

Effects of fish farm effluents on kelp forest ecosystems

Kelp performance, associated species, and habitats

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Preface

When I applied for this PhD, it was not because of a great interest in seaweeds or kelp forests, but because I wished to work with human impacts on the environment. In fact, my knowledge of kelp and seaweed communities was rather limited, and I had never really understood that we have these amazing underwater forests in our backyard. But as I began working and learning my interest grew, and was followed by fascination when I started to experience this marine habitat up close through free diving. Now, four years later, I have spent many hours with my head under water in between kelp laminas, and I never get tired of looking at the beautiful colors of all the algae growing along the kelp stipes, or the gentle movements of nudibranchs.

My time as a PhD student has been filled with excitement, exhaustion, frustration, fun, lots of laughter and some tears – and today I am grateful for all of it, and happy and proud that I followed through. But I couldn't have done it without all the co-workers, friends, and family that have supported me along the way, through all the ups and downs.

I especially want to thank my supervisors Vivian, Tina, Stein, Kjell Magnus, and Raymond firstly for giving me the opportunity to do this work, for their encouragement and guidance along the way, for always being available and ready to give me feedback and advise, and for their friendship. I was lucky enough to become a part of the best research group at IMR, the 8th floor. I will greatly miss the coffee breaks and talks, and always value the friendships I have made within this group. Tore and Øivind, thank you for introducing me to free diving, and for all the great days we've had with our heads under water. Ellen, Ann-Lisbeth and Tina, thank you for bringing me along to lønningspils and showing me the ropes, and for green jackets. Siri, thank you for being the best officemate and for always being ready to help and offer 'non-academic' support.

I also especially want to thank my mother, for believing in me and always supporting me in the choices that I make (even though you don't always agree). And all my friends and family for your enthusiasm, encouragement and support through these years, you believing in me made me able to push through.

Barbro T. Haugland
Bergen, June 2019

Scientific environment

This doctoral work was carried out as a member of the Benthic Resources and Processes research group at the Institute of Marine Research, and in the Section for Aquatic Biology and Toxicology at the Department of Biosciences, Faculty of Mathematics and Natural Sciences, University of Oslo.

My project was part of the research project ERA, *Environmental Responses to Aquaculture* (NFR project number 228871) which was funded through The Research Council of Norway.



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Summary

Sea cage production of salmonid fish delivers a large amount of waste products to the marine environment including dissolved nutrients, organic particles, and chemicals. Recent developments in the Norwegian aquaculture industry have led to large-scale fish farms being increasingly situated in dispersive environments where kelp forests dominate the shallow hard-bottom habitats. The potential impacts of farm effluents on kelp forests are poorly understood, yet important to clarify with kelp forests currently declining worldwide. To assess the overall effect of fish farming effluents on the performance of first-year kelp, I conducted an *in-situ* translocation study with *Saccharina latissima*. I found that growing near fish farms did not improve or reduce the growth performance of first-year *S. latissima*, or affect the plants' nutrient-reserves. I tested the toxicity of a common therapeutant used to treat salmon for sea lice infections (hydrogen peroxide) and found that juvenile *S. latissima* plants are highly sensitive to this chemical. By comparing determined toxicity potentials (e.g. LC₅₀) with model predictions I found that *S. latissima* forests growing near fish farms may be exposed to lethal concentrations of hydrogen peroxide. In a dispersive coastal system, I evaluated the presence of large-scale fish farms during high and low production on three different communities associated with *Laminaria hyperborea* plants; the lamina epiphytic community, the stipe epiphytic community, and the holdfasts macrofaunal community. Where the lamina and stipe communities would receive the farm-nutrients from the water column, the holdfasts were anticipated to trap farm-derived organic particles. For both the epiphytic load on laminas and the macrofaunal community in holdfasts, I found no differences between reference sites and farm sites that could be directly related to fish farm effluent loads, suggesting that these communities are resilient to farm-impacts in dispersive environments. The stipe epiphytic community showed a clear response to fish farm effluents, with an increase in bryozoan and opportunistic macroalgae biomass, lower habitat heterogeneity and a shift in community structure near farms that were at high production. This demonstrates that proximity to fish farms can result in a small but significant alteration of *L. hyperborea* as a habitat.

This thesis reveals the impacts that different waste products from salmonid production can have on kelp forests. The impact of fish farm effluents on the performance and health status of kelp species and communities associated with the kelp forest is, however, far from fully understood. Results gained from this thesis should be utilized to set management directions but also to set the path for future focus areas in research into the environmental effects of fish farming.

List of papers

This PhD thesis is based on the following papers, which will be referred to in text by their Roman numerals:

- Paper I:** Haugland B.T., Fredriksen S., Norderhaug K.M., Skogen M.D., Kutti T., Bannister R.J., and Husa V. ***Saccharina latissima* cultivated in salmonid farm effluents: a case study from a cold temperate fjord shows no effect on growth and nutrient status.** Under revision, *Aquaculture*
- Paper II:** Haugland B.T., Rastrick S.P.S., Agnalt A.-L., Husa V., Kutti T., and Samuelsen O.B., 2018. **Mortality and reduced photosynthetic performance in sugar kelp *Saccharina latissima* caused by the salmon-lice therapeutant hydrogen peroxide.** *Aquaculture Environment Interactions* **11**:1-17. doi: 10.3354/aei00292.
- Paper III:** Haugland B.T., Armitage C., Kutti T., Husa V., Skogen M.D., Bekkby, T., Carvajalino-Fernandez M., Bannister R.J, White C.A., Norderhaug K.M., and Fredriksen S. **High-intensity finfish farming and kelp forests at dispersive locations. I. Effects on *Laminaria hyperborea* stipe and lamina epiphytic communities.** Manuscript in preparation
- Paper IV:** Haugland B.T., Husa V., Kutti T., Bekkby T., Bannister R.J, White C.A., Fredriksen S., and Norderhaug K.M. **High-intensity finfish farming and kelp forests at dispersive locations. II. Resilience in macrofauna communities in *Laminaria hyperborea* holdfasts exposed to farm emissions.** Manuscript in preparation

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1 Introduction

The archipelago on the west coast of Norway that includes the islands Frøya and Smøla is a unique marine environment. Thousands of islets and skerries on a shallow hard-bottomed seafloor are exposed to strong currents and heavy waves, creating an ideal environment for kelp forests to thrive. Due to its environmental qualities, this area has seen a developing aquaculture industry over the past 10 years, and is today the most productive region of salmonid biomass along the entire Norwegian coastline. Many large-scale, shallow-water fish farms are now scattered among these native kelp forests (Fig. 1), but the interactions between farm waste and the kelp ecosystem are poorly understood. Concerns have been raised regarding the potential negative impacts on these ocean forests. As similar locations will likely be favored during the projected expansion of aquaculture over the next decades, the effect of fish farm effluents on the kelp ecosystem must be studied to inform management decisions today and in the future.

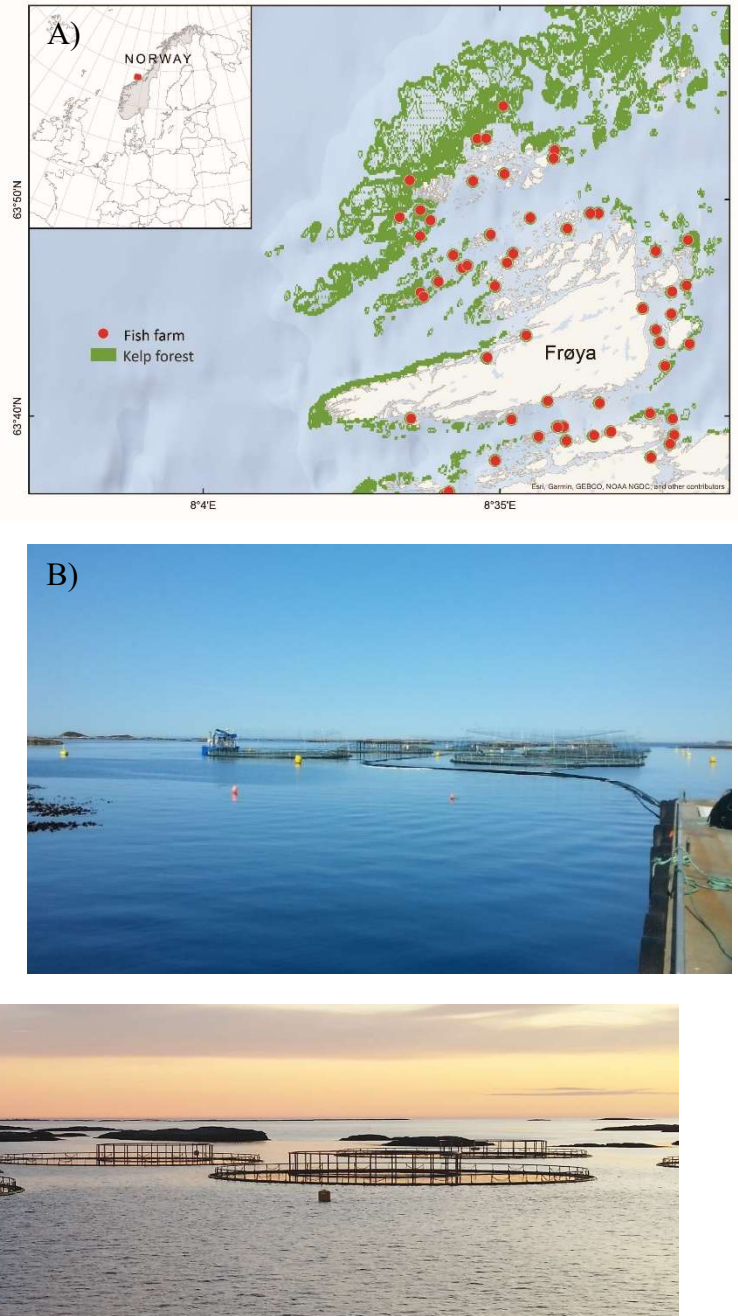


Figure 1. Kelp forests and aquaculture sharing the environment; (A) mapped kelp forest and aquaculture sites in the Frøya archipelago, (B) photo from a fish farm fleet, the kelp forest can be seen bulging up from the water to the left in the picture, (C) 5 net pens (ca. 60 m in diameter) in between skerries. Photos by Nigel B. Keeley, IMR. Map produced by Trude H. Thangstad, IMR.

1.1 Kelp forests and ecoservices under pressure

Kelp forests (order Laminariales) thrive in cold nutrient-rich waters in temperate coastal zones, covering about one quarter of the world's coastlines (Filbee-Dexter & Wernberg 2018). The services provided by our kelp forests are not to be underestimated, and the recent review showing that more than one third of kelp forests worldwide have been in decline over the past 50 years (Krumhansl et al. 2016) should alarm us all.

Laminaria forests supports a plethora of other species, ranging from epiphytic macroalgae and amphipods, to commercially important fish (e.g. Atlantic cod *Gadus morhua* Linnaeus, 1758) and various seabirds (e.g. cormorants) (Røv et al. 1990, Bustnes et al. 1997, Christie et al. 2003, 2009, Fredriksen 2003, Norderhaug et al. 2005). Primary production is exceptionally high (Charpy-Roubaud & Sournia 1990), competitive with both marine phytoplankton and terrestrial rainforests (Mann 1973), providing a major source of organic carbon to coastal ecosystems (Mann 2000). Along the Norwegian coastline alone, yearly carbon (C) fixation by *Laminaria hyperborea* (Gunnerus) Foslie is estimated to range between 1200 and 1900 g C m² (Christie et al. 2009). Particulate organic matter (POM) produced by kelp is a significant food source for local secondary production (Fredriksen 2003, Norderhaug et al. 2003), and the effects also reach other habitats. Approximately 80% of local kelp forest production is estimated to be transported away as drift kelp (Krumhansl & Scheibling 2012), and can be an important facilitator for enhancing secondary production in less productive, deeper areas (Bustamante et al. 1995, Krumhansl & Scheibling 2012, Filbee-Dexter & Scheibling 2014, Ramirez-Llodra et al. 2016). Kelp forests also serve as shoreline protection through wave reduction (Løvås & Tørum 2001, Smale et al. 2013), and create economic revenue via kelp harvesting, commercial fisheries and recreation (Beaumont et al. 2008, Smale et al. 2013, Gundersen et al. 2017). Changes in our kelp forests could therefore have cascading effects on ecosystem function and services, both locally and far beyond the coastal zone.

The stressors that our kelp forests are facing today include ocean warming, more frequent storm-events, overgrazing, overfishing, harvesting, competitive exclusion and eutrophication (extensive reviews given in: Steneck et al. 2002, Araújo et al. 2016, Filbee-Dexter & Wernberg 2018), most of which are related to human activities. Often several stressors occur at the same time, with interactions having different effects on kelp populations in different areas, making it difficult to assign the exact cause (Strain et al.

2014). Some of the anthropogenic pressures, such as impacts of effluents released from fish farming, may however be controlled through sustainable management.

1.2 Pressures on Norwegian kelp forests

The coastline along Norway is dominated by hard bottoms, providing support for extensive kelp forests stretching from subtidal depths and as far down as 20 to 30 m (Christie et al 2009). The kelp species focused on in my studies, *Laminaria hyperborea* and *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders are two of the most common kelp species, and also the kelp populations which (to our knowledge) have suffered the most severe deforestations.

Along the coastline of mid- and northern Norway, extensive grazing by the green sea urchin *Strongylocentrotus droebachiensis* (O.F. Müller, 1776) decimated kelp forests nearly five decades ago (reviewed in Norderhaug & Christie 2009). The result was a habitat dominated by barren grounds from 63°30'N in the south (Sivertsen 1982) and into Russian waters in the north (Norderhaug & Christie 2009). This mainly affected *L. hyperborea* forests, though *S. latissima* forests most likely also disappeared at the same time in this area (mapping of previous *S. latissima* forests in this area is limited). The *S. droebachiensis* populations have been retreating northward since the 1980s (Norderhaug & Christie 2009), probably due to increasing water temperatures having a negative effect on sea urchin recruitment (Fagerli et al. 2013). A gradual regrowth of *L. hyperborea* forests have followed (Norderhaug & Christie 2009), and kelp forests are again dominating as far north as 65°40'N (Steen et al. 2018). Approximately 160 000 tonnes of *L. hyperborea* are harvested annually along the coastline, an activity that is monitored and managed to secure recovery of the kelp ecosystem (see e.g. Steen 2017, Steen 2018). *L. hyperborea* forests are currently listed as near threatened (NT; Gundersen et al. 2018a) and *S. latissima* forests as endangered (EN; Gundersen et al. 2018c) along the mid- and northern Norwegian coastline.

Along the south and south-west coast, *S. latissima* was decimated in the early 2000s (Moy et al. 2006, 2008). By 2008, the forest had recovered again along the south-west coast (Moy & Christie 2012, Husa et al. 2014). Along the southern coastline, an estimated 51 to 80 % of previous *S. latissima* forests are lost (Bekkby & Moy 2011, Moy & Christie 2012) and replaced with turf-forming algae (Moy & Christie 2012). Likely stressors were a synergistic effect of high summer temperatures and eutrophication (caused by increased runoff), which both reduced the resilience of *S. latissima* and gave turf forming algae a

competitive advantage (Moy & Christie 2012). Heavy fouling by lamina epiphytes, sedimentation by particles and persisting turf mats, i.e. also present during winter when *S. latissima* spores are released and settle, have been suggested as causes preventing forest recovery (Andersen 2013a). *S. latissima* forests are currently listed as an endangered nature type in this area due to the large area reduction (EN; Gundersen et al. 2018b). In the last couple of years, signs of struggling *L. hyperborea* have been observed in the same area, with laminas overgrown by filamentous turf-algae (Trine Bekkby, pers. comm.), specifically at semi-exposed locations.

Better management strategies of kelp forests are being called for, and is necessary due to combinations of multiple global and local stressors (Teagle et al. 2017). Global stressors, such as climate change, can be difficult to regulate on a country-level. Local stressors however, such as eutrophication, can be controlled via management. Historically, agriculture has been the human activity contributing the most to coastal nitrogen levels in Norway. In 2005 it was surpassed by aquaculture emissions and concerns regarding impacts on macroalgae were raised. In the latest report (2017 emissions), 55% of the total anthropogenic nitrogen emissions were aquaculture-related (Selvik & Sample 2018). The largest source depends on the region; along the southern coast (Sweden to Lindesnes) agriculture is the largest contributor, whereas aquaculture dominates to varying degrees in all other regions all the way to the Russian border. There are also indications that other fish farm effluents, i.e. medicinal treatments, might have unpredicted impacts on non-target species (Escobar Lux 2016, Bechmann 2017).

1.3 Norwegian aquaculture: a brief history on monitoring

The breakthrough in Norwegian salmonid farming came in the beginning of the 1970s with the construction of the first open sea cage (Gjedrem 1993). The preferred localities during the first 10 years were shallow fjord sites (10-30 m) characterized by calm waters (Braaten et al. 1983). However, the shallow water depth and low water flow resulted in an accumulation of organic particles on the seafloor under the net pens, often leading to anoxic sediment conditions, accompanied by a decrease in faunal biodiversity and abundance, and the release of methane gas (Braaten et al. 1983, Ervik et al. 1985, Samuelsen et al. 1988). Water flow was found to be a central mechanism for sediment oxygenation and dispersal of fish farm effluents, and a gradual relocation of fish farms to deeper fjord sites with higher water currents began (Kutti 2008). A national, standardized, monitoring tool for

benthic soft-bottom impacts was also developed and implemented (the “MOM” monitoring system: Ervik et al. 1997, Hansen et al. 2001) to ensure that impacts from organic waste from fish farms did not exceed the assimilation capacity of the surrounding environment. The “MOM” system is now well established and has also been implemented in other countries (Iceland and the Faroe Islands), but its limitations lies within being designed to monitor the response of soft-bottom benthic habitats.

Since 2000, the Norwegian aquaculture industry has grown rapidly, with a doubling of salmonid production from ca. 490 000 tonnes in year 2000 (Gullestad et al. 2011) to ca. 1 340 000 tonnes in 2018 (Fiskeridirektoratet 2019), making Norway one of the leading countries worldwide in marine aquaculture production (FAO 2018). A continuous restructuring of the industry has occurred along with this rapid expansion. Technological advances have allowed for relocations to more wave-exposed coastal sites where a higher salmonid biomass production is permitted due to their dispersive nature; up to 14 000 tonnes produced in an 18-month period compared to the typical 3-5000 tonnes for farms located in sheltered waters. Indeed, the majority of the growth seen in aquaculture industry over the last decade can be attributed to the growth of large-scale farms in the Frøya-Smøla archipelago. This new arrangement increases the likelihood of impacts on *L. hyperborea* forests which are typically found at exposed sites (Kain 1979, Lüning 1990, Bekkby et al. 2009), and is the most dominant kelp species in this specific area (Steen 2017, 2018). Future expansion of salmonid aquaculture, both in Norway and globally, will include further relocations to dispersive coastal environments due to their higher resilience (Keeley et al. 2013b, Valdemarsen et al. 2015). When it comes to monitoring environmental impacts at exposed localities, where kelp forests dominate the hard-bottom benthic habitats, the efficiency of “MOM” is highly limited. How kelp forests respond to high-intensity fish farming is poorly understood, yet vital, if we are to be able to monitor, detect potential changes, and ensure healthy kelp forest ecosystems in the future.

1.4 Aquaculture emissions and potential effects on kelp forests

1.4.1 Dissolved nutrients and particulates

Salmonid production in open net pens results in the discharge of large amounts of organic and inorganic carbon (C), nitrogen (N), and phosphorous (P) in the form of particulate organic matter (POM, [from feces and excess fish feed pellets]) and dissolved

nutrients (salmonid metabolic by-products) to the surrounding environment. For 2017, the total estimated amount of POM released from Norwegian fish farms amounted to 540 000 - 670 000 tonnes (Husa et al. 2018). For dissolved nutrients, different models and mass-balance budgets exist for estimating emissions. The lowest estimated values (using the ANCYLYS model) for dissolved inorganic nitrogen (DIN) and phosphorous (DIP) were 26 000 tonnes and 3900 tonnes, respectively, whereas the TEOTIL-model estimated higher values of 45 240 tonnes DIN and 6630 tonnes DIP (Husa et al. 2018). Both POM and dissolved nutrients have the potential of affecting kelp performance and the associated community, both directly and indirectly (Fig. 2).

The kelp thallus consists of an annual lamina, and a perennial holdfast and stipe. For both *L. hyperborea* and *S. latissima* some degree of epiphytic fouling on the lamina is common in natural communities, where fouling decreases with increasing wave exposure (Jorde 1966, Røv et al. 1990, Pedersen et al. 2012). For *L. hyperborea* an extensive epiphytic community is associated with the stipe (Whittick 1983, Marstein 1997, Norderhaug et al. 2012, Pedersen et al. 2014). Nutrient-enrichment often benefit opportunistic species (Pedersen & Borum 1996, Worm & Sommer 2000, Karez et al. 2004), many of which are epiphytic on kelp, and the nutrient-emissions from fish farms may therefore enhance epiphytic growth. The lamina is the main site for photosynthesis and gas exchange (Bartsch et al. 2008) and an increased lamina epiphytic load could reduce kelp performance (Andersen 2013a). Encrustation by the bryozoan *Membranipora membranacea* (Linnaeus, 1767) can leave the lamina more prone for breakage (Krumhansl et al. 2011), which have led to large-scale kelp defoliation in areas where *M. membranacea* is an introduced species (Scheibling et al. 1999, Saunders & Metaxas 2008, Krumhansl et al. 2011). An increase in epiphytic bryozoan cover has been observed for Mediterranean seagrass communities with increasing nutrients (Balata et al. 2010). Enhanced epiphytic load on the stipes may support a higher amount of associated fauna but nutrient-enrichment tend to homogenize communities (Balata et al. 2010, Oh et al. 2015), which could have the opposite effect on the faunal community. Changes in the epiphytic community could thus affect both kelp performance and kelp ecosystem functioning.

In some scientific environments, DIN from fish farms has been suggested to have a positive effect on kelp, by stimulating kelp performance. Theoretically, the additional nitrogen could be used either directly for growth, or to increase lamina nitrogen storage (Hadley et al. 2015) and used later for growth (Lüning & tom Dieck 1989, Sjøtun 1995),

i.e. in the summer months when nitrogen is limited. This idea has encouraged the cultivation of macroalgae together with fish farming, so-called integrated multi-trophic aquaculture (IMTA) (Chopin et al. 2001, Troell et al. 2009, Chopin et al. 2012), and *S. latissima* has been proposed as an excellent IMTA-candidate for cold temperate waters (Petrell et al. 1993, Barrington et al. 2009, Handå et al. 2013). However, as the farm-nutrients could also promote epiphytic growth on the lamina, the benefits could be outweighed. For both *S. latissima* forests and cultivated *S. latissima*, it is important to understand if the effluents from fish farming have an overall negative or positive effect on their performance. This especially relates to first-year plants, as this is the age-group being cultivated, and for *S. latissima* forests an overall negative impact could have implications for recruitment and hence the development of the adult population.

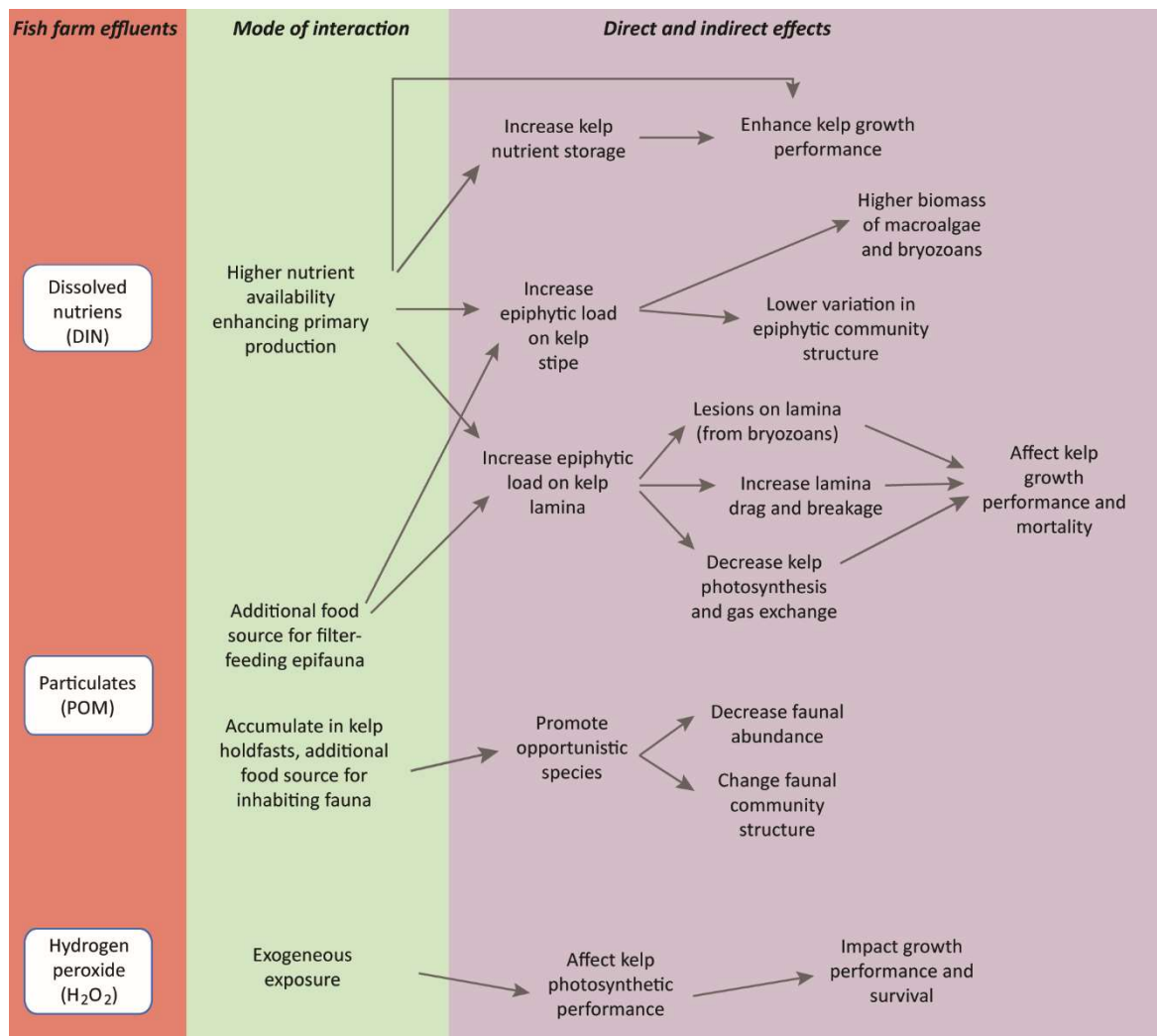


Figure 2. Overview of possible modes of interaction between fish farm effluents and kelp, and the potential subsequent effects that are assessed in this thesis.

