



Impact of aquaculture and agriculture nutrient sources on macroalgae in a bioassay study

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ABSTRACT

Eutrophication is a major threat to aquatic ecosystems, because excessive nutrient enrichment may result in the loss of ecosystem services. Fjord systems are specifically under pressure due to nutrient input from land (agriculture) and sea (aquaculture). In this bioassay study, we have analyzed the effect of different nutrient sources, as well as their combination, on growth, nutrient composition and recruitment of habitat-forming and ephemeral macrophytes. We found that agricultural fertilizer increased growth for all algae (except *Fucus*), while the fish farm effluents mainly increased growth of *Ulva*. The C:N ratio was hardly affected by the fish farm, but decreased significantly in all algae when agriculture fertilizer was added. Most interestingly, however, distance to the fish farm modulated the algal response to the fertilizer. Our results demonstrate the importance of studying effects of multiple stressors in aquatic ecosystems to sustainably manage the consequences of anthropogenic impacts.

1. Introduction

For several decades, coastal ecosystems received anthropogenic inorganic nutrients mainly through atmospheric deposition, fertilizer use and wastewater disposal (Howarth et al., 2002; Valiela et al., 1997). Specifically partly-enclosed systems, for example the Baltic Sea and the Gulf of Mexico, have suffered from severe eutrophication events with large areas of hypoxia (Conley et al., 2009; Dybas, 2005; NOAA, 2017). Accordingly, the effects of fertilizer use on algal growth have been tested widely and today knowledge on how agricultural fertilizer benefits algal growth is extensive (e.g. Bucolo et al., 2008; Hillebrand and Kahlert, 2001; Korpinen et al., 2007; Mörk et al., 2009; Worm et al., 2000).

However, a more recent source of nutrient input into coastal systems has received much less attention – marine aquaculture in open cages. Asia is, with 57% of the global production in 2016, the largest producer of marine and coastal finfish from aquaculture (FAO, 2018; Venvik, 2005). However, Norwegian aquaculture has been continuously growing since 1983 and aquaculture accounted for 35% of the total fish production in Norway and almost 50% of global salmon production in 2018 (Directorate of Fisheries, 2021; FAO, 2020). Although the industry has already reduced environmental impacts, for example through reduction of food spill (Price et al., 2015), open cage fish farms still release vast amounts of dissolved nutrients and particulate matter.

Ecological effects of the particulate organic matter from fish farms

are relatively well studied and are usually characterized by inter alia high abundances of opportunistic species and a decrease in benthic diversity (Kutti et al., 2007; Price et al., 2015; Zhulay et al., 2015). In contrast, knowledge on the ecological effects of dissolved nutrients from fish farms, particularly on primary producers is very scarce (Price et al., 2015). Nutrient concentrations around fish farms and potential effects on macroalgae communities are currently also not monitored as part of the Environmental Monitoring Programme for fish farms in Norway.

Approximately 45% of nitrogen, and 18% of phosphorous contained in feed are excreted as dissolved inorganic nutrients (Wang et al., 2012), resulting in the release of 26,000 t nitrogen and 3960 t phosphorus annually in Norway (Torrissen et al., 2016). Despite those high loads of dissolved nutrients that are released from a farm, nutrient concentrations in the surrounding seawater decline rapidly after release and are often not detectable at distances greater than 400 m (Oh et al., 2015). This rapid decline in nutrient concentrations is often attributed to dilution in large water bodies making it very unlikely to detect nutrient changes (García-Sanz et al., 2011; Pitta et al., 2009). This is thought to be even more significant at exposed sites, where fish farms often are placed. Accordingly, many studies failed to link high nutrient loads from fish farms to for example increased phytoplankton production, particularly in oligotrophic waters (Husa et al., 2014b; Price et al., 2015). In the oligotrophic Mediterranean, however, Pitta et al. (2009) found that part of the released nutrients are immediately assimilated by

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phytoplankton and transferred to higher trophic levels by grazers.

In marine systems, nitrogen is more often limiting the growth of primary producers than phosphorus (Howarth and Marino, 2006), and the Norwegian coastal areas are normally nitrogen limited in the euphotic zone in summer (Aure and Johannesen, 1997). Thus, input of nitrogen may significantly increase macroalgal production and affect community composition particularly in summer, when also salmon growth and thus nutrient effluents are highest (Husa et al., 2014b).

Macroalgae constitute important primary producers and habitat formers in coastal rocky shore ecosystems. Especially perennial canopy-forming algae, such as *Fucus* and other brown algae, provide shelter and food for a highly diverse invertebrate fauna and can act as a nursery ground for fish (Fredriksen et al., 2005; Lorentsen et al., 2010; Smale et al., 2013). However, filamentous algal species often respond more quickly to nutrient changes, for example from fertilizer, than perennial algae (Karez et al., 2004). While the ratio of nitrogen and phosphorus in agricultural fertilizer is designed to optimize growth of plants with almost equal ration of nitrate and ammonium, fish farm effluents consist largely of fish excretions and faeces with high ammonium concentrations (Wang et al., 2013). Due to their different composition, both agricultural and aquaculture derived nutrients may accordingly have varying impacts on the algal community (Lotze and Schramm, 2000) and possibly the structure and even trophic interactions in macroalgal communities. Specifically, the interactive effects of both nutrient sources on primary producers and further indirect effect on coastal ecosystems are largely unknown (García-Sanz et al., 2011; Price et al., 2015).

This lack of knowledge is in stark contrast to the expansion plans of the aquaculture industry along the Norwegian coast. At the same time no monitoring scheme of eutrophication effects on primary production is implemented within the Norwegian regulations either (Standard Norge, 2016). Most eutrophication monitoring methods are based on a combination of physico-chemical and biological indicators (Ferreira et al., 2011), with the assessment of canopy forming macroalgal communities over longer time spans often being resource- and time-consuming. The more short-term responses by fast growing filamentous algae could be used as a more efficient indicator for eutrophication (Ferreira et al., 2011 and references therein). Especially potential interactive effects of different nutrient sources can only be properly assessed by monitoring schemes that include eutrophication indicators.

In this study, we investigated the combined effect of nutrients from aquaculture and agriculture on the growth of benthic macroalgae. We assessed the performance of both slow-growing (*Fucus vesiculosus*, *Palmaria palmata*) and fast-growing algae (*Ulva* spp., *Cladophora rupestris*) exposed to different levels of aquaculture and/or agriculture-derived nutrients in a bioassay experiment. With this approach, we addressed the following research questions: What are the combined effects of agriculture and aquaculture-based nutrients on the growth, recruitment and nutrient composition of the different macroalgal species? Our findings have relevance for monitoring and management of eutrophication in coastal marine ecosystems and we discuss the use of standardized bioassays as a monitoring approach.

