.0001 ** | 0.0267 * |
| N 36 |  |  |  |  |  |  | 0.5159 |
| BN 36 |  |  |  |  |  |  |  |

# 4. Discussion

Juvenile stickleback grew faster with nutrient-enrichment, in both clear and brown water treatments. In contrast, they had a lower body condition and a tendency for slower body growth with browning, unless nutrients were added. These differences can be explained by prey responses to browning and nutrient-enrichment. Large-bodied zooplankton and chironomid larvae were common both in the environment and in the diet in nutrient-enriched treatments, while when there was browning-only stickleback stomachs predominantly contained rotifers at the end of the experiment. These findings support and add explanation to previous results on that eutrophication (Jamet & Desmolles 1994; Moran et al. 2010; Hayden et al. 2019) and browning (Hayden et al. 2019; Leech et al. 2020; van Dorst et al. 2020) can affect fish performance through shifts in prey composition. My findings highlight the potential for substantial but antagonistic effects of eutrophication and browning on coastal food webs, including fish.

The positive effect of nutrient-enrichment on fish body growth can be explained by the higher availability of preferred food items (chironomid larvae and large-bodied zooplankton; Welker et al. 1994; Graeb et al. 2004; Sohel et al. 2017) in these treatments before fish were added. The higher densities of chironomid larvae in the nutrient-enriched mesocosms are in line with findings in natural systems where chironomid larvae generally are more abundant in eutrophic waters, due to the higher availability of organic detritus (Burkholder & Glibert 2013). The higher abundance of large-bodied zooplankton with nutrient-enrichment is likely explained by higher resource availability, as indicated by the much higher concentrations of chl *a* in treatments with added nutrients. This was expected, as an increase in nutrient concentration generally promotes primary productivity (Carpenter et al. 1998; Bonsdorff et al. 2002; Fleming-Lehtinen et al. 2008). In contrast, primary productivity, although mostly benthic, is often negatively affected by browning (Jansson et al. 2000; Mustaffa et al. 2020). In this study, however, browning did not negatively affect chl *a* concentrations or large-bodied zooplankton biomass but rather had a slight positive effect on both. An explanation for this could be that the chlorophyll pigment concentration in phytoplankton tends to increase with decreasing light, meaning that although there was a slight positive effect of browning on chl *a*, the effect on phytoplankton might still have been neutral or negative (Fennel & Boss 2003; van Dorst et al. 2020). However, the lack

of a decrease in zooplankton biomass with browning suggests that the effect, if any, on phytoplankton biomass was minor. The combination of browning and nutrient-enrichment resulted in a high chl *a* concentration and zooplankton biomass, suggesting that browning did not change the positive effect of nutrient-enrichment.

The abundance of large-bodied prey (such as copepods, cladocerans, and chironomids) decreased considerably in all treatments after fish addition, suggesting that the sticklebacks depleted most of their preferred prey. Although chironomid larvae decreased over time in all treatments, they were highest in abundance in mesocosms with nutrient-enrichment, which likely contributed to increased fish body growth in these treatments. Zooplankton community composition underwent major changes from the start of the experiment both until and after fish addition, and between treatments. Before fish addition, large-bodied zooplankton dominated in all treatments, although the biomass was higher in nutrient-enriched mesocosms. After fish addition, however, large-bodied zooplankton could no longer be found in the browning-only treatment, which instead was dominated by small zooplankton, such as rotifers and nauplii. In contrast, nutrient-enriched mesocosms still contained some large-bodied zooplankton at the end of the experiment, although nauplii now dominated. In addition to the treatment-induced changes in biomass before fish addition, an explanation for the change in zooplankton community composition after fish addition could be stickleback prey selection. Most planktivores, including stickleback, prefer to prey on large-bodied zooplankton rather than more abundant small-bodied prey, as they have higher nutritional value and are easier to see (Hangelin & Vuorinen 1988; Ljunggren 2002; Jakobsen et al. 2003). Therefore, as there were fewer large-bodied prey with browning (before fish addition), the sticklebacks most likely depleted them earlier and had to go for smaller-bodied and less nutritious prey such as rotifers, and thus grew slower in brown waters. Although large-bodied zooplankton biomass decreased considerably after fish addition, chl *a* concentration did not change in any of the treatments after fish addition. This was not expected, because a decrease in zooplankton biomass should decrease the grazing pressure on algae (Carpenter & Kitchell 1988; Akihiro et al. 1997; Carpenter et al. 1998). An explanation for this lack of a trophic cascade could be a too short timeframe or that the phytoplankton community was dominated by grazing resistant algae (Lürling 2021). It is possible that identification of the phytoplankton taxa could have provided more answers. Another explanation could be that the abundant rotifers might have maintained a high grazing pressure on phytoplankton throughout the experiment.