The POM that is released from farms could potentially reduce light attenuation both for the kelp itself, and for the epiphytic community. It may also stimulate growth of filter feeding epiphytic fauna, such as bryozoans, via increased food-supply (Lojen et al. 2005). Furthermore, kelp forests have a reducing effect on waves and water movement, leading to higher sedimentation rates beneath the canopy (Eckman et al. 1989). It is therefore anticipated that a high amount of the farm-derived POM will settle in nearby *L. hyperborea* forests. Structurally complex holdfasts, such as the holdfast of *L. hyperborea*, act as a passive sediment trap for particles (Moore 1972, Smith 1996), and the diverse faunal community inhabiting the holdfasts will likely be affected by the composition and amount of particles (Moore 1972, 1973, Edwards 1980). Faunal communities are known to respond to increased loadings of organic particles in a predictable manner by increasing the abundances of opportunistic species and an overall reduced species richness (e.g. Pearson & Rosenberg 1978, Kutti 2008). The organic material in farm-derived particles (60 – 80 %, Kutti et al. 2007b) is assimilated by invertebrates in both soft-bottom (Kutti et al. 2007b) and hard-bottom dispersive environments (White et al. 2017, Woodcock et al. 2017, 2018), and could therefore subsidize an opportunistic macrofaunal community. Kelp holdfasts could thus potentially be used as a tool for monitoring ecosystem health (Smith 2000). The ability to detect a farm-effect will however depend on the background variation, which generally is found to be very high in holdfast communities (review in Teagle et al. 2017).

1.4.2 Medicinal treatments

Fish cultivated under the high-density conditions that occur in the net pens, are susceptible to bacterial-, viral- and parasitic infections. Salmonid farming effluents therefore include a range of chemicals administered to the fish for medicinal purposes, either orally via the feed or as a bath treatment (Samuelsen & Agnalt 2018). Of the medicinal therapeutants that are used, the majority either act on the nervous system (Azametiphos, Deltamethrin, Cypermethrin and Emamectin) or inhibit chitin synthesis (diflubenzuron and teflubenzuron) (Samuelsen & Agnalt 2018), i.e. chemicals that cannot directly affect kelp or other algae. However, the chemical that is the most used in terms of volume is hydrogen peroxide (H₂O₂); a strong oxidizing agent with inherent potential to be harmful to algae. The annual consumption of H₂O₂ was 31 577 tonnes, 43 246 tonnes, and 26 597 tonnes respectively for the years 2014, 2015, and 2016 (www.fhi.no).

H₂O₂ is used to treat the salmon for sea lice infections and is administered to the fish as a bath treatment, commonly at a concentration of 1700 mg H₂O₂ l⁻¹ (range: 1500 – 2100 mg H₂O₂ l⁻¹, www.felleskatalogen.no). After treatment, the H₂O₂-solution is released directly into the environment and disperses. Previous studies have shown negative effects on the photosynthetic performance of macroalgae when exposed to much lower H₂O₂-concentrations than the recommended bath-treatment dose (Collén & Pedersén 1996, Collén & Davison 1999b, Dummermuth et al. 2003), and H₂O₂-emissions related to aquaculture thus have the potential of being harmful to nearby kelp forests. At what concentrations H₂O₂ is toxic to kelp in terms of concentrations and durations that are possible from aquaculture emissions, was unknown until this work.

2 Scope of the synthesis

2.1 Objectives

This doctoral thesis was part of the research project ERA, *Environmental Responses to Aquaculture*, working to increase knowledge of how benthic habitats in dispersive environments responds to high-intensity fish farming. The contribution of my studies to this project was to evaluate how fish farming effluents may interact with kelp forests in dispersive environments. To achieve this, I addressed the effects of H₂O₂ on the performance of the vulnerable juvenile life-stage of kelp, the potential effects of farm effluents on the performance of first-year kelp plants, and the potential effects of farm effluents on the communities associated with established kelp forests. Specifically, I set out to answer the following questions:

- 1) Can farm effluents have an overall positive (via increased nutrient supply) or negative (via e.g. increased lamina fouling) effect on first-year *Saccharina latissima* plants' growth performance? (**Paper I**)
- 2) Are juvenile *Saccharina latissima* plants sensitive to the H₂O₂-levels they might be exposed to in natural communities due to fish farm emissions? (**Paper II**)
- 3) Does the epiphytic community on *Laminaria hyperborea* stipes respond to farm effluents in dispersive environments? (**Paper III**)
- 4) Can an increased farm effluent load in a dispersive environment increase the epiphytic loading on *Laminaria hyperborea* lamina? (**Paper III**)
- 5) Will holdfasts of *Laminaria hyperborea* act as organic particle traps for farm-derived POM in dispersive environments to a level that affects the macrofaunal community inhabiting the holdfasts? (**Paper IV**)

To answer these questions, a multiple methodological approach was adopted. Fieldwork studies were conducted in two different areas, the coastal Frøya-Smøla archipelago and the fjord system Hjeltefjorden, in addition to a laboratory study. The

different study areas called for work with different species, and the two kelp species *Saccharina latissima* and *Laminaria hyperborea* were chosen to focus on. A description and reasoning for the study species and study areas are given below.

2.2 Study species

Along European coasts, *S. latissima* dominates the sublittoral zone on sheltered to semi-exposed sites (Kain 1979, Bekkby & Moy 2011) while *L. hyperborea* thrives at wave-exposed localities (Bekkby et al. 2009) (Table 1). Longevity of both kelp species increases with latitude (Lee & Brinkhuis 1986, Sjøtun et al. 1993). *S. latissima* commonly reaches 3 years of age (Parke 1948, Sjøtun et al. 1993) except for at its most southern geographical range where it is considered an annual species (Lee & Brinkhuis 1986). For *L. hyperborea*, the stipe and holdfast typically becomes 10-15 years old (Rueness 1977) with a maximum age of 18 years being recorded in northern Norway (Sjøtun et al. 1993). Both kelp species have a pronounced seasonal growth pattern that corresponds to the natural fluctuations of nutrient availability in coastal waters, and a new lamina is formed during each growth period (Bartsch et al. 2008).

Table 1. General characteristics of the two study species (From Rueness 1977 unless otherwise stated)

	Preferred habitat	Geographical distribution	Lifespan	Lamina size and form	Stipe size and form
<i>Laminaria hyperborea</i>	Wave exposed, sublittoral to 30 m	From Murmansk (Russia) to the coast of Portugal (Tyler-Walters 2007)	Perennial, up to 18 years (Sjøtun et al. 1993)	1 m long, split	2-3 m long, 7-8 cm in diameter at bottom, rigid with rough surface
<i>Saccharina latissima</i>	Sheltered to semi-exposed, sublittoral to 30 m	From Svalbard to the coast of Portugal (White & Marshall 2007)	Perennial, up to 3 years	1-3 m long, 10-30 cm wide	5-30 cm long, 0.5-0.8 cm in diameter, soft with smooth surface

The kelp species differ in their morphology (Table 1). The rigid and long stipe produced by *L. hyperborea* supports a diverse epiphytic community of both flora and fauna (Whittick 1983, Marstein 1997, Christie et al. 2003), that develops over time (Christie et al. 1998). The holdfasts of *L. hyperborea* are also larger and more complex than that of *S. latissima*, and forms an intricate three-dimensional structure that is inhabited by a diverse macrofaunal community (Sheppard et al. 1980, Schultze et al. 1990, Christie et al. 1998, 2003).

2.3 Choice of methods

The Frøya and Smøla archipelago on the west coast of Norway stands out as an area where high-intensity fish farming and extensive *L. hyperborea* kelp beds share the environment (Fig. 1). The salmonid farms in this area are typically large-scale, shallow-water (30 m) farms producing up to 14 000 tonnes during a production cycle (ca. 18 months). The kelp forest in this area has been re-establishing since the 1990s after the extensive grazing by *Strongylocentrotus droebachiensis* (see section 1.2) (Norderhaug & Christie 2009), and is considered recovered. Sampling sites within this area was therefore chosen to assess the research questions related to **Paper III** and **Paper IV** (Fig. 3). Other work packages in the ERA project also conducted fieldwork in this area, yielding results that contributed to the understanding of the system, e.g. how effluents are dispersed, settle, and affect benthic macrofaunal communities (Brager et al. 2016, Woodcock et al. 2017, 2018, Keeley et al. 2019). A great effort has also been put towards producing and validating models for dissolved and particulate farm effluents in this system, which contributed to interpretation of my results (see **Paper III** for detailed description of the models and output).

At the time of sampling (August 2015), fish farm production cycles were synchronized within areas depending on county (regulations were revised in 2017; Grefsrud et al. 2018). Farms in the Frøya archipelago (Trøndelag county) were in the beginning of the production cycle (5-6 months) with low fish biomass (referred to as low effluent farms), and farms in the Smøla archipelago (Møre og Romsdal county) were at the end of the production cycle (ca. 17 months) with high fish biomass (referred to as high effluent farms). Feed use increase with fish biomass, and resulted in mean monthly feed use at the high effluent farms being 3.5 - 11 times higher than at the low effluent farms in the 6 months preceding sampling. Selecting sampling sites in both the Frøya and Smøla areas created the opportunity to compare effects of a high effluent load versus a low effluent load.

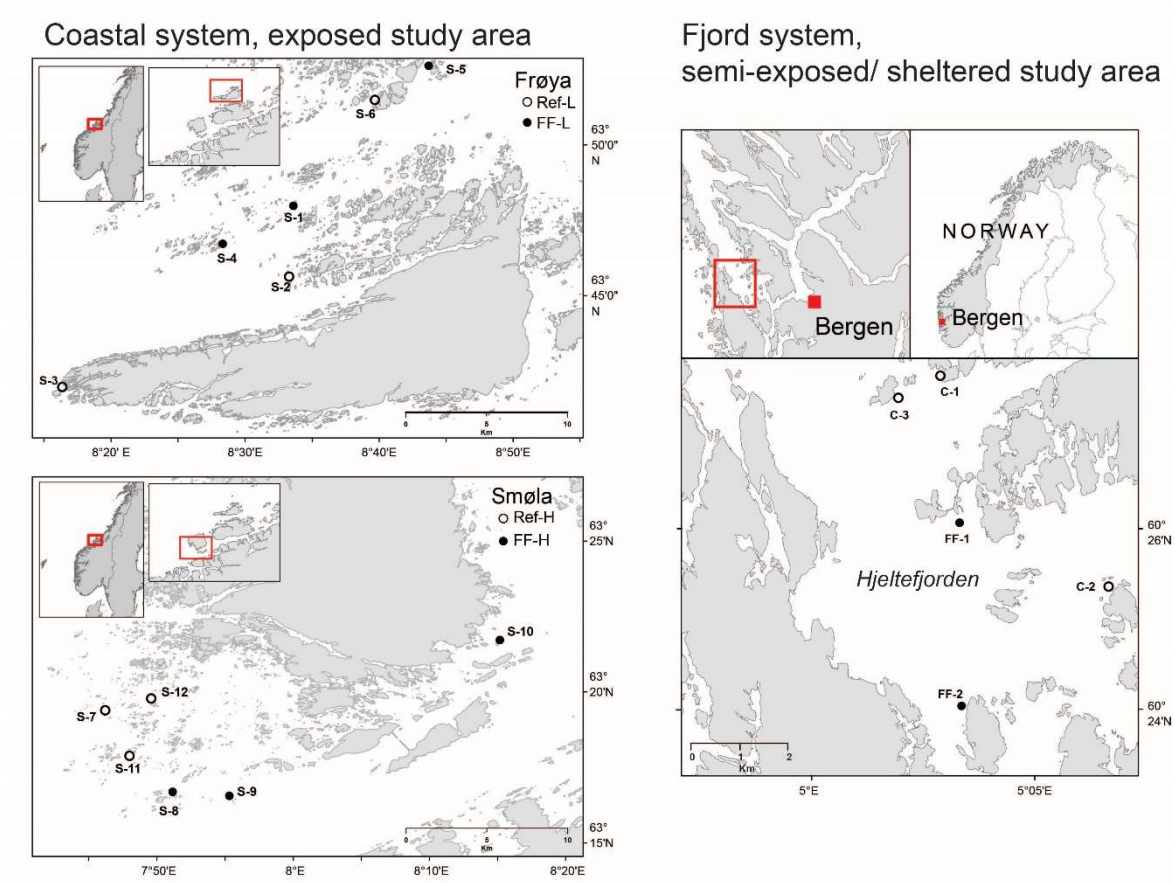


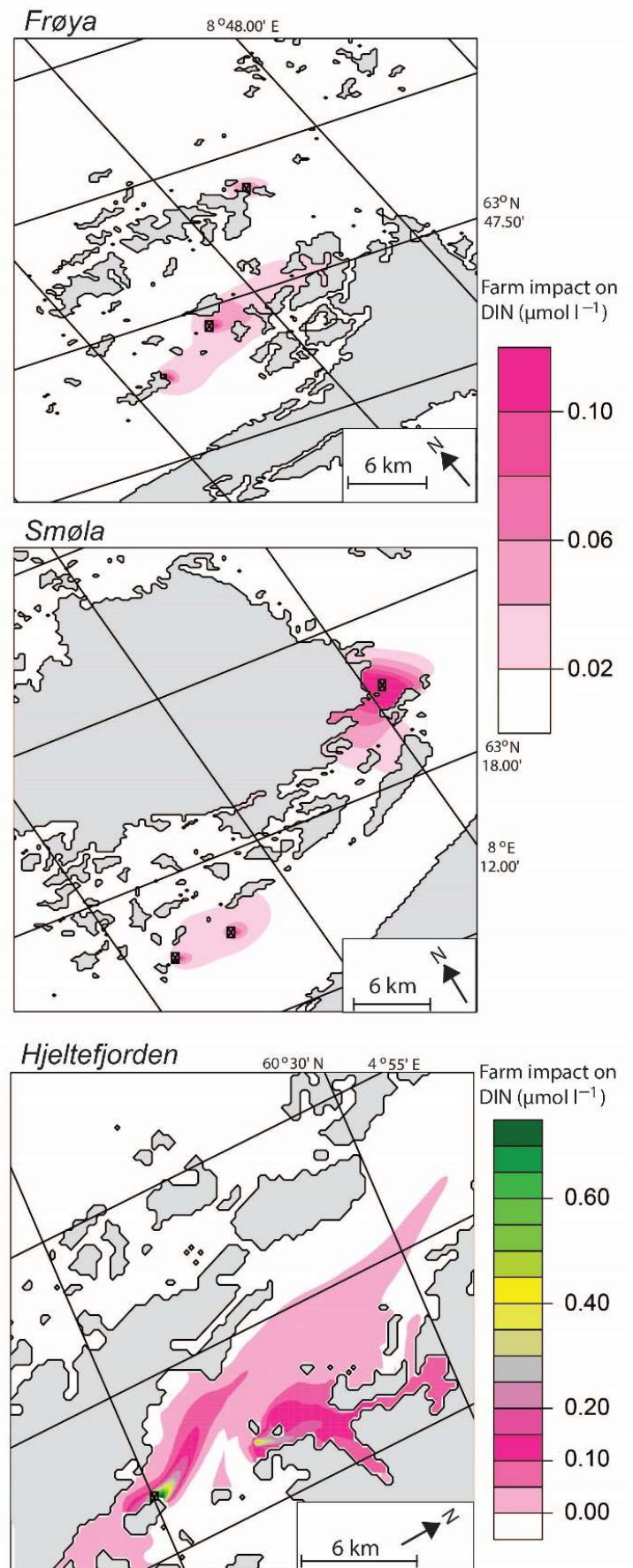
Figure 3. Geographical location of study areas; the coastal Frøya and Smøla archipelago (left), where *Laminaria hyperborea* plants were collected (see **Paper III** and **Paper IV** methods for details), and the fjord system Hjeltefjorden (right) where an *in-situ* translocation experiment with *Saccharina latissima* was conducted (open circles: control sites, closed circles: farm sites, see **Paper I** methods for details).

Due to wave exposure and ocean current patterns in the sampling area, the impact of effluents was not believed to follow a clear gradient as is usually the case at deep and calm fjord locations (Kutti et al. 2007a, b), but rather in patches in the prevailing current direction (see particle modelling **Paper III**) (Keeley et al. 2019). The location of sampling sites at farm locations was therefore chosen by selecting the closest skerry to the farm that was in the main effluent-plume. None of the study farms reported using hydrogen peroxide during the 6 months preceding sampling (www.barentswatch.no). Kelp harvesting is conducted in parts of the study area, with different sections being open for harvesting in different years. The exact location of harvesting is not recorded, though none of the sampling sites within sections that had recently been open (within the last 2 years) showed signs of harvesting. In dynamic environments, such as the Frøya and Smøla archipelago, the plume of the dissolved nutrients disperses and spreads at a fast rate (Fig. 4). In sheltered locations, the main plume

is highly localized and can be found down-current of the fish farms, with the highest concentrations occurring within 60 m followed by a rapid decrease away from the farm (Neofitou & Klaoudatos 2008, Sanderson et al. 2008, Handå et al. 2013). To answer the research question of **Paper I**, a translocation study was conducted to assess the effect in a natural environment. As I wanted to make sure the translocated kelp plants were exposed to elevated levels of fish farm nutrient effluents, fish farms in a semi-exposed to sheltered fjord were selected (Fig. 3, Fig. 4). *S. latissima* was selected as the study species as translocation is a common and successful methodology for studying the biology of this kelp species (see e.g., Gerard & Du Bois 1988, Lyngby & Mortensen 1996, Andersen et al. 2011, Armitage et al. 2017), and it has a habitat preference comparable to that present in Hjeltefjorden.

Translocation was first conducted with rigs deployed at the seafloor. Implications

► **Figure 4.** Predicted spatial impact of the dissolved inorganic nitrogen (DIN) plume in the exposed coastal systems Frøya and Smøla, and in the sheltered fjord system Hjeltefjorden (see Fig. 5 and text for modelling details). Note the higher dispersal of higher DIN concentrations from the study farms in Hjeltefjorden. Maps are constructed by calculating an average of the difference in nutrient level (simulation 2 - simulation 1; Fig. 5) for 4 months (Produced by Morten D. Skogen, IMR).



arose with this method, including a high loss of plants (detached and grazed), rigs becoming too entangled in other sublittoral vegetation to be able to be retrieved, difficulties with placement regarding deploying the rigs as close to the farm as preferred (within 60 m), and rigs being deployed at the same depth at all sites. The methodology was therefore improved by instead suspending plants on ropes (see **Paper I** Methods for details). This modification also gave the opportunity to use the results to evaluate *S. latissima* as an IMTA-candidate (see section 1.4.1).

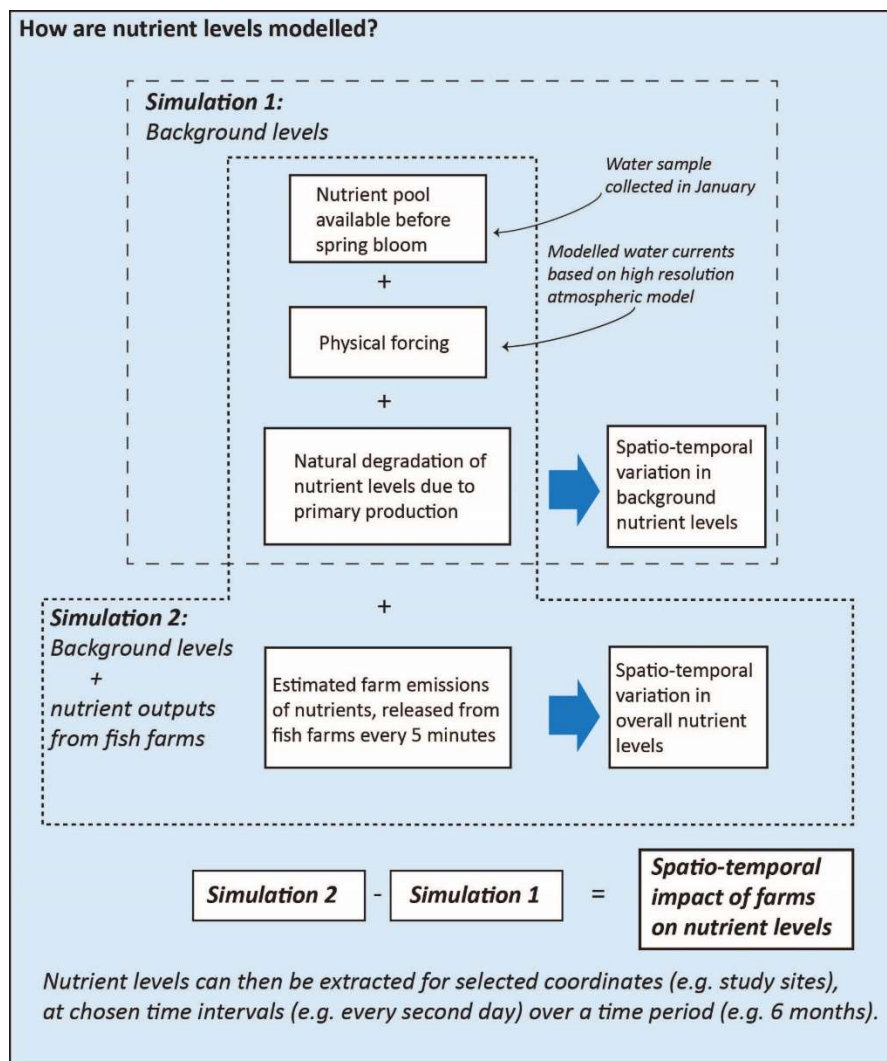


Figure 5. Simplified overview of the modelling procedure used to predict the impact of fish farming effluents on natural (i.e. background) nutrient levels at the study sites for **Paper I** and **III**. Two simulations are run; one predicting the spatial variation (using physical forcing) in natural nutrient concentrations over time if there were no farms present in the study area, and the second simulation predicting the spatial variation in nutrient concentrations over time when fish farms are releasing nutrients every 5 minutes. The difference between the two simulations give the relative increase in nutrients caused by fish farm emissions over time (e.g. Fig. 3, Paper I).

The effluent plume released from a fish farm will vary both daily and seasonally, depending on feeding regime, fish activity, fish biomass, tidal cycles and wind-induced current patterns. The spatial-temporal variations of the effect of a fish farm on background nutrient concentrations is therefore extremely challenging to capture by sampling (Jansen et al. 2016, 2018). The NORWECOM model (see **Paper I** methods for details) was instead used to predict the dispersion and relative effect on ambient concentrations of dissolved nutrients for all study-farms in **Paper I** and **Paper III**. Nutrient modelling also has its limitations; it will for example not incorporate variations over short time scales (days to weeks) in farm feeding regimes which can occur due to e.g. off-feeding events during medicinal treatments (see Jansen et al. 2016). Exact output of nutrients will also depend on the conversion estimate that is used, for which several exists and there is no scientific consensus regarding which one is more accurate (see Husa et al. 2018 for comparisons). The ANCYLUS model was used in these studies.

H₂O₂ emitted from fish farms is likely to disperse slower in calm waters compared to at exposed locations (Refseth et al. 2017, O. Samuelsen unpubl. data), thus non-target species inhabiting sheltered locations have a higher chance of being exposed to high concentrations. Results from the translocation field-study conducted in Hjeltefjorden (**Paper I**) indicated that *S. latissima* was sensitive to farm-related H₂O₂-emissions, which encouraged the research question related to **Paper II**. *S. latissima* is one of the kelp species more likely to be exposed to high concentrations due to its habitat preference (Table 1). Juvenile *S. latissima* sporophytes was anticipated to be the most vulnerable life-stage to such emissions, as they are more sensitive, and have a slower recovery rate than adults when exposed to high light stress (Hanelt et al. 1997). Obtaining baseline knowledge regarding which H₂O₂-levels that could be harmful to *S. latissima* required a controlled laboratory experiment, where juvenile sporophytes were exposed for 1 hour to different dilutions of the commonly used treatment dose (1700 mg l⁻¹).

3 Synthesis and discussion

3.1 Effect of fish farm effluents on kelp performance

3.1.1 Nutrient availability

The growth performance of first-year *Saccharina latissima* plants was not significantly affected, positively or negatively, by growing near fish farms (**Paper I**). These results challenge the idea behind cultivating *S. latissima* together with salmonid production in an IMTA-scenario, namely that *S. latissima* can readily take up ammonium (Ahn et al. 1998) - the main dissolved inorganic nutrient released from fish farming (Hall et al. 1992, Pitta et al. 1999) - and enhance their growth, resulting in higher yields.