2. Material and methods

A bioassay field experiment was set up near a salmon farm (*Salmo salar*) in Morsdalsfjorden (67° 01.654N, 14° 07.657E), Northern Norway, to study the effects of agriculture and aquaculture-derived nutrients on algal growth. The experiment was carried out over the course of 5 weeks from June until July 2017. The salmon farm had a fish biomass of 1500 t at the beginning of the study period. Nutrient enrichment from agriculture was imitated by adding artificial slow-release fertilizer (Plantacote® Depot 6M, 40 g; 9% N from nitrate, 10% N from ammonium, 1.8% P₂O₅ neutral-ammoniumcitrate-soluble phosphate, 4.2% P₂O₅ water-soluble phosphate, 12% K₂O water-soluble potassium oxide, 4.5% S water-soluble sulphur) in mesh bags. The macroalgal species used in the experiments were: the perennial algae *Fucus vesiculosus* Linnaeus

(Phaeophyceae) and *Palmaria palmata* (Linnaeus) F.Weber & D.Mohr (Rhodophyta), and the annual algae *Ulva* spp. (Chlorophyta) and *Cladophora rupestris* (Linnaeus) Kützting (Chlorophyta) (hereafter named *Fucus*, *Palmaria*, *Ulva* and *Cladophora*). The algae were collected outside of the university's research station from natural communities (67° 16.628N, 14° 33.305E) and stored in a flow-through tank in the dark for 6 to 8 days before deployment. This was done to acclimatize the algae under standardized background nutrient levels and allow the different individuals of each species to enter the experiment with similar levels of nutrient storage. Thalli from either *Fucus*, *Palmaria* or *Cladophora* were tied together in standardized clusters of similar size (of about 1.9 g, 1.9 g and 2.6 g respectively) for the deployment in the experiment (see below). *Ulva* spp. was deployed as discs with a diameter of 5.4 cm.

2.1. Experimental design

Bioassay panels were deployed in varying distances to the fish farm to obtain a gradient of aquaculture effluents: at the fish farm ("farm"; 67° 01.654N, 14° 07.657E), 300 m from the farm ("intermediate"; 67° 01.879N, 14° 07.287E) and 9 km from the farm in a neighbouring fjord ("control"; 67° 02.269 N, 14° 13.397E; Fig. 1).

Agricultural fertilizer was added to every other panel, while the other half of the panels remained at ambient conditions (empty nutrient bags). Thus, fertilizer treatments were randomly distributed within panels but blocked across panels to avoid spill-over effects. In total, six bioassay panels were deployed, two at each distance to the fish farm, of which one carried additional fertilizer and the other did not (Fig. 1B). The two panels at each site were approximately 10-20 m apart to avoid spill over effects from the fertilizer treatment.

Each bioassay panel contained 15 acrylic glass tubes (20 cm long, 8 cm in diameter, of which only 10 tubes were used) that contained algae on both sides of the panel (Fig. 1C). The algae were distributed such that *Fucus* and *Palmaria* or *Ulva* and *Cladophora* shared one tube and these algae pairs were replicated 5 times in each panel. The distribution and density of algae within the tubes was mainly due to the spatial limitations of this setup and to avoid interference between the more robust perennial algae and more fragile annual algae. Simulating potential inter-species interactions similar to natural communities, was therefore not in the scope of this study. Tubes were closed off with a fine mesh (1 mm). In addition, algal recruitment was estimated from recruitment tiles (5 × 5 cm² ceramic sandstone, fully vitrified unglazed tiles) placed in the centre of each tube (Fig. 1C).

This resulted in replication of 5 for effects of the fish farm (3 levels), fertilizer (2 levels) and their combination on all four algal species. Accordingly, replication regarding the recruitment tiles was 10.

The bioassay panels were positioned vertically at ca. 1.6 m water depth, kept in position by a buoy at the top and weights at the bottom, secured with an anchor line (control and intermediate site) or a mooring line (farm site) (Fig. 1B). The bioassay panels at the farm site were deployed in an un-used cage inside the farm.

2.2. Analysis of algal responses

Algal growth was measured by comparing biomass (wet weight) before and after the experiment for *Fucus*, *Palmaria* and *Cladophora*. The procedure of wet weighing was standardized as far as possible to reduce variation based on attached surface water, which was carefully removed with tissue paper. For *Ulva* we measured growth by comparing surface area of the discs before and after the experiment from photos using ImageJ 1.53e (Graiff et al., 2015; Lüning, 1990; Schneider et al., 2012; Rasband, 1997-2018). *Ulva* discs were sampled already after 13-15 days to avoid sporulation due to stress. Relative growth rates (RGR) of all macroalgae were calculated using a logarithmic formula for both wet weight and area (Lüning, 1990):