The greater abundance of preferable stickleback prey (such as copepods and chironomid larvae) with nutrient-enrichment, i.e. in eutrophic waters, suggests that early stages of eutrophication may have a positive effect on juvenile stickleback performance via bottom-up processes, negating the negative effects of browning.

The higher abundance of preferable stickleback prey with nutrient-enrichment was mirrored in the sticklebacks' diet. At the end of the experiment, sticklebacks in mesocosms with eutrophic waters fed on a larger variety of prey, including adult copepods, benthic cladocerans (mainly *Chydorus sp.*), nauplii, and chironomid larvae. Interestingly, a large part of their diet consisted of benthic prey, potentially suggesting they were abundant, or selected for, in mesocosms with nutrient-enrichment. However, as I did not sample benthic cladocerans I cannot know for sure if this was the case. Previous studies have, however, found benthic cladocerans, such as *Chydorus sp.* and *Alona sp.*, to be more abundant in eutrophic waters (Whiteside 1970). Similar to what has been found in many natural systems (Hall & Gerhardt 2002; Jakubavičiūtė et al. 2017), I found chironomid larvae to be an important prey item for sticklebacks (based on relative biomasses) across all treatments. It should be noted, however, that the diet content at the end of the experiment may not reflect preferences during the whole experiment as some prey items had already been completely or partly depleted in the environment. For example, the numerically dominating taxa in fish diets at the end of the experiment in treatments without nutrient additions was the rotifer *Keratella sp.*, but this is unlikely to be the preferred food item and rather reflect that most preferred zooplankton had already been consumed. Interestingly, even though the browning-only treatment did not decrease overall prey availability, the fish grew slower in brown waters (without nutrient-enrichment). This may be because browning can negatively affect foraging ability due to reduced visibility (Ranåker et al. 2012a; Hedström 2016; Weidel et al. 2017; van Dorst et al. 2020). Thus, reduced visibility rather than reduced prey availability might cause the negative effect of browning on YOY stickleback body condition.

There is a general lack of previous studies on how the combination of eutrophication and browning, which we know are affecting water bodies worldwide (Leech et al. 2018; Bergström & Karlsson 2019), are affecting consumers via prey responses. This is especially problematic concerning sticklebacks exposed to a large natural and human-caused variation in trophic status and water colour in the Baltic Sea (Candolin 2009; Gagnon et al. 2017), where the sticklebacks in some areas have undergone a drastic increase in population biomass over the last years (Bergström et al. 2015; Olsson et al. 2019). As these pressures often occur simultaneously it is important to understand the effects of combined browning and eutrophication on fish, which, on their own, we know to be important for other components of the food web (Taipale et al. 2018; Hayden et al. 2019). Furthermore, the difference in stickleback body growth found in my experiment between eutrophic, browned waters, and the combination, could have important ramifications for species interactions and, thus, the function of coastal food-webs in the Baltic Sea. For instance, piscivorous fish might be affected by changes in stickleback growth rate depending on their feeding preferences and gape size

(Nilsson & Brönmark 2000). Vice versa, sticklebacks themselves may switch diet from zooplankton to zoobenthos and fish prey earlier given faster body growth. In fact, this could potentially worsen the suggested negative interaction between sticklebacks and important coastal piscivores such as perch and pike (Ljunggren et al. 2010; Nilsson et al. 2019; Eklöf et al. 2020). Especially if a faster stickleback body growth rate in eutrophic areas results in increased predation rates on fish eggs and larvae. Changes in light and nutrient availability, leading to altered growth patterns, may also affect other stages of the sticklebacks life history, e.g. leading to sexual maturity at a younger age (Dieckmann & Heino 2007).

In conclusion, I found that early stages of eutrophication increases the availability of preferred prey for sticklebacks and, therefore, facilitates their body growth. In contrast, I found that browning might impede body growth of sticklebacks, due to reduced visual conditions. Interestingly, nutrient-enrichment negated the negative effects of browning on prey availability and fish body growth and condition. Overall, this study provides new understandings of the combined impact of browning and early eutrophication on coastal food webs, especially on mesopredatory fish, and can aid in predictions about how they may respond to future changes in water colour and nutrient supply following changes in land use and climate.