Although nutrients are important for kelp growth, several environmental conditions also affect kelp performance, including light (Peteiro & Freire 2013), temperature (Andersen et al. 2013), and waves and currents via affecting the diffuse boundary layer (reviewed in Hurd 2000). As the variation in these environmental conditions can be broad in coastal environments, both on regional and local scales, it can result in a high variation in kelp growth performance across short distances, as seen in this study (**Paper I**). This variation could potentially be taken advantage of to cultivate *S. latissima*. Though not significant, there was a higher cumulative growth and a lower mortality at control sites, indicating monoculture or cultivation with other low trophic species will provide higher yields. The kelp plants might not enhance their growth performance compared to in natural communities, but a higher amount of kelp biomass in a fjord system via large-scale cultivation could still have an overall positive effect on regional nutrient budgets, as suggested for mussel cultivation on regional particle loadings by Sanz-Lazaro & Sanchez-Jerez (2017). The optimum abiotic conditions for high growth are not fully understood (Bartsch et al. 2008), and interactions between several might be important (Hurd 2000), e.g. water velocity and light. More knowledge regarding these interactions could be obtained with multifactorial experiments.

The elongation rates of *S. latissima* plants cultivated both at control sites and at farm sites were high during winter, and peaked between April and May, followed by a slower growth, hence a similar growth pattern as natural populations (Sjøtun 1993). The absence of a prolonged period of high elongation rates indicates that the reduced growth that sets in during late spring is not driven by nutrient limitation at this depth (5 to 6 m) for first-year plants (**Paper I**). Daylength is the governing environmental signal for sorus formation in *S.*

latissima (Lüning 1988), and could likely also contribute to the growth reduction in late spring for first-year plants (**Paper I**), as shown for the kelp species *Alaria esculenta* (Buggeln 1978) and *Laminaria hyperborea* (Schaffelke & Lüning 1994) (see also: Lüning 1993, Bartsch et al. 2008).

The DIN effluent plume released from farms is not constant but varies temporally depending on e.g. feeding regimes and flushing (Jansen et al. 2016), making a fast response vital to be able to utilize the excess DIN. My finding that first-year kelp plants are not utilizing this highly fluctuating nutrient resource that fish farm effluents may provide to enhance their performance (**Paper I**), corresponds to the population biology theory of different life-history strategies (Pianka 1970). Two strategies (for terrestrial systems) were termed by MacArthur and Wilson in 1967; *r*-strategy and *K*-strategy (Pianka 1970), which were later related to seaweeds based on thallus-structure (e.g. Littler & Littler 1980, Rosenberg & Ramus 1984). Having the ability to quickly respond to and exploit new and temporarily available resources, such as the additional DIN from fish farms, are characteristics associated with *r*-strategists, commonly referred to as opportunists. Examples of these in the marine flora include species belonging to the Ectocarpaceae family and *Ulva* spp. (Littler & Littler 1980, Lüning 1990). These opportunistic seaweed species are ephemeral, and have a filamentous or thread-like thallus structure giving them a high surface area to volume ratio. This structure favors a fast uptake of nutrients and subsequently a rapid growth rate. Kelp are perennial, have a thick and more complex lamina structure, and a strongly seasonal growth pattern. These are traits more similar to that of *K*-strategists.

An extensive *Membranipora membranacea* encrustation has been identified as an issue when cultivating *S. latissima* close to salmonid farms (Handå et al. 2013, Førde et al. 2015), though this is not related to a higher food-supply (**Paper I**). *M. membranacea* encrustation does however have a negative impact on growth performance of first-year plants, and prolonged encrustation can result in mortality (**Paper I**). Lamina epiphytic fouling can affect kelp nutrient uptake (Hurd et al. 1994, Hurd et al. 2000), and reduce pigment concentrations (Hepburn et al. 2006) and light availability (Andersen 2013a) - all factors which can negatively affect photosynthetic performance, and could have contributed to the lower growth performance observed for encrusted plants. Prolonged encrustation of bryozoans has shown to form tissue lesions (Krumhansl et al. 2011), which also could have contributed as encrusted kelp plants might divert more energy into repair, leaving less for growth.

Extending experimental results to natural situations is never trivial. Kelp in this field study (**Paper I**) were suspended on ropes in the water column, and different mechanisms may have affected their performance compared to in natural kelp communities. Uptake of nutrients might be higher when suspended in the water column in sheltered to semi-exposed areas due to increased water motion that reduce the diffusive boundary layer (see e.g. review by Hurd 2000). Other than that, there are (to my knowledge) no indications that the uptake of DIN should be different, and these results can therefore most likely be transferred to first-year plants in natural kelp communities. First-year *L. hyperborea* plants grow continuously through the first summer, a growth that is also suggested to not be nutrient limited (Sjötun et al. 1996). This means that the one positive effect that fish farm effluents potentially could have on first-year kelp plants, i.e. to enhance kelp growth performance, is unlikely. Nutrient uptake may however differ for older (2nd and 3rd year) *S. latissima* plants, as age-dependent nutrient uptake have been found for other Laminariales species (Bartsch et al. 2008).

For the bryozoan cover, more caution should be made when extending the conclusion from **Paper I** to natural communities. It has been argued that dislodgement and translocation of plants for experimental purposes does not affect bryozoan cover (Marzinelli et al. 2009), though this has not been tested for suspended translocation. It has however been observed that *S. latissima* growing on suspended rigs, in contrast to rigs placed on the sea bed, tend to become more covered by bryozoans (pers. comm. Caroline Armitage, Vivian Husa). This could be related to the laminas being less prone to abrasion against other structures such as other algae and/ or the forest floor. Abrasion have shown to affect settlement of sessile invertebrates in understory communities (Jenkins et al. 1999, Connell 2003), and could effectively reduce larvae settlement and development of bryozoan colonies on kelp lamina. Higher water motion in the upper water column could also provide these filter-feeders with more nutrients, such as phytoplankton. Suspension may also indirectly lead to a higher bryozoan cover by reducing the abundance and diversity of epifaunal grazers compared to in natural communities, e.g. reduce the accessibility for grazing sea urchins as suggested by Plesner et al. (2015). Furthermore, different abiotic conditions in the water column compared to on the seafloor may influence characteristics of the bacterial biofilm of the lamina (Bengtsson et al. 2010), which again can influence the settlement of bryozoan larvae (Dahms et al. 2004, Rao et al. 2007).

3.1.2 Hydrogen peroxide exposure

Hydrogen peroxide (H_2O_2) caused mortality of juvenile *S. latissima* plants at concentrations of only 5% (85 mg l^{-1}) of the dose commonly used and released to the environment (1700 mg l^{-1}) (**Paper II**), suggesting that H_2O_2 emissions from aquaculture could be lethal to *S. latissima* growing nearby.

The lethal H_2O_2 concentration ($\geq 85 \text{ mg l}^{-1}$) immediately effect photosynthetic performance, reducing both the maximum photosynthetic rate (P_{MAX}) and photosynthetic efficiency (α) of juvenile *S. latissima* plants by $>90\%$ (**Paper II**). Algae produce H_2O_2 in low rates as a metabolic by-product, which is readily scavenged by antioxidant systems (Bischof & Rautenberger 2012). Oxidative stress can however occur if there is an imbalance between the accumulation of H_2O_2 and the algae's antioxidant capacity (Bischof & Rautenberger 2012). This can in turn lead to damage on the photosynthetic apparatus (Asada & Takahashi 1987), both reversible and irreversible. The dramatic drop in photosynthetic performance reported in **Paper II** indicates that a concentration of $85 \text{ mg H}_2\text{O}_2 \text{ l}^{-1}$ exceeds the scavenging capacity of juvenile *S. latissima*'s antioxidant system. For 67% of the *S. latissima* plants exposed to this concentration, the damage to the photosynthetic system appeared to be irreversible, resulting in mortality (**Paper II**).

The high sensitivity of *S. latissima* to H_2O_2 could be related to it being a subtidal species. High interspecific sensitivity of exogenous exposure to H_2O_2 on macroalgal photosynthetic performance have been reported (Collén & Pedersén 1996, Collén & Davison 1999b, Dummermuth et al. 2003), and is suggested to be related to species' shore zonation (Aguilera et al. 2002a, 2002b, Dummermuth et al. 2003). Seaweed species inhabiting the upper intertidal zone have generally been found to be more stress-tolerant compared to low-intertidal and subtidal seaweed species. This is believed to be an adaptation, as high-shore species experience a more stressful environment in terms of e.g. desiccation, wave action, freezing and UV radiation (all of which may lead to oxidative stress), and therefore greatly benefit from having a more efficient antioxidant system (Davison & Pearson 1996, Collén & Davison 1999a, Aguilera et al. 2002a, 2002b, Dummermuth et al. 2003, Huovinen & Gómez 2013).

The photosynthetic response seen in *S. latissima* (**Paper II**) is comparable to that observed for other sublittoral kelp species when exposed to H_2O_2 (Dummermuth et al. 2003). Studies assessing mortality caused by H_2O_2 are however limited to intertidal species.

In the first study by Åsnes (2019), the sea lettuce *Ulva lactuca* Linnaeus and red dulse *Palmaria palmata* (Linnaeus) F. Weber & D. Mohr were found to be tolerant to concentrations 5 to 10 times higher than what caused *S. latissima* mortality, hence consistent with the shore zonation theory of littoral species being more tolerant to stress than sublittoral species. In the second study by Collén & Pedersén (1996), the reported sensitivity of the green macroalgae *Ulva rigida* C. Agardh was however much lower than that of *S. latissima*. I suggest that the higher sensitivity found for *U. rigida* in the latter study is related to the use of tissue discs (2 cm in diameter), compared to the use of whole plants in Åsnes (2019) and **Paper II**. Use of whole plants could increase the endurance, as a higher plant biomass might increase the chance of healthy unaffected tissue being left post-exposure, which could aid in thallus regeneration (**Paper II**). Such reserves could also be vital in terms of multiple exposures. For future studies, use of whole plants is recommended as this method is more representative of the effect H₂O₂ exposure can have in natural seaweed communities.

To properly assess the environmental risk associated with an emission, both knowledge regarding the sensitivity of non-target species and the concentrations that they might be exposed to in their natural habitat is needed. Based on the dose-response relationship obtained from the experimental results, I report on determined toxicity potentials of H₂O₂ on *S. latissima* (e.g. the lethal concentration for 50% of the population [LC₅₀]; **Paper II**), though knowledge regarding what concentrations natural kelp communities might be exposed to is still uncertain. In the translocation study with first-year *S. latissima* plants (**Paper I**), high kelp mortality was recorded on one of the two farms sites on two occasions. These mortality events could not be related to bryozoan cover or any of the other recorded variables (**Paper I**), but were found to coincide with on-site H₂O₂ treatment of the salmon (data from study-farms), and the low LC₅₀ value ($80.7 \pm 53.5 \text{ mg l}^{-1}$) determined for *S. latissima* (**Paper II**) suggests that H₂O₂ emissions could have been the cause. The risk of mortality will depend on the distance between kelp forests and farm location, and the hydrodynamic conditions at time of emission (**Paper II**). Fish farms are generally located close to land or skerries, thus often overlapping with *S. latissima* habitats. Dispersion models predicting the spatial and temporal movement of H₂O₂-plumes released from fish farms indicate that natural kelp beds at sheltered locations can be exposed to harmful concentrations (Refseth et al. 2017, O. Samuelsen unpubl. data, Fig. 6).

The total proportion of nearby *S. latissima* forests that would experience lethal or sublethal concentrations of H₂O₂ from *one emission* might not result in implications for the

local population. However, delousing operations usually involve multiple bath-treatments (1 net pen at a time) over several days (Grefsrud et al. 2018), and for some farms several times during the production-cycle (12 to 18 months). Successive exposures can lower the sensitivity of non-target species (Burrige et al. 2000, 2008), and several exposures over a few days could certainly decrease the sensitivity of *S. latissima*, potentially resulting in juvenile mortality also when the concentrations are lower than the determined LC₅₀.

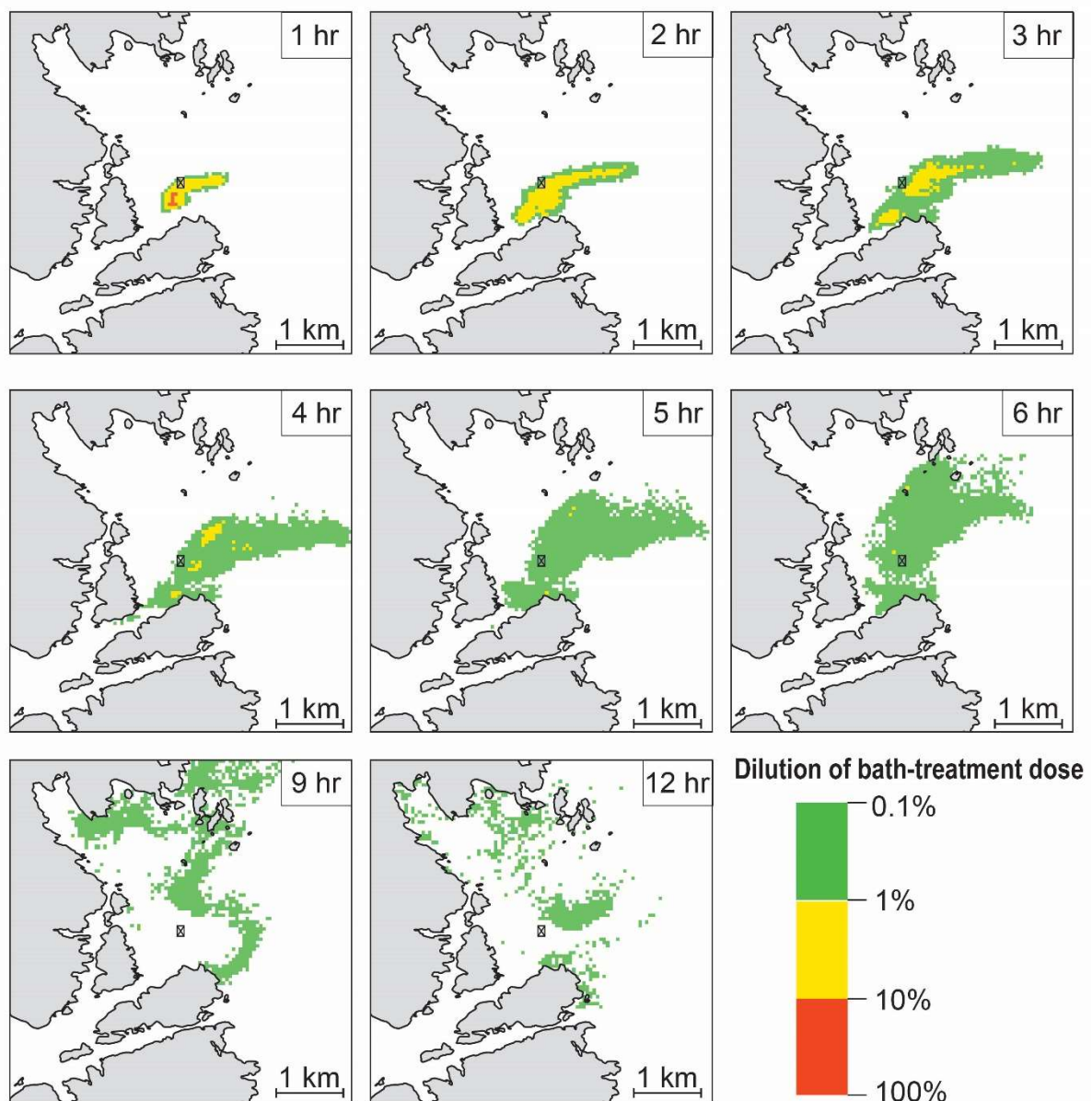


Figure 6: Model simulation predicting the dispersal of a hydrogen peroxide plume over 12 hours post-emission from a randomly selected fish farm (☒) located at a sheltered location (Preliminary results; Pål Næverlid Sævik, IMR).

Alternatively, a larger part of the surrounding population could be exposed if the hydrodynamic conditions are changing between emissions, spreading the main plume in different directions and to different parts of the nearby shoreline, successively exhaust small proportions of the population. The number of H₂O₂ prescriptions given to fish farms in 2016 was highest for the months January to March (Samuelsen & Agnalt 2018), which overlaps with the recruitment period (October to March) for sugar kelp (Andersen et al. 2011, Andersen 2013b). If a delousing-operation in early winter exhaust 30% of that year's local recruits, and a second delousing-operation in early spring exhaust another 50%, this could have large implications for the development of the adult population. Sublethal effects in terms of a reduced carbon budget (see **Paper II** discussion for details) could lead to lower fitness, making the kelp plants more vulnerable to other stressors (Wernberg et al. 2010), such as epiphytism or elevated temperatures. In that way, it could indirectly lead to mortality of local *S. latissima* forests. Another potential implication arises if intertidal turf-species are more tolerant to H₂O₂ exposure than *S. latissima* (as outlined above). Turf-forming species replacing kelp beds is an issue both on a global (Strain et al. 2014, Filbee-Dexter & Wernberg 2018) and national scale (see section 1.2). If H₂O₂ emissions exhaust kelp recruits while other turf-species survive, this could give turf-species an upper hand in the competition for space. These are however scenarios that needs further testing.

Laboratory experiments have shown that some faunal species also have a high sensitivity to H₂O₂ (*Calanus* spp.: Escobar Lux 2016, *Pandalus borealis*: Bechmann 2017). To increase the understanding of the actual implications that emissions may have on kelp forests and how the H₂O₂ plume can affect non-target species, I suggest the use of juvenile *S. latissima* as an indicator organism for future field studies. They have a high H₂O₂ sensitivity, are easy to check for lethal effects (i.e. detachment of lamina), and are cost-efficient to use as no maintenance is required after deployment. Plants can easily be attached to long ropes that are suspended vertically (see **Paper I** methods), allowing for assessment of several depths of the water column at the same time. Until we understand more of the potential impacts on marine communities, I encourage the fish farming industry to consider hydrodynamic conditions when releasing H₂O₂ to the environment, and to avoid emissions at sheltered locations when there is onshore wind. Fish farms located at more exposed locations likely have a lower potential of impacting nearby kelp beds due to a faster dispersion and dilution of the plume.

3.2 Effect of fish farm effluents on kelp forest communities in dispersive environments

3.2.1 *Stipe epiphytic community*

I found a small but clear response of the stipe epiphytic community associated with *L. hyperborea* to fish farm effluents (**Paper III**), showing that the interaction between aquaculture and kelp forests in dispersive environments includes an alteration of the kelp habitat. Bryozoan biomass was significantly higher at high effluent farm-sites compared to low effluent farm-sites and reference sites. The response of macroalgae biomass was less clear, though there were indications of an increased total biomass with increasing effluent level, and a clear increase in biomass of opportunistic thread-forming *Ectocarpus* spp. with farm effluent load (**Paper III**). The stronger response of total biomass of bryozoans compared to macroalgae, could be related to different factors. Epiphytic macroalgae are generally *r*-strategists, and can take up DIN from farms (Carballeira et al. 2013) including ammonium (Ahn et al. 1998), while bryozoans may be able to utilize both the dissolved and particulate fraction released from the farms, although this has not yet been shown (see **Paper III** discussion). The method used to group the stipe community species was likely contributing to this difference; bryozoan-covered macroalgae were counted as ‘Bryozoa’ (see **Paper III** methods for details), hence any extra growth of macroalgal epiphytes that later became extensively covered by bryozoans would have contributed to bryozoan differences rather than macroalgal differences. Phytoplankton may also have played a role; by having a higher growth rate than macroalgae, phytoplankton could potentially have taken up the DIN before the macroalgae had time to respond (Hadley et al. 2015). Higher phytoplankton biomass could also potentially be an additional food source for the bryozoans, though in dispersive environments such as the investigated area, it is unlikely that this would have only benefitted bryozoans at the farm-sites.

Changes in community structure is a known response of marine floral communities under enhanced nutrient levels (e.g. Oh et al. 2015, Balata et al. 2010), and was also found at sites receiving a high farm effluent load (**Paper III**). The macroalgae community structure was less heterogeneous at these sites, and the high bryozoan biomass further reduced the habitat heterogeneity, in addition to resulting in a significantly different stipe epiphytic community structure. An important question following these results is how this could affect the associated faunal community. Mobile macrofauna are well known to be affected by the community structure of different macroalgae habitats (Bégin et al. 2004, Schmidt & Scheibling 2006, Wikström & Kautsky 2007, Tuya et al. 2008). In the kelp

forest, one *L. hyperborea* stipe supports on average 80-90 macrofaunal species (Christie et al. 2009), where the faunal diversity increases with habitat diversity (Norderhaug et al. 2014). The reduced complexity of the stipe community as a habitat could thus reduce the associated invertebrate community that depends on the kelp forest for e.g. food (Norderhaug et al. 2003) or refuge (Norderhaug 2004) from predators and wave action. Furthermore, the structure and form of stipe epiphytic macroalgae have been suggested to be the most important property for the associated invertebrates (Norderhaug 2004), and bryozoan-covered macroalgae could be less favoured. The changes caused by farm effluents could thus have profound implications for the mobility patterns of the faunal community that directly depends on the stipe community. This in turn could affect the food availability for higher trophic levels, such as Atlantic cod *G. morhua*, Saithe *Pollachius virens* (Linnaeus, 1758), and seabirds that forage in the kelp forest (Røv et al. 1990, Fredriksen 2003, Norderhaug et al. 2005).

During short-term increases in nutrients, grazing can mediate the growth of epiphytes (Balata et al. 2010) and macroalgae communities (Karez et al. 2004, Russell & Connell 2007). Large grazing fronts of the sea urchin *Echinus esculentus* Linnaeus, 1758 were regularly observed at the sampling sites and are known to feed on algae and associated epiphytes (Jorde & Klavestad 1963, Comely & Ansell 1988). Very little sea urchins were however observed in the holdfasts in this area (**Paper IV**). Top-down control of epiphytes via grazing could, therefore, have limited any epiphyte biomass increase in response to farm effluents, making impacts harder to detect. Sea urchins have been found to be able to exploit and assimilate farm waste (White et al. 2017, 2018), and may benefit from intensive farming.

3.2.2 *Lamina epiphytic community*

Macroalgal biomass and bryozoan cover on the lamina of *L. hyperborea* did not increase with fish farm effluents (**Paper III**), suggesting that the kelp lamina is more resilient to an increased epiphyte load than the kelp stipe in dispersive environments. The statistical model predicted that higher kelp density had a negative effect on macroalgae biomass and bryozoan cover (**Paper III**). This relationship is also put forth as a possible explanation for the higher bryozoan cover often seen on suspended *S. latissima* (i.e. lower density, see chapter 3.1.1) but needs further investigations to be confirmed and clarified.

Along the southern coast of Australia, macroalgal epiphyte load on lamina of the kelp *Ecklonia radiata* (C. Agardh) J. Agardh increased significantly under high and constant nitrogen conditions (1.8 times higher than ambient waters) (Russell et al. 2005). The elevation in nutrient levels was comparable to the maximum that was predicted at the high effluent farm sites in **Paper III**, but the contrasting results could be related to the nature of fish farms' nutrient pulse. Longer, constant nitrogen pulses have a higher impact on epiphytic communities than several shorter-duration pulses (Worm & Sommer 2000), and the nitrogen plume from fish farms would be much more variable compared to the artificial nutrient source used in Russell et al. (2005). However, the dominant lamina epiphyte was thread-forming Ectocarpales spp. (**Paper III**), thus including the stipe algae that was found to respond to farm effluents. An alternative is that sampling earlier in the summer (than August, as in **Paper III**) could have yielded different results for the epiphyte load; a higher lamina epiphyte diversity is present in May compared to in August for undisturbed *S. latissima* and *Laminaria digitata* (Hudson) J.V. Lamououx (Carlsen et al. 2007), and this could also be true for *L. hyperborea*. Still, faunal abundance and diversity associated with *L. hyperborea* lamina is higher in August compared to May (Christie et al. 2003), indicating a higher epiphyte load is present at that time. If lamina epiphyte load varies seasonally in a different pattern than the stipe epiphyte load, this could have contributed to the difference in response to farm effluents seen in these two communities.

For the range of wave exposure levels that were modelled at the investigated sites, stipe epiphyte load decrease with increasing exposure (Norderhaug et al. 2012), whereas lamina epiphyte load decrease (Pedersen et al. 2012). The latter is anticipated to be due to a whiplash-effect of the lamina causing settled algae and faunal larvae to be dislodged (Kiirikki 1996). I therefore suggest that laminas could be more resilient towards fish farming impacts in dispersive environments compared to in inner-coastal systems.

3.2.3 *Holdfast macrofaunal community*

The macrofaunal community inhabiting *L. hyperborea* holdfasts displayed no clear response-patterns that could be associated with farm-induced enrichment (**Paper IV**). This demonstrates that holdfast particle accumulation rates are not exceeding the assimilation capacity of the holdfast community under the current level of farming in this area. The high resilience of the macrofaunal community is likely related to the dispersive nature of the area, as suggested for the lamina epiphyte load.

The particle load that was predicted to reach the sampled kelp forest near Frøya farms (low effluent load farms) was approximately 1.0 – 1.5 tonnes day⁻¹ for three months (May through July 2015) preceding sampling. For the kelp forest near Smøla farms (high effluent load) the predicted loads were 3.7 – 5.6 tonnes day⁻¹ (**Paper III, IV**). A significant amount of particles thus had the potential to end up trapped in the kelp holdfasts. It was therefore surprising that there was no accumulation of particles in any of the sampled holdfasts (**Paper IV**). Multiple theories emerged from this finding. The method used to collect and process the holdfasts in this study has been well documented and regularly results in findings of sediment content (e.g. Smith et al. 1996, Tuya et al. 2011, Teagle et al. 2018). The methodology is therefore considered to be an unlikely causal factor. A more credible explanation is related to the dispersive and wave-exposed nature of the sampling area. Moore (1972) found that wave height and sea swell are the most important factors explaining the amount of suspended particles in the water column and further suggested that the accumulation of material in holdfasts at exposed locations could be low due to resuspension. Resuspension of settled particles has also been found to be an important factor determining accumulation areas for farm-derived particles in shallow and dispersive environments (Keeley et al. 2013a), and ‘flushing events’ of seafloor accumulations have been observed in the specific area where the holdfasts were collected (N. B. Keeley, pers. comm.). Considering these findings, it is possible that particles settle in the holdfasts, but are readily flushed out again. A rapid consumption by the inhabiting holdfast community may also contribute and prevent particle accumulation. To clarify which species are utilizing the farm-derived food source, chemical tracing techniques using soya DNA (soya being a common ingredient in fish feed), fatty acids, and stable isotopes have been successful (White et al. 2017, Woodcock et al. 2017, 2018). In either case, the particle settling rate in the holdfasts was not high enough to stimulate an opportunistic community.

