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Coastal darkening exacerbates eutrophication symptoms through bottom-up and top-down control modification

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Abstract

Coastal eutrophication due to excessive anthropogenic nutrient loading is a major threat worldwide, and especially in estuaries and semi-enclosed waterbodies, like the brackish Baltic Sea. In addition, coastal waters may become darker (coastal darkening) due to increased input of colored compounds from terrestrial run-off and sediment resuspension. Still, the effects of darkening on coastal food web responses to eutrophication are unknown. In a mesocosm experiment with benthic and pelagic habitats, we manipulated nutrient loading, presence of fish and light availability to disentangle bottom-up and top-down control of eutrophication symptoms in ambient and darkened waters. Overall, we found higher pelagic Chlorophyll *a* concentrations (a proxy of algal biomass) with darkening and with nutrient enrichment in both clear and dark waters. Albeit fish had a strong impact on zooplankton and zoobenthos, they had no cascading effect on algae. We conclude that coastal darkening due to changes in land use and climate change can pose an additional challenge concerning the recovery of coastal waters from eutrophication.

Worldwide, coastal habitats are threatened by multiple human pressures (Halpern et al. 2008; Blenckner et al. 2021), especially eutrophication and overuse of biological resources (e.g., overfishing). Eutrophication by nutrient enrichment can lead to excess growth of phytoplankton, filamentous and toxic algae, oxygen depletion of bottom waters, loss of macrophytes, and increased fish mortality (Smith and Schindler 2009; Cloern et al. 2014). Excess algal growth and other eutrophication symptoms are not only caused by high nutrient supply, but also by lack of top-down regulation by predatory fish on planktivorous fish and then invertebrates (e.g., zooplankton, bivalves, gastropods) that, in turn, control pelagic and benthic algae (Faithfull et al. 2011; Östman et al. 2016; Donadi et al. 2017). Management to control nutrient inputs, and to a lesser extent also to restore the top-down control of predatory fish (Elser et al. 2000), are therefore common in eutrophic waters, such as in the highly eutrophic semi-enclosed Baltic Sea (HELCOM 2007, 2021).

Alongside nutrients, runoff brings dissolved organic matter, which often has a colored light-absorbing fraction that reduces light penetration and makes the water darker (Harvey et al. 2015). This process, mostly described in freshwaters, is often referred to as "browning" or "brownification" (Kritzberg et al. 2020). However, "browning" also suggests an additional carbon input with (possible) effects on the aquatic food web (Roulet and Moore 2006). In this study, we will focus on light extinction and thus use the term coastal "darkening". Reduced light penetration and thus less light reaching the sea floors (de Wit et al. 2016) can, in turn, reduce primary production, especially in benthic habitats reliant on high light availability (Ask et al. 2009; Solomon et al. 2015; Puts et al. 2022). Darkening of coastal waters may therefore not only affect overall productivity but also shift the proportion of benthic vs. pelagic production (Ask et al. 2009). Consequently, due to its effects on primary producers, darkening can play an important, but unknown, role in modifying eutrophication symptoms (Duarte and Krause-Jensen 2018;

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Additional Supporting Information may be found in the online version of this article.

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Deininger and Frigstad 2019). Previous studies in freshwaters suggest that pelagic production is mainly controlled by nutrient availability (Jäger and Diehl 2014) and benthic production by light availability (Ask et al. 2009). Thus, it can be speculated that the effect of nutrient loading on total and relative (pelagic vs. benthic) algal biomass and production may differ depending on light availability (darkening).

To which extent the bottom-up effects of darkening manifest higher up the food chain may, in turn, depend on top-down processes in the food web. Darkening can affect predators such as fish, both indirectly via effects on invertebrate prey composition and availability (van Dorst et al. 2020; Bell et al. 2022) and directly via effects on feeding efficiency. Fish that are visual hunters may therefore have lower prey intake rates and grow slower in dark waters (van Dorst et al. 2020), which may dampen the top-down effect of fish predation on invertebrate prey.

Still, how shallow coastal food webs, which are highly dependent on benthic production, respond to concurrent variation in nutrient concentrations, presence of fish and light supply is unknown. The understanding of how darkening modifies eutrophication symptoms, caused by high nutrient loads and weakened top-down control (i.e., through trophic cascades), is relevant to achieve efficient coastal management for improved ecological status. If darkening reduces the biomass and production of primary producers, and thus the strength of bottom-up regulation via nutrient supply, nutrient reductions may be less efficient in reducing eutrophication symptoms relative to fish management (top-down regulation) in darker waters.

Here, we experimentally address the role of light limitation for bottom-up and top-down processes in shallow coastal food webs and thereby their role in modifying eutrophication symptoms. To this end, we set up a factorial mesocosm experiment, with tanks inoculated with natural assemblages of pelagic and benthic organisms from the Baltic Sea archipelago. We manipulated nutrient supply, light availability ("darkening") and presence/absence of juvenile Eurasian perch (*Perca fluviatilis*) feeding on invertebrates. Our hypotheses are: (H1) nutrient enrichment will boost pelagic algal biomass in both clear and dark water treatments, but benthic algae only in clear waters due to the stronger effect of light extinction on benthic vs. pelagic habitats; (H2) juvenile perch will indirectly increase pelagic and benthic algal biomass in clear but not in dark waters due to lower feeding efficiency when dark.

Methods

Study site and experimental set up

We set up 24 outdoor mesocosms at a field station in Forsmark at the Swedish coast of the Baltic Sea. Mesocosms consisted of 400-liter blue foldable plastic tanks (FlexiTank, 1.1 m deep × 0.68 m diameter) filled with a 7 cm deep bottom substrate consisting of 3 and 4 cm layers of sand and natural soft sediments, respectively, and 350-liter unfiltered seawater. We collected the sediment in the surrounding area (Supporting Information Fig. S1, 60° 24′16.7976″N, 18° 11′ 2.3748″E), sieved it through a 5-mm mesh to remove debris and large zoobenthic organisms. The sediment was bubbled with air to oxygenate it prior to adding it to the mesocosms. On three occasions (1st, 2nd, and 5th of August 2020), for 1 h at low speed, we collected zooplankton from about 0.1–1 m depth with 20- and 70-μm mesh nets in the surrounding archipelago close to the shore. We pooled all zooplankton in a 700-liter tank from which we randomly added 10 liters to each mesocosm (rendering an initial zooplankton biomass of about 20 μg dw L^{-1}). We also collected benthic invertebrates (in addition to those in the sediment) at nearby locations on the 9th of August. For each mesocosm we added (similar in size and composition): five bivalves (e.g., *Macoma balthica* and *Cerastoderma glaucum*) sampled from the sediment using a 5-mm sieve, and 10 hand-picked gastropods (e.g., *Radix balthica* and *Theodoxus fluviatilis*) sampled from rocks. Before the start of the experiment (Day 0—11th of August), we randomly added sediments (−7 to −3 d), seawater (−7 to −2 d), zooplankton (−2 d), and zoobenthos (−1 d) at similar amounts to all mesocosms.

Experimental design

We conducted a full-factorial experiment with two levels (i.e., a control and one treatment level) manipulating nutrients, light availability and fish presence, rendering eight treatments with three replicates of each (i.e., 24 mesocosms in total).