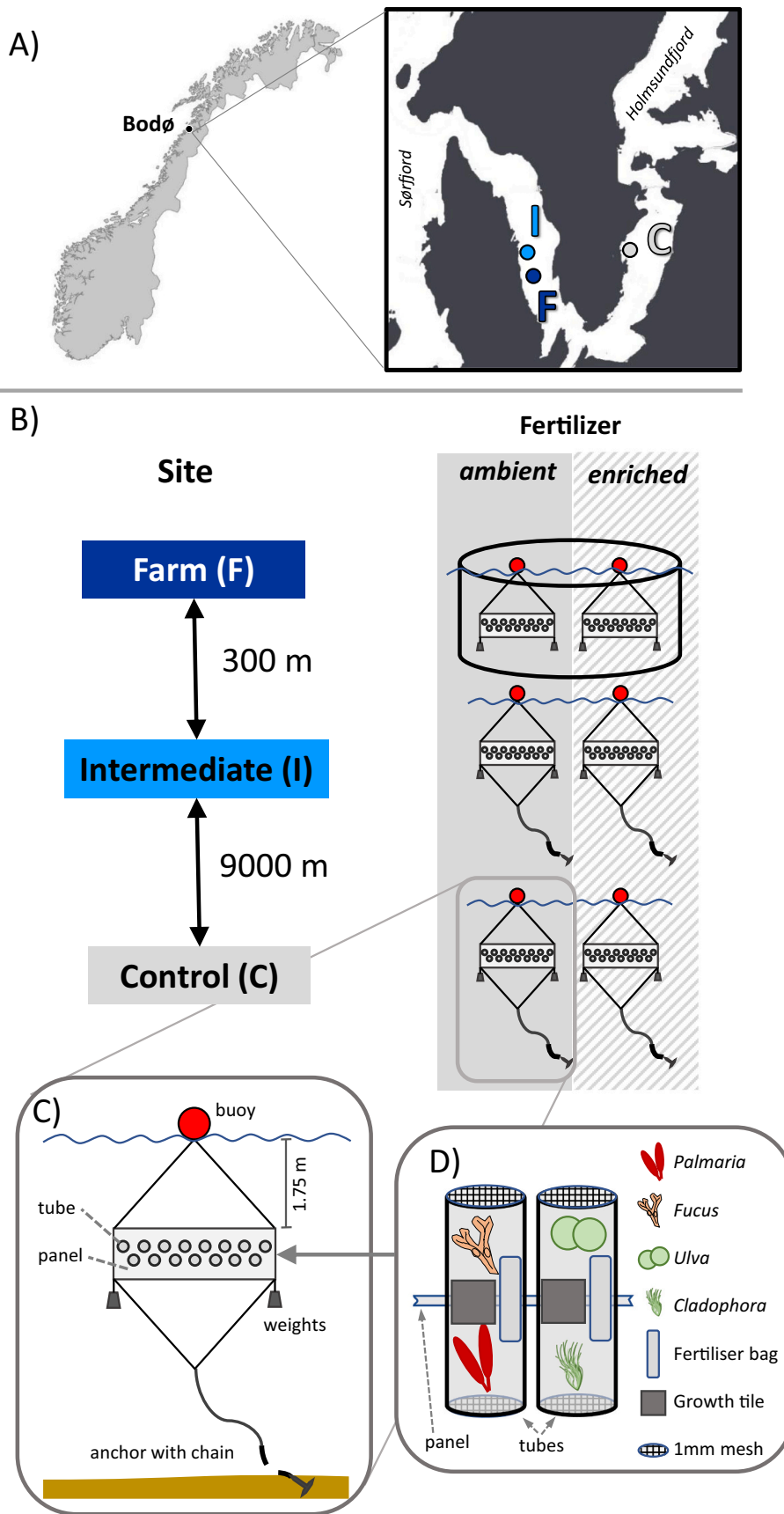


Fig. 1. Overview of the experimental setup. A) location of the control ("C", white), intermediate ("I", light blue) and farm sites ("F", dark blue); B) experimental set up at the three sites with one panel containing fertilizer (enriched, striped pattern) and without (ambient, solid pattern); C) setup of a panel containing 15 tubes; D) detailed configuration of the tubes containing the different algae species, a recruitment tile and a fertilizer bag (empty or filled). The colour and pattern coding is the same for Figs. 1 to 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$$RGR(\%d^{-1}) = \frac{\ln\left(\frac{x_2}{x_1}\right)}{t_2 - t_1} \cdot 100\%$$

where x_1 = wet weight [g] or area [cm^2] at t_1 and x_2 = wet weight [g] or area [cm^2] at t_2 , $t_2 - t_1$ is the time difference in days [d].

Algal nutrient composition (C:N) was analyzed from tissue samples taken before and after the experiment. A small portion of the of algal tissue (between 0.3 and 0.8 g) was frozen at $-20^\circ C$, later dried (24–48 h at $30^\circ C$), ground to a fine powder using a ball mill grinder (Retsch MM 400 Mixer Mill), filled in silver capsules, decalcified with hydrochloric acid (2 N HCL) and analyzed using a C:N Analyser.

The ceramic tiles to estimate algal recruitment were frozen at $-20^\circ C$ at the end of the experiments. For analysis, they were defrosted, carefully cleaned from epifauna (hydrozoans and bivalve recruits) and photographed. Algal recruits were very small filamentous taxa and could not be further identified. Algal coverage per tile was measured using CoralPointCount (Kohler and Gill, 2006), using a stratified random distribution points (5×5 cells with one point per cell).

Water samples of each site were taken and frozen at $-20^\circ C$ for later analysis of dissolved nutrients (PO_4^{3-} , NO_2^- , NO_3^- and NH_4^+) with a SAN++ Segmented Flow Analyser.

2.3. Statistical analyses

Treatment effects on algal growth rates and algal recruits were tested using generalized least squares models (Pinheiro et al., 2018) and backward selection including the fixed factors “farm” (control, intermediate, farm), “nutrient” (ambient, fertilized). Most of our data did not show homogeneity, and we therefore chose analysing the raw data with GLS rather than transforming the data to achieve homogeneity (Zuur et al., 2009). A generalized least squares model allows for heterogeneity by selecting a variance structure in the model (Zuur et al., 2009). Backward selection was used to find the optimal model with only significant terms by step-wise dropping interactions that were not significant.

For treatment effects on algal C:N ratios, similar generalized least squares models were used for all except for *Palmaria*. Here, a linear mixed-effects model (Pinheiro et al., 2018) was used (model selection based on the lowest AIC). The reason for this was a better fit of a model that included both the fixed factors (farm and nutrient) but also a random factor (tube). The random factor tube (number/position of tube in the panel) was first included in all models, but only optimized the model for the C:N-ratios of *Palmaria*.

For $p < 0.05$ differences were considered significant. Significant treatment effects were further tested with post hoc Least-Squares Means test (Lenth, 2016). All statistical analyses were run in R version 3.5.1 (R Development Core Team, 2014).

3. Results

3.1. Site characteristics

The study site is relatively exposed with an average flowrate of 5 cm/s and a maximum of 26 cm/s (at 5 m depth). The currents are tidal driven and alternate between a northward and southward flow. The southward current is on average 3 m/s stronger than the northward current. The salinity at the control site was slightly lower with 29.25 compared with more than 32 at the farm and intermediate site, while temperature was relatively constant during the deployment ranging between 10.05 and 11.12 $^\circ C$ at the control and intermediate site respectively (Table 1).

The ambient nutrient concentrations measured prior to the experiment revealed a gradient from the control towards the farm site. Phosphate concentrations increased from 4.8 $\mu g/l$ at the control site to 11.69 $\mu g/l$ at the farm site and the same trend was found for ammonium

Table 1

Environmental conditions at the experimental sites.

	Control	Intermediate	Farm
Salinity	29.25	32.08	32.60
Temperature [$^\circ C$]	10.05	11.12	11.01
Phosphate [$\mu g/l$ P]	4.8	10.76	11.69
Ammonium [$\mu g/l$ N]	1.28	2.51	16.19
Nitrite and nitrate [$\mu g/l$ N]	0.48	1.51	1.01

increasing from 1.28 to 16.19 $\mu g/l$ at the consecutive sites. Only for nitrate and nitrite combined the values were highest at the intermediate site (Table 1).