Previously reported responses of holdfast macrofauna to turbidity (Sheppard et al. 1980, Jones 1973), sediment content (Moore 1972) and domestic effluents (Smith & Simpson 1992) have led to the suggestion of using this microhabitat as a monitoring tool. The absence of a farm-effect in dispersive environments (**Paper IV**) known to have high resilience to farm-impacts (Keeley et al. 2013b, Valdemarsen et al. 2015) could indicate that the holdfasts work along an enrichment gradient. However, some factors have presented themselves in this study which could complicate the inclusion of holdfasts in a monitoring scheme. The monitoring program used today classifies the ecological status of the benthic

environment based on, among others, the complex multi-metric Norwegian Quality Index (NQI1) (Direktoratsgruppen vanndirektivet, 2018). The NQI1 is calculated based on the sensitivity of the identified infauna. Extensive databases have been compiled listing macrofaunal species in marine waters and their relative sensitivity to pollution (e.g. NSI: Rygg & Norling 2013, AMBI: <http://ambi.azti.es/>). However, the focus has been on soft-bottom macrofauna, and holdfast communities comprise many species not included in these databases, e.g. of the 149 species identified in my study information was only available for 25%, not including the majority of the dominant species (**Paper IV**). Hence more information regarding the sensitivity of the species constituting this habitat would be required to determine the ecological condition. Secondly, a high natural variation was found in the study area on both local (between sites) and regional (between reference sites at Frøya and Smøla) scales. This variation was suggested to be related to, among others, stochastic events that affect recruitment patterns both spatially and temporally. This indicates that a high number of holdfasts would need to be sampled to fully understand the background variation and detect farm-effects. And furthermore, that sampling over a temporal scale might be required as the background variation could change from year to year. The presumably intensive sampling regime suggests this might be an unsuitable monitoring tool.

The use of artificial holdfasts could be an option (Smith 2000, Norderhaug et al. 2002). Artificial structures mimicking the holdfast habitat could provide a more standardized method needed for monitoring, as some factors contributing to the natural variation e.g. holdfast structural complexity, age, as well as interactions with the stipe community, could be controlled for. However, as the characteristic of an opportunistic species (*r*-strategists) is to exploit newly available resources, they are also often the first species to colonize a new area (Gray 1979). Hence the artificial structures may disproportionately sample more opportunistic species.

3.3 Sustainable management in dispersive environments

For soft-bottom macrofaunal communities in fjord systems, the magnitude of impact generally follows the fish farm production cycle; some biological parameters (e.g. abundance of opportunistic species) increase with farm effluent load and normalize again during the following period, while impacts on faunal community structure can persist (e.g. Macleod et al. 2004, Kutti et al. 2008). The same response pattern has been found for the macrofaunal community of a soft-bottom area in the Frøya archipelago (Keeley et al. 2019). In the hard-bottom *L. hyperborea* community, shifts in the community structure of associated organisms was only observed near farms that were at the end of the production cycle (17-18 months in), while no clear response was detected near farms that were in the beginning of the production cycle (5-6 months in). While a significant shift was apparent for the stipe epiphytes, there was no clear farm impact on the lamina epiphytic- or holdfast macrofaunal communities (**Paper III, IV**) indicating an overall high resilience of kelp-associated communities to fish farm effluents. Furthermore, **Paper III** indicated that when significant shifts in community structure takes place in kelp communities, it normalizes between production cycles. Overall, the studies upon which this thesis is based indicate that kelp communities are less impacted by fish farming effluents than soft-bottom communities in dispersive environments, and that an incremental degradation is unlikely under the current pressures. However, to understand the full extent of the impacts high-intensity farming can have in dispersive environments, and hence achieve sustainable management, more studies are needed. In particular studies directed toward clarifying the assimilation pathways for farm waste in the kelp forest. About 30-40 % of the particulate matter that is released from fish farms in this area are assimilated by the soft-bottom habitat, but the remaining 60-70 % is unaccounted for (Keeley et al. 2019). Dietary tracing (e.g. fatty acids) could be used to identify the direct links between farm waste and kelp forest species. Potential interesting species include sessile and mobile fauna sampled from the kelp holdfasts, and also sea urchins. Sea urchins are seen as a key stone species, and there are indications that these might be interacting with the epiphytic community on the kelp stipes (**Paper III**) and with the farm waste.

3.4 Northbound: projected aquaculture and kelp forest recovery

Infestations with the salmon lice *Lepeophtheirus salmonis* (Krøyer, 1837) on wild salmonid fish is currently of great concern in Norway. In 2017 new regulations were implemented to mitigate the salmon lice pressure caused by intensive fish farming. The new regulations have separated the Norwegian coastline into 13 production zones, where the request to increase biomass production at a farm locality will depend on the salmon lice pressure within that zone (Grefsrud & Svåsand 2018). In 2018, mainly fish farms located within zones along the northern part of the coast (from 64°N) were given the opportunity to increase salmonid production biomass. With its vast areas suitable for fish farming it is expected that future aquaculture growth will be centered here. This is also the part of the coastline where *L. hyperborea* and *S. latissima* have started re-establishing from the extensive sea urchin *Strongylocentrotus droebachiensis* grazing events in the 1970's (Norderhaug & Christie 2009) (see section 1.2).

The results in this thesis gives an overview of how large-scale high-intensity farming are interacting with established and recovered kelp forests in highly dispersive environments in mid-Norway. The interactions between aquaculture and kelp forest in the northern areas where kelp forests are recovering, and where there is still a grazing pressure from *S. droebachiensis* (Steen et al. 2018), might be different than that found in this thesis. For example, kelp in less wave-exposed areas are more prone to *S. droebachiensis* grazing (Sivertsen 1997), and also tend to have a higher lamina epiphyte load (Pedersen et al 2012). Recovering kelp forests also often have a lower density (Sivertsen 1997) which could leave the lamina more prone to a higher lamina epiphyte load (**Paper III**). Fish farming effluents will disperse in slower rates in such areas (Fig. 4) which could further promote epiphytic growth on the laminas in the same manner as for stipe epiphytes at exposed locations (**Paper III**), and as observed by Russell et al. (2005) along the Australian coast. Furthermore, the additional nutrients are not likely to enhance the growth of juvenile kelp (**Paper I**), but organic fish farming waste can boost the reproductive output of sea urchins by 5 times and promote dense aggregations (White et al. 2018). An increased pressure brought on by fish farming effluents in these areas could thus stimulate barren grounds, while also reduce kelp growth via increased lamina epiphyte load. Aquaculture related emissions of H₂O₂ could be an additional factor stabilizing the barren ground state, as the potential impacts of H₂O₂ on juvenile *S. latissima* - loss of tissue, juvenile mortality and low recruitment (**Paper II**) - are factors that have been found to negatively impact recovery of *S. latissima* forests in the

Northwest Atlantic (O'Brien & Scheibling 2018). Sea urchins on the other hand, are much more tolerant to high H₂O₂ exposure (Ø. Hansgård Gjelsvik unpubl. data). The abovementioned factors could contribute to a lower resilience of kelp forests to fish farming effluents in northern areas compared to in mid-Norway.

4 Conclusions

The studies that this thesis is based on contributes to the understanding of how fish farm effluents can interact with kelp forests in dispersive environments. It also brings new knowledge of the toxicity of the medicinal therapeutant H₂O₂ used in fish farming, that until recently has been viewed as an environmentally friendly, non-harmful chemical. The main conclusions are summarized below.

- Growing near fish farms does not have an overall positive (in terms of enhanced growth performance or nutrient reserves) or negative (in terms of increased lamina fouling) effect on first-year *Saccharina latissima* plants. This indicates that first-year *S. latissima* plants are not nutrient limited, and that cultivation of *S. latissima* is likely to produce similar yields if conducted separated from salmonid farms.
- Extensive lamina fouling of the bryozoan *Membranipora membranacea* negatively affects the growth performance of first-year *S. latissima* plants, though fish farm effluents do not significantly affect *M. membranacea* cover on *S. latissima* lamina in a cultivation scenario.
- Juvenile *S. latissima* plants are highly sensitive to H₂O₂ exposure, where a concentration as low as 5% of the dose commonly used at farms and emitted to the environment causes mortality. *S. latissima* populations can therefore be negatively affected when fish farms in the vicinity release H₂O₂-solutions.
- The epiphytic community on *Laminaria hyperborea* stipes showed a clear, though small, response to high loads of fish farm effluents in dispersive environments. Impacts include an increased bryozoan biomass, lower stipe habitat complexity, and a shift in community structure. This could have further implications for the faunal community that depends on the stipe epiphytes for food and refuge, and for higher trophic levels that are affected by food availability. The impacts appear to follow the salmon production cycle, where impacts increase with salmonid biomass and recover between production cycles.

- Lamina of *L. hyperborea* are probably resilient towards increased epiphyte load in dispersive environments, due to mechanisms related to high wave exposure and / or perhaps kelp density.
- Macrofaunal communities inhabiting *L. hyperborea* holdfasts are highly variable and appear resilient to impacts from fish farming effluents in dispersive environments. The resilience is suggested to be related to an efficient dispersal of organic particles in the kelp forest, which prevents an accumulation in the holdfasts.

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1 ***Saccharina latissima* cultivated in salmonid farm effluents: a case study**
2 **from a cold temperate fjord shows no effect on growth and nutrient**
3 **status.**

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Mortality and reduced photosynthetic performance in sugar kelp *Saccharina latissima* caused by the salmon-lice therapeutant hydrogen peroxide

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ABSTRACT: Sugar kelp *Saccharina latissima* is one of the dominating kelp species on sheltered sublittoral sites along the Norwegian coastline, providing important habitats and ecosystem services. In finfish aquaculture in Norway, infections caused by salmon lice *Lepeophtheirus salmonis* are one of the biggest challenges the industry is currently facing, and presently the most-used therapeutant is hydrogen peroxide (H_2O_2). Commonly, this treatment involves bathing the fish in a solution of approximately $1700\text{ mg } H_2O_2\text{ l}^{-1}$ seawater before releasing the solution into the surrounding waters. The present study was conducted to increase the knowledge on how these H_2O_2 emissions from farm operations could impact juvenile *S. latissima*. This was achieved by determining the effects of a 1 h exposure to realistic H_2O_2 levels on *S. latissima* mortality and photosynthesis. Effects on photosynthesis were determined by incubating plants at 3 time intervals post-exposure. Toxicity potentials including lethal concentration for 50% of the population (LC_{50}) and effective concentration (EC_{50}) for photosynthetic capacity (P_{MAX}) and efficiency (α) were determined based on these data. Juvenile *S. latissima* was highly sensitive, having an LC_{50} of $80.7\text{ mg } H_2O_2\text{ l}^{-1}$, which is less than 5% of the dose commonly used at farms and emitted to the environment. A concentration of 85 mg l^{-1} caused an immediate 90% reduction in both P_{MAX} and α . The EC_{50} was found to be 27.8 and 35.4 mg l^{-1} for P_{MAX} and α , respectively. This may indicate that natural *S. latissima* populations in the vicinity of fish farms can be negatively affected by H_2O_2 bath treatments.

KEY WORDS: Kelp mortality · Kelp photosynthesis · Aquaculture effluents

1. INTRODUCTION

Production of salmonid fish is a major industry in Norway, with >1.3 million tons produced in 2016, a 3-fold increase over the last 15 yr (Directorate of Fisheries Norway 2018b). Fish in the high-density conditions of modern aquaculture are susceptible to bacterial, viral, and parasitic diseases. At present, infestation with salmon lice *Lepeophtheirus salmonis* is a major problem for the Norwegian fish-farming industry, increasing the requirement for effective antiparasitic therapeutants. Several antiparasitic thera-

peutants are currently in use to mitigate the effects of sea lice on cultured Atlantic salmon (Grefsrud et al. 2018). These are administered as a bath treatment or orally via feed and are ultimately released into the environment. Recent studies have shown that the release of these chemical therapeutants can have significant consequences for non-target organisms in marine ecosystems (e.g. Samuelsen et al. 2014, Van Geest et al. 2014).

Hydrogen peroxide (H_2O_2) is used in salmonid aquaculture in Norway, as well as in Chile, Canada, and Scotland (Burrige et al. 2010). H_2O_2 is adminis-

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tered as a 15–20 min bath treatment of approximately 1500–2100 mg H₂O₂ l⁻¹, depending on water temperature (www.felleskatalogen.no). This is conducted either at the farm by wrapping a tarpaulin around the net pen or using a well-boat (Andersen & Hagen 2016). After treatment, the tarpaulin is removed, and the H₂O₂ disperses into the surrounding water or is pumped out from the well-boat directly into the sea. H₂O₂ dissociates into oxygen and water, and is therefore seen as being 'environmentally friendly' (BurrIDGE et al. 2010), and H₂O₂ emissions are currently not included in any environmental risk assessments in relation to fish-farm activities. However, the half-life is approximately 7 d at 15°C and longer at colder temperatures (Bruno & Raynard 1994), leaving a window of time where this highly reactive oxidizer may interact with non-target species. As the H₂O₂ solution has similar density to the surrounding seawater, the effluent plume is likely to be concentrated in the upper ~30 m of the water column (Andersen & Hagen 2016, Refseth et al. 2017), making subtidal zones the most likely to be affected. In Norway, H₂O₂ is currently the most-used antiparasitic therapeutant, with an annual consumption of 31 577, 43 246, and 26 597 t respectively for the years 2014–2016 (www.fhi.no). Nonetheless, more knowledge regarding potential environmental effects is required to decide if and how these H₂O₂ emissions should be included in future environmental risk assessments.

To be able to make inferences regarding the potential effects H₂O₂ emissions from fish farms may have on population levels of subtidal species, it is necessary to have knowledge of the toxicity potentials toward non-target species, as well as which doses these species may be exposed to in the natural environment. Two field studies (Andersen & Hagen 2016, Fagereng 2016) and 2 dispersion models (Refseth et al. 2017, O. Samuelsen unpubl. data) have looked at how the H₂O₂ plume may disperse post-emission under conditions found in Norwegian waters. Results from the field studies give a snapshot of the concentrations in very close vicinity (≤60 m) to the release point, whereas the dispersion models give indications of how the plume may move locally through time and space. For sheltered locations within 1 km of the emitting farm, it is predicted that remaining concentrations 24 h post-emission will range from 18% to <1% of the H₂O₂ bath-treatment dose used (Refseth et al. 2017, O. Samuelsen unpubl. data). The toxicity potential of these concentrations on subtidal species has not been assessed to date.

Along the Norwegian coastline, one of the most dominating macroalgal habitats are kelp (order Laminariales) forests, estimated to cover an area >10 000 km², from the shore down to a depth of 20–30 m (Christie et al. 2009). Macroalgae are important primary producers on the continental shelf (Charpy-Roubaud & Sournia 1990) and key components in coastal ecosystems (Araújo et al. 2016). On sheltered and medium-exposed locations, the sublittoral vegetation is commonly dominated by the perennial sugar kelp *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl, & G. W. Saunders (Andersen et al. 2011, Moy & Christie 2012). Kelp forests are biodiversity hotspots, serving key functions in the ecosystem by providing refuge, habitat, nursery grounds, and feeding grounds for >100 marine faunal species (Christie et al. 2003, 2009, Fredriksen 2003), including economically important fish such as Atlantic cod *Gadus morhua* and saithe *Pollachius virens* (Norderhaug et al. 2005), and seabirds such as sea ducks, cormorants, and black guillemots *Cephus grylle* (Bustnes et al. 1997).

Healthy photosynthetic activity is essential for algae both in terms of nutritional uptake and in maintaining a positive carbon budget. As in all living cells, H₂O₂ and other oxygen intermediates (superoxide radical, singlet oxygen, and hydroxyl radical) are produced in low rates in algae as metabolic by-products and commonly grouped as reactive oxygen species (ROS) (Bischof & Rautenberger 2012). Algae have antioxidative systems that scavenge ROS, but stress (e.g. suboptimal temperature or light conditions) may cause an imbalance between the accumulation of ROS and the system's antioxidative capacity, leading to oxidative stress (Bischof & Rautenberger 2012). This in turn may lead to oxidation of cellular components (lipids, nucleic acids, and proteins) and thus damage the photosynthetic apparatus and lead to photoinhibition (Asada & Takahashi 1987). Concentrations as low as 2 mg H₂O₂ l⁻¹ within chloroplasts have shown to cause a 90% reduction of photosynthetic activity (Kaiser 1979). As H₂O₂ has the ability to readily diffuse across membranes (Apostol et al. 1989), exogeneous exposure could therefore have detrimental effects on macroalgae.

High interspecific sensitivity of the photosynthetic system to H₂O₂ has been reported both for macroalgae (Collén & Pedersén 1996, Collén & Davison 1999) and within the Laminariaceae family (Dummermuth et al. 2003), but H₂O₂ sensitivity of *S. latissima* has not been assessed to date. The lethal effect-dose of H₂O₂ has been determined for one macroalgae (Collén & Pedersén 1996). For microalgae,

H₂O₂ has previously been evaluated as a growth inhibitor related to nuisance blooms (European Communities 2003 and references therein, Southard 2005). However, studies reporting on toxicity potentials required for environmental risk assessments—such as lethal concentration for 50% of the population (LC₅₀), no-effect concentration (NEC), or effective concentration affecting photosynthesis in 50% of the population (EC₅₀)—of H₂O₂ on macroalgae, in terms of concentrations and durations comparable to emissions from aquaculture, are lacking.

Reversible and irreversible damage to the photosynthetic apparatus caused by a stressor, such as photosynthetic toxicity potential of H₂O₂, can be observed by measuring different photosynthetic parameters. The algae's ability to utilize available light for carbon fixation (photosynthetic efficiency) and the maximum potential rate of carbon fixation (photosynthetic capacity) can be reduced during suboptimal temperature (Davison et al. 1991, Andersen et al. 2013) and light conditions (Borum et al. 2002). Use of fixated carbon (respiration) however, tends to increase, as shown for both temperature (Davison et al. 1991, Andersen et al. 2013) and light (Borum et al. 2002). A reduced photosynthetic efficiency and/or increased respiration will result in an increase in the minimum amount of light required to maintain a balanced carbon budget (compensation irradiance) and could thus reduce the amount of carbon available for growth (Davison et al. 1991). A lower photosynthetic capacity and/or efficiency will reduce the saturation irradiance of the algae (Davison et al. 1991, Andersen et al. 2013) and could consequently affect their ability to take advantage of high light conditions. Exogenous exposure to H₂O₂ has been shown to reduce photosynthetic efficiency (Collén & Pedersén 1996, Collén & Davison 1999, Dummermuth et al. 2003, Vega et al. 2006), while respiration is less sensitive (Collén & Pedersén 1996, Vega et al. 2006). The effects of exogenous exposure on photosynthetic capacity, saturation, or compensation depth have, however, not been assessed to date for macroalgae.

Aquaculture is projected to expand dramatically in the near future, and thorough risk assessments which include all potential impacts associated with the industry are essential to maintain healthy coastal ecosystems. Furthermore, *S. latissima* has been proposed as an excellent species for integrated multi-trophic aquaculture (IMTA) in cold-temperate waters (e.g. Petrell et al. 1993, Barrington et al. 2009, Handå et al. 2013), and the best practices for cultivation integrated with salmonid farming are currently being

intensely investigated in Norway (reviewed in Stévant et al. 2017). Mortality of *S. latissima* cultivated at fish farms has been observed, and coinciding H₂O₂ treatments may have been a possible factor (B. T. Haugland et al. unpubl. data).

Consequently, the aim of the present study was to evaluate the toxicity of H₂O₂ on *S. latissima*, mainly to (1) determine potential risks associated with emissions for natural *S. latissima* populations, and, additionally, to (2) determine if treatment with H₂O₂ at IMTA farms is innocuous to cultivated *S. latissima*. To achieve this, the toxicity potential of a 1 h exposure to ecologically relevant levels of H₂O₂ on mortality and photosynthetic activity of juvenile *S. latissima* was assessed at 3 time points post-exposure. Novel to effects of H₂O₂ exposure on macroalgae, photosynthetic capacity, compensation depth, and saturating irradiance were assessed, in addition to photosynthetic efficiency and dark respiration. LC₅₀, EC₅₀ and NEC values were also determined.

2. MATERIALS AND METHODS

2.1. Collection and experimental design

First-year sugar kelp *Saccharina latissima* was collected by free-diving in the upper subtidal zone (1–3 m depth) at Hjeltestad, SW of Bergen, Norway (60° 15' 40.4" N, 5° 12' 31.7" E) in March 2017 and transported to the Institute of Marine Research, Bergen, in dark plastic bags to avoid exposing the plants to high irradiances (after Dummermuth et al. 2003). Prior to initiation of the experiment, plants were kept at their collection temperature of 8°C in 15 l aquariums for minimum 24 h. Six fluorescent daylight lamps (OSRAM L 58W/954) provided irradiance of approximately 100 μmol photons m⁻² s⁻¹ (photosynthetically active radiation, PAR) at a natural seasonal regime of 12 h light: 12 h dark. A submersible micropump (flow rate: 150 l h⁻¹) maintained circulation of the water. Seawater in the aquarium was changed every other day, and no growth medium was added.

A preliminary study was conducted to define the dose–response relationship between H₂O₂ concentrations and lethal effects on *S. latissima*, ranging from 100% mortality to no effect (see Appendix). In addition to identifying the concentrations to be applied in the main experiment, the preliminary study also indicated that H₂O₂ affected the lamina tissue in patches, and not uniformly across the whole lamina. Whole-plant exposure and incubation (after

Andersen et al. 2013) was therefore chosen over lamina-disc incubation to better simulate how *S. latissima* plants would be impacted in nature in terms of exposure effects, post-exposure effects, and survival.

Consequently, following lab acclimatization, 5 healthy plants without wounds or fouling were chosen, numbered individually, measured by volume, and randomly assigned to 1 of 5 H₂O₂ exposure concentrations: 170 mg l⁻¹ (10% of the bath-treatment dose), 85 mg l⁻¹ (5%), 17 mg l⁻¹ (1%), 1.7 mg l⁻¹ (0.1%), or a seawater control (i.e. no H₂O₂). These concentrations were based on our preliminary dose–response study (see Appendix) and ranged from 10% to 0.1% of the bath-treatment dose of 1700 mg H₂O₂ l⁻¹ recommended by the producer (www.felleskataloge.no) for a temperature of 8°C. Plants were exposed in individual 2 l beakers for 1 h under low (50 PAR) light conditions. A total of 30 plants were included in the main study and divided equally between the 5 H₂O₂ concentrations (i.e. 6 replicate plants per concentration). A randomized schedule was prepared *a priori* to eliminate potential day effects and incubation chamber effects in the dataset. Following H₂O₂ exposure, plants were flushed with fresh seawater for a couple of minutes to remove any H₂O₂ on the surface before being transferred to individual incubation chambers per the randomized schedule. Exposures and incubations immediately after exposure were conducted over 9 consecutive days, as 5 plants were exposed at a time. As the preliminary study indicated that effects may not be apparent until several hours post-exposure, incubations to determine the effects on photosynthesis were conducted at 3 post-exposure time points: immediately after exposure (Day 0), 24 h post-exposure (Day 1), and 15 d post-exposure (Day 15), giving a total of 90 individual incubations.

2.2. Mortality

Determining time of mortality in macroalgae is challenging, and to date there are no standardized methods. From the preliminary study, it was found that it is essential to keep the plants in the laboratory for at least 7 d post-exposure to be able to determine mortality with certainty (see Appendix). The endpoint 'mortality' was determined to be when the lamina disintegrated and/or detached from the stipe when the plant was lifted out of the aquarium by the holdfast. In the main study, each individual was inspected daily and pictures were taken at the end of the 15 d post-exposure period or when the lamina showed visible signs of disintegration.

2.3. Effects on photosynthesis

To assess effects on photosynthetic activity (photosynthetic efficiency, α ; photosynthetic capacity, P_{MAX} ; dark respiration, R_D ; compensation irradiance, I_C ; and saturating light intensity, I_{SAT}), the methodology of Gerard (1988), Henley & Dunton (1997), and Andersen et al. (2013) was followed. Plants were incubated to construct a photosynthesis–irradiance (PI) curve based on measured oxygen (O₂) production or consumption over 11 different light intensities (0–350 PAR), starting with R_D (Table 1).