Nutrient enrichment was done by adding nitrogen and phosphorus at levels high enough to reach concentrations like those observed in highly eutrophic areas of the Baltic Sea (i.e., areas exhibiting algal blooms, 30–50 μg P L^{-1}; HELCOM 2018; Wasmund et al. 2001). We initiated nutrient enrichments with a first pulse of ammonium nitrate (60 μmol N L^{-1} NH_4NO_3) and potassium dihydrogen phosphate (3.8 μmol P L^{-1} KH_2PO_4). Then we added nutrients every 2nd or 3rd day (before sampling) with 10% of the concentration of the first addition. In total, the mesocosms with nutrient enrichment received 108 μmol N L^{-1} (= 1512 μg N L^{-1}) and 6.84 μmol P L^{-1} (= 212 μg P L^{-1}).

We manipulated the light availability by adding 80 mL (i.e., 0.23 mL L^{-1}) of Sera Blackwater Aquatan water conditioner (Sera GmbH) to each tank. This water conditioner is not harmful or toxic in any way and it is commonly used in aquariums and has also been used in some previous experiments about impacts of darkening (van Dorst et al. 2020). This concentration rendered a light attenuation coefficient K_d(PAR) ≈ 5 (*see* "Sampling and laboratory analyses" section for information about its derivation) and, on average, 5% (± 8% SD) of the light reached the bottom in dark water treatments compared to 14% (± 6% SD) in clear water treatments (with $K_{d\_control}$ ≈ 2). To maintain the light effects, we

added 10% of the initial concentration (i.e., 8 mL) on the 11th day of our experiment. The day after the addition of the conditioner, we measured nutrients and dissolved organic carbon (DOC). There were no immediate effects of the conditioner on nutrient concentrations ("Dark" treatment effect from an ANOVA on nitrogen and phosphorus concentrations: $F_{1,13} = 0.030$, $p = 0.866$ and $F_{1,13} = 0.003$, $p = 0.954$, respectively). However, the conditioner increased the DOC concentration by 2.18 mg C L^{-1} ("Dark" treatment effect from ANOVA: $F_{1,13} = 16.949$, $p = 0.001$) with an ambient value of 4.88 mg C L^{-1} in the control tanks. The low amount of nutrients and relatively low amount of labile organic carbon contrasts to other browning substances (HuminFeed®, SuperHume® or using reverse osmosis) that primarily aim at increasing the DOC concentration (and an associated light reduction). In addition, HuminFeed® might negatively affect zooplankton abundance and reproduction (Scharnweber et al. 2021). Darkening the system did not increase the water temperature (ANOVA on the mean temperature in clear vs. dark water tanks: $F_{1,15} = 0.9$, p-value $= 0.4$; for other abiotic parameters see Supporting Information Fig. S2).

We added young-of-the-year Perch (*Perca fluviatilis*) of an average standard length of 3.5 cm (± 0.3 cm SD). We collected the perch 2 d prior to the start of the experiment in a nearby shallow coastal bay (60°18′38.6″N 18°19′59.7″ E) and kept them in a 600-liter oxygenated tank with zooplankton. We chose perch as fish species because it is highly abundant in the brackish coastal waters of the Baltic Sea (HELCOM 2018) and is known to feed on both zooplankton and zoobenthos (Byström et al. 2012; Jacobson et al. 2019). Note that perch in this experiment serves as a treatment and not as response variable. We report on perch survival in the Supporting Information (Fig. S3; Table S1). The experiment and handling of perch 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 and 5.2.18-06022/2020).

Sampling and laboratory analyses

At each sampling occasion, we took three water samples with a 0.66-liter Ruttner water sampler at 40 cm depth to collect organisms of the pelagic community (i.e., zooplankton, phytoplankton, and bacteria) and to measure nutrient concentrations (total nitrogen and total phosphorus). Only the upper half of the water column was gently stirred with the probe and the water sampler to mix the top layer without disturbing the sediments. We also measured other abiotic parameters (temperature, salinity, dissolved oxygen [DO] saturation and concentration, pH) at 40 cm depth with a portable multiparameter Aquaprobe AP-2000 with a GPS Aquameter (AP 2000, AquaReadLtd). Photosynthetically active radiation (PAR) was measured at 10, 40, and 65 cm depth with a LI-250A light meter with a LI-193SA spherical underwater quantum sensor (LI-COR Biosciences-Biotechnology). From these PAR measurements, we calculated the light attenuation coefficient (K_d, m^{-1}) as the slope of the linear regression of the natural logarithm of PAR vs. depth.

We filtered the water samples through a 70-μm mesh to collect the zooplankton and then rinsed the mesh with filtered water in 100-mL dark bottles. The samples were immediately stored in darkness with 2 mL of iodine Lugol's solution ($I_2 = 3.4$ g L^{-1} and KI = 6.8 g L^{-1}). When analyzed in the laboratory, we refiltered the samples on a 70-μm mesh to sort out the zooplankton. The zooplankton consisted of copepods (calanoid, cyclopoid, and nauplii), cladocerans (*Bosmina* sp. and *Polyphemus* sp.), and rotifers (mainly *Keratella quadrata*, and less frequently *Keratella cochlearis*, *Brachionus* sp., *Asplanchna* sp., *Bdelloida* sp., *Euchlanis* sp., *Notholca* sp.). We counted at least 50 individuals per taxa (all if fewer), and measured the length of 15 random individuals per taxa (all if fewer) using a stereomicroscope (Leica M125C), to the nearest 0.01 mm. We converted the lengths to dry mass using taxa-specific size-mass regressions. For the rotifers, we used the regression estimated for *Brachionus calyciflorus* (Dumont et al. 1975; Bottrell et al. 1976; Supporting Information Table S2).

To estimate the pelagic Chlorophyll *a* (Chl *a*) concentration, we filtered 500 mL of the water sample after filtration (< 70 μm) onto a Whatman GF/F glass fiber filter and stored the filters in aluminum foil at −20°C until they were processed. We extracted the Chl *a* with 10 mL of 96% ethanol for 12 h and measured fluorescence in darkness with a spectrofluorometer (LS 30 Perkin Elmer) with $\lambda_{excitation}$ 433 nm and $\lambda_{emission}$ 673 nm.

We added 2 mL of iodine Lugol's solution to water samples (filtered through 70-μm mesh and stored in dark bottles) containing phytoplankton. To estimate the phytoplankton abundance and composition, we concentrated 10 or 50 mL (depending on the nutrient treatment) of the water sampled using an Utermöhl sedimentation chamber. We identified and counted phytoplankton in a fraction of the chamber using an inverted microscope with 100×, 200×, or 400× magnification (Olympus CK2). Note that this was only done for a subset of treatments sampled at the end of the experiment.

We sampled pelagic bacteria from the water sample after filtration (< 70 μm, see above) and stored them with 2% glutaraldehyde at 4°C until processing. Bacteria densities were estimated using a flow cytometer, as described in Rulli et al. (2022).

Between 20 and 40 cm depth, we took two pictures of the wall per tank (at opposite sides) to estimate the coverage of periphyton. We processed the pictures with ImageJ (Schneider et al. 2012) and automatized it with R (R Core Team 2021; *see* details in Supporting Information). Overall, we (1) cropped the picture to obtain an area of 5 cm^2; (2) converted the colored picture to black and white; (3) estimated the percentage of coverage and took the mean of the two pictures per tank.