3.2. Algal growth rates

Algal growth rates were affected by both nutrient sources, fish farm effluents as well as agricultural fertilizer (Fig. 2, Table 2).

Fish farm effluents affected algal growth depending on algal species (Fig. 2A). Specifically, growth rates of the annual green algae *Ulva* doubled close to the fish farm, from 1.23% d^{-1} at the control site to 2.73% d^{-1} and 2.83% d^{-1} at the intermediate and farm site ($p < 0.001$ for both, Fig. 2A). Similarly, growth rates of the perennial brown alga *Fucus* increased significantly at the intermediate site (1.49% d^{-1}) compared to the control site (1.28% d^{-1} , $p = 0.016$). *Fucus* growth rates at the farm site (1.42% d^{-1}) were similar to the intermediate site, but this was not significant. In contrast, relative growth rates of the red alga *Palmaria* was highest at the control site (2.38% d^{-1}), significantly reduced at intermediate distance to the farm (1.95% d^{-1} , $p = 0.039$) and slightly reduced at the farm (2.31% d^{-1} , $p = 0.21$). The green alga *Cladophora* lost similar amounts of biomass in all treatments.

Agricultural fertilizer had positive effects on algal growth rates of all species except *Fucus*. Growth rates of *Palmaria* and *Ulva* increased quite equally from 1.97% and 2.05% d^{-1} to 2.34% and 2.47% d^{-1} , respectively, under enriched conditions ($p = 0.038$ and 0.045, Fig. 2B). However, the thalli of the red *Palmaria* changed colour to green at ambient conditions (at all distances to the farm). *Cladophora* lost significantly less weight under enriched conditions ($p = 0.012$). Only growth rates of the brown alga *Fucus* was not affected from agricultural fertilizer.

The combination of both nutrient sources, fish farm and agricultural fertilizer, had no significant interaction effect on algal growth rates (Fig. 2C). Note, however, that effects of agricultural fertilizer on *Ulva* growth rates seem to be more pronounced in the vicinity of the fish farm, compared to the control site.

3.3. Algal C:N ratios

Algal nutrient content was strongly affected by enrichment from agricultural fertilizer, while the vicinity to the fish farm only affected *Ulva* (Fig. 3, Table 3).

Ulva tissue C:N ratios significantly increased at the fish farm (25.52 ± 1.20) compared to both control (22.75 ± 2.27 , $p < 0.001$) and intermediate distance (21.66 ± 0.99 , $p = 0.006$, Fig. 3A).

Agricultural fertilizer, in contrast, significantly reduced C:N ratios of all algae but most notably in the perennial species (Fig. 3B). C:N ratios of *Fucus* and *Palmaria* were reduced by 69% (from 56.7 to 17.8) and 61% (from 31.1 to 12.1), respectively, of the values at ambient conditions. In contrast, C:N ratios of the annual species were only 29% (*Ulva*, from 27.2 to 19.4) and 26% (*Cladophora*, from 12.8 to 9.5) lower compared to ambient conditions.

Most interestingly, we found significant interaction effects of both nutrient treatments on the nutrient content of all algal species (Fig. 3C). Thus, effects of fish farm effluents on algal C:N ratios changed depending on the agricultural fertilizer. Both perennial species, *Fucus* and *Palmaria*, had significantly lower C:N ratios in fertilizer treatments, but ratios increased again when algae were at the same time close to the fish farm