Incubations were conducted in 600 ml transparent, gas-tight, rectangular prisms (width: 5 cm, length: 15 cm, height: 15 cm) using magnetic stirring for water circulation. The magnetic stirrers were set to the same level (1250 rpm) to ensure the boundary layers around the thallus would be similar for all plants. Filtered seawater (glass-fibre filter, 1 μ m) was bubbled with N₂ gas to ~60% air saturation to prevent oversaturation of O₂ in the chambers during incubation. One thallus was hung within each incubation chamber from the lid. The incubation chambers were separated by partitions and illuminated directly from the front by one LED lamp (V-Light E14 dimmable HALED bulb; 230 V, 7 W). The rectangular shape of the chambers allowed for the thallus to be fixed in a perpendicular position to the light source

Table 1. Light levels used to generate photosynthesis–irradiance curves for juvenile *Saccharina latissima*. Values presented are the range (min. to max.) of measured light intensity, and time held, at each light level. α : photosynthetic efficiency, I_C : compensation irradiance, PAR: photosynthetically active radiation, P_{MAX} : photosynthetic capacity, R_D : dark respiration

Light level	Light intensity (PAR, μ mol photons m ⁻² s ⁻¹)	Time interval (min)
0 ^a (R_D)	0	120
1 ^a	10–12	30
2 ^a	19–22	30
3 ^a	29–32	30
4 ^a	42–45	30
5	64–66	20
6	84–88	20
7	125–128	10
8	146–150	10
9 ^b	169–174	10
10 ^b	230–235	8
11 ^b	347–350	8

^aBelow-saturating light levels used for mixed linear regression to determine α and I_C
^bAbove-saturating light levels used to determine P_{MAX}

throughout the incubation, independently of the water circulation.

The 11 different light levels (Table 1) were attained using a dimmer and density screens while measuring the PAR at every light level. Using separate light sources for each chamber increased control and allowed for adjustments to be made during incubations to ensure that all chambers were exposed to the same amount of irradiance at the different light levels for every incubation. Irradiance was measured as the maximum PAR hitting the front of the chamber at the centre point of the lamina. PAR was measured with a LI-190R quantum cosine-corrected sensor (up to 82° angle of incidence) coupled to a LI-250A light meter (LI-COR).

At each light level, changes in O₂ within the incubation chamber were determined using an optical non-invasive methodology (modified from Rastrick & Whiteley 2011). Each incubation chamber was internally equipped with an O₂ sensor spot and aligned with an exterior fibre optic sensor probe connected to a PreSens® OXY-10 mini sensor. OXY-10 software converted fluorescent readings to changes in seawater O₂ levels, and logged the values every 15 s. The plants were therefore held at each light level for a *priori* set time intervals (determined from test runs) until a ~5% change in O₂ was observed (Table 1), ensuring that all individuals were exposed to the same conditions. For all incubations, 1 control chamber was kept empty, from which the temperature was recorded (LabQuest® 2 multimeter, Vernier) and logged every 15 s. Relative humidity (RH %), barometric pressure (kPa), and salinity were also recorded for each incubation (LabQuest® 2 multimeter and Logger Lite software, Vernier). Immediately after incubation, the plants were blotted, weighed (fresh weight; FW), and transferred back to the aquarium.

2.4. Data processing

To obtain the rate of O₂ production or consumption for each light level, the following calculations were conducted. O₂ concentration (µmol O₂) over time in the incubation chambers was calculated from % O₂ values transformed to *p*O₂ using measured barometric pressure (adjusted for vapour pressure and relative humidity), multiplied by the solubility coefficients (after Benson & Krause 1980, 1984, adjusted for temperature and salinity) and the volume of seawater within each incubation chamber (adjusted for the volume of the sample). The values from incubation chambers containing a sample were then

corrected for changes in the control chamber. FW-standardized rate of O₂ consumption or production at each light level was calculated from the linear regression of O₂ concentration (µmol O₂) against time (h) divided by the FW of samples, giving rates expressed in µmol O₂ gFW⁻¹ h⁻¹. The first 2 min were excluded from each linear regression to allow the plants to adjust to the new light level, resulting in a minimum of 24 data points being used in all regressions (i.e. to determine net rate of photosynthesis for each light level). Rates of oxygen production or consumption plotted against light intensity yielded 1 PI curve for each individual plant for each of the 3 time points (Days 0, 1, and 15). Photosynthetic parameters (α , P_{MAX} , R_D , I_C , and I_{SAT}) could subsequently be calculated from the plotted curves.

α , defined as the initial slope of the PI curve at below-saturating light intensities, was obtained by fitting a linear mixed model (LMM) to a subset of the data including only the first light levels (see Table 1). I_C , defined as the light requirements for a net photosynthetic rate of zero, was obtained from the same LMM. P_{MAX} was calculated by averaging 3 values from above-saturating light levels (see Table 1). Subsequently, I_{SAT} was determined as the intercept between P_{MAX} and α . R_D was determined as O₂ consumption in darkness. Some of the plants exposed to 85 and 170 mg H₂O₂ l⁻¹ demonstrated negative P_{MAX} and/or negative α values. These were excluded (number of included plants is indicated in the figures) when calculating the light parameters (I_{SAT} and I_C), as inclusion would have produced unrealistic values.

2.5. Fitting dose–response curves to determine toxicity potentials

The extension package *drc* version 3.0-1 (Ritz et al. 2015) for R software (R Core Team 2017) was used to model the dose–response relationships. The statistical package provides a range of different regression models, a model-select function, and methods to extract model parameters such as EC₅₀, LC₅₀, and NEC using the delta method (Ritz et al. 2015). To determine the lethal toxicity potentials, mortality of *S. latissima* plants on Day 15 was regressed over H₂O₂ concentration using the model that gave the best fit: a 2-parameter log-logistic model with a binomial distribution and with the upper and lower asymptotes set to 1 and 0, respectively:

$$Y = \frac{1-0}{1 + \exp[b(\log(x) - \log(e))]} \quad (1)$$

where Y is the response, x is the dose, b is the slope of the line, and e is the inflection point of the curve (in this case also the LC_{50} value) (Ritz et al. 2015). To determine the photosynthetic toxicity potentials, the photosynthetic parameters that showed potential for being indicators for immediate effect (P_{MAX} and α) were regressed over H_2O_2 concentration. The models that gave the best fit were the 3-parameter log-logistic model (P_{MAX} , Eq. 2) and the 3-parameter Weibull 1 model (α , Eq. 3), both models with the lower asymptote set to 0:

$$Y = 0 + \frac{d - 0}{1 + \exp[b(\log(x) - \log(e))]} \quad (2)$$

$$Y = 0 + (d - 0) \exp\{-\exp[b(\log(x) - \log(e))]\} \quad (3)$$

where Y is the response, x is the dose, d is the upper asymptote, b is the slope of the line, and e is the inflection point of the curve (Ritz et al. 2015).

2.6. Statistical analysis

The effect of H_2O_2 concentration on photosynthesis (α , P_{MAX} , R_D) was tested by fitting generalized least squares (GLS) models using the package *nlme* (Pinheiro et al. 2017), where H_2O_2 concentration (5 levels: control, 1.7, 17, 85, and 170 mg l⁻¹) and time (2 levels: Day 0 and Day 1) were used as fixed effects. Loss of plants in the 85 and 170 mg l⁻¹ concentrations on Day 15 prevented the running of a full statistical model on all time points. As each plant was repeatedly measured over time, ‘plant’ was treated as a random factor and incorporated with a correlation term, allowing dependency between the response variables from the same plant (Zuur et al. 2009). Heteroscedasticity was obtained by allowing differing variances across H_2O_2 concentration for α and P_{MAX} and across time for R_D . The significance of the interaction H_2O_2 concentration \times Time and the 2 fixed effects were tested with the likelihood ratio test, and only significant terms were included in the final model (Zuur et al. 2009).

3. RESULTS

3.1. Mortality

Results from the preliminary study showed 100% mortality when exposing juvenile sugar kelp *Saccharina latissima* for 1 h to the commercially recommended bath-treatment dose of 1700 mg l⁻¹ and for

concentrations down to 10% (170 mg l⁻¹) of this dose (see Appendix).

In the main experiment, no mortality was observed on Day 0 or Day 1 in any of the concentrations ranging from no added H_2O_2 (seawater control) to 170 mg H_2O_2 l⁻¹. At the end of the 15 d post-exposure period, mortality had increased to 100% in the 170 mg l⁻¹ concentration and to 67% in the 85 mg l⁻¹, while there was no mortality in plants exposed to 17 mg l⁻¹, 1.7 mg l⁻¹, or in the control (Fig. 1). On Day 15, the control plants showed no fragmentation or sign of pigment loss. Plants in the 170 mg l⁻¹ concentration, however, had lost pigments in larger parts of the lamina after 3 d (Fig. 2A). This was followed by continuous fragmentation until all plants in the 170 mg l⁻¹ concentration were completely disintegrated within 10 d. The same course of events was observed for the 4 plants that died in the 85 mg l⁻¹ concentration: loss of pigments followed by fragmentation and complete disintegration. The LC_{50} for juvenile *S. latissima* was determined to be 80.7 ± 53.5 mg l⁻¹ and the NEC to be 72.9 ± 0.4 mg l⁻¹ (Table 2).

3.2. Effects on photosynthesis

The PI relationship of control plants followed a typical pattern at all 3 time points: a linear increase in net photosynthetic rate for below-saturating light intensities (0 to 43.5 PAR) followed by a gradual decrease for above-saturating intensities (43.5 to 348 PAR) (Fig. 3). Exposure for 1 h to concentrations of 170 and 85 mg H_2O_2 l⁻¹ resulted in immediate negative impacts on the net photosynthetic rate on Day 0, reducing the median (\pm SD) rate compared to

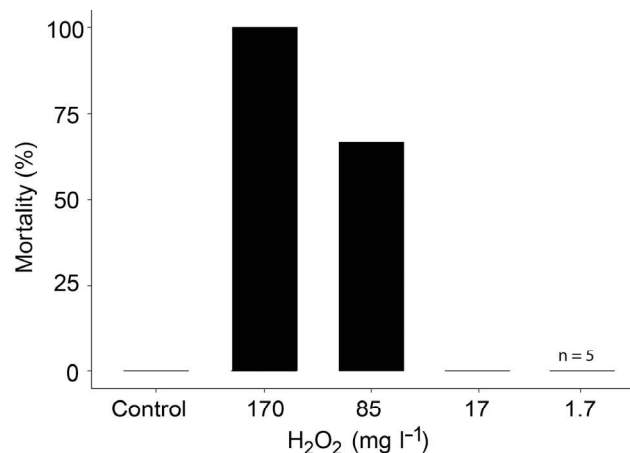


Fig. 1. Mortality of juvenile *Saccharina latissima* plants 15 d after 1 h exposure to 5 different H_2O_2 concentrations, including control (n = 6 unless otherwise stated)

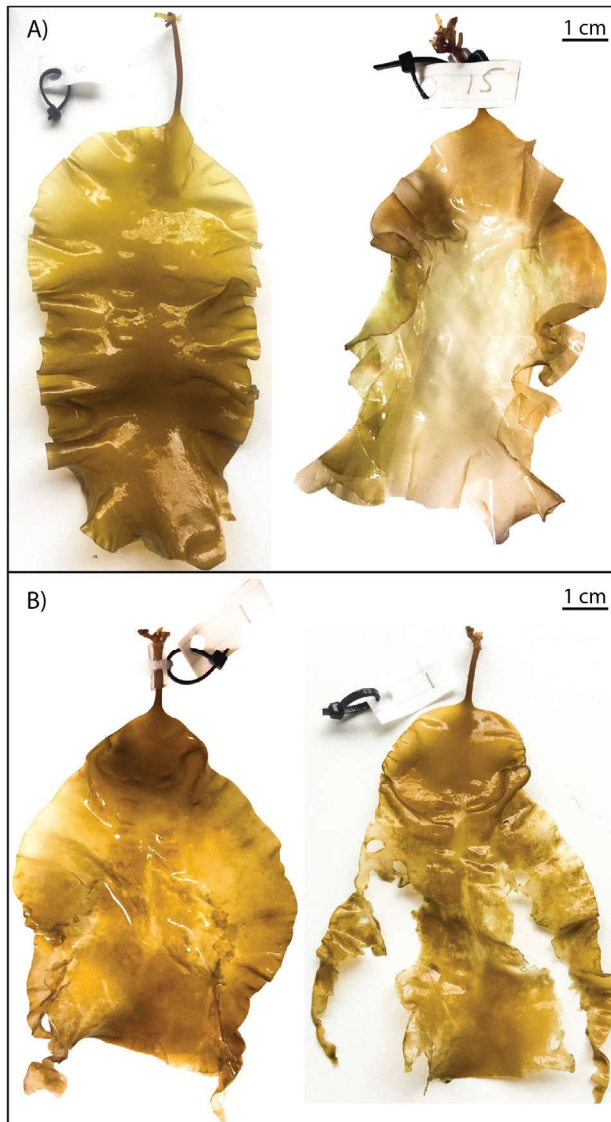


Fig. 2. Effects of different concentrations of H₂O₂ on lamina of juvenile *Saccharina latissima* plants at different time intervals. (A) Normal lamina with no visible damages 15 d post-exposure to concentrations ≤ 17 mg l⁻¹ (left), and discoloured and pale lamina 3 d post-exposure to 170 mg l⁻¹ (right); (B) discoloured lamina due to pigment loss 8 d post-exposure to 85 mg l⁻¹ (left), and lamina with loss of tissue due to cell death 15 d post-exposure to 85 mg l⁻¹ (right)

that of the control plants at both below-saturating irradiance (43.5 PAR; control: 13.99 ± 3.73 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 170 mg l⁻¹: -0.72 ± 1.01 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 85 mg l⁻¹: 0.17 ± 1.27 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹) and at the highest irradiance (348.5 PAR; control: 45.62 ± 8.77 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 170 mg l⁻¹: -0.77 ± 3.64 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 85 mg l⁻¹: 0.59 ± 3.37 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹). A slightly lower median (\pm SD) net photosynthesis was demonstrated by plants in the 17 mg H₂O₂ l⁻¹

Table 2. Toxicity potentials ($\pm 95\%$ CI, mg l⁻¹) of a 1 h exposure to H₂O₂ on juvenile *Saccharina latissima* for mortality and photosynthesis (α : photosynthetic capacity, P_{MAX} : photosynthetic efficiency). LC₅₀ and LC₉₀: median lethal concentrations and EC₅₀ and EC₉₀: median effective concentrations, for 50% and 90% of the population, respectively; NEC: no-effect concentration

	LC ₅₀	LC ₉₀	NEC
Mortality	80.7 \pm 53.5	95.1 \pm 134.1	72.9 \pm 0.4
	EC ₅₀	EC ₉₀	NEC
α	35.4 \pm 13.4	77.7 \pm 40.7	13.1 \pm 11.2
P_{MAX}	27.8 \pm 9.1	57.5 \pm 39.8	13.1 \pm 11.2

concentration immediately post-exposure at high light intensities (148 PAR: 31.92 ± 7.15 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 348.5 PAR: 33.47 ± 4.46 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹) compared to the control (148 PAR: 37.22 ± 5.85 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹), though not at the lower intensities. Plants exposed to 1.7 mg l⁻¹ concentration showed comparable median net photosynthesis across all irradiances to the control.

On Day 1, photosynthetic rates of *S. latissima* plants exposed to ≥ 85 mg H₂O₂ l⁻¹ were still heavily impacted at all light intensities, showing highly reduced maximum rates compared to the control plants (control: 41.87 ± 5.40 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 170 mg l⁻¹: 0.40 ± 3.02 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 85 mg l⁻¹: 8.15 ± 5.07 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; median \pm SD). Net photosynthetic rates of plants in the 17 and 1.7 mg l⁻¹ concentrations were similar to that of the control for all light intensities.

On Day 15, control plants showed a lower median net photosynthesis compared to Day 0: 9.65 ± 1.77 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ at 43.5 PAR, 18.14 ± 3.84 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ at 148 PAR, and 24.87 ± 4.64 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ at the highest light intensity (348.5 PAR). The 2 surviving plants in the 85 mg l⁻¹ concentration still showed signs of impact from the H₂O₂ exposure under light intensities up to 171.5 PAR, demonstrating a 50% reduction in net photosynthesis, while the response to high light > 200 PAR appeared to have recovered more.

The reduced net photosynthetic rate found for plants exposed to concentrations ≥ 85 mg l⁻¹ resulted in a drop in α and P_{MAX} on Day 0 compared with the control plants (Fig. 4). Median (\pm SD) α -value for the 2 highest concentrations (170 mg l⁻¹: 0.00 ± 0.01 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ / $\mu\text{mol m}^{-2} \text{s}^{-1}$; 85 mg l⁻¹: 0.02 ± 0.03 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ / $\mu\text{mol m}^{-2} \text{s}^{-1}$) were significantly different ($p < 0.001$, Table 3) from the control

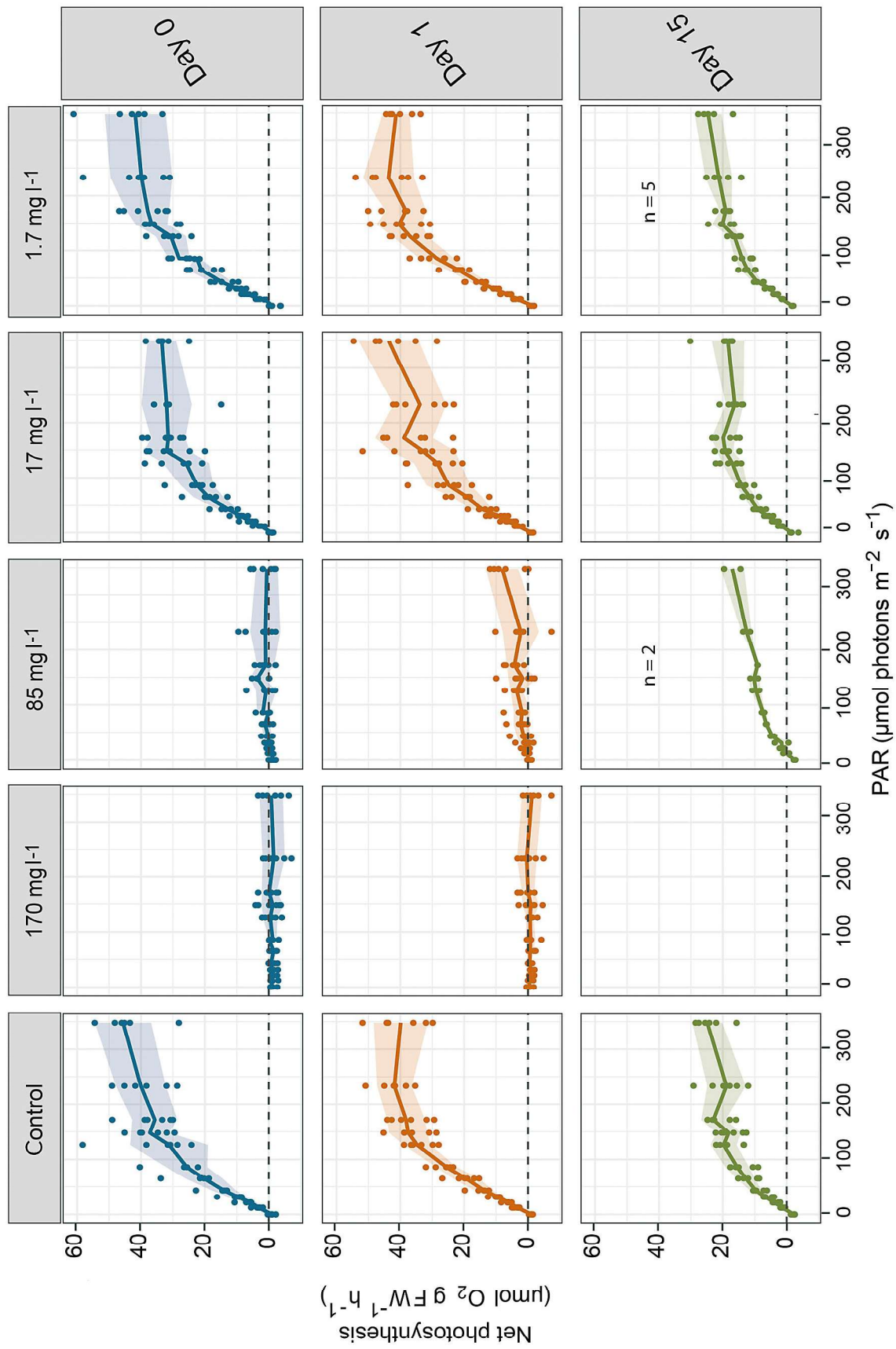


Fig. 3. Photosynthesis–irradiance curves for juvenile *Saccharina latissima* plants exposed for 1 h to 5 different concentrations of H_2O_2 , including control. Raw data and median (± 1 SD) for immediately post-exposure (Day 0), 24 h post-exposure (Day 1), and 15 d post-exposure (n = 6 unless otherwise stated). FW: fresh weight, PAR: photosynthetically active radiation

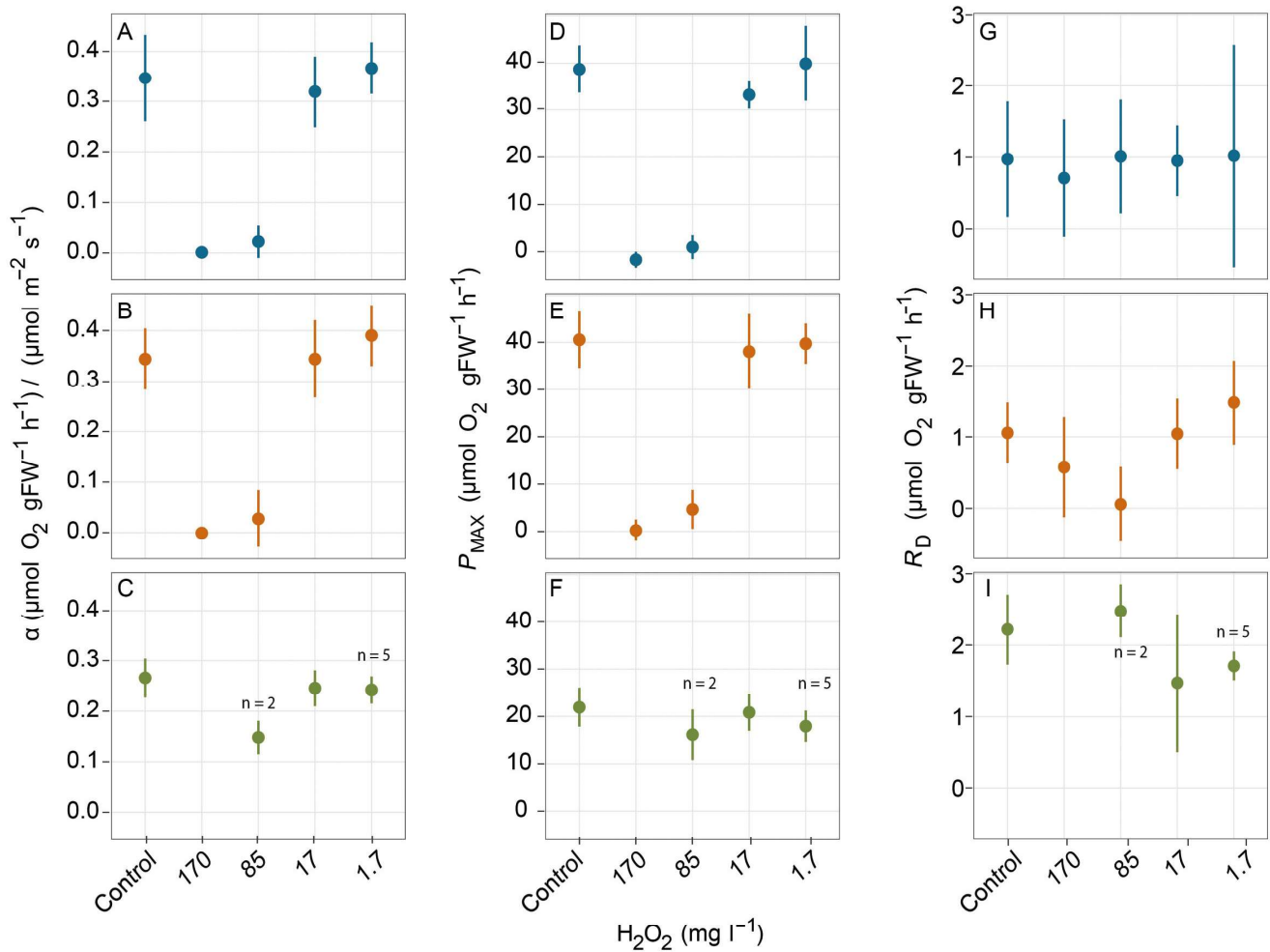


Fig. 4. Effect after 1 h exposure to 5 different concentrations of H₂O₂, including control, on photosynthetic parameters (A–C: α : photosynthetic efficiency, D–F: P_{MAX} : maximum photosynthetic rate at high light, G–I: R_{D} : respiration rate in the dark) of juvenile *Saccharina latissima* plants. Median (± 1 SD) response (A,D,G) immediately post-exposure (Day 0), (B,E,H) 24 h post-exposure (Day 1), and (C,F,I) 15 d post-exposure ($n = 6$ unless otherwise stated). FW: fresh weight

($0.35 \pm 0.09 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$). For concentrations $\leq 17 \text{ mg l}^{-1}$, however, there were no significant differences compared to the control (17 mg l^{-1} : $0.32 \pm 0.07 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$, $p = 0.429$; 1.7 mg l^{-1} : $0.37 \pm 0.05 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$, $p = 0.543$; median \pm SD). No significant H₂O₂ concentration \times time interaction ($p = 0.078$, $\text{df} = 2$) or significant differences between Day 0 and Day 1 ($p = 0.615$, $\text{df} = 11$) were found; hence α was equally unaffected by time in all concentrations.

The median (\pm SD) P_{MAX} for plants in the 170 and 85 mg l^{-1} concentrations (-1.70 ± 1.69 and $0.97 \pm 2.50 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$, respectively) were significantly different to that of the control ($38.71 \pm 5.04 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$) on Day 0 ($p < 0.001$, Table 3). For plants exposed to 17 mg l^{-1} , the median P_{MAX} (\pm SD) was lower ($33.15 \pm 2.88 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$)

than that of the control plants, but neither this nor the P_{MAX} of plants exposed to 1.7 mg l^{-1} were significantly different to the control (17 mg l^{-1} : $p = 0.077$; 1.7 mg l^{-1} : $p = 0.594$). No significant H₂O₂ concentration \times time interaction ($p = 0.531$, $\text{df} = 2$) or significant differences between Day 0 and Day 1 ($p = 0.125$, $\text{df} = 11$) were found; hence P_{MAX} was equally unaffected by time in all concentrations.