# Popular Science Summary

Climate change, leading to increased rainfall and runoff, combined with human activities such as forestry and agriculture, may simultaneously cause browning and eutrophication of water bodies, including coastal areas of the Baltic Sea. Browning is caused by coloured organic matter from land reaching the coastline, reducing light availability for plants and algae, and visibility for fish. Eutrophication occurs when water bodies receive high nutrient loadings, causing, for example, algal blooms.

The effects of browning and eutrophication could affect the performance of coastal fish. For instance, less food and reduced visibility caused by browning can reduce fish body growth and condition, whereas eutrophication may increase fish body growth and condition if it leads to higher food production. Although we know a lot about the effects of browning and especially eutrophication separately, there is a lack of knowledge concerning how the combination of these factors affect fish and their prey.

Using three-spined stickleback, which has increased dramatically in many areas of the Baltic Sea in recent years, as the study species, I set up a so-called mesocosm (tank) experiment and tested how the fish would grow in waters that were browned, enriched with nutrients (i.e. eutrophic), or both. Besides comparing fish growth, I also studied responses of algae and invertebrates, as well as fish gut content. This was to see how the pressures changed the availability of prey and provide a possible explanation for differences in fish growth.

I found that the fish grew faster with eutrophication, because of higher food availability. In contrast, fish grew slower with browning, probably because of the lower visibility reducing their foraging ability. Interestingly, when combined, the positive effects of increased food availability with eutrophication negated the negative effects of reduced visibility with browning.

My findings add new understanding about the potential for both eutrophication and browning to affect coastal food webs and fish body growth in the Baltic Sea.

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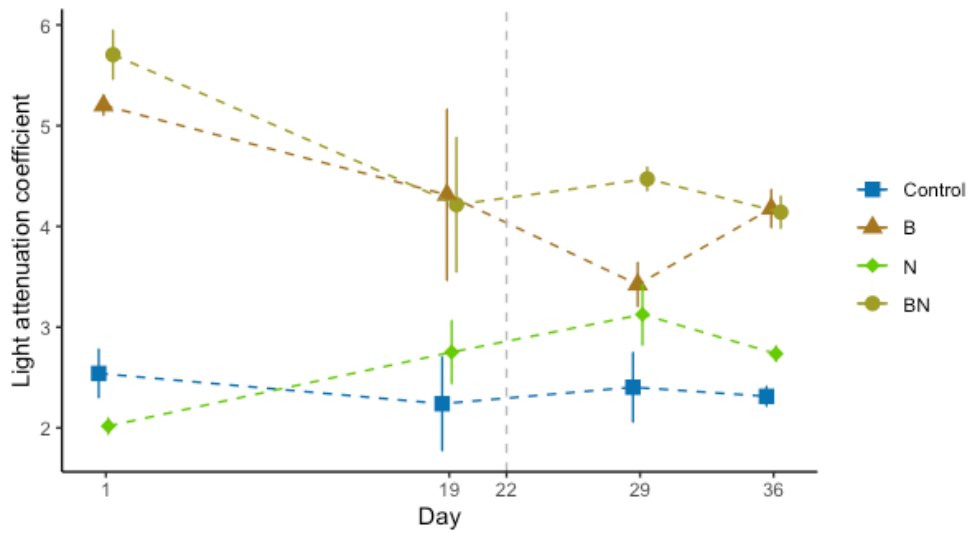
# Acknowledgements