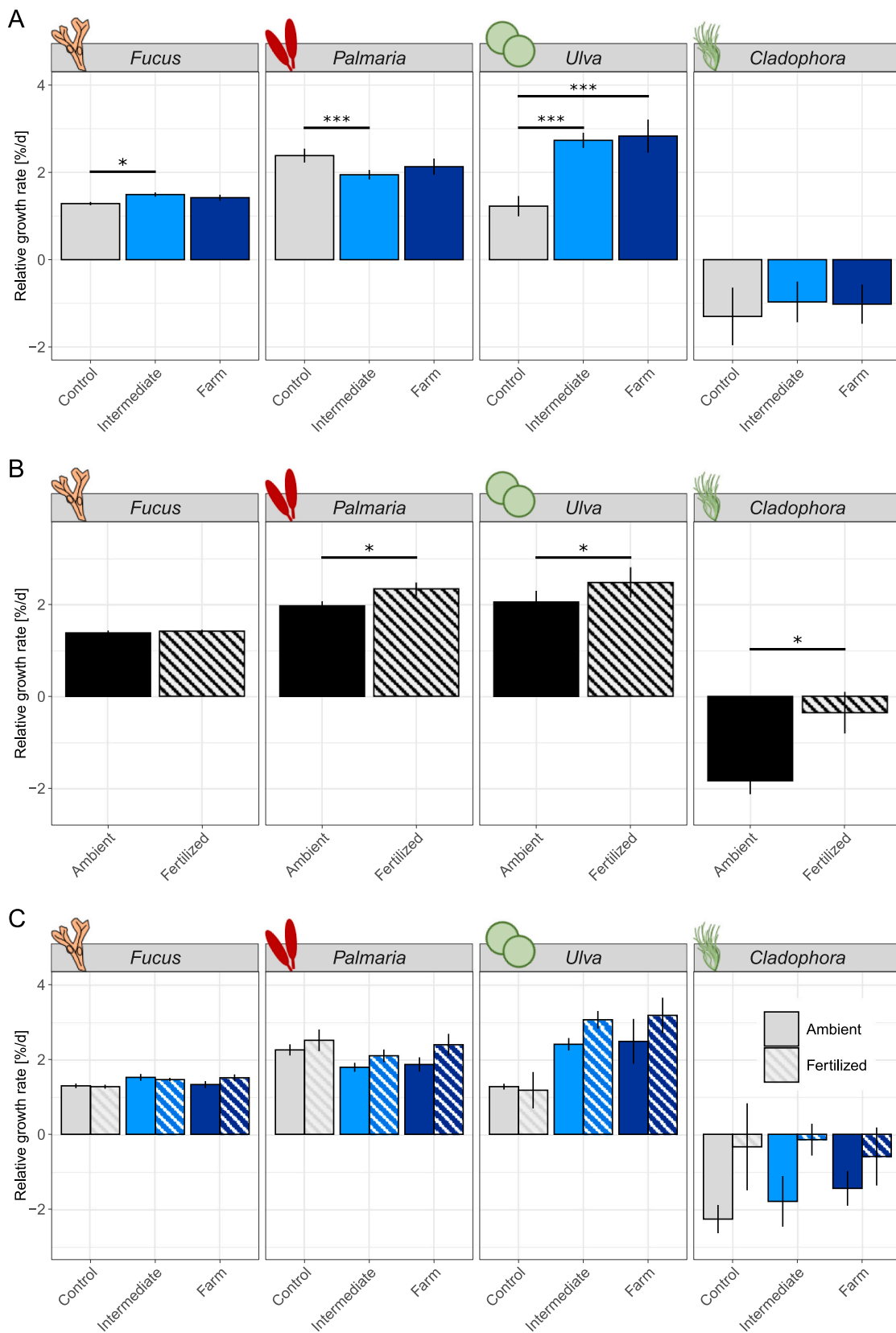


Fig. 2. Effects of A) distance to the fish farm (control, intermediate, farm) and B) additional fertilizer (ambient or fertilized) and C) nutrient enrichment with fertilizer (ambient or fertilized) at the different distances to the fish farm (control, intermediate, farm) on relative growth rates (% per day) of the four algae species. Means \pm SE. Black lines indicate significant difference between two bars. Significance levels: * $p < 0.05$, *** $p < 0.001$. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

Table 2

Treatment effects (fish farm, fertilizer, grazers) on relative growth rates of the four algae. Results from gls models after backward model selection.

		df	F-value	p-Value	
<i>Fucus</i>	Farm	2	3.94	0.032	*
<i>Palmaria</i>	Farm	2	3.14	0.060	.
	Fertilizer	1	4.76	0.038	*
<i>Ulva</i>	Farm	2	13.92	<0.001	***
	Fertilizer	1	4.42	0.045	*
<i>Cladophora</i>	Fertilizer	1	7.50	0.11	**

Significance levels: p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001.

(post-hoc test: control vs. farm at enriched, p < 0.0001 for both *Fucus* and *Palmaria*, Fig. 3C). Without additional fertilizer, the fish farm had no effect on the perennial species nutrient content. Similarly, agricultural fertilizer alone decreased the C:N ratio of *Ulva*, but a simultaneous exposure to the farm increased C:N ratios compared to the control site (post-hoc test: control vs. farm at enriched, p < 0.0001). *Cladophora* C:N ratios were inconclusive when both nutrient sources were combined.

3.4. Algal recruits

Growth of filamentous algal recruits was significantly higher when agricultural fertilizer was added (F = 22.38, p < 0.001), while the vicinity to the fish farm had no clear effect (Fig. 4). Under ambient conditions, coverage of algal recruits ranged between 1.14% (farm) and 3.6% (intermediate). When agricultural fertilizer was added, coverage of algal recruits was on average 10-fold higher than in the ambient assemblages (control: 10.1-fold, intermediate: 3.5-fold, farm: 16.4-fold, p < 0.001). Thus, enhancing effects of agricultural fertilizer on recruits seem to be mitigated at intermediate distance to the fish farm, but this was not significant.

There was no significant interaction effect between agricultural fertilizer and distance to the fish farm.

Moreover, we noted higher abundances of epifauna, mainly hydrozoans, at the farm and at intermediate distance, compared to the control site.

4. Discussion

This is one of the first field studies investigating effects of multiple nutrient sources, in this case from aquaculture and agriculture, on macroalgal growth and performance.

We found that fish farm effluents and agricultural fertilizer had varying effects on algal growth, and that effects depended on algal species. While fertilizer mostly increased algal growth, close proximity to the fish farm increased growth of the green annual *Ulva*, but slightly decreased growth of the red perennial *Palmaria*. The perennial brown alga *Fucus* had higher growth rates only at intermediate distance to the farm.

Most interestingly, however, the distance to the fish farm modulated the algal response to nutrient enrichment from the fertilizer. Although exposure of the algae to fertilizer strongly decreased their C:N ratios, a simultaneous exposure to fish farm effluents slightly increased those C:N ratios. Thereby this field study shows that macroalgal species are differently affected by nutrient enrichment from aquaculture and agriculture, and more importantly, that effects from both nutrient sources on algae were depended on another.

4.1. Nutrient effects on algal growth rates

Nutrient enrichment generally favours ephemeral species over perennial algal species because of their more efficient nutrient uptake and faster growth (Karez et al., 2004; Wallentinus, 1984). Dissolved nutrients as effluents from fish farms were already found to increase cover of epiphytic or opportunistic species for example in the Baltic Sea

(Rönnerberg et al., 1992; Worm and Sommer, 2000), the Tasman Sea (Fowles et al., 2018a, 2018b; Oh et al., 2015), and Norwegian fjords (Haugland et al., 2021). Thereby, macroalgal species assemblages changed towards more green, fast-growing or filamentous algal species near fish farms (Boyra et al., 2004; Fowles et al., 2018a; Rönnerberg et al., 1992). A recent study on natural macroalgal reef communities found similarly that cover of *Ulva* spp. and opportunistic green and filamentous algae were significantly higher closer to the fish farms (Oh et al., 2015).

Dalsgaard and Krause-Jensen (2006) were among the first to use bioassays to monitor effects of dissolved nutrients from fish farms, who found enhanced growth of *Ulva* spp. up to 152 m and lower C:N ratios up to 310 m away from a fish farm (Dalsgaard and Krause-Jensen, 2006). Phytoplankton growth was even increased up to almost 1000 m downstream a fish farm (Dalsgaard and Krause-Jensen, 2006), while another study in Spain detected effects from fish farm effluents at distances up to almost 2.5 km (García-Sanz et al., 2011). The differences in dispersal distances of the nutrient emissions were in this study mainly attributed to characteristics of the fish farm, e.g. farmed species, produced biomass and used amount of feed (García-Sanz et al., 2011). However, also species-specific characteristics of the algae such as nutrient physiology and growth play a role for algal response on nutrient enrichment (Deutsch and Voss, 2006; Gartner et al., 2002; Pedersen and Borum, 1996).

We found that algal growth in response to higher nutrient availability was strongly dependent on algal species and the nutrient source. The annual species *Ulva*, for example, showed more than doubled growth rates at the farm and at 300 m distance, while addition of agricultural fertilizer resulted in much weaker response of *Ulva*'s growth rate. *Ulva* species are known for their quick nutrient uptake (Martínez et al., 2012) and can fill their N-reserves within two days (Lubsch and Timmermans, 2018), which might explain the weaker response to additional agriculture fertilizer. The other annual alga in our study, *Cladophora*, was not significantly affected by the fish farm effluents, but increased growth from agricultural fertilizer. More precisely, the negative 'growth rates' observed in *Cladophora*, which was probably caused by tissue loss, was reduced significantly compared to the non-fertilizer treatment. Here the addition of nutrients seemed to have partially compensated the tissue loss through enhanced growth rates.