Based on these results, the EC₅₀ for α for juvenile *S. latissima* plants to H₂O₂ exposure was determined to $35.4 \pm 13.4 \text{ mg l}^{-1}$, while the EC₅₀ for P_{MAX} was determined to $27.8 \pm 9.1 \text{ mg l}^{-1}$ (Table 2). The determined NEC value for both photosynthetic parameters was $13.1 \pm 11.2 \text{ mg l}^{-1}$.

Median (\pm SD) respiration rate (R_{D}) was not affected in any concentration on Day 0 compared to the control ($0.97 \pm 0.81 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$) (Fig. 4). On Day 1, me-

Table 3. Estimated regression parameters (H_2O_2 concentration, and time), standard error (SE), t -values, and p -values from generalized least squares (GLS) models fitted to the photosynthetic response variables. Only significant terms were included in the final models

Source of variation	Estimate	SE	t	p
α (photosynthetic efficiency)				
Intercept	0.36808	0.024391	15.09055	<0.001
1.7 mg l^{-1}	0.021754	0.035521	0.612437	0.543
17 mg l^{-1}	-0.02804	0.035201	-0.79665	0.429
85 mg l^{-1}	-0.33031	0.029399	-11.2355	<0.001
170 mg l^{-1}	-0.3708	0.024876	-14.906	<0.001
P_{MAX} (photosynthetic rate at high light)				
Intercept	39.88723	1.625547	24.53773	<0.001
1.7 mg l^{-1}	1.36651	2.545536	0.536824	0.594
17 mg l^{-1}	-4.48864	2.49097	-1.80197	0.077
85 mg l^{-1}	-36.8761	1.956721	-18.8459	<0.001
170 mg l^{-1}	-40.7071	1.746224	-23.3115	<0.001
R_{D} (respiration rate at low light)				
Intercept	1.2415940	0.2714216	4.574411	<0.001
1.7 mg l^{-1}	0.4078867	0.3196865	1.275896	0.208
17 mg l^{-1}	0.1167600	0.3196865	0.365233	0.716
85 mg l^{-1}	-0.7006096	0.3196865	-2.191552	0.033
170 mg l^{-1}	-0.3700247	0.3196865	-1.157461	0.252
Day 1	-0.2961013	0.1466637	-2.018913	0.049

dian (\pm SD) R_{D} was significantly reduced to $0.06 \pm 0.53 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$ for plants exposed to 85 mg l^{-1} ($p = 0.033$, Table 3). The R_{D} of plants in concentrations 170 and $\leq 17 \text{ mg l}^{-1}$ were not significantly different to that of the control (170 mg l^{-1} : $p = 0.252$; 17 mg l^{-1} : $p = 0.716$; 1.7 mg l^{-1} : $p = 0.208$; Table 3). Unlike P_{MAX} and α , there was a significant difference over time from Day 0 to Day 1 ($p = 0.049$, Table 3), most likely related to the change in the 85 mg l^{-1} concentration. The interaction H_2O_2 concentration \times time was non-significant ($p = 0.130$, $df = 2$).

Median (\pm SD) I_{SAT} was reduced to $57 \pm 36 \text{ PAR}$ for plants exposed to 85 mg l^{-1} on Day 0 compared to the control ($106 \pm 16 \text{ PAR}$) (Fig. 5). This reduction was caused by the observed drop in P_{MAX} and α . In the 170 mg l^{-1} concentration, only 1 plant met the criteria (see 'Materials and methods') to calculate the I_{SAT} (9861 PAR). On Day 1, the variation (SD) around the median was highly inflated for plants exposed to these 2 concentrations (170 mg l^{-1} : $511 \pm 459 \text{ PAR}$; 85 mg l^{-1} : $89 \pm 305 \text{ PAR}$) compared to the control ($108 \pm 13 \text{ PAR}$). Due to the high variation, and in 1 case, lack of replicates, further interpretation of this response parameter for plants exposed to $\geq 85 \text{ mg l}^{-1}$ on Day 0 and Day 1 may be misleading and is thus avoided (Fig. 5). For concentrations $\leq 17 \text{ mg l}^{-1}$, the median (\pm SD) I_{SAT} values were similar to the control both on Day 0 (17 mg l^{-1} : $102 \pm 13 \text{ PAR}$; 1.7 mg l^{-1} :

$107 \pm 17 \text{ PAR}$) and Day 1 (control: $108 \pm 14 \text{ PAR}$; 17 mg l^{-1} : $102 \pm 19 \text{ PAR}$; 1.7 mg l^{-1} : $101 \pm 13 \text{ PAR}$) (Fig. 5).

Median I_{C} (\pm SD) was 98 and 9 times higher on Day 0 for plants in concentrations 170 mg l^{-1} ($297 \pm 2034 \text{ PAR}$) and 85 mg l^{-1} ($29 \pm 141 \text{ PAR}$), respectively, compared to the control ($3 \pm 2 \text{ PAR}$) (Fig. 6). The higher I_{C} was attributed to the drop in α values, as there was no acute effect on R_{D} (Fig. 4). Due to the high variation (SD), the values were deemed uncertain, as was the I_{C} for the same concentrations on Day 1 (170 mg l^{-1} : $231 \pm 245 \text{ PAR}$; 85 mg l^{-1} : $14 \pm 14 \text{ PAR}$), and further interpretation was not conducted. The median (\pm SD) I_{C} for the 17 mg l^{-1} ($3 \pm 1 \text{ PAR}$) and 1.7 mg l^{-1} ($3 \pm 3 \text{ PAR}$) concentrations were comparable to the control. On Day 1, the I_{C} showed no change for the $\leq 17 \text{ mg l}^{-1}$ concentrations, including for the control (control: $3 \pm 1 \text{ PAR}$; 17 mg l^{-1} : $3 \pm 1 \text{ PAR}$; 1.7 mg l^{-1} : $3 \pm 1 \text{ PAR}$).

3.3. Prolonged effects of H_2O_2

On Day 15, median (\pm SD) α of control plants was $0.27 \pm 0.04 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Fig. 4). The 2 plants that survived the 85 mg l^{-1} concentration demonstrated a 50 % reduction in α ($0.15 \pm 0.03 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$) compared to the control (Fig. 4), causing a 50 % higher I_{C} (85 mg l^{-1} : $15 \pm 6 \text{ PAR}$, control: $7 \pm 2 \text{ PAR}$) (Fig. 6). The other parameters (P_{MAX} , R_{D} , and I_{SAT}) were comparable to the control for all concentrations. The parameters of the control plants had changed over time; the P_{MAX} and α showed a reduction of 24 % and 20 %, respectively, while R_{D} had increased from 0.97 ± 0.81 to $2.22 \pm 0.49 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$, leading to a higher I_{C} of $7 \pm 1 \text{ PAR}$ and a lower I_{SAT} of $81 \pm 30 \text{ PAR}$.

The 2 plants that survived the 85 mg l^{-1} concentration had the highest initial biomass compared to other plants in the same concentration. Some loss of pigments was observed after 8 d, shown as paler patches on the lamina (Fig. 2B). Reduced structural integrity of the lamina was observed in the same areas as the pigment loss, leading to tearing. After 15 d, these patches had disintegrated, leaving a degraded lamina (Fig. 2B). A 20 % reduction in biomass from Day 0 to Day 15 was demonstrated by these 2 plants, whereas plants in the control showed an average increase of 105 % (Fig. 7). Plants in the 17 and 1.7 mg l^{-1} concentrations showed respectively 88 % and 110 % increase in biomass in the same period (Fig. 7).

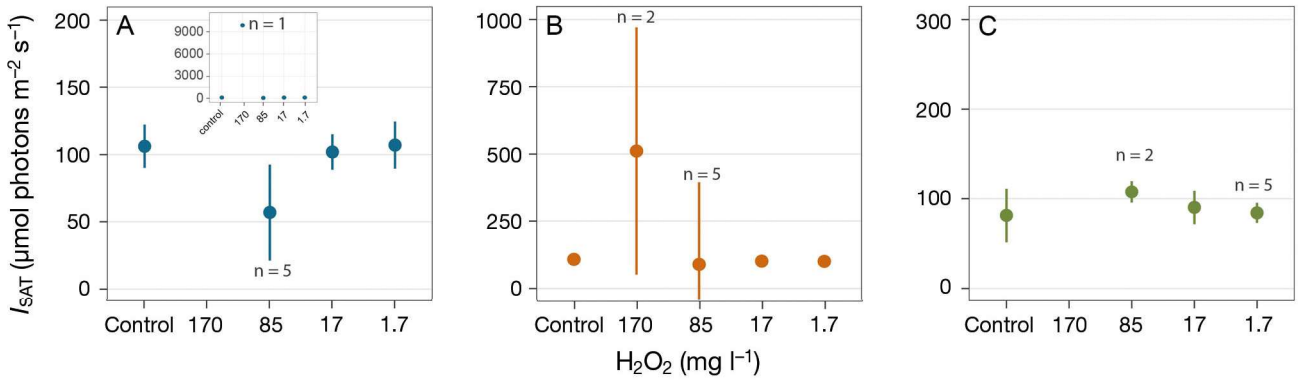


Fig. 5. Effect of 1 h exposure to 5 different concentrations of H₂O₂, including control, on the saturating irradiance (I_{SAT}) of juvenile *Saccharina latissima* plants. Median (± 1 SD) response (A) immediately post-exposure (Day 0), (B) 24 h post-exposure (Day 1), and (C) 15 d post-exposure ($n = 6$ unless otherwise stated). Note different y-axis scales. Inset: zoomed-out view of plant I_{SAT}

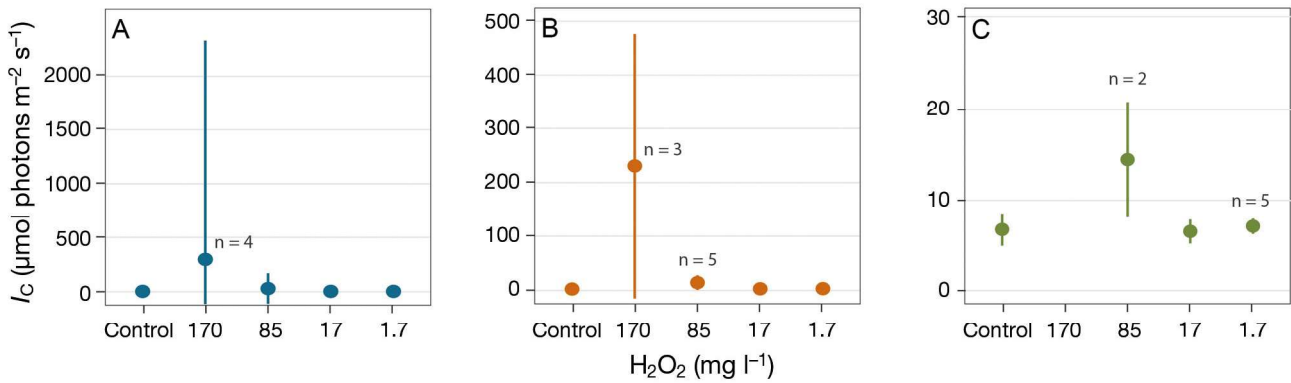


Fig. 6. Effect of 1 h exposure to 5 different concentrations of H₂O₂, including control, on the light compensation irradiance (I_C) of juvenile *Saccharina latissima* plants. Median (± 1 SD) response (A) immediately post-exposure (Day 0), (B) 24 h post-exposure (Day 1), and (C) 15 d post-exposure ($n = 6$ unless otherwise stated). Note different y-axis scales

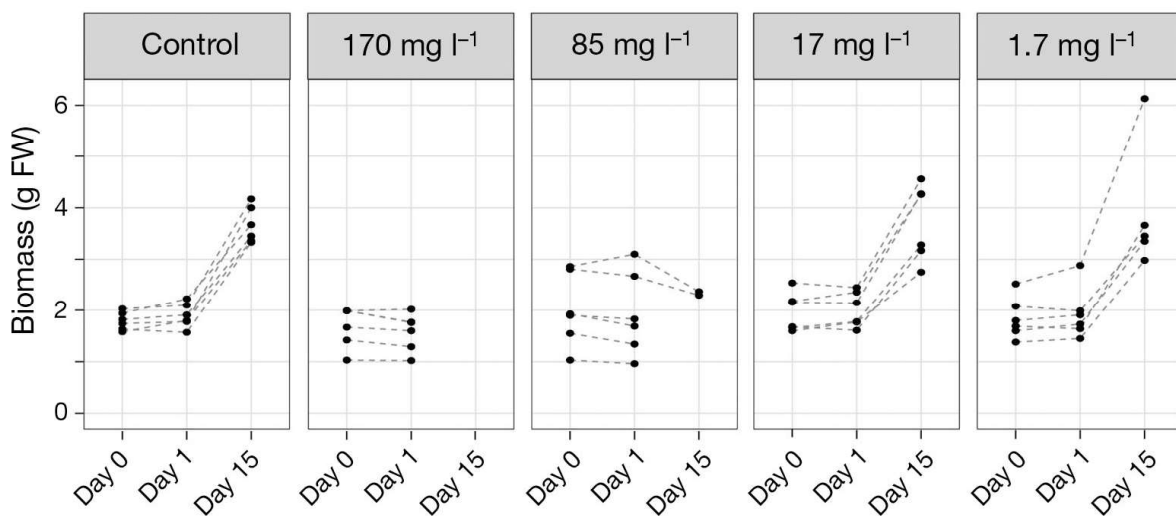


Fig. 7. Effect of 1 h exposure to 5 different concentrations of H₂O₂, including control, on biomass of individual juvenile *Saccharina latissima* plants over time at intervals immediately post-exposure (Day 0), 24 h post-exposure (Day 1), and 15 d post-exposure. FW: fresh weight

4. DISCUSSION

This study demonstrates for the first time the potential consequences of emissions of H_2O_2 , a common sea-lice therapeutant in salmon farming, on the survival and photosynthetic performance of the habitat-building sugar kelp *Saccharina latissima* in marine ecosystems. Mortality of juvenile *S. latissima* was observed for plants exposed to concentrations as low as 85 mg l^{-1} , equivalent to only 5% of the treatment dose commonly used in net pens (1700 mg l^{-1}). The impact of an H_2O_2 concentration of 85 mg l^{-1} and higher was immediate, reducing both P_{MAX} and α by >90%. Furthermore, we saw prolonged effects 15 d post-exposure for individuals that survived the 85 mg l^{-1} concentration, both in terms of decreased biomass and reduced α and I_C . The LC_{50} and EC_{50} values indicate that *S. latissima* is highly sensitive to H_2O_2 levels that natural local populations could be exposed to from aquaculture emissions.

4.1. Lethal effects of H_2O_2

The highly reduced photosynthetic performance and subsequent mortality of 83% of the plants exposed to concentrations $\geq 85 \text{ mg l}^{-1}$ strongly indicates that the antioxidative scavenging system of juvenile *S. latissima* was not able to cope with H_2O_2 concentrations this high, and that oxidative stress occurred. Oxidative stress caused by H_2O_2 may lead to irreversible damages through the auto-destruction of cells (e.g. membrane leakage, oxidation of lipids) and inhibition of photosynthesis (Davison 1991, Collén & Pedersén 1996, Dummermuth et al. 2003). The observed pigment loss and more flaccid lamina that preceded mortality suggest that oxidation of pigments, membrane leakage, and cell death are possible main causes for the reduced photosynthesis and ultimate plant mortality seen in this study. Inhibition and destruction of photosystem II may also have played a role, as has been suggested as a cause for cell death post- H_2O_2 exposure of the macroalgae sea lettuce *Ulva rigida* (Collén & Pedersén 1996), though this was not tested in this study.

A much lower H_2O_2 concentration caused mortality for *U. rigida* (10.2 mg l^{-1} ; Collén & Pedersén 1996) than for *S. latissima* (85 mg l^{-1} ; present study). The difference could be related to plant structure, as a higher stress tolerance has been linked to the thickness of the thallus (Roleda et al. 2007). *S. latissima* has a thicker, corticated, and thus more complex thallus structure than *U. rigida*, which has distro-

matic (2 cell-layers thick) thallus (Bold & Wynne 1978). Furthermore, the Laminariales contain the carbohydrate mannitol that can act as an antioxidant and stabilize proteins (Bartsch et al. 2008). The use of whole plants in the present study compared to tissue discs (2 cm in diameter) in the *U. rigida* study might have also influenced mortality; H_2O_2 always affected the *S. latissima* lamina in patches, and one could infer that the larger the lamina, the larger the chance that healthy, unaffected tissue is left post-exposure that could aid in lamina regeneration. Older *S. latissima* sporophytes have been shown to be more tolerant than juveniles to high light stress, and to recover faster after damage to the photosystem (Hanelt et al. 1997). It is therefore likely that older sporophytes are also more tolerant to H_2O_2 stress than juveniles, both due to physiology and lamina size. The position of the patch, i.e. a non-affected meristem, will most likely also increase chance of survival, as has been observed in relation to bryozoan cover on the lamina of *S. latissima* (pers. obs.). Both higher initial biomasses and unaffected meristems might have increased the endurance and led to survival of 2 of the plants in the 85 mg l^{-1} concentration.

4.2. Effects of H_2O_2 on photosynthesis and carbon budget

Our results demonstrate that the antioxidative system of *S. latissima* plants can cope with exogeneous exposure to low ($\leq 17 \text{ mg l}^{-1}$) concentrations of H_2O_2 , most likely through scavenging before any significant oxidative stress and irreversible damage are caused. Exposure to high ($\geq 85 \text{ mg l}^{-1}$) concentrations, however, caused an immediate and dramatically reduced P_{MAX} and α , indicating that the oxidative stress was too high for the photosynthetic system to function. This can prevent plants from maintaining a positive carbon budget and consequently have no surplus carbon available for cell repair and growth. The response is comparable to that of *U. rigida* (Collén & Pedersén 1996) post-exposure to a lower concentration ($10.2 \text{ mg H}_2\text{O}_2 \text{ l}^{-1}$), and to *S. latissima* plants exposed to extreme temperature stress (Andersen et al. 2013). Dummermuth et al. (2003) studied antioxidative potentials of several species of seaweed by exposing them to different concentrations of H_2O_2 . For an H_2O_2 concentration of 68 mg l^{-1} , they found a 90 to 95% reduction in photosynthetic efficiency (in F_v/F_m) for the kelp species bladderlocks *Alaria esculenta* and arctic kelp *Laminaria solidungula*, equivalent to the α -reduction we observed for *S. latissima*.

After 15 d, only 2 plants were left in the 85 mg l⁻¹ concentration, and the following discussion on the prolonged impact should therefore be interpreted with care. Paler patches on the lamina were still visible on the plants at this time, indicating non-recovery in pigment concentration. Under low light, photosynthetic rate is limited by the rate of photochemical reactions (Steemann Nielsen 1975), which depends on light-harvesting pigments. Correlations between lower pigment concentration and lower α have previously been reported for *S. latissima* (Gerard 1988, Davison et al. 1991, Hanelt et al. 1997) as well as for higher plants (Pastori & Trippi 1993). The still reduced α found for these plants may therefore have been caused by a prolonged effect on their pigment concentration. P_{MAX} of these 2 plants was similar to that of the control plants, indicating recovered response to high light conditions.

The slow recovery of α prolongs the negative impact on both I_C and I_{SAT} , and under natural light regimes, this could result in an overall reduced daily carbon fixation for *S. latissima* (Bruhn & Gerard 1996). Firstly, by having a higher I_C , plants may be more susceptible to light limitation and more vulnerable to additional factors, such as shading by epiphytic growth, which could result in an imbalance of the plants' carbon budget (Andersen et al. 2013). Secondly, having a reduced I_{SAT} may prevent plants from taking advantage of occasional high light conditions. *S. latissima* inhabits the sublittoral, where the light conditions are often sub-saturating (Davison et al. 1991), and to be able to take advantage of any available light, it is important to maintain a viable carbon budget to support growth and reproduction. A reduced daily carbon fixation, in addition to a continuous loss of tissue, are possible causes for the lack of growth observed 15 d post-exposure for these 2 plants. Lack of growth may also have been affected by energy being diverted from growth due to the demand for repair (Roleda et al. 2007). The rate of carbon fixation might have also been too low for the plants to replace the lost pigments, preventing α and consequently I_C and I_{SAT} to recover. Based on the above-mentioned results, we propose that exposure to 85 mg l⁻¹ is either lethal to juvenile *S. latissima*, or may have a prolonged negative effect on their physiology and carbon budget.

An increase in R_D is typically observed for algae during stress (Davison et al. 1991, Andersen et al. 2013), although impacted plants demonstrated a reduction in the present study, as was observed by Vega et al. (2006) for microalgae. A reduced R_D would be an advantage for the plant, as it would

reduce carbon loss during dark periods (Davison et al. 1991). However, as P_{MAX} and α at this time indicate that the photosynthetic system was not only reduced, but barely functioning; it may be the same for respiration. The delayed effect on R_D compared to photosynthesis indicates that respiration is less sensitive to H₂O₂ and oxidative stress, as shown for *U. rigida* (Collén & Pedersen 1996) and microalgae (Vega et al. 2006). H₂O₂ primarily targets and oxidizes enzymes of the Calvin cycle, such as fructose-1,6-bisphosphatase (Asada 1992), enzymes that are essential for photosynthesis to take place, but not respiration. In addition, photosynthesis is reliant on chlorophyll as a catalyst, which has been shown to decrease with H₂O₂ exposure, whereas no catalyst is required for respiration (Campbell & Reece 2002). It may be that R_D is not negatively affected until the photosystem is severely inhibited, hence no response is seen immediately post-exposure.

For the control plants, the values for P_{MAX} , α , and R_D were within the natural variation found for *S. latissima* in other studies (Davison et al. 1991, Borum et al. 2002, Andersen et al. 2013), indicating the method used to measure and calculate the parameters was suitable. Using photosynthetic performance as an indicator of physiological stress is a well-established method in the field of phycology (Dummermuth et al. 2003), and has been shown in this study to be suitable for studying the toxicity potentials of a chemical. The change in P_{MAX} and α observed for the control plants after 15 d may have been due to different levels of environmental conditions in the laboratory compared to what the plants were adapted to in nature. Possible explanations include: lower water flow which could reduce the boundary layer and thus lead to a decreased gas exchange and accessibility to nutrients, lower nutrient levels as no medium was added to the seawater, and/or lower light conditions. However, this does not affect the reliability of the results in this study, as interpretative caution of the results has already been taken due to the low number of surviving plants in the high (85 mg H₂O₂ l⁻¹) concentration.

4.3. Potential effects on natural populations

The dispersion models (Refseth et al. 2017, O. Samuelsen unpubl. data) offer a more complete picture of the spatial and temporal movements of the H₂O₂ plume compared to the field studies (i.e. by Andersen & Hagen 2016, Fagereng 2016) and are hence considered more suitable for discussing the

potential concentrations that *S. latissima* may be exposed to and the subsequent impacts. Simulations done by Refseth et al. (2017) on the dilution and spread of H_2O_2 in surface waters (0–3 m depth) predicted that areas within 1000 m of the emitting fish farm can experience concentrations almost twice as high as the concentration found in this study to cause 100% mortality. Within 2000 m, simulated concentrations were higher than the herein-determined LC_{90} . The second dispersion model (O. Samuelsen unpubl. data) predicted a higher dilution rate of H_2O_2 and that the highest concentration of H_2O_2 within 5600 m post-emission would be lower than the current study's determined LC_{50} , but still higher than the EC_{50} values for both α and P_{MAX} . Both models thus indicate that exposure to H_2O_2 levels, that in the current study were found to negatively affect the photosynthesis of *S. latissima*, near farms are possible, while lethal exposures to *S. latissima* juveniles (within 2000 m) are only predicted in the Refseth et al. (2017) model. Delousing operations usually involve multiple bath-treatments (1 net pen at a time), hence non-target species will most likely experience several H_2O_2 exposures over several days (Grefsrud et al. 2018), which may further decrease the LC_{50} and EC_{50} values, as reported for American lobster *Homarus americanus* (Burrige et al. 2000, 2008). Fish farms are generally located close to land or skerries, thus often overlapping with the distribution of *S. latissima* beds. Furthermore, the number of H_2O_2 prescriptions given to fish farms in 2016 was highest for the months January to March (Grefsrud et al. 2018), which overlaps with the recruitment period (October to March) for sugar kelp (Andersen et al. 2011, Andersen 2013). A high mortality or reduced photosynthesis of the local juvenile *S. latissima* population during the recruitment period could lead to reduced recruitment and affect the development of the demography of the adult *S. latissima* population. Furthermore, a reduced carbon budget may lead to lower fitness and make them more susceptible to other stressors (Wernberg et al. 2010), such as grazing, storm surges, or elevated temperatures, and in that way indirectly lead to mortality of local *S. latissima* beds.

Monitoring programmes are regularly executed at all aquaculture sites in Norway (MOM system: Ervik et al. 1997), to ensure environmental impacts are not exceeding set thresholds (Grefsrud et al. 2018). However, these do not include monitoring of marine flora, including kelp beds. The total proportion of local *S. latissima* beds that would experience lethal or sub-lethal concentrations of H_2O_2 from 1 emission will be

highly dependent on the specific location of the farm, farm practice, treatment dose used, stratification of the water body, and current velocity and directions at the time of release (Andersen & Hagen 2016, Refseth et al. 2017). There is thus a need for further field studies on the distribution of H_2O_2 in the vicinity of fish farms and well-boats during treatment operations to improve the models. Juvenile *S. latissima* may be a good indicator organism for future field studies of H_2O_2 distribution around farms, as they have demonstrated a high sensitivity, are easy to check for lethal effects (i.e. detachment of lamina), and are cost-effective to use as no maintenance is required after deployment. Nonetheless, based on the high sensitivity of *S. latissima*, the available dispersion models (Refseth et al. 2017, O. Samuelsen unpubl. data) and the importance of kelp forests in coastal ecosystems (Araújo et al. 2016), it is recommended that H_2O_2 use and emissions are taken under consideration in future risk assessments and monitoring programmes to ensure that these habitats remain healthy.