To estimate nutrient concentrations (total nitrogen and total phosphorus), we filtered 50 mL of sampled water using a

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prerinsed 0.2-μm-pore syringe and then stored the samples in Falcon tubes at −20°C until analysis on a segmented flow analyzer (QuAAtro 39, Seal Analytical, method no: Q-115-10 Rev. 4).

To avoid disturbances of the sediments and risk of nutrient release we sampled benthic primary production and respiration, and benthic Chl *a*, only three times during the experiment. The sediment was sampled using a 20-mL syringe (area of 3.14 cm^2 and 8.6 cm in height), from which we removed the plain tip, attached to a PVC tube to be able to reach the bottom (*see* Supporting Information for details). This way, we created two "incubation chambers" including a sediment core (2 cm diameter) and overlaying water. One of the chambers was incubated in light to obtain net ecosystem production (NEP), and the other one in darkness to obtain respiration, where the gross primary production (GPP) here is the difference between NEP and respiration (*see* Supporting Information).

We used the sampled sediment from the benthic incubation chambers to estimate benthic Chl *a* concentrations. After incubation, we removed the remaining water from the sediment samples before storing them at −20°C. In the laboratory, we first freeze-dried the sediment for 24 h, then took 3 g of a mixture from the dried sediment, added 30 mL of 96% ethanol, and stored them for 24 h in the dark before measuring fluorescence in the same way as for pelagic Chl *a*. To get the total concentration (mg m^{-2}), we took into consideration the total weight of the sediment sampled and the inner area of the chamber. The relative biomass of pelagic over benthic algae (pelagic : benthic Chl *a*) was estimated as pelagic Chl *a* concentrations multiplied by the total volume of the tank (350 liters) divided by benthic Chl *a* multiplied with the area (0.36 m^2). On the last day of the experiment, we sampled the benthic invertebrates with a 66-mm-diameter core sampler (repeated three times per tank), added water and mixed the sediment thoroughly before filtering the pooled sample with water, first through a 500-μm mesh and then a 70-μm mesh. We disregarded the bivalves and gastropods in the analyses because of their low abundance and because they would not reflect the fish diet. Instead, we focused our sampling and analyses of benthic invertebrates on chironomids (Diptera: Chironomidae). We hand-picked the chironomids and stored them in 95% ethanol until we counted and measured them with a stereomicroscope. We converted the head width into dry mass using taxa-specific size-mass regressions (Méthot et al. 2012).

Statistical analysis

All data processing and analyses were done with the statistical software R 4.2.0 (R Core Team 2021), data will be available on Zenodo (10.5281/zenodo.7314091).

For the response variables total zooplankton biomass, pelagic and benthic Chl *a*, pelagic : benthic Chl *a*, bacterial abundance and DO, we performed a repeated-measures ANOVA (RM-ANOVA) with the *afex* R package (Singmann et al. 2016). Treatments were categorical between-subject factors, and "Date" a within-subject factor. We checked the assumptions of residuals being normally distributed (QQ-plot or a Shapiro–Wilk's test) and sphericity in the observations (i.e., homogeneity of variance with a Mauchly's sphericity test). When normality was not observed, we transformed the data with natural logarithm or logarithm base 10 transformations (specified in the tables). When the sphericity criterion was not met (e.g., for pelagic Chl *a* and DO), we included a Greenhouse–Geisser correction term. We removed the interaction terms if non-significant (p-value > 0.05). However, we kept the interaction term when (1) higher-order interaction terms were significant or when (2) the within-subject factor "Date" was significant (e.g., for $p\text{-value}_{D:N:Date} < 0.05$ and $p\text{-value}_{D:N} > 0.05$, we kept the interaction "D : N"). We computed effect sizes as partial eta-squared (η^2).

For the light attenuation coefficient (K_d), the dark treatment had much different values than the others, creating a bimodal data distribution. Therefore, to be able to detect effects and potential interactions of other treatments, we split the experimental design based on this treatment, and perform two RM-ANOVAs. For the periphyton, we analyzed the proportion of cover with a zero-inflated beta regression model (link = "logit") using the R package *gamlss* (Rigby and Stasinopoulos 2005). For chironomid biomass and phytoplankton abundance, we performed a three-way and two-way ANOVA (Type 3), respectively, with the treatments as categorical factors, using the R package *car* (Fox and Weisberg 2019). The assumptions of normality of residuals and homoscedasticity of variance were met, despite of what may seem like a non-normal distribution of the observations. For the zooplankton and phytoplankton composition (only on the last day of the experiment), we performed a permutational multivariate ANOVA (PERMANOVA), using the R package *vegan* (Oksanen et al. 2007). To test our treatments' effects on the (dis)similarity of zooplankton composition, we used the Bray–Curtis dissimilarity index in the PERMANOVA.

On Day 8, we noticed we had mistakenly attributed the wrong fish treatment to three tanks (#15, #19, and #22). We removed the fish in the tank with nutrient addition ("N"—#15) and added two new fish to the two tanks that were supposed to be "Fish" (#19 and #22). For the statistical analyses, we handled the mistaken treatment by removing all the data for tank #15 (because the response observed would no longer answer the intended research question, but rather the recovery after fish removal) but used data from the tanks we added fish to (as this did not change the research question). Note that, in presence of NA in the repeated measures, the *afex* R package discard all observations (i.e., all dates) of that replicate from the analysis. For the benthic Chl *a*, two samples were missing (tanks #1 (control) and #2 (nutrient treatment) on the first sampling event), therefore these two additional replicates were excluded in the analysis concerning this response variable and thus also for the relative pelagic : benthic Chl *a*.

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Table 1. Results of RM-ANOVA on the effects of nutrient additions, darkening and fish on nutrient and light availability (total nitrogen, total phosphorus, light attenuation coefficient). Significant results are highlighted in dark yellow and marginally significant results in light yellow. Total nitrogen and total phosphorus were natural-log (ln) transformed prior to analyses to fit a normal distribution. Due to the bimodal distribution of light attenuation coefficients, we split the data between clear and dark treatments and performed RM-ANOVA without and with Greenhouse–Geisser correction for clear and dark water treatments, respectively.

	Total nitrogen (ln)			Total phosphorus (ln)			Light attenuation coefficient (clear water)			Light attenuation coefficient (dark water)		
	RM-ANOVA			RM-ANOVA			RM-ANOVA			RM-ANOVA with GG correction		
Test	F	η^2_G	p-value	F	η^2_G	p-value	F	η^2_G	p-value	F	η^2_G	p-value
Main effect												
Nutrient	$F_{1,16} = 116{,}38$	0.693	< 0.001	$F_{1,16} = 154.56$	0.806	< 0.001	$F_{1,6} = 43.68$	0.173	< 0.001	$F_{1,9} = 0.02$	< 0.001	0.892
Fish	$F_{1,16} = 0.18$	0.004	0.675	$F_{1,16} = 0.10$	0.003	0.756	$F_{1,6} = 10.11$	0.046	0.019	$F_{1,9} = 0.75$	0.020	0.410
Dark	$F_{1,16} = 13.78$	0.211	0.002	$F_{1,16} = 14.91$	0.286	0.001	—	—	—	—	—	—
Nutrient : Dark	$F_{1,16} = 0.68$	0.013	0.423	$F_{1,16} = 4.07$	0.098	0.061	—	—	—	—	—	—
Temporal effect												
Date	$F_{2,32} = 14.73$	0.388	< 0.001	$F_{2,32} = 7.52$	0.212	0.002	$F_{4,24} = 3.41$	0.355	0.024	$F_{1.88,16.94} = 11.99$	0.499	< 0.001
Nutrient : Date	$F_{2,32} = 6.18$	0.210	0.005	$F_{2,32} = 14.80$	0.346	< 0.001	$F_{4,24} = 1.79$	0.225	0.164	$F_{1.88,16.94} = 1.04$	0.080	0.371
Fish : Date	$F_{2,32} = 0.17$	0.007	0.846	$F_{2,32} = 0.68$	0.024	0.515	$F_{4,24} = 3.30$	0.348	0.027	$F_{1.88,16.94} = 0.47$	0.037	0.624
Dark : Date	$F_{2,32} = 1.31$	0.054	0.283	$F_{2,32} = 5.50$	0.164	0.009	—	—	—	—	—	—
Nutrient : Dark : Date	$F_{2,32} = 3.16$	0.120	0.056	$F_{2,32} = 9.67$	0.257	< 0.001	—	—	—	—	—	—