Thus, among the annual species specifically, *Ulva* could increase growth when exposed to fish farm effluents. Highest growth rates of *Ulva* close to fish farms were already shown by Dalsgaard and Krause-Jensen (2006) and, consequently, *Ulva* species are nowadays discussed to be used as biofilters in integrated multi-trophic aquaculture (IMTA) (Shpiguel et al., 2019) and other bioremediation approaches (Tremblay-Gratton et al., 2018).

The perennial brown algae species *Fucus* also showed higher growth rates at 300 m distance from the fish farm, and slightly higher growth rates at the farm, while agricultural fertilizer did not significantly affect growth rates of *Fucus*. Similar to *Ulva*, *Fucus* also seemed to benefit from higher nutrient availability from the fish farm. In natural macroalgal communities however, *Fucus* might not show this response, because epiphytic ephemeral algae can assimilate the enhanced nutrient load much quicker than *Fucus* and often overgrow their thalli and can suppress *Fucus* growth (Berger et al., 2004; Wahl et al., 2015).

At last, growth rates of the perennial red algal species *Palmaria*, responded in opposite ways to both nutrient sources. While agricultural fertilizer increased *Palmaria*'s growth rate, the vicinity of the fish farm resulted in reduced growth, especially at 300 m distance. We also observed discoloration of *Palmaria* thalli, thus a deteriorated state, but only in samples without additional agricultural fertilizer and at all distances to the fish farm. This was surprising, since bleached tissue in *Palmaria* was found to be an indication for too high ammonium concentrations (Grote, 2016), which was unlikely the case in our study. But bleaching of red algae was also observed under e.g. increased water temperatures or through bacterial diseases (Campbell et al., 2011). While temperature was relatively similar at collection and experimental

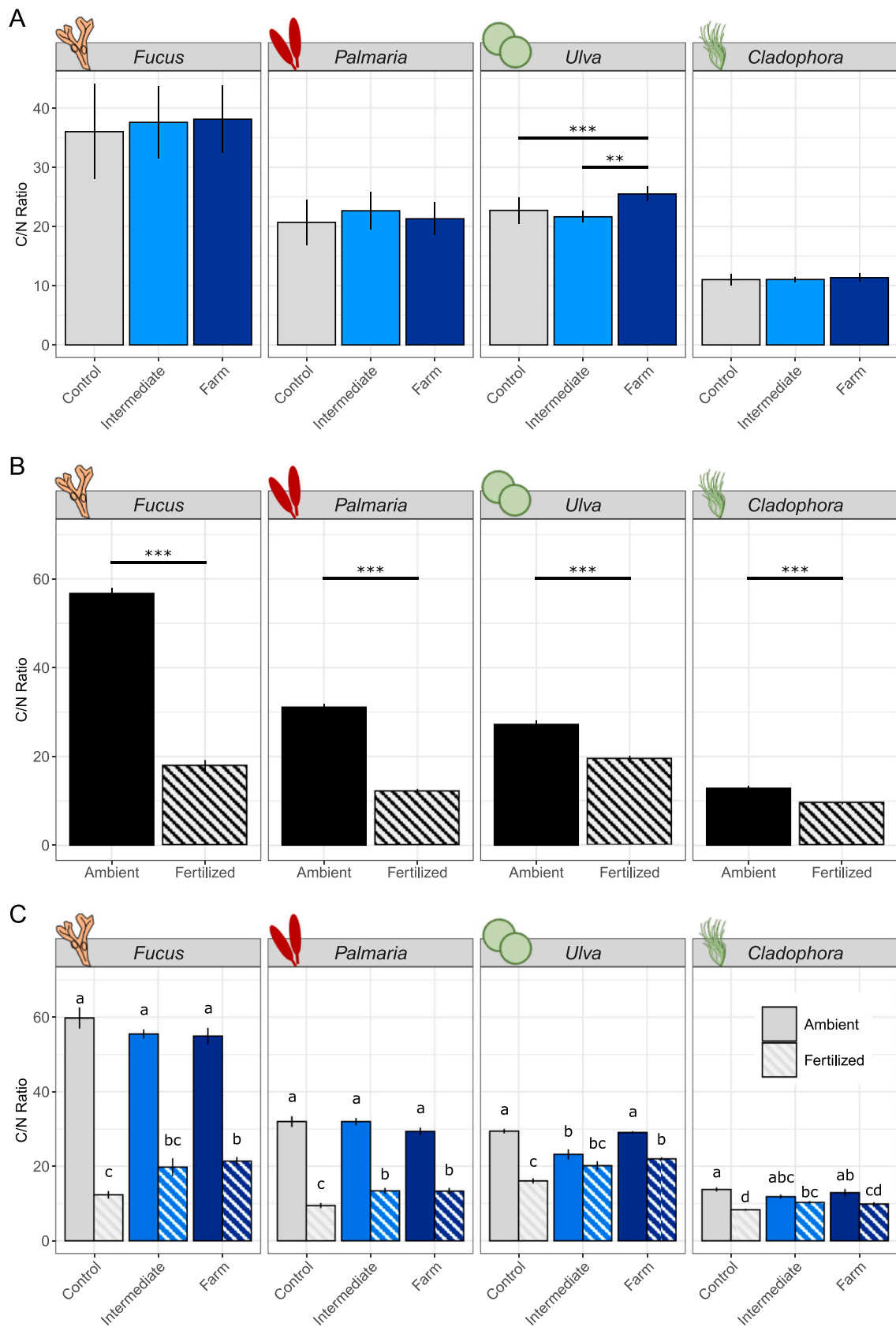


Fig. 3. Effects of A) distance to the fish farm (control, intermediate, farm), B) additional fertilizer (ambient or fertilized) and C) nutrient enrichment with fertilizer (ambient or fertilized) at the different distances to the fish farm (control, intermediate, farm) on C:N ratios in the tissue of the four algae species. Means \pm SE. Black lines indicate significant difference between two bars. Significance levels: * $p < 0.05$, *** $p < 0.001$. Bars with the same letter marked above are not significantly different ($p < 0.05$). (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

Table 3

Treatment effects (fish farm, fertilizer, grazers) on the C:N ratio of tissue of the four algae. Results from gls models (lme for *Palmaria*) after backward model selection.