4.4. Implications for H_2O_2 treatments at IMTA farms

The increased interest for developing an industry in Norway based on *S. latissima* cultivation in integration with salmonid fish is evident from the rapid increase in permits awarded to the private sector over the last couple of years (Stévant et al. 2017). In 2017, a total amount of 135 000 tons was harvested, with a value of 355 000 NOK (Directorate of Fisheries Norway 2018a). Development of the cultivation technology to optimize yield and make the industry as cost-efficient as possible is currently the main focus (MACROSEA: www.sintef.no/projectweb/macrosea/, Stévant et al. 2017). Our results may have implications for the production yield of cultivated *S. latissima* next to fish farms, as on-site H_2O_2 emissions will most likely harm the cultured seaweeds, and it is therefore recommended that these emissions are considered. Sporophytes are commonly deployed in the sea when they are smaller than the ones exposed in this study. Emissions should therefore especially be avoided in the beginning of the sea-cultivation cycle to minimize chances of negative impacts on the yield.

This study clearly demonstrates that *S. latissima* is highly sensitive, with a high mortality found down to a concentration of $85 \text{ mg } H_2O_2 \text{ l}^{-1}$ seawater. Moreover, a prolonged negative effect of the H_2O_2 exposure was demonstrated in surviving plants in the form of reduced photosynthetic efficiency and lack of new

growth. The current extensive use and emission of H₂O₂ from fish farms in shallow areas is therefore likely to have negative impacts on the kelp community.

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APPENDIX. Preliminary H₂O₂ dose–response study in juvenile *Saccharina latissima* plants (n = 6). Plants were exposed for 1 h to different dilutions of the common bath-treatment dose used in net pens for treating salmon for sea-lice infestations. Visible effects were recorded over time, from 0 h to 7 d post-exposure: discoloured lamina (Dis), lamina covered in mucus (Muc), soft and flaccid lamina (Fla), no visible effects (NVE), lamina had disintegrated and detached from stipe (Dead)

Concentration (mg H ₂ O ₂ l ⁻¹)	Dilution of bath treatment (%)	0 h	8 h	22 h	2 d	3 d	6 d	7 d
1700	100	NVE	Lamina white, Dead	–	–	–	–	–
1250	75	NVE	NVE	Dis, Muc	Dis, Muc, Fla	Dis, Muc, Fla	Dis, Muc, Fla	Dead
850	50	NVE	NVE	Dis, Muc	Dis, Muc, Fla	Dis, Muc, Fla	Dis, Muc, Fla	Dead
475	25	NVE	NVE	Dis, Muc	Dis, Muc, Fla	Dis, Muc, Fla	Dis, Muc, Fla	Dead
170	10	NVE	NVE	Dis, Muc	Dis, Muc, Fla	Dis, Muc, Fla	Dis, Muc, Fla	Dead
Control	Control	NVE	NVE	NVE	NVE	NVE	NVE	NVE

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1 **High-intensity finfish farming and kelp forests at dispersive locations. I.**
2 **Effects on *Laminaria hyperborea* stipe and lamina epiphytic communities**

3

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16

17 Abstract

18 Intensive fish farming in open net pens releases large amounts of particulate and dissolved
19 nutrients, effluents that are known to substantially affect soft-bottom benthic communities in
20 fjord-systems. A common perception is that coastal, dispersive locations have a higher resilience
21 to such effluents, and large-scale farms are currently located in hard-bottom environments where
22 *Laminaria hyperborea* kelp forests dominate. The interactions between farm effluents and kelp
23 forests are however poorly understood, and with the current decline of kelp forests worldwide,
24 potential negative impacts are important to clarify. Stimulation of fast-growing epiphytic algae
25 and faunal species on *L. hyperborea* stipes can have impacts on the composition of the kelp forest
26 community, and increased epiphytic growth on the kelp lamina can have negative effects on the
27 kelp itself. The effects of fish farming effluents on *L. hyperborea* epiphytic communities were
28 therefore assessed by sampling from 12 sites in two dispersive environments (area 1: 3 low effluent
29 farms, 3 reference sites; area 2: 3 high effluent farms, 3 reference sites). On the kelp lamina, the
30 association of epiphytes and effluent load was difficult to interpret as the statistical results
31 deviated from trends in the raw data, likely because the lamina epiphytes were also associated
32 with other environmental factors. As the post-hoc tests of differences between effluent levels were
33 also insignificant, we suggest further study is needed to clarify the results. On the stipes, however,
34 bryozoan biomass was significantly higher at high effluent farm-sites compared to low effluent
35 farm-sites and reference sites. This increase in bryozoan biomass resulted in a significantly
36 different stipe epiphyte community. An increase in stipe macroalgal biomass was also observed
37 with increasing effluent levels, but the relationship was less clear than for bryozoans. There was
38 however an increase in opportunistic *Ectocarpus* spp. with farm effluent load, and the macroalgae
39 community was less heterogeneous at high-effluent farm sites. The habitat heterogeneity was
40 further reduced by the high bryozoan biomass at these sites. These changes in the epiphyte
41 community could potentially influence the faunal community that relies on the epiphytes for food
42 and refuge and have cascading effects on the kelp forest food-web.

43

44

45 1. Introduction

46 Kelps (Laminariales) are important seaweed species in temperate coastal seas; they have high
47 primary production (Charpy-Roubaud & Sournia 1990, Steneck et al. 2002), facilitate a diverse
48 community of associated organisms (e.g. seabirds: Bustnes et al. 1997, macrofauna: Christie et al.
49 2003, Fredriksen 2003, fish: Norderhaug et al. 2005), and provide important ecosystem services
50 (Smale et al. 2013, Gundersen et al. 2017, Teagle et al. 2017, Belgrano et al. 2018). Kelp forest
51 primary production is estimated to be 1200 to 1900 g C m⁻² yr⁻¹ (Christie et al. 2009), with resulting
52 kelp detritus providing a significant source of food for invertebrates within the kelp forest
53 (Fredriksen 2003, Norderhaug et al. 2003) and subsidizing other habitats (Bustamante & Branch
54 1996, Krumhansl & Scheibling 2012). Kelps are also foundation species, creating habitat for a large
55 abundance and diversity of associated organisms both directly and indirectly through ecosystem
56 engineering. For example, *Laminaria hyperborea* (Gunnerus) Foslie forests in Norway can support
57 over 500 000 animals per m², with a single kelp thallus supporting 80-90 faunal species on average
58 (Christie et al. 2009).

59 *L. hyperborea* is also known for supporting many species of epiphytic algae and sessile fauna, with
60 different communities associated with the stipe, holdfast or lamina of the kelp (Marstein 1997,
61 Christie et al. 2003). Epiphytes can have impacts on both the composition of the kelp forest
62 community and on the kelp itself. Habitat complexity tends to be positively associated with the
63 number of associated epiphytic species, with positive relationships between epiphytic algae and
64 fauna abundance on large macrophytes (Hall & Bell 1988, Martin-Smith 1993, Christie et al. 2003,
65 2009, Norderhaug et al. 2014, Armitage & Sjøtun 2016). Some faunal species also have preferences
66 for epiphytic algae with specific morphologies, influenced by characteristics such as interstitial
67 space, surface and colour, which respectively affect habitat use and detritus accumulation, ability
68 to grip, and crypsis (Meyer & Bell 1989, Hacker & Steneck 1990, Norderhaug 2004, Christie et al.
69 2007, 2009, Eilertsen et al. 2011). However, if algae and encrusting fauna cover the kelp lamina,
70 this can reduce growth and survival of the kelp (Levin et al. 2002) through affecting photosynthetic
71 performance and nutrient uptake (Andersen et al. 2011), and through weakening the lamina
72 leaving it more prone for breakage (Krumhansl et al. 2011). Changes in the epiphytic community
73 of *L. hyperborea* forests could, therefore, have effects on ecosystem functioning via changes to the
74 fauna community, as well as affect the health of the kelp if epiphytes on the lamina increase. Given
75 the role of *L. hyperborea* as a foundation species, and the importance of its associated fauna in the
76 food web (Norderhaug et al. 2005), any human activities which can potentially drive changes in
77 epiphytic communities associated *with L. hyperborea* should be assessed.

78 Increased loading of nitrogen and particulate matter from anthropogenic activities in coastal
79 waters are two such potential drivers of change. Along the Norwegian coast, fish farming adds the
80 largest amount of nutrients to coastal waters, contributing 55% of the total anthropogenic nitrogen
81 emissions (Selvik & Sample 2018). The aquaculture industry has grown rapidly, with salmonid
82 production more than doubling from approx. 490 000 tons in the year 2000 (Gullestad et al. 2011)
83 to approx. 1 340 000 tons in 2018 (Fiskeridirektoratet 2019). Fish waste and excess feed release
84 particulate organic matter (POM, approx. 540 000 to 670 000 tons), dissolved phosphorus (P,
85 approx. 3900 tons) and dissolved inorganic nitrogen (DIN, approx. 26 000 tons) into coastal waters
86 (estimates from 2017, Husa et al. 2018), with DIN mostly as ammonium (Hall et al. 1992, Pitta et
87 al. 1999). The amount and dispersal of effluents from individual farms will depend on
88 characteristics of both the farm (biomass and feed levels, related to the stage of the production
89 cycle) and of the location (depth, current strength, and direction) (Jansen et al. 2016).

90 Over the past 20 years, there has been a shift from small farms in sheltered fjords (3-5000 tons
91 maximum total biomass [MTB]), to larger farms based in more exposed coastal locations (ca. 6000-
92 14 000 tons MTB). Indeed, the majority of the growth in Norwegian aquaculture industry over the
93 last 10 years can be attributed to a developing aquaculture industry in the archipelago on the west
94 coast, which includes the islands Frøya and Smøla. This new arrangement increases the likelihood
95 of impacts on *L. hyperborea* forests, as they are typically found at exposed sites to about 30 m
96 depth (Kain 1979, Lüning 1990, Bekkby et al. 2009), and are the most dominant kelp species in this
97 area (Steen 2017, 2018). It most likely also increases the dispersion of farm effluents, and
98 environmental responses may be more diffuse and more complicated to detect compared to in
99 fjord-systems. Currently, there have been few previous studies examining the effect of salmon
100 farms in exposed coastal locations, with most previous work on soft-bottom habitats (Holmer
101 2010) or in fjords (Kutti et al. 2007a, b).

102 Both POM and DIN have the potential to affect *L. hyperborea* forests, both directly and indirectly.
103 Nutrient enrichment is thought to benefit ephemeral algae (which include many epiphytic species)
104 compared to large perennial macroalgae (such as *Laminaria*) because the former tend to have a
105 higher surface area to volume ratio (SA: V) and growth rate, and may thus be able to respond faster
106 to increased nutrient availability (Pedersen & Borum 1996, Karez et al. 2004). Several studies have
107 documented effects on ephemeral and epiphytic species or communities of perennial macroalgae
108 with nutrient enrichment (e.g. Rönnerberg et al. 1992, Worm & Sommer 2000, Karez et al. 2004,
109 Russell et al. 2005, Worm & Lotze 2006, Sanderson et al. 2012, Oh et al. 2015), and in some cases
110 this has contributed to ephemeral algae-mats replacing perennial macrophytes (Kraufvelin et al.
111 2006, Worm & Lotze 2006, Burkholder et al. 2007, Filbee-Dexter & Wernberg 2018). Filter feeders

112 may also be affected by increased nutrients or particulate organic matter (POM) as this can
113 increase food supply, directly in the case of POM (Lojen et al. 2005) or via an increased abundance
114 of phytoplankton with nutrients (Worm & Lotze 2006). This could potentially affect *L. hyperborea*
115 if encrusting bryozoans, which tend to feed on small phytoplankton (Winston 1977), become more
116 abundant on the lamina. Furthermore, increased particle load can directly affect both stipe
117 epiphytes and kelp by reducing light levels. Although *Laminaria* spp. are relatively tolerant of low
118 light conditions, turbidity affects their lower depth limit (Bartsch et al. 2008). Epiphyte growth on
119 the lamina can further reduce light available to the kelp by 80-100 % (Andersen et al. 2013). Thus,
120 a combination of particles and epiphytes could attenuate light considerably.

121 Due to the importance of *L. hyperborea* to ecosystem function, a better understanding of the
122 interaction between aquaculture and *L. hyperborea* forests is essential. Furthermore, as the coast
123 of western Norway is typically a low nitrogen system from late spring to early autumn (Strömberg
124 1986), enrichment could have particularly strong effects on ecosystems via increased primary
125 production at a time of year that is typically nutrient limited. Finally, *L. hyperborea* forests along
126 the mid and northern coast are currently listed as a near threatened (NT) habitat type (Gundersen
127 et al. 2018), and can be affected by several stressors such as urchin overgrazing, climate change,
128 and harvesting (reviewed in: Steneck et al. 2002, Araújo et al. 2016). Therefore, as the industry
129 continues to operate and expand in coastal environments, it is critical to quantify any potential
130 effects it may have on forest condition.

131 The aim of this study was to assess the effect of wave exposed salmon farms on *L. hyperborea*
132 forest functioning; specifically, whether the presence of a farm has an impact on the epiphytic
133 community (algae and colonial tunicates and bryozoans) living on the kelp. As epiphytes can
134 affect the associated faunal community and kelp health, changes could result in cascading effects
135 on wider phenomena such as food webs and forest structure.

136
137

138 2. Materials and methods

139 2.1 Sampling sites

140 A total of 12 sites were chosen for this study; 6 sites near fish farms (FF) and 6 reference sites (Ref).
141 Sites were chosen in two geographical areas on the west coast of Norway, separated by
142 approximately 40 km; the first area in the Frøya archipelago (3 Ref, 3 FF: Trøndelag county) and
143 the second in the Smøla archipelago (3 Ref, 3 FF: Møre og Romsdal county) (Fig. 1). Both counties
144 are subject to a high-intensity of salmonid farming, producing more than 420 000 tonnes of
145 salmonid biomass in the sampling year (fiskeridir.no). This part of the coast is characterized by
146 ocean swells, waves, and strong currents resulting in a highly dynamic area. The terrain is mostly
147 hard-bottom mixed with shell sand, with *Laminaria hyperborea* dominating the sublittoral flora
148 down to about 30 m (Steen 2017, 2018). Salinity ranges from 31 to 34 ‰ (IMR, 2017).

149 Two areas were chosen so that two levels of farm effluent load could be studied; at the time of
150 sampling (2015), fish farm production cycles were synchronized within areas depending on the
151 county (regulations revised in 2017; Grefsrud & Svåsand 2018). A typical farm cycle lasts 12-18
152 months, with the farms in the present study having cycles around 18 months (excluding the
153 fallowing period). Farms in the Frøya archipelago were early in the production cycle (5-6 months)
154 with low biomass and feed levels (sites referred to as FF-L [low]), and farms in the Smøla
155 archipelago were at the end of their production cycle (ca. 17 months) with high biomass and feed
156 levels (sites referred to as FF-H [high]). Mean monthly feed use at the FF-H farms was 3.5 - 11 times
157 higher than at the FF-L farms in the 6 months preceding sampling (Table 1). Sampling in both areas
158 gave the opportunity to compare the effects of a high versus a low effluent load, and to assess
159 whether potential impacts were short term (i.e. if impacts were only observed on FF-H sites and
160 not FF-L sites) or long term (i.e. if the same level of impacts were found on both FF-H and FF-L
161 sites), as the effluent load level varies with farm cycle stage.

162 Wave exposure levels varied at the FF locations, therefore the study location at Ref sites was
163 chosen based on two criteria: similar wave exposure levels as the FF locations, and > 2 km from
164 any fish farm or other major industries. Within each study location, a skerry with *L. hyperborea*
165 forest was chosen as the sampling site itself. This was based on two criteria. The first was that there
166 should be *L. hyperborea* forest at 5 m LAT, as depth can have an impact on kelp communities (Kain
167 1977). The second was that, at farm locations, the skerry should be within the main effluent plume
168 of the farm. Due to wave-exposure and ocean current patterns in the sampling area, the impact of
169 effluents is not believed to follow a clear gradient as is usually the case in calm fjords (Kutti et al.
170 2007a, Kutti et al. 2007b), but rather impact in patches in the prevailing current direction (particle

171 modelling conducted herein), with benthic effects observed up to 1000 m away (Keeley et al.
172 2019). To ensure that *L. hyperborea* plants at FF locations were sampled from effluent-impacted
173 kelp forest, the direction of the main effluent plume was determined from ocean current models,
174 and the closest suitable skerry to the farm within the plume was selected as the sampling site.
175 Maps of modelled particle movement and dissolved nutrients were consulted after the study to
176 check that the farm sites were in the area of effect of the farm (see section 2.5 for details, Fig. 2,
177 Fig.3).

178 2.2 Sampling design

179 Sampling was conducted over 6 consecutive days from the 8th to the 14th of August 2015, the
180 time of year where epiphyte abundance on *Laminaria hyperborea* is normally near its highest
181 (Christie et al. 2003). At each site, a 25 m transect was laid out along the sea floor by divers,
182 following contours so the depth remained at 5 m LAT. The diver swam along the transect and
183 collected the closest living canopy plant to the meter mark every 2 m for 20 m, giving a total of 10
184 thalli (holdfasts were collected along the last 5 m, see Haugland et al. in prep). The density of the
185 *L. hyperborea* forest was determined by counting the number of living canopy thalli within five
186 randomly selected 1 m² quadrants along the transect. Collected *L. hyperborea* plants were kept in
187 fresh seawater until processed. The thallus of the collected plants was divided into lamina and
188 stipe, where the lamina was cut off one cm below the stipe-lamina transition zone, and the stipe
189 right above the holdfast-stipe transition zone. A summary of sampled kelp variables is given in
190 Supp. Table 1.

191 2.3 Stipe measurements and epiphytes

192 Epiphytic macroalgae and bryozoans were collected by scraping them off the stipe and frozen until
193 processed in the laboratory. In some cases, large bundles of *Desmarestia acuelata* were tangled
194 around the top of the stipe, or a second *Laminaria hyperborea* (several years old) was growing out
195 from the sampled thallus. These were identified and weighed *in situ*, but excluded from further
196 data analysis. To determine area available for epiphytic growth, the surface area (SA) of the stipes
197 was calculated by recording the length and the diameter at the top and bottom of the stipe. As
198 stipes can be oval in cross-section, two diameters perpendicular to each other were recorded and
199 the average used. Grazing by urchins was common in the study area (75-100 % of the stipe grazed
200 in most samples). Stipe epiphytes were standardized to 10 cm² ungrazed SA, to allow comparisons
201 across sites independent of stipe length, and as grazing would reduce epiphyte biomass
202 irrespective of the effect of farm effluents on epiphyte growth. Grazed SA was calculated using the
203 same method (using the diameters of the upper and lower grazing-boundary and length of the
204 grazed stipe) where whole sections of the stipe had been grazed, which was mostly the case. When

205 grazing was only apparent on smaller sections, a percentage in relation to total stipe SA was
206 estimated to the nearest 10%. The age of the stipes was determined by counting growth rings (Kain
207 1963).

208 In the laboratory, epiphytic macroalgae > 0.5 cm in length were identified to the lowest possible
209 taxonomic level, and the biomass of each species was determined (fresh weight, FW) after spinning
210 the sample in a salad spinner for a consistent number of rounds to remove excess water.
211 Macroalgae < 0.5 cm were grouped and blotted to determine FW and included in the univariate
212 analysis of total biomass. Bryozoans (encrusting [thin sheets] and erect [plant/bush-like]), and
213 macroalgae which had bryozoans covering > 40 % of their surface, were grouped as 'Bryozoa'.
214 These were not identified further as different bryozoan species were frequently overgrowing each
215 other. One level of epiphytic growth was identified and quantified, i.e. except for the case of
216 bryozoans, epiphytes growing on epiphytes on the stipe were not included in the study.

217 2.4 Lamina measurements and epiphytes

218 The biomass (FW) and maximum length of the lamina were recorded after removal of epiphytes.
219 Presence of dominant epiphytes was quantified using different methods depending on the group;
220 SA for encrusting colony-forming fauna (bryozoans and tunicates), the number of colonies for erect
221 bryozoans, or biomass (FW) for macroalgae. SA and the number of erect colonies were determined
222 by laying the lamina out on a flat surface and taking photographs. Five randomly selected
223 photographs from each site were later processed using the program ImageJ (Schneider et al. 2012),
224 by tracing the edge of the lamina and subsequently the edge of the attached fauna. Macroalgae
225 biomass was determined by gently scraping them off the lamina, and freezing until processing in
226 the laboratory at Institute of Marine Research in Bergen, Norway. Biomass in FW was determined
227 in the same way as the stipe epiphytes.

228 2.5 Modelling of abiotic variables and farm effluents

229 Five different models were run to obtain site information of abiotic data (see Supp. Table 1 for
230 summary of abiotic data), particle settlement and DIN.

231 The first model was on *wave exposure*, modelled with a spatial resolution of 25 m using data on
232 fetch (distance to nearest shore, island or coast), averaged wind speed and wind frequency. Wind
233 data were delivered by the Norwegian Meteorological Institute and averaged over ten years (1995-
234 2004). The model was developed by Isæus (2004), has been applied to the whole of the Norwegian
235 coast by the National program for mapping biodiversity – coast (Bekkby et al. 2013) and has been
236 applied in several research projects in Norway (e.g. Bekkby et al. 2009, Norderhaug et al. 2012,
237 2014, Pedersen et al. 2012, Bekkby et al. 2014a, b, Rinde et al. 2014), Sweden (Eriksson et al. 2004),

238 Finland (Isæus & Rygg 2005), the Danish region of the Skagerrak coast and the Russian, Latvian,
239 Estonian, Lithuanian and German territories of the Baltic Sea (Wijkmark & Isæus 2010).

240 The second model was predicting *mean current speed*, by using a 3-D numerical ocean model
241 ROMS (Shchepetkin & McWilliams 2005) in a two-level nesting procedure. Level 1: large-scale
242 ocean currents, atmospheric forcing (wind, temperature, pressure, cloud cover, humidity,
243 precipitation, and solar radiation), river flow rates and bathymetry were used to drive an ocean
244 model at an 800 m spatial resolution (NorKyst-800, Albretsen et al. 2011). Level 2: in combination
245 with higher-resolution bathymetry, the fields from the 800 m model were used to drive a series of
246 inner models, resulting in a model of 500 m spatial resolution. ROMS has shown good results when
247 compared with field observations (Lacasce et al. 2007) and has users worldwide.

248 The third model was on *light exposure*, calculated at a spatial resolution of 25 m scale, as the
249 deviance from an optimal influx of light based on estimates of vertical slope and orientation
250 (aspect). This index was developed for terrestrial vegetation (Parker 1988); discussed and
251 developed further by Økland (1990, 1996), and is suitable for use in the shallow most areas of
252 marine environments (e.g. to model kelp distribution, Bekkby et al. 2009), as light attenuation with
253 depth is not considered. The light exposure is optimal (index value = 1) at slope (45°) and aspect
254 (202.5°) (Økland 1990, 1996). The index is positive at aspects $202.5 \pm 90^\circ$ (regardless of slope) and
255 negative at $(202.5-180^\circ) \pm 90^\circ$.

256 The Lagrangian transport model LADIM (Ådlandsvik & Sundby 1994) was used to predict
257 movements of the particle plume for the study area to verify that the sampling sites were within
258 the effluent plume of the nearby farm. The model used ocean currents, simulated using the same
259 3D hydrodynamic model (*NorFjords*) used for the second model described above. At the time of
260 this implementation, a higher resolution of 160 m for the modeled currents was available. LADIM
261 calculates active transport of individual particles using the modeled currents as inputs, as well as
262 particle specific dynamics including settling and lifespan via an Individual-Based module specifically
263 designed for the salmon fish farming industry. Constant settling velocity of 7.5 cm/s and a particle
264 lifespan of approximately 12 days were used as model parameters based on previous research
265 (Bannister et al. 2016; M. C. Fernandez unpubl. data). Particle distribution at the study sites was
266 calculated to the same grid used for the hydrodynamic model and presented as particle
267 accumulation maps in order to identify the plume trajectories for the periods of interest. This
268 model does however not allow for comparisons of particle accumulation between locations.

269 The fifth model was used to model dissolved nutrients, as using sampling to adequately capture
270 the effect of fish farming effluents on dissolved nutrient concentrations can be extremely

271 challenging, due to the high spatial-temporal variation (Jansen et al. 2016). The NORWECOM
272 model (Skogen et al. 1995, Skogen & Sjøiland 1998) was therefore used to predict the dispersion
273 and ambient concentrations of DIN at the all study sites (see Haugland et al. in prep for details). To
274 highlight the effect of fish farm effluents on natural DIN levels at all 12 sampling sites, the
275 difference in predicted DIN concentrations between two simulations are presented (Fig. 2); one
276 reference simulation without any fish farms present, and one simulation including nutrient inputs
277 from the six study farms. The amount of DIN (ammonium, nitrate, and nitrite) released from the 6
278 farms (nutrient inputs for the second simulation) were calculated based on monthly feed data by
279 using conversion estimates from the ANCYLUS model (Bergheim & Braaten 2007, Husa et al. 2016).
280 Predicted concentrations were extracted daily for all sites.