We summarize the statistical results in tables and guide readers in the text by modulating the confidence using the language of evidence (Muff et al. 2021). Note that the "language of evidence" is based on p-values gradient and thus without a strict significant vs. nonsignificant effect. Therefore, for a p-value < 0.001, there is a "very strong evidence"; for p-values between 0.001 and 0.01, there is a "strong evidence"; for p-values between 0.01 and 0.05, there is a "moderate evidence"; for p values between 0.05 and 0.1, there is a "weak evidence"; and for p-values between 0.1 and 1, there is "little or no evidence."

Results

In summary, we manipulated three environmental drivers: nutrient addition, fish presence, and light availability, with the potential to induce or modify eutrophication symptoms. The highest pelagic Chl *a* concentrations, a proxy of phytoplankton biomass, and our indicator of eutrophication, was observed with nutrient addition in dark waters. However, there was no evidence that fish had an effect on pelagic Chl *a* in contrast to moderate evidence for a temporal effect on benthic Chl *a*, despite a strong impact of fish on zooplankton and zoobenthos.

Community feedback on abiotic treatments

Nutrient additions initially increased nutrient concentrations across treatments, but concentrations were later reduced. The reduction in nutrient concentrations was, however, less pronounced in dark water tanks (*see* Nutrient : Dark : Date interaction in Table 1). There was a linear decrease in nutrient concentrations over time in dark water, contrasting to the more abrupt decrease in clear water (Fig. 1). Toward the end of the experiment, there was an increase in phosphorous concentrations in dark but not in clear waters (Fig. 1b; Table 1). As intended, we increased the light attenuation coefficient (K_d) by adding the darkening conditioner. In clear, but not dark water treatments, nutrient additions and the presence of fish also increased K_d (Fig. 1, Table 1).

Bottom-up effects

The data provide strong evidence of a bottom-up effect of nutrient addition on the pelagic community in both clear and dark water (Figs. 2 and 3, Tables 2 and 4). In clear water, the pelagic Chl *a* concentration increased by three times within the first week after the first nutrient addition, and then remained stable throughout the experiment (Fig. 2a). The change in pelagic Chl *a* was associated with differences in both total phytoplankton abundance and composition on the last day of the experiment, with the highest abundance found in the dark-nutrient treatment (Supporting Information Figs. S4, S5; Table 2). Although the presence of fish seemed to delay the peak in pelagic Chl *a* concentrations and stabilize them at elevated levels in the nutrient and dark-nutrient treatments (Fig. 2a), there was no evidence that fish affected the

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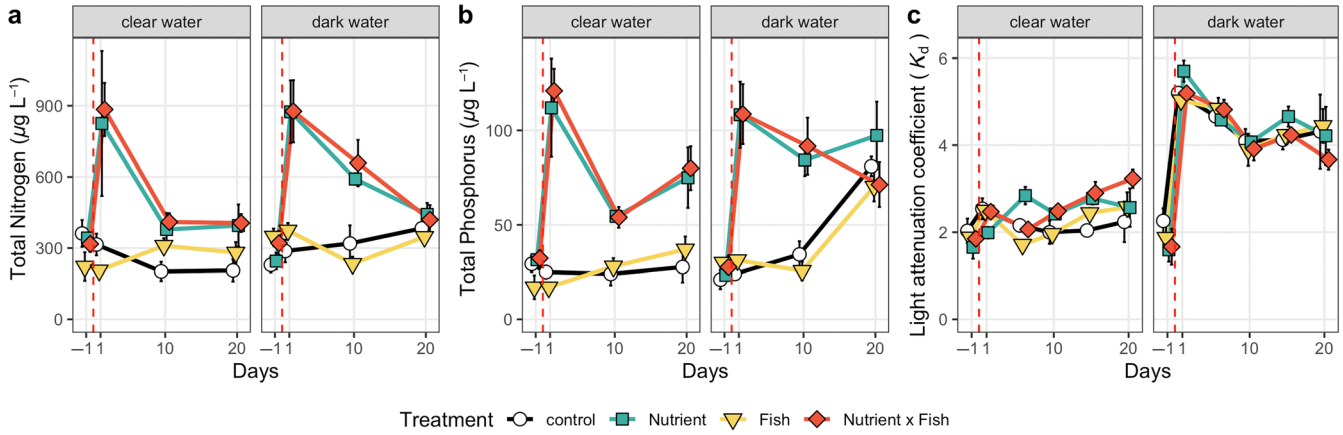

Fig. 1. Treatment effects on nutrient and light availability. Changes in (**a**) total nitrogen, (**b**) total phosphorus, and (**c**) light attenuation during the experiment in our four treatments: the control (white dots), with nutrient additions (green squares), with fish (yellow triangles) and the combination of nutrient additions and fish (orange diamonds). Large symbols represent the means (± SE). The red hatched line indicates the addition of the treatments, which thus corresponds to Day 0.

Chl *a* (Table 2). Pelagic Chl *a* was slightly higher in dark than in clear water (Fig. 2a; Table 2). In contrast to pelagic Chl *a*, we did not observe any effect of nutrient addition on the benthic Chl *a* concentration, whereas darkening reduced the benthic Chl *a* concentrations (Fig. 2b; Table 2). Thereby, there was a strong positive effect of nutrient addition on the ratio pelagic : benthic Chl *a* concentration and especially so in dark water, due to an increase in pelagic and a decrease in benthic Chl *a* concentrations (Table 2; Supporting Information Fig. S6).

In contrast to benthic Chl *a* concentrations, there was no evidence for an effect of darkening on benthic primary production at the end of the experiment, but an antagonistic interaction between darkening and fish only on benthic community respiration, with a more negative effect of darkening in the absence of fish (Supporting Information Fig. S7; Table S3). From pictures of the tank's walls, we also observed that both nutrient addition and darkening increased the cover of periphyton, with the highest cover observed in dark water tanks with added nutrients (Table 3; Supporting Information Fig. S8).

Nutrient enrichment had a positive effect on total zooplankton biomass, mostly related to positive effects on copepods and cladocerans, in contrast to a moderate negative effect on rotifers (Supporting Information Fig. S9; Table S4). With darkening, the positive effect of nutrient enrichment on the total zooplankton biomass strengthened toward the end of the experiment (Table 4). Darkening itself had no effect on total zooplankton biomass but opposite effects on copepods (moderate evidence for a positive effect) and rotifers (weak evidence for a negative effect; Supporting Information Fig. S9; Table S4). An antagonistic interaction between nutrient enrichment and darkening was only evident for the copepods (Supporting Information Table S4), highlighting that darkening affected some taxonomic groups of zooplankton differently depending on if the nutrients were added or not.