		df	F-value	p-Value	
<i>Fucus</i>	Farm	2	0.64	0.534	
	Fertilizer	1	610.24	<0.001	***
	Farm:Fertilizer	2	7.53	0.003	**
<i>Ulva</i>	Farm	2	19.10	<0.001	***
	Fertilizer	1	337.78	<0.001	***
	Farm:Fertilizer	2	21.42	<0.001	***
<i>Cladophora</i>	Farm	2	3.57	0.044	*
	Fertilizer	1	4.90	<0.001	***
	Farm:Fertilizer	2	5.60	0.010	*

		numDF	denDF	F-value	p-Value	
<i>Palmaria</i>	Farm	2	11	0.72	0.507	
	Fertilizer	1	11	893.53	<0.001	***
	Farm:Fertilizer	2	11	8.14	0.007	**

Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

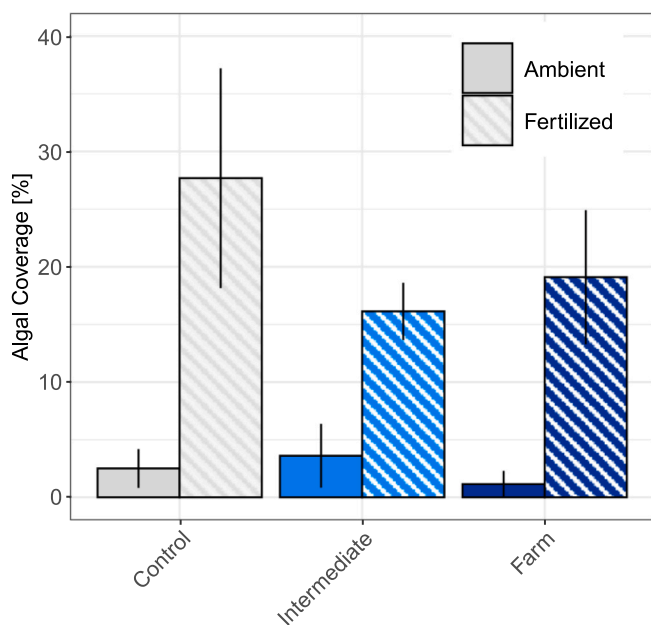


Fig. 4. Effects of nutrient enrichment with fertilizer (ambient or fertilized) at the different distances to the fish farm (control, intermediate, farm) on the algal coverage [%] of the growth tiles (algal species were not identified). Agricultural fertilizer significantly increased algal coverage ($p < 0.001$), while distance to the fish farm had no effect. There was no significant interaction effect. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

locations, potential diseases could not be evaluated in our study. Thus, although the reason for the observed *Palmaria* bleaching remains unknown, this effect seemed to be compensated by addition of agriculture fertilizer in all treatments.

In the Baltic Sea, *Fucus* epiphyte assemblages changed from more brown and red towards green algae species closer to a fish farm (Rönnerberg et al., 1992). The ephemeral green algae often possess a higher ability to faster utilise the available nutrients (Boyra et al., 2004; Eriksson et al., 2002; Pedersen and Borum, 1996; Rönnerberg et al., 1992). Our study showed, in line with previous studies, that green annual species such as *Ulva* may profit most from higher nutrient availability from fish farms.

4.2. Nutrient effects on algal nutrient ratios

The majority of the nitrogen added to fish farms (as fish feed) is released in dissolved form (48%) (Hall et al., 1992) and mainly in the form of ammonium (Wang et al., 2012).

Agricultural fertilizer releases dissolved nitrogen in the forms of nitrate (47% of total nitrogen added) and ammonia (53% of total nitrogen added) (Worm et al., 2000). Most macroalgae take up ammonium faster than nitrate, but preferences and uptake ability depend on the algal species (Cohen and Fong, 2004; D'Elia and DeBoer, 1978; DeBoer et al., 1978; Naldi and Wheeler, 2002; Pedersen and Borum, 1997; Runcie et al., 2003). For example, the uptake and assimilation of nitrate requires more energy compared with ammonium and it was suggested that it is a more efficient source for slow growing macroalgae (Pritchard et al., 2015). Differences in uptake rates of dissolved nitrogen released from fish farms or agricultural fertilizer across algal species may therefore result in changes in species composition of algal communities (Masterson et al., 2008; Mörk et al., 2009; Pedersen and Borum, 1997; Teichberg et al., 2008), but also in changed nitrogen and carbon content within the macroalgae tissue (Pedersen and Borum, 1997; Teichberg et al., 2008). Indeed, the C:N ratios of the perennial macroalgae in our study decreased significantly with agriculture fertilizer added by a factor of approximately 2.5-3 for *Fucus* and 2 for *Palmaria*, while the annual algae showed a much less pronounced reduction (Fig. 3). The resulting differences in nutrient composition can have further effects on the algal palatability for herbivores (Hemmi and Jormalainen, 2002) and may therefore have interactive effects on algal communities and the associated food web through higher preference of herbivores for nutrient-enriched algae (Hemmi and Jormalainen, 2002; Worm and Sommer, 2000).

Benthic algae have an average C:N:P ratio of 550:30:1 (Atkinson and Smith, 1983; Harrison and Hurd, 2001), thus they require 30 times more nitrogen than phosphorous. *Ulva* sp. has been found to have a C:N ratio between 15 and 20 when exposed to aquaculture compared to 30 to 40 under control conditions (Dalsgaard and Krause-Jensen, 2006). Both *Ulva lactuca* and *Palmaria palmata* had C:N value even lower than 10 (around 6.9 and 5.6 respectively) in an environment where enough nutrients for storage were present (Tremblay-Gratton et al., 2018). In our experiment, C:N ratios of the annual algae were around 23 for *Ulva* and 11 for *Cladophora* under control conditions, indicating oligotrophic conditions. However, aquaculture effluents did not affect the C:N ratio of these species significantly (with the exception of slightly lower values for *Ulva* at the intermediate site). Also the C:N ratios of the perennial species at the control site were relatively high for *Fucus* (approximately 36) and *Palmaria* (approx. 21), and did not significantly change with distance to the fish farm (Fig. 3). Thus, exposure to fish farm effluents alone seem to affect growth of some macroalgae such as *Ulva*, but not the nutrient composition in any of the tested species.

Our study shows further that agricultural fertilizer decreased C:N ratios of all algae, through higher N-uptake. The fertilizer, however, had a much stronger effect on the tissue N of the perennial species (C:N ratio on average 65% lower), while C:N ratios of the annual species decreased on average by only 22%. The high C:N ratios of *Fucus* at ambient conditions suggest that this species was the most N-deprived of all algal species, the C:N ratios we measured exceeded those generally found in *Fucus* (Faganeli et al., 1986; Graiff et al., 2015; Young et al., 2009). The extreme decrease of the C:N ratio due to addition of agriculture fertilizer in the perennial species might also be related to the more efficient uptake of nitrate found in *Fucus* compared to the annual species *Ulva* and *Cladophora* (Bracken and Stachowicz, 2006).