# Appendix



*Figure A1. Light attenuation ($k_z$, $m^{-1}$) over time without ("Control") or with browning ("B"), nutrient-enrichment ("N"), or the combination of both browning and nutrient-enrichment ("BN"). Values are means ($\pm$ SE).*
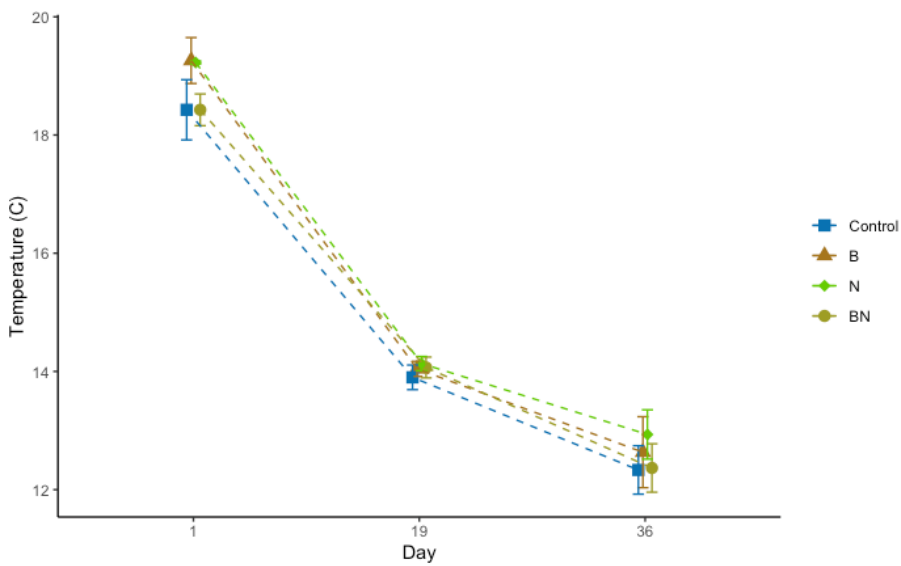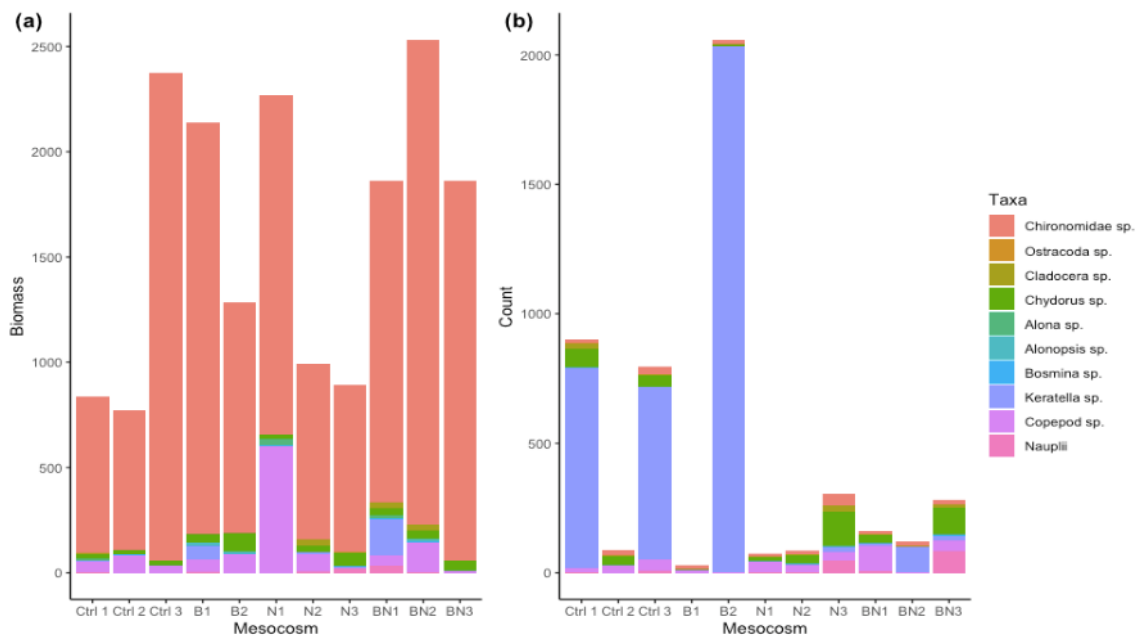


*Figure A2. Temperature ($C^o$) over time without ("Control") or with browning ("B"), nutrient-enrichment ("N"), or the combination of both browning and nutrient-enrichment ("BN"). Values are means ($\pm$ SE).*