DO concentrations decreased within the first week of the experiment in all treatments, but at a faster rate if nutrients were added (Fig. 2c, Table 2). During the second half of the experiment, the DO instead increased with nutrient additions and reached over 100% saturation in clear water (Fig. 2c). Even though darkening increased the Chl *a* concentration (Fig. 2a) it decreased the DO, and thus along with increased bacterial abundance in dark treatments (Fig. 2d), reduced the positive effect of nutrient enrichment on DO in dark treatments (i.e., an antagonistic interaction).

Bacterial abundance increased over time in all treatments with nutrient additions, and initially also with darkening (Fig. 2d; Table 2). The initial positive effect of darkening diminished over time, unless nutrients were added (as indicated by the interaction between those treatments and time; Table 2). The lowest levels of bacterial abundance were found in the control and with fish in absence of nutrient additions or darkening.

Top-down control

The data revealed no evidence that fish had any effect on the DO, bacterial abundance or Chl *a* concentrations (Table 2; Fig. 2), despite a strong negative effect on the zooplankton biomass and composition (Fig. 3). Without fish, the zooplankton communities were dominated by copepods and cladocerans whereas rotifers dominated when fish were present. Presence or absence of fish was the main factor influencing the zooplankton composition and biomass (Table 4). The nutrient treatment was the second strongest driver of zooplankton community dissimilarity (Table 4) with higher total zooplankton biomass (Fig. 3a; Table 4). However, in presence of nutrient enrichment and fish, the zooplankton biomass increased toward the end of the experiment, indicating a time dependent interaction between the two factors (Fig. 3a; Table 4). Presence of fish mostly affected the benthic taxa with

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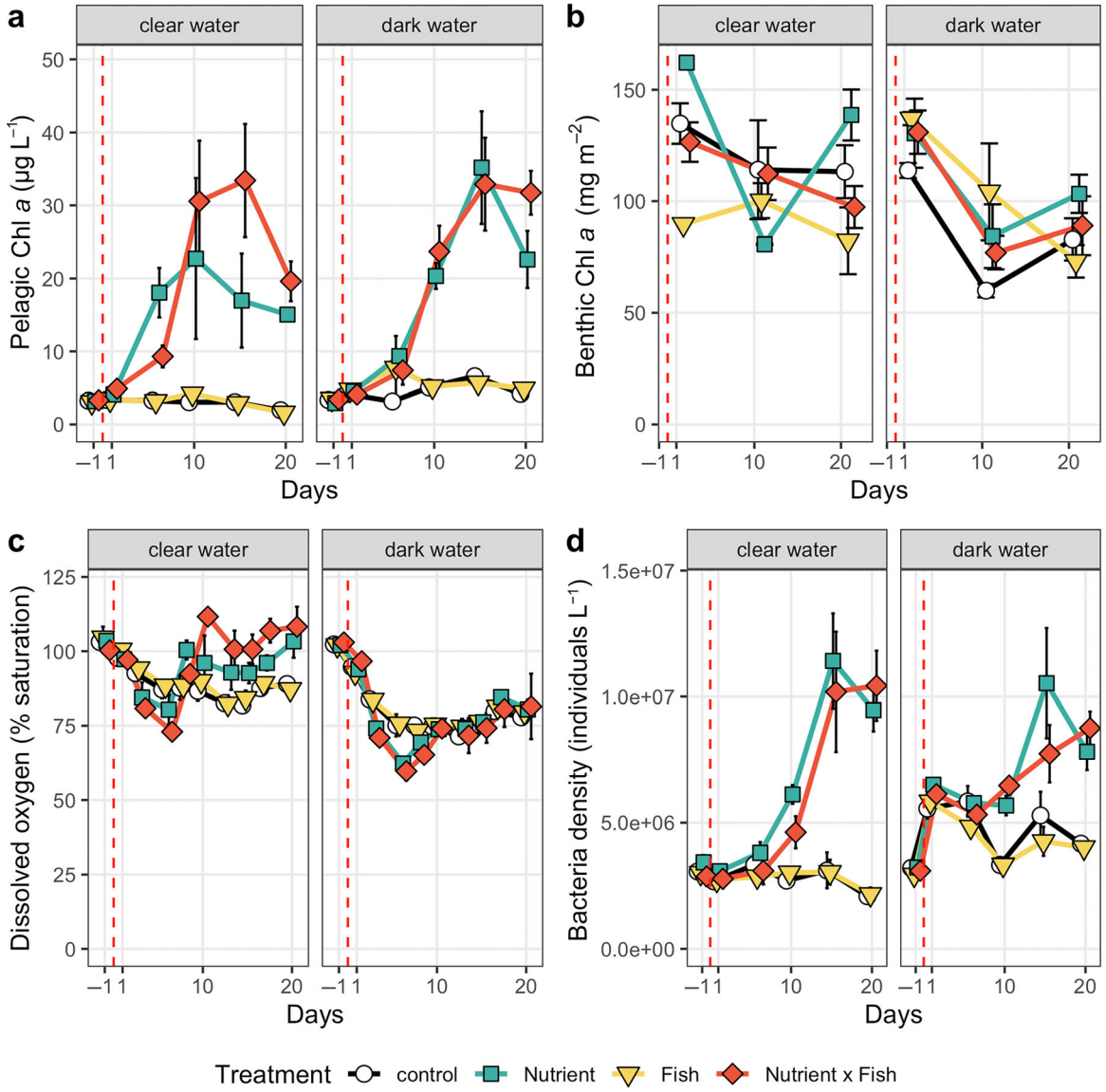

Fig. 2. Changes in (**a**) pelagic and (**b**) benthic Chl *a* concentrations, (**c**) DO, and (**d**) bacterial abundances over time in the four treatments: the control (white dots), with nutrient additions (green squares), with fish (yellow triangles) and the combination of nutrient additions and fish (orange diamonds). Large symbols represent means (± SE). The red hatched line indicates the addition of the treatments, which thus corresponds to Day 0.

a negative effect over time on benthic Chl *a* (Table 2). Thus, the presence of fish increased the pelagic : benthic algal ratio (Table 2; Supporting Information Fig. S4). Fish had a strong negative effect on chironomid biomass (Fig. 4; Table 4) and abundance (Supporting Information Fig. S10; Table S5).

Discussion

With increased inputs of terrestrial organic matter and sediment resuspension, coastal waters are becoming darker (Fleming-Lehtinen and Laamanen 2012; Dupont and Aksnes 2013; Deininger and Frigstad 2019), adding to the threat to coastal ecosystems already facing eutrophication. By experimentally studying how light limitation affect bottom-up and top-down drivers of eutrophication, we find that reduced light availability (i.e., darkening) can worsen eutrophication symptoms and increase the relative abundance of pelagic vs. benthic algae. We find the highest pelagic Chl *a* concentrations with nutrient enrichment and presence of fish in dark water. Our results thus suggest that actions to reduce eutrophication and accompanying symptoms may be hindered by darkening of coastal waters, but that the outcome can vary depending on the relative importance of pelagic vs. benthic primary production.