The differences in the decrease of C:N ratios at high nutrient supply may be further explained by different growth rates and internal storage capacities of the algal species (Harrison and Hurd, 2001; Pedersen and Borum, 1996; Teichberg et al., 2008). Fast-growing ephemeral species have higher nitrogen demands, than slow-growing species, to sustain high growth rates, and less long-term storage capacities (Fujita, 1985;

Pedersen and Borum, 1997, 1996; Wallentinus, 1984). These ephemeral species in some cases still have higher maximum tissue N-concentrations, from surging nutrients (Fujita, 1985; Harrison and Hurd, 2001; Pedersen and Borum, 1997, 1996). However, the internal N-pools are used up more rapidly in fast-growing species to support their high growth rates (Fujita, 1985; Pedersen and Borum, 1997, 1996).

Most interestingly, both perennial species *Fucus* and *Palmaria* as well as *Ulva* spp. exhibited increased C:N ratios when exposed to both fish farm effluents and fertilizer, compared to assemblages with only fertilizer. Thus, fertilizer alone decreased C:N ratios of all algae, but when algae were exposed to both aquaculture and fertilizer nutrients, the respective C:N ratios increased compared to fertilizer alone treatments. An earlier bioassay study, similar to our study, found that the fast-growing *Ulva* assimilated N from a near fish farm and that C:N ratios strongly decreased towards the fish farm (Dalsgaard and Krause-Jensen, 2006). In contrast, in our study the fish farm effluents only changed C:N ratios of algae that were simultaneously exposed to fertilizer, while distance to the fish farm alone did not have on C:N ratios. This may mean that 1) nitrogen is mainly assimilated as nitrate (and not ammonium) or that 2) too high ammonium concentrations inhibit the N-uptake. Increasing C:N ratios at near nutrient saturation may be caused by higher tissue C as algae grow, but the relatively low growth rates of *Fucus* and *Palmaria* don't support this assumption.

Thus, although the mechanism behind remains unclear, this interaction effect of both nutrient sources may have implications specifically in environments with several nutrient sources, where macroalgal communities may be differentially affected depending the nutrient context.

4.3. Bioassays as bioindicators for monitoring fish farms

Biomonitoring of water pollution with the help of macroalgae can be a useful tool for environmental management (Ferreira et al., 2011; García-Seoane et al., 2018a), mainly because macroalgae are sessile species and are available most of the year (García-Seoane et al., 2018b). Specifically, *Ulva* and *Fucus* are the most widely used algae genera in biomonitoring (García-Seoane et al., 2018b) and might even be used for other emerging contaminants such as microplastics (Feng et al., 2020).

The release of dissolved nutrients from open cage fish farms is known to be enormous but usually assumed to have minor impacts on the environment due to dilution effects of the large surrounding water bodies and high currents (García-Sanz et al., 2010; Karakassis et al., 2001; Price et al., 2015). Accordingly, the release of dissolved nutrients is often not monitored (García-Sanz et al., 2010; Price et al., 2015). However, algal assemblages and their recruitment may be particularly susceptible to nutrient pulses especially in oligotrophic systems (Eriksson et al., 2002; Russell et al., 2005; Worm and Sommer, 2000). Nutrient concentrations have been shown to strongly affect recruitment of macroalgae (Gao et al., 2018, 2017; Lotze, 1998; Lotze et al., 2001, 1999; Lotze and Schramm, 2000). Specifically these early life stages can benefit from nutrient enrichment more than adults (Lotze, 1998; Lotze et al., 2000, 1999) making them specifically suitable for monitoring fish farm impacts. In contrast, recruitment success of slow-growing algae, such as *Fucus* and *Ceramium tenuicorne* is reduced under nutrient enriched conditions most likely due to competition with filamentous algae (Korpinen et al., 2007; Korpinen and Jormalainen, 2008). Our study showed higher recruitment of algae (predominantly filamentous algae) when the panels were enriched with agricultural fertilizer. Proximity to the fish farm, however, had only very weak effects on recruitment, slightly reduced under combined fertilizer and farm conditions (Fig. 4). Thus, although we found a clear response of algae recruitment to nutrient load, the concentrations around the fish farm seem to be too low to clearly affect algae recruitment. In contrast, macroalgae have been used by several studies to detect the effects of dissolved nutrients from fish farms (Price et al., 2015). They accumulate dissolved nutrients and allow integrating the nutrient loading over time e.g. the duration of a bioassay (Cohen and Fong, 2005; Cole et al., 2005;

García-Sanz et al., 2011; Jones et al., 2001; Lyngby and Mortensen, 1994).

Our results indicate that bioassay experiments have the potential to play a role as bioindicator for fish farm impacts on macroalgae, although the response to the farm effluents alone was equivocal. It was observed previously that some macroalgae species are less tolerant to the experimental procedures in a bioassay study (García-Sanz et al., 2010). But most of the macroalgae in our experiment seemed to thrive within the bioassay set up, with the exception of *Palmaria* under ambient condition, showing signs of bleaching, and tissue loss in *Cladophora*. Especially *Fucus* and *Ulva* did not show any signs of decomposition and seemed to generally tolerate the experimental procedures, but did not seem to have ideal growth conditions either. However, for *Ulva* it is known that sudden changes in environmental parameters can trigger the reproductive cycle of the algae as a stress signal (Tremblay-Gratton et al., 2018), which was not the case in our study.

Compared to the locally monitored impact of particulate farm waste (Bannister et al., 2016; Giles, 2008; Kutti et al., 2007), the potentially more far reaching effects through dissolved nutrients are neither monitored based on water measurements (see above), nor by regular monitoring of hard substrate macrofauna or macroalgae (Husa et al., 2014a). Since the natural settings of the hard substrate communities might be highly variable and influenced by local environmental conditions (García-Sanz et al., 2010; Price et al., 2015), bioassay monitoring approaches could provide a standardized method to assess these impacts across space and time. Especially the combined effects by both aqua- and agriculture nutrient sources are often difficult to assess and might be less relevant in northern Norway with limited agriculture activities, but are of specific importance for the management of other coastal regions with multiple human uses.

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CRedit authorship contribution statement

Michael D. Streicher: Conceptualization, Investigation, Methodology, Writing – original draft. **Henning Reiss:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Katrin Reiss:** Conceptualization, Investigation, Methodology, Writing – review & editing.

Declaration of competing interest

Publishing of this paper will cause no conflict of interest with any people, institutions or legislation.

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