*Figure A3. Stickleback diet composition as (a) biomass and (b) numbers/count of different taxa (benthic taxa: Chironomidae sp., Ostracoda sp., cladocera sp., Chydorus sp., Alona sp., and Alonopsis sp.; pelagic taxa: Bosmina sp., Polyphemus sp., Keratella sp., copepod sp., and nauplii). for without ("Ctrl") or with browning ("B"), nutrient-enrichment ("N"), or the combination of both browning and nutrient-enrichment ("BN").*
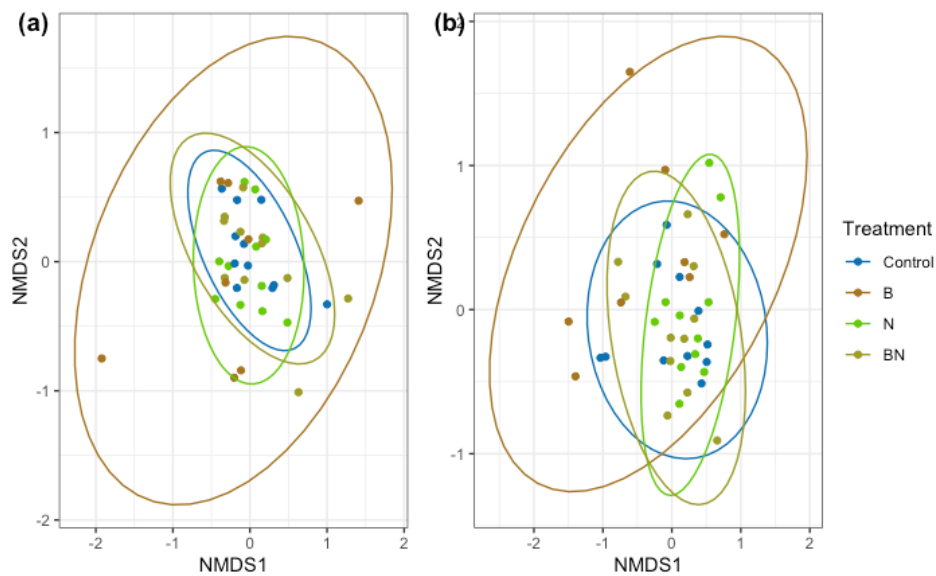


*Figure A4. Non-metric multi-dimensional scaling (NMDS) plots for the diet content composition for (a) biomass and (b) count without ("Control") or with browning ("B"), nutrient-enrichment ("N"), or the combination of both browning and nutrient-enrichment ("BN").*
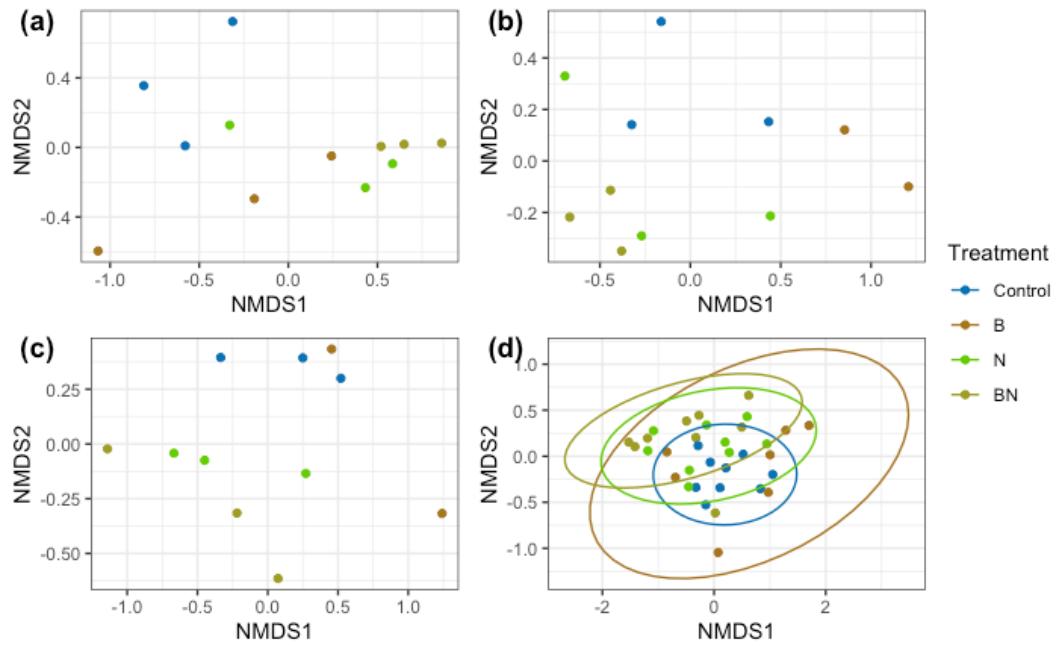
*Figure A5. Non-metric multi-dimensional scaling (NMDS) plots for zooplankton community composition on (a) day 19, (b) day 29, (c) day 36, and (d) over time without ("Control") or with browning ("B"), nutrient-enrichment ("N"), or the combination of both browning and nutrient-enrichment ("BN"). Fish were added on day 22.*