Our first hypothesis stated that *nutrient enrichment will boost pelagic algae in both clear and dark water but benthic algae in clear*

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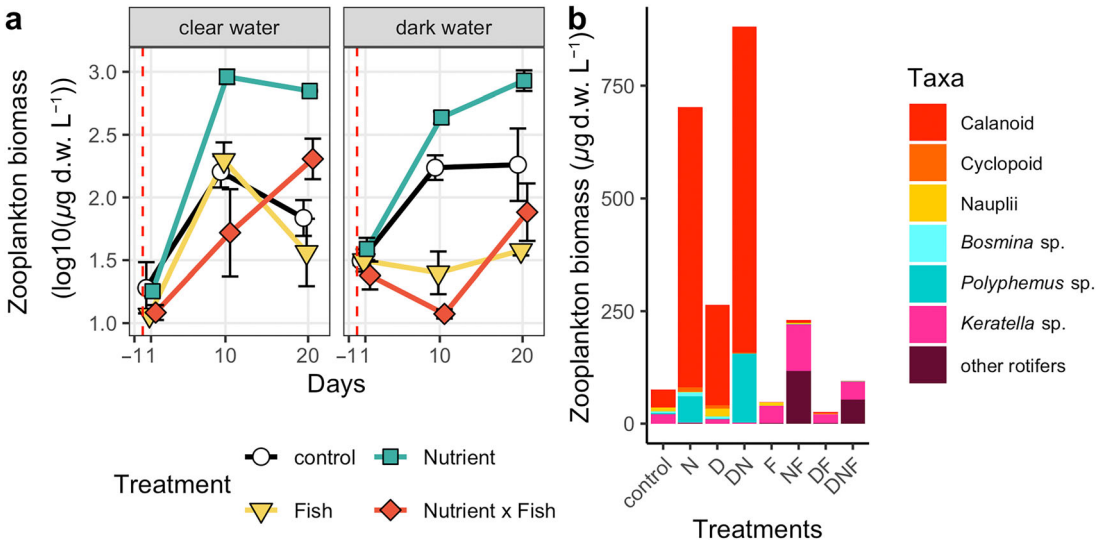

Fig. 3. Responses of (**a**) total zooplankton biomass and (**b**) zooplankton community composition on the last day of the experiment (Day 20) to our four treatments: the control (white dots), with nutrient additions (green squares), with fish (yellow triangles) and the combination of nutrient additions and fish (orange diamonds). Large symbols represent means (± SE). The red hatched line indicates the addition of the treatments, which thus corresponds to Day 0. In (**b**), each treatment is referred to with a word or letter: no perturbation ("control"), nutrient addition ("N"), dark water ("D"), presence of fish ("F"), and the combinations of them.

water only. In line with the hypothesis, we observed higher pelagic Chl *a* concentrations in both clear and dark water (Fig. 2; Table 2). Rather than an increase of benthic algae in clear water following nutrient additions, we observed reduced benthic Chl *a* concentrations in dark water (Fig. 2, Table 2), which is expected if benthic algae are light limited (Ask et al. 2012). The latter suggests that the extent to which coastal darkening has a negative or positive effect on primary producers depends on the relative importance of pelagic vs. benthic algae and nutrient availability. In systems dominated by pelagic production (i.e., eutrophic waters; Krause-Jensen et al. 2012), darkening may have no or even positive effects on total primary production. However, in nutrient-poor systems with high light-availability and thus a higher benthic rather than pelagic production, darkening would benefit pelagic relative to benthic production but likely decrease total production.

An increase in pelagic Chl *a* concentrations in darker water, as we found, has also been observed in lacustrine ecosystems with natural variation in light availability (Leach et al. 2019) and in previous experiments (van Dorst et al. 2020). However, also the opposite pattern has been observed (Bartels et al. 2012; Gall et al. 2017; Mustaffa et al. 2020), with reduced phytoplankton biomass following darkening. There are several mechanisms that can lead to increased Chl *a* concentrations in darker waters: (1) different phytoplankton composition in clear and dark waters (Hanson et al. 2003); (2) phytoplankton species having more chloroplasts to compensate for less light (Geider et al. 1997); and (3) a vertical compression of the phytoplankton community into a narrower surface layer rather than being equally distributed across the whole water column (Carpenter and Pace 2018). We cannot fully disentangle the extent to which each of the above mechanisms contributed to the moderate increase in pelagic Chl *a* concentration with darkening in our experiment.

Nevertheless, by analyzing a subset of treatments at the end of the experiment, we could observe that darkening changed the phytoplankton composition (Soulié et al. 2022; Table 2). Nanoflagellates dominated in dark water, whereas nutrient enrichment increased the abundance of diatoms (based on the taxa we could identify; Supporting Information Fig. S5). From that same subset of treatments, we found an increased in the number of phytoplankton cells with nutrient enrichment and to a lesser extent also with darkening, which correlated with pelagic Chl *a* concentrations (adjusted $R^2 = 0.86$; Supporting Information Fig. S4b). In addition, there was a stronger increase in phytoplankton cells compared to Chl *a* concentration in the dark treatment, mainly driven by an increase in unidentified small phytoplankton (< 20 μm; Supporting Information Fig. S5a). This observation could explain the increase in zooplankton toward the end of the experiment in dark waters (Table 4), potentially with an additional trophic niche consisting of microzooplankton such as protozoa (Novotny et al. 2021). However, the Chl *a* concentration and abundance might still not fully reflect phytoplankton biomass. Combining multiple methods: pigment analyses for the community composition (Soulié et al. 2022) and image analyses for the community size structure (Dunker et al. 2018) could be alternative approaches in future studies to provide faster and more reliable estimation of

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Table 2. Results of the effects of nutrient additions, darkening and fish on primary producers (pelagic and benthic Chl *a*), relative pelagic/benthic Chl *a*, DO, and bacterial abundance (RM-ANOVA). Significant results are highlighted in dark yellow. The variables (pelagic Chl *a*, relative pelagic/benthic Chl *a*, DO, and bacterial abundance) were natural-log (ln) transformed prior to analyses to fit a normal distribution. The zero-inflated beta regression was used to analyze proportional data bounded between 0 and 1.

Test	Pelagic Chl *a* (ln)			Benthic Chl *a*			Relative pelagic/benthic Chl *a* (ln)			DO (ln)			Bacteria (ln)		
	RM-ANOVA with GG correction			RM-ANOVA			RM-ANOVA			RM-ANOVA with GG correction			RM-ANOVA with GG correction		
	F	η^2_G	p-value	F	η^2_G	p-value	F	η^2_G	p-value	F	η^2_G	p-value	F	η^2_G	p-value
Main effect															
Nutrient	$F_{1,16} = 260.91$	0.820	< 0.001	$F_{1,15} = 1.42$	0.033	0.252	$F_{1,14} = 238.44$	0.812	< 0.001	$F_{1,16} = 0.30$	0.007	0.592	$F_{1,16} = 138.28$	0.741	< 0.001
Fish	$F_{1,16} = 1.96$	0.033	0.181	$F_{1,15} = 0.08$	0.002	0.783	$F_{1,14} = 2.42$	0.042	0.142	$F_{1,16} = 0.07$	0.002	0.789	$F_{1,16} = 2.72$	0.053	0.0118
Dark	$F_{1,16} = 9.24$	0.139	0.008	$F_{1,15} = 5.83$	0.124	0.029	$F_{1,14} = 25.86$	0.319	< 0.001	$F_{1,16} = 106.57$	0.703	< 0.001	$F_{1,16} = 61.37$	0.560	< 0.001
Nutrient : Dark	$F_{1,16} = 5.94$	0.094	0.027	—		—	$F_{1,14} = 9.25$	0.144	0.009	$F_{1,16} = 10.92$	0.195	0.004	$F_{1,16} = 11.07$	0.187	0.004
Temporal effect															
Date	$F_{2.12,33.86} = 31.11$	0.583	< 0.001	$F_{2,30} = 14.58$	0.382	< 0.001	$F_{2,28} = 63.88$	0.773	< 0.001	$F_{2.41,38.56} = 35.19$	0.586	< 0.001	$F_{2.37,37.97} = 15.89$	0.399	< 0.001
Nutrient : Date	$F_{2.12,33.86} = 27.53$	0.553	< 0.001	$F_{2,30} = 1.39$	0.056	0.265	$F_{2,28} = 35.35$	0.653	< 0.001	$F_{2.41,38.56} = 15.45$	0.384	< 0.001	$F_{2.37,37.97} = 40.58$	0.629	< 0.001
Fish : Date	$F_{2.12,33.86} = 0.76$	0.033	0.482	$F_{2,30} = 3.90$	0.142	0.031	$F_{2,28} = 4.12$	0.180	0.027	$F_{2.41,38.56} = 1.59$	0.060	0.213	$F_{2.37,37.97} = 1.83$	0.071	0.169
Dark : Date	$F_{2.12,33.86} = 3.57$	0.139	0.037	$F_{2,30} = 1.65$	0.065	0.209	$F_{2,28} = 6.02$	0.243	0.007	$F_{2.41,38.56} = 8.29$	0.250	< 0.001	$F_{2.37,37.97} = 10.76$	0.310	< 0.001
Nutrient : Dark : Date	$F_{2.12,33.86} = 0.029$	0.013	0.764	—		—	$F_{2,28} = 0.42$	0.022	0.662	$F_{2.41,38.56} = 2.28$	0.084	0.107	$F_{2.37,37.97} = 8.24$	0.256	< 0.001

phytoplankton biomass and composition, as manual counting requires expertise and time.

Although the general effect of darkening on pelagic Chl *a* was much less than that of nutrient enrichment, we observed a synergistic interaction between the two with the highest pelagic Chl *a* concentrations in dark nutrient rich waters (with and without fish). In clear water, nutrient additions are quickly used both by the pelagic phytoplankton and periphyton on the tank walls (*see* Table 2; Supporting Information Fig. S6). Regarding our method to estimate the periphyton coverage, we noticed that using pictures of tank walls underestimated the periphyton growth, as it reduced the analysis to two dimensions while the periphyton can grow in three dimensions. The walls were covered by a thin (close to 2D) algal layer in dark water tanks, but with a 3D-expension with nutrient enrichment. When combined, that is, in dark nutrient enriched water, the periphyton covered close to 100% of the wall surface with a thick layer (Supporting Information). In dark enriched water, without a 3D-expension, resulting in a lower algal growth, we suggest that nutrients were instead mainly used by the pelagic phytoplankton leading to a slower decline in nutrient concentrations (Fig. 1a,b). Overall, these observations suggest that periphyton growth was more limited by nutrient availability than by light, leading to more nutrients being available for pelagic algae in dark water.

Given a higher nutrient availability for phytoplankton in dark than clear waters, a subsequent difference of nutrient uptake can explain why we observed higher pelagic Chl *a* concentrations (and abundance at the end of the experiment) in dark water. The difference in relative abundance of phytoplankton (as indicated by our analyses of community composition), and their specific nutrient uptake rates could also explain the delay of the Chl *a* peak (Mercado et al. 2014). The decline in Chl *a* after the peak likely resulted from an increased abundance of zooplankton. Another potential mechanism for the delayed phytoplankton peak in dark water is reduced top-down control by the zooplankton. Copepods, the dominant zooplankton taxa, showed an antagonistic interaction from nutrient addition and darkening with a lower biomass in nutrient rich dark water than expected from the response to darkening and nutrient additions alone. Thus, we speculate that darkening—in enriched water—may limit copepod population growth, which in turn reduced the predation pressure on phytoplankton. The latter may, in turn, relate to difference in phytoplankton community composition between treatments at the end of the experiment, although better resolved data on species composition across treatments and time would be required to show this is the case. We also observed a similar delay of the Chl *a* peak in clear water in the presence of fish and nutrient enrichment, where fish may have initially reduced the predation pressure by zooplankton on phytoplankton.

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Table 3. Results of the effects of nutrient additions and darkening on the final phytoplankton abundance (ANOVA) and composition (PERMANOVA); and of all treatments (i.e., the full-factorial design) on the periphyton coverage (zero-inflated beta regression). Significant results are highlighted in dark yellow. The total phytoplankton abundance was natural-log (ln) transformed prior to analyses to fit a normal distribution. The zero-inflated beta regression was used to analyze proportional data bounded between 0 and 1.

	Phytoplankton abundance (ln)		Phytoplankton composition (last day)		Periphyton coverage	
Test	ANOVA		PERMANOVA (Bray–Curtis dissimilarity index)		Zero-inflated beta regression link = logit	
Main effect	*F*	*p*-value	*F*	*p*-value	*t*	*p*-value
Nutrient	$F_{1,11} = 73.04$	< 0.001	$F_{1,10} = 35.26$	0.005	6.311	< 0.001
Fish	—	—	—	—	0.689	0.5
Dark	$F_{1,11} = 48.26$	< 0.001	$F_{1,10} = 15.92$	0.005	6.287	< 0.001
Nutrient : Dark	$F_{1,11} = 12.60$	0.009	$F_{1,10} = 12.38$	0.011	—	—

Table 4. Results of the effects of nutrient additions, darkening and fish on total zooplankton biomass (RM-ANOVA), zooplankton composition (PERMANOVA), and chironomid biomass (ANOVA). The variables were log10 transformed prior to analyses to fit a normal distribution. Significant results are highlighted in dark yellow.

	Total zooplankton biomass (log10)			Zooplankton composition (last day)		Chironomid biomass (log10)	
Test	RM-ANOVA			PERMANOVA (Bray–Curtis dissimilarity index)		ANOVA	
	F	η^2_G	*p*-value	*F*	*p*-value	*F*	*p*-value
Main effect							
Nutrient	$F_{1,16} = 14.56$	0.304	< 0.001	$F_{1,16} = 9.13$	0.001	$F_{1,19} = 2.81$	0.11
Fish	$F_{1,16} = 50.94$	0.605	< 0.001	$F_{1,16} = 16.00$	0.001	$F_{1,19} = 9.60$	0.006
Dark	$F_{1,16} = 0.01$	< 0.001	0.933	$F_{1,16} = 1.45$	0.221	$F_{1,19} = 0.11$	0.743
Nutrient : Fish	$F_{1,16} = 3.05$	0.084	0.1	$F_{1,16} = 6.68$	0.001	—	—
Nutrient : Dark	—	—	—	$F_{1,16} = 0.80$	0.522	—	—
Dark : Fish	—	—	—	$F_{1,16} = 1.09$	0.351	—	—
Temporal effect							
Date	$F_{2,32} = 50.05$	0.619	< 0.001	—	—	—	—
Nutrient : Date	$F_{2,32} = 16.04$	0.342	< 0.001	—	—	—	—
Fish : Date	$F_{2,32} = 16.87$	0.354	< 0.001	—	—	—	—
Dark : Date	$F_{2,32} = 9.18$	0.23	< 0.001	—	—	—	—
Nutrient : Fish : Date	$F_{2,32} = 4.08$	0.117	0.026	—	—	—	—

In contrast to hypothesized (H1), we did not find a positive effect of nutrient enrichment on benthic Chl *a*, irrespective of light availability. This might be explained by initially high nutrient concentrations in the sediment and high initial Chl *a* concentrations ($\sim 130\ \mu g\ m^{-2}$), meaning little scope for benthic algae to respond to further nutrient additions. Another potentially contributing factor could be that the zoobenthos may have incorporated much of the nutrients (Blumenshine et al. 1997). However, based on our data on chironomid biomass, there is no statistical support for the latter mechanism.

Our second hypothesis stated that *juvenile perch will* (indirectly through a trophic cascade) *increase pelagic and benthic algal biomass in clear water but not in dark water due to lower feeding efficiency in low-visibility conditions*. The fish did, as expected, impose strong top-down control on zooplankton and zoobenthos, but this effect did not cascade down to affect the pelagic and benthic algal biomass. The zooplankton community changed from being dominated by copepods in absence of fish to dominance of rotifers in the presence of fish, reflecting their selection for larger-sized high nutritional food (Hangelin and Vuorinen 1988; Jakobsen et al. 2003). The sustained high biomass of rotifers, also in presence of fish, likely maintained the predation pressure on phytoplankton, impeding the expected increase in phytoplankton biomass following fish predation on zooplankton. It is also possible that the length of the experiment (3 weeks with juvenile fish) was

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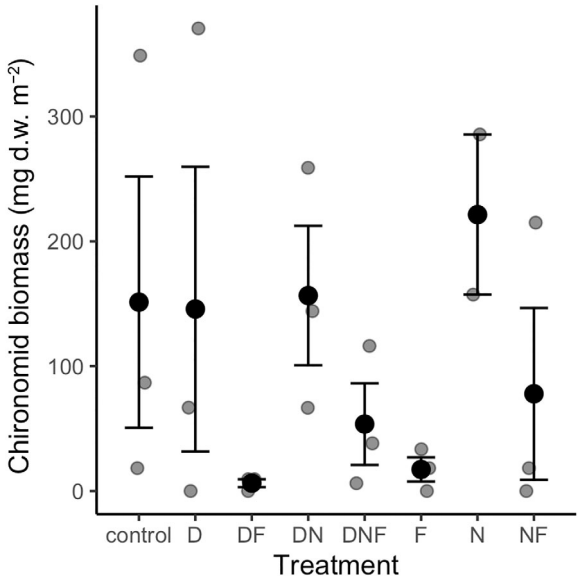

Fig. 4. Chironomid biomass in the benthic habitat on the last day of the experiment (Day 21). Large symbols represent means (± SE), and small dots represent observations. Each treatment is referred to with a word or letter: no perturbation ("control"), nutrient addition ("N"), dark water ("D"), presence of fish ("F"), and the combinations of them.

not long enough to observe top-down control across several trophic levels as the pathway from fish to phytoplankton may take a longer time than the bottom-up effect of nutrient enrichment on phytoplankton (Elser et al. 2000). In darker water, we expected a higher zooplankton biomass due to lower feeding efficiency for fish in low-light conditions. However, we did not observe any significant interactions between fish and darkening on zooplankton. The lack of clear support for a top-down effect of darkening linked to lower predator feeding efficiency is in line with some previous studies (Jönsson et al. 2012; Weidel et al. 2017; van Dorst et al. 2020). The extent to which low visibility affects fish feeding and growth may vary depending on species (van Dorst et al. 2020) and may rather be linked to shifts in prey composition (van Dorst et al. 2020; Leech et al. 2021; Bell et al. 2022).

In the present study, we focused on the link between nutrient enrichment and Chl *a* concentrations. However, eutrophication involves symptoms other than high Chl *a* concentrations, such as excessive growth of filamentous and toxic algae, oxygen depletion of bottom waters, loss of macrophytes, and fish mortality (Carpenter et al. 1999). Thus, more research is needed to understand how variation in light availability affect the full range of eutrophication symptoms. Also, we measured Chl *a* but did not directly estimate phytoplankton biomass and only to a limited extent abundance and composition with microscopy methods. Temporal changes in the phytoplankton composition, including the abundance of toxic cyanobacteria, are therefore unaccounted for here. As for oxygen depletion of bottom waters, we did not observe this in our experiment. Our tanks were not deep enough to allow for stratification in the water column. Still, we observed a synergistic interaction between nutrient enrichment and darkening on DO at 40 cm depth. Toward the end of the experiment, nutrient addition had a positive effect on oxygen concentration, likely reflecting a high algal production. In contrast, darkening had a negative effect on oxygen concentration. This suggest that primary production relative to the biomass of primary producers was lower in darker waters or that the observation of higher bacterial abundance in dark waters can explain the depletion of oxygen. Decreased oxygen is often associated with an increase in total phosphorus released from sediments, exacerbating eutrophication symptoms (Søndergaard et al. 2003; Carpenter 2005). We did observe higher phosphorus concentrations toward the end of the experiment in dark water, but not any severe oxygen depletion.

The set up with two juvenile perch were not meant to capture long term effects of eutrophication and darkening on fish mortality. Still, we did see a negative effect of darkening on fish survival in absence of nutrient additions. The likely explanation for higher fish mortality in dark water tanks is the lack of visible large-bodied zooplankton prey toward the end of the experiment. Mortality likely happened toward the end of the experiment, given the observed strong negative effect on zooplankton throughout the experiment also in the dark water treatment, and variation in fish mortality among treatments therefore likely had minor effects on our results concerning other response variables.

Our experiment gives support for strong bottom-up effects on pelagic food-web components in shallow coastal waters following nutrient enrichment, irrespective of light availability, but as hypothesized not with respect to the benthic part of the food web. Fish presence–absence had no significant impact on pelagic or benthic algal biomasses in neither clear nor dark waters. Thus, despite fish having a strong impact on invertebrate prey biomass and composition, the top-down effect of fish on Chl *a* was weak relative to the effect of nutrient enrichment and darkening. We conclude that eutrophication management is likely to impact both pelagic and benthic food webs in shallow clear waters, but mainly the pelagic part in darker waters. In fact, both nutrient levels and pelagic Chl *a* concentrations were higher in dark treatments, which suggest that nutrients are less efficiently incorporated into the benthic part in dark water food webs (as suggested by Vasconcelos et al. 2019). It is important to stress that the generally associated increase in the load of dissolved organic matter with coastal darkening, but not the focus in this study, may have to be considered in eutrophication management. The absence of significant top-down effects of fish on pelagic algae but a negative effect on benthic algae also implies that fish management as a tool to combat eutrophication symptoms may be more effective in dark enriched relative to clear enriched waters, but that nutrient management still is key. Darkening of coastal waters may thus not only exaggerate

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eutrophication symptoms but also call for more elaborate mitigation actions to combat eutrophication symptoms, taking indirect effects caused by trophic interactions into account, and future management of coastal waters should thus adapt solutions accordingly.

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Conflict of Interest

All authors declare that they have no conflicts of interest.

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