281 2.6 Statistical analysis

282 Before analysis, the lamina-associated algal epiphytes were standardized according to the weight
283 of the lamina and the faunal epiphytes (encrusting and bush-forming bryozoans, and colony-
284 forming tunicates) according to the SA of the lamina. No statistical analysis was conducted for
285 bush-forming bryozoans and colony-forming tunicates due to low abundances and large variation
286 between sites. The stipe epiphytes (algal and bryozoan biomass) were standardized according to
287 the ungrazed SA of the stipe. Note that for stipes recorded as 100 % grazed (5 thalli), 5 % of their
288 total surface area was used for standardization as this was considered a relevant margin of error.

289 All statistical analyses were done using R (R Core Team 2017) with plots created using package
290 ggplot2 (Wickham 2016). The effect of farming on the abundance of epiphytes was analysed by
291 constructing linear mixed-effects models (R package nlme; Pinheiro et al. 2017). These allow for
292 the inclusion of a random effect, site (12 levels), which accounts for the potential inter-dependence
293 of thalli sampled from the same site. The effect of farming was examined using the variable *group*
294 (categorical, 4 levels: Ref-L, Ref-H, FF-L, FF-H). A number of control variables were chosen for
295 inclusion, based on biological theory that they could influence the response variables: *kelp age*
296 (continuous), *kelp density* (continuous), *wave exposure* (continuous, modelled), *current speed*
297 (continuous, modelled), and *light* (continuous, modelled). Variables were tested for collinearity by
298 calculating the variance inflation factor (VIF) and examining pairwise correlations. All of the VIF
299 values were low (≤ 3.2 ; Supp. Table 2).

300 Because there were only 3 sites within each of the four *group* levels (Ref-L, FF-L, Ref-H, FF-H), there
301 were only 3 levels of *kelp density*, *wave exposure*, *current speed* and *light* in each *group*. This was
302 not enough data to include interactions. It also means that the dataset is limited for making
303 conclusions about the impact of these factors on epiphyte communities. One site had much higher

304 wave exposure than the rest (site 7, Ref-H; > 2.5x higher than any other site), which could unduly
305 influence the analyses. The analyses were therefore run once with, and once without this site.

306 Before any testing, plots of residuals for the models were examined to check for homogeneity of
307 variance and normality. All models had issues with these, so to correct this some response variables
308 were square-root transformed, and variance structure functions were included to allow the
309 residual variation to vary along certain variables (selected based on plots of residuals and AIC and
310 likelihood ratio testing, as according to Zuur et al. 2009). Details of the models are presented in
311 Table 2. Significance testing of variables was done by carrying out likelihood ratio tests (LRT;
312 comparisons of the model with and without the variable of interest, Zuur et al. 2009). To test for
313 differences between levels of *group* Dunnett contrasts were done, which compare a treatment (FF-
314 H or FF-L) to controls (Ref-H and Ref-L averaged), using an approximation of the Dunnett p-value
315 adjustment for multiple comparisons (R package lsmeans; Lenth 2016). The epiphytic algae were
316 grouped by morphology (corticated, foliose, uniseriate, other) according to Karez et al. (2004), and
317 by higher taxon (Rhodophyta, Phaeophyceae, Chlorophyta) to inspect any shifts caused by fish
318 farming effluents.

319 Non-metric multi-dimensional scaling (NMDS) was chosen to graphically present the stipe
320 epiphytic community. NMDS ordination plots (2-dimensional) were applied to Bray-Curtis distance
321 matrix calculated from biomass data (R package vegan; Oksanen et al. 2018). Prior to the analyses,
322 the data were square-root transformed to prevent very abundant species having an overwhelming
323 effect, and stipes with a total biomass of zero omitted as calculating a difference between samples
324 with no result is not viable (Clarke & Warwick 2001). Vectors of scaled control variables (*kelp age*,
325 *kelp density*, *wave exposure*, *current speed*, and *light*) were generated utilizing data positioning
326 obtained in the NMDS (envfit function). Vectors' relative lengths correspond to their strength (i.e.,
327 magnitude of change and variability) in the positioning of samples relative to each other in NMDS
328 plots and can thus be used to aid in NMDS interpretations. The significance of vectors and the *a-*
329 *priori* set *group* were tested via 999 permutations. Vector fitting was conducted both with and
330 without the high wave exposure site (site 7). Ellipses representing 95% confidence intervals around
331 the centroid for each level within *group* (Ref-L, FF-L, Ref-H, FF-H) were plotted to aid graphical
332 representation (ordiellipse function), where a shift in centroid location indicates a different species
333 composition.

334 To test if the stipe community composition differed between levels within *group*, a pairwise
335 PERMANOVA was run (R package pairwiseAdonis; Martinez Arbizu 2017). A 5-way PERMANOVA (R
336 package vegan; Oksanen et al. 2018) was used to test the significance of the chosen control

337 variables on the epiphytic community, and to assess the amount of variation explained by these
338 compared to the *a priori* set factor. P-values ($\alpha = 0.05$) for both were based on 999 permutations
339 and adjusted for multiple testing in the pairwise PERMANOVA. SIMPER (R package vegan; Oksanen
340 et al. 2018) was run to investigate which species that contributed to potential differences between
341 *group* community compositions.

342 PERMDISP (betadisp function) was conducted to assess the multivariate dispersion of the stipe
343 epiphytic community within the four different *groups* (i.e. beta-diversity), where a lower average
344 distance to centroid indicates a more homogeneous community. The significance was determined
345 with an ANOVA. Based on statistical results obtained so far, the analysis was conducted twice to
346 assess if the observed patterns were caused by the macroalgae community, and/ or caused by
347 bryozoan biomass.

348

349

350 3. Results

351 3.1 Dissolved inorganic nitrogen and abiotic variables

352 Modelled dissolved inorganic nitrogen (DIN) and particle movements predicted that all farm
353 sampling sites were within the main effluent plume of the respective farms. Modelled DIN
354 predicted a smaller elevation in DIN levels at low effluent farm sites (FF-L; up to 1.4 times higher
355 than at control sites) compared to at high effluent farm sites (FF-H; up to 1.8 times higher than at
356 control sites) (Fig.2). The difference in DIN concentrations between farm sites (FF-L, FF-H) and
357 control sites (Ref-L, Ref-H) was consistent, although small compared to predicted weekly
358 fluctuations. Modelled particle trajectories predicted that farm sampling sites were located within
359 40-60 % of the particle-plume from the respective fish farms (Fig. 3).

360 Predicted wave exposure levels ranged from 7206 to 700 125 across sites, and predicted mean
361 current speed from 0 to 0.07 m s⁻¹ across sites. The Ref-H sites all had relatively high wave exposure
362 (Supp. Fig. 1). Modelled light exposure was similar between all sites, ranging from -0.14 to 0.07 (1
363 being optimal). Kelp density varied, ranging from 1 to 14 canopy plants per m² at the sampled sites.
364 Mean kelp density was slightly higher at reference sites (Ref-L, Ref-H) compared to farms (FF-L, FF-
365 H; Supp. Table 1).

366 3.2 Stipe epiphyte biomass

367 Macroalgae and bryozoans were the dominating stipe epiphytes, with a total of 67 macroalgal taxa
368 identified, where the majority was red algae (Supp. Table 3). Sponges and non-colonial tunicates
369 commonly grow on *L. hyperborea* stipes, but the former was not observed, and the latter only
370 sporadically observed, and therefore not included further. Total epiphytic biomass on stipes
371 ranged from an average of 0.01 to 34.4 g FW between sites. Standardized to 10 cm² ungrazed stipe,
372 average macroalgal and bryozoan epiphytes were 1.25 g FW and 0.48 g FW respectively. Bryozoans
373 were predominantly *Membranipora membranacea*, *Electra pilosa*, and species in the genera
374 *Scruparia* and *Crisia*.

375 Epiphytic biomass of algae was higher at farm sites than at reference sites and increased with wave
376 exposure level and kelp age (Fig. 4). *Group* (levels: Ref-L, FF-L [low effluent load], Ref-H, FF-H [high
377 effluent load]) was a significant explanatory factor in the model ($p = 0.014$, Table 2). However,
378 post-hoc contrasts failed to find significant differences between reference sites and farm sites (FF-
379 H vs Ref-L/H: $p = 0.14$, FF-L vs. Ref-L/H: $p = 0.38$). Of the control variables, kelp age and wave-
380 exposure had positive significant relationships with epiphyte biomass (Table 2, Supp. Fig. 1),
381 though the significance of wave exposure was mainly driven by the one site with a higher level.
382 When the macroalgal epiphytes were divided into morphological and taxonomic groups (Fig. 5),

383 they all showed the same trend of increasing biomass with increasing effluents (except for
384 Phaeophyceans, which were constantly at very low abundances). Foliose species and Rhodophytes
385 were the most abundant morphological and taxonomic groups, respectively.

386 For epiphytic bryozoans on the stipe, the biomass increased with farm effluent load (Fig. 4), and
387 with wave exposure level and light exposure. *Group* was significant ($p < 0.001$, Table 2), and the
388 post-hoc contrasts showed that FF-H sites had a significantly higher biomass than reference sites
389 (FF-H vs Ref-L/H: $p = 0.034$, FF-L vs. Ref-L/H: $p = 0.50$). Wave exposure level and light exposure
390 were significantly positively related to biomass (Supp. Fig. 2), though the relationship was mainly
391 driven by the one site with much higher wave exposure level (Table 2). In general, the biomass of
392 epiphytes could be very variable between sites and between individual stipes at the same site
393 (Supp. Fig. 3). Most stipes were heavily grazed. The lowest levels of grazing were recorded at two
394 of three FF-H sites (Supp. Fig. 4).

395 3.3 Stipe epiphyte composition

396 The NMDS indicated that both the community composition and the community similarity between
397 stipe (i.e. multivariate dispersion), were affected by farm effluents (Fig. 6). This was confirmed by
398 the statistical analysis. The community composition on stipes in the high effluent load (FF-H) *group*
399 was significantly different compared to stipes exposed to low effluent load (FF-L vs FF-H: $p = 0.008$)
400 and the reference (Ref-H vs FF-H: $p = 0.006$, Table 3). SIMPER analysis revealed this difference was
401 primarily driven by the higher bryozoan biomass at FF-H sites. Bryozoan biomass contributed 61%
402 and 47% to the dissimilarity between FF-H vs Ref-H and FF-H vs FF-L, respectively (Table 4). Among
403 the few other species that contributed to the difference between *groups*, *Ecotcarpus* spp. showed
404 an increase in biomass with increasing effluent load, whereas there was no consistent pattern for
405 the other contributing species (*Palmaria palmata*, *Membranoptera alata*, *Phycodryis rubens*,
406 *Polysiphonia stricta*) (Table 4).

407 The highest amount of the variation in the epiphytic community was explained by the *group* factor
408 (PERMANOVA: 13%, Table 3). All control variables except wave exposure level were found
409 significant by the PERMANOVA, though each explained only 4% or less of the community variation
410 (Table 3). In the constricted 2-D dimensional space, the control variables were found to explain
411 more of the variation in the community than the *group* factor (Fig. 6, Supp. Table 4).

412 The multivariate dispersion of the macroalgae community was significantly different between
413 *group* levels, with FF-H sites having the lowest dispersion (PERMDISP: $F = 6.64$, $p < 0.001$, Supp.
414 Table 5). The dispersion was also significantly different between *groups* when including the

415 bryozoans (PERMDISP: $F = 21.12$, $p = 0.010$, Fig. 6), with the high bryozoan biomass at FF-H sites
416 (Fig. 4) further decreasing the dispersion at these sites by 30% (Supp. Table 5).

417 3.4 Lamina epiphytes

418 For epiphytic algal biomass on the lamina, *group* was a significant factor in the model ($p = 0.011$,
419 Table 2), although post-hoc comparisons of farm vs. reference were insignificant (FF-H vs Ref-L/H:
420 $p = 0.304$, FF-L vs. Ref-L/H: $p = 0.322$). The model predicted that farm sites would have lower levels
421 of epiphytic algae than the reference sites if all other variables were equal. This was not apparent
422 from a plot of epiphytes by *group* (Fig. 7), but this was most likely caused by the varying kelp
423 density, wave exposure and light between the sites, all of which were significantly negatively
424 associated with epiphyte biomass (Supp. Fig. 5). Again, the importance of wave exposure level was
425 mainly driven by the one high exposure site. The epiphytic biomass was mostly made up of brown
426 uniseriate algae in the order Ectocarpales (on average 77 % of the lamina macroalgal epiphytes by
427 weight).

428 Cover of encrusting bryozoans on the lamina (predominately *M. membranacea* and *E. pilosa*) was
429 found to be significantly different between *group* levels ($p < 0.001$, Table 2). Again, the model
430 predicted a lower cover at farm sites (Supp. Fig. 6), and kelp density was found to be a significant
431 factor (Table 2). FF-L was found to have a significantly lower bryozoan cover compared to the other
432 *groups* (post-hoc: FF-H vs Ref-L/H, $p = 0.736$; FF-L vs. Ref-L/H, $p = 0.011$), but had a higher presence
433 of colony-forming tunicates (Fig. 7). The density of erect bryozoan tufts (identified to *Crisia* spp.)
434 was slightly higher at FF-L and Ref-H compared to FF-H and Ref-L (Fig. 7). The natural variation in
435 epiphytic fauna was in general high and could be very variable both within and between sites.

436

437

438 4. Discussion

439 We have demonstrated that some components of the epiphytic community on *Laminaria*
440 *hyperborea* are influenced by the presence of fish farms in dispersive environments. There was a
441 clear association between high bryozoan biomass on stipes and proximity to high effluent fish
442 farms, resulting in an altered and more homogeneous stipe community composition. The epiphyte
443 load on kelp lamina was not positively impacted by farm effluents, and relationship with several
444 factors (fish farm effluent load, wave exposure, kelp density) complicated interpretation, calling
445 for further studies to clarify the relationship. In general, natural variables, such as wave exposure,
446 kelp age, and kelp density had a very strong influence on variation within epiphyte communities.
447 Our results suggest that an effect is present, however, further explorations of the interaction
448 between epiphytic communities on *L. hyperborea* and aquaculture is necessary to understand the
449 extent of the impacts.

450 4.1 Stipe biomass and community structure

451 The epiphyte response on stipes was consistent with the expectation that dissolved nutrients and
452 particulate matter released from fish farms may supply extra nutrition to the surrounding
453 community. Macroalgae can take up DIN from farms (Carballeira et al. 2013) including ammonium
454 (Ahn et al. 1998), and it was anticipated that this should benefit small, fast-growing epiphytic algae
455 species particularly. While we did not detect a strong effect on total macroalgae biomass, the
456 increase in thread-forming opportunistic *Ectocarpus* spp. with increasing farm effluent load, in
457 addition to a more homogeneous macroalgae community at the high effluent load sites, shows
458 that there is a farm-impact. The predicted farm-effect on local DIN levels suggest that dissolved
459 nutrients are diluted at a much faster rate in this dispersive area compared to reports for smaller
460 farms at less exposed locations (Sanderson et al. 2008, Jansen et al. 2018, Haugland et al. in prep).
461 This could explain why the trend on total macroalgae biomass was not clearer, and why the
462 patterns within morphological and taxonomic groups were not clearly affected. Bryozoans may be
463 able to utilize both the POM and the dissolved nutrients generated by fish farming, which could
464 explain the strong response of bryozoan biomass to effluents. Higher bryozoan cover with
465 increasing dissolved nutrients have also been observed on seagrass *Posidonia oceanica* (Balata et
466 al. 2010), though studies looking at uptake of farm-derived DIN or POM for these animals is limited.
467 Bryozoans (species not specified) at a seabream farm in the Red Sea was found to not take up much
468 nitrogen from POM compared to other filter feeders (Lojen et al. 2005), though food selection and
469 uptake rates vary between species (Winston 1977, Riisgård & Maríquez 1997) and could differ for
470 the bryozoans in this study. Phytoplankton may also have played a role; by having a higher growth
471 rate than macroalgae, phytoplankton might take up DIN before the macroalgae have time to

472 respond (Hadley et al. 2015). Higher phytoplankton biomass could potentially also benefit the
473 bryozoans, though in this dispersive environment it is not likely that this would only benefit local
474 bryozoan growth. The chosen methodology could also have contributed to the larger response of
475 bryozoans; bryozoan-covered macroalgae were counted as 'Bryozoa', therefore any extra growth
476 of macroalgal epiphytes that later became extensively covered by bryozoans would have
477 contributed to bryozoan differences rather than macroalgal differences.

478 A more homogeneous epiphytic community, as observed in this study on sites exposed to high
479 effluent levels, is a known response of communities exposed to stress, and has been reported for
480 macroalgae communities (Oh et al. 2015) and the epiphytic community on seagrass (Balata et al.
481 2010). Faunal diversity in kelp forests increases with habitat diversity (Norderhaug et al. 2014),
482 hence lower heterogeneity could affect the wide range of fauna species that depend on the kelp
483 forest as a habitat. The change in community composition caused by the high bryozoan biomass
484 could further alter the faunal composition of kelp forests, as different communities tend to support
485 different faunal assemblages (Wikström & Kautsky 2007). However, the fact that the impacts on
486 community and bryozoan biomass were only found at the sites at the end of their production cycle
487 (FF-H) could indicate that this effect was short-term and that epiphyte presence and biomass can
488 respond quickly to effluent levels within a year. Compared to that seen in benthic soft-bottom
489 macrofauna communities exposed to fish farm effluents, this would be a faster response (Macleod
490 et al. 2004, Keeley et al. 2019). This could imply that *L. hyperborea* forest as habitat is more resilient
491 to fish farming than other systems. The natural seasonal fluctuation in epiphytic biomass, which
492 peaks in the summer and decreases in winter (Whittick 1983) could mediate recovery. It is likely
493 also related to the dispersive nature of the study area, as more dispersive sites generally show
494 higher resilience (Keeley et al. 2013, Valdemarsen et al. 2015). Recovery speed should be further
495 explored, e.g. by including samples from farms at the end of their fallowing period, or examining
496 areas where farms are being moved.

497 Other factors than farm effluents also play a role in epiphyte abundance and composition. Wave
498 exposure and kelp age are reported to positively affect stipe epiphyte density (Norderhaug et al.
499 2012, Bekkby et al. 2014a, Steen et al. 2016) and composition (Pedersen et al. 2014), which was
500 mostly supported by our results for both macroalgae and bryozoans. The total stipe epiphytic
501 biomass reported here was low compared to other reports for *L. hyperborea* stipes (Norton et al.
502 1977, Whittick 1983, Steen et al. 2016), most likely caused by the observed high grazing activity in
503 the study area. Large grazing fronts of the sea urchin *Echinus esculentus* were regularly observed
504 and are known to feed on algae and associated epiphytes (Jorde & Klavestad 1963, Comely & Ansell

505 1988). This grazing may have affected our results in two ways. As *L. hyperborea* stipe communities
506 show vertical zonation (Whittick 1983, Christie et al. 2007), the upper-stipe epiphyte community
507 could have been disproportionately represented in this study (as the lower part of stipes were
508 often grazed). However, this likely did not affect the main results, as zonation patterns have only
509 been reported for Rhodophytes, and not for bryozoans and *Ectocarpus* spp. Secondly, during short-
510 term increases in nutrients, grazing can mediate the growth of epiphytes (Balata et al. 2010) and
511 macroalgae communities (Karez et al. 2004, Russell & Connell 2007). Top-down control of
512 epiphytes via grazing could, therefore, have limited any epiphyte biomass increase in response to
513 farm effluents, making impacts harder to detect. Given this, the low level of replication at the site
514 level in this study (n=3), and the substantial influence of natural variables on epiphyte
515 communities, our detection of a significant relationship between fish farm effluents and kelp
516 epiphytes is highly noteworthy.

517 4.2 Lamina epiphytes

518 In contrast to the stipe, there were no overall increases in lamina epiphyte abundance that could
519 be related to fish farming. Lamina epiphyte biomass decrease with increasing wave exposure
520 (Pedersen et al. 2012) which was also observed for macroalgae biomass in the present study when
521 including the site with the highest wave exposure level. The lowest cover of encrusting lamina
522 bryozoans was found at the sites with low effluent fish farming. A higher cover of colony forming
523 tunicates was however observed at these sites, which could indicate competition for space
524 between epiphytic invertebrates. Space-competition between colony-forming invertebrates on
525 host-plants is common (Boaden et al. 1976, O'Connor et al. 1980). Other factors not examined in
526 the present study could also have played a role in epiphyte abundance, for example, the growth
527 rate of kelp (Andersen et al. 2011) or grazer density (Worm & Lotze 2006, Balata et al. 2010). In
528 addition, both lamina bryozoans and epiphytes showed a negative relationship with kelp density.
529 One potential explanation for this relationship is that higher kelp density could directly reduce
530 epiphyte settlement via increased mechanical abrasion by sweeping laminas, as shown for
531 settlement of sessile invertebrates in the understory community (Jenkins et al. 1999, Connell
532 2003). Sampling time could also have had an effect; sampling was conducted in August, and a
533 higher lamina epiphyte diversity is present in May compared to August for *Saccharina latissima*
534 and *Laminaria digitata* (Carlsen et al. 2007), and could also be true for *L. hyperborea*. Hence,
535 sampling laminas earlier in the summer could have yielded different results.

536 Although we sampled from relatively many sites for this type of study, the variability in the dataset
537 was high. Clearly, the epiphytic community of kelp is variable both within and between sites at
538 small scales, as has also been found in previous work (Oh et al. 2015, Karez et al. 2004). For impact

539 studies, this means that a high number of sites are needed to detect impacts, especially when
540 abiotic and biotic factors differ between sites. In the present study, some factors could be
541 controlled for (e.g. the abiotic modelled factors, kelp density, depth), though the resolution of the
542 abiotic modelled factors could miss small-scale variations that have an influence.

543 4.4 Future perspectives

544 Changes in epiphytic communities on perennial algae can be seen as early warning signs of
545 eutrophication (Balata et al. 2010), and can over time be followed by a degradation of the perennial
546 species itself (e.g. Worm & Sommer 2000). The natural variation is high in these communities,
547 which often requires a rigorous sampling regime to be able to identify real changes. At the level of
548 sampling conducted herein, impacts were found on the epiphytic stipe community, which could
549 have implications for the fauna associated with this habitat and should be further explored. Though
550 no increase in lamina epiphytes was detected with increasing fish farm effluents, eutrophication
551 from fish farming may interact with other stressors in unpredictable ways. Climate change is one
552 potential stressor, as higher temperatures have negative impacts on kelp (Araújo et al. 2016,
553 Krumhansl et al. 2016) while promoting recruitment and growth rates of bryozoans such as *M.*
554 *membranacea* (Saunders & Metaxas 2008, Scheibling & Gagnon 2009). The Norwegian fish-farming
555 industry is expected to expand quickly in the future (NFD 2014), and dispersive coastal sites will
556 most likely be favoured due to their higher resilience. More understanding of the interactions
557 between kelp forest and aquaculture is needed to be able to monitor, detect potential changes,
558 and ensure healthy kelp forest ecosystems in the future.

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884 *Marine Science Symposia 1994*, Copenhagen, Denmark: International Council for the
885 *Exploration of the Sea*, 379-392.

886 **Table 1.** Overview of sampling sites for *Laminaria hyperborea* plants collected from farm sites (low
 887 effluent load: FF-L, high effluent load: FF-H) and reference sites (Ref-L, Ref-H) in the Frøya (Ref-L,
 888 FF-L) and Smøla (Ref-H, FF-H) archipelagos, Norway, August 2015. Information on the distance to
 889 nearest farm for all sites, and feed use at farms for the 6 months prior to sampling. MTB =
 890 maximum total biomass permitted at the farm.

Site	Group	MTB (tons)	Mean feed (kg/month) Jan-Jul 2015	Distance to farm (m)
<i>Frøya</i>				
S-1	FF-L	10920	122000	380
S-2	Ref-L			3850
S-3	Ref-L			12600
S-4	FF-L	8580	131000	60
S-5	FF-L	7800	79000	200
S-6	Ref-L			2890
<i>Smøla</i>				
S-7	Ref-H			7100
S-8	FF-H	8580	857000	150
S-9	FF-H	6240	466000	200
S-10	FF-H	5460	577000	520
S-11	Ref-H			3530
S-12	Ref-H			6020

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893 **Table 2.** Model structure and results of likelihood ratio tests. All models included *site* as a random
 894 effect. Below each response variable is given its transformation (if applicable), and the variance
 895 structure functions used in that model. For each predictor, the likelihood ratio (L) is given with
 896 degrees of freedom in subscript. Italics show the results when the site with high wave-exposure (in
 897 Ref-H) was excluded; bold indicates significant results ($p < 0.05$); a dash indicates variable not
 898 included. R^2 is calculated as a comparison of the residual variance of the full model against the
 899 residual variance of an intercept-only null model (Xu 2003).

Response variable and model info	Predictors						R^2
	Group	Kelp age	Kelp density	Current speed	Wave exposure	Light exposure	
Algal epiphytes (g per 10 cm ² stipe) <i>Square-root; varIdent(Group), varExp(Kelp age)</i>							
n = 110	L₃ = 10.6, p = 0.014	L₁ = 16.4, p < 0.001	L ₁ = 0.9, p = 0.337	L ₁ = 0.6, p = 0.430	L₁ = 5.0, p = 0.026	L ₁ = 0.6, p = 0.43	0.15
n = 100 (-site 7)	L₃ = 9.9, p = 0.020	L₁ = 12.2, p < 0.001	L ₁ = 0.9, p = 0.347	L ₁ = 0.2, p = 0.693	L ₁ = 1.3, p = 0.249	L ₁ = 0.2, p = 0.650	0.13
Bryozoan epiphytes (g per 10 cm ² stipe) <i>Square-root; varIdent(Group), varExp(Kelp age)</i>							
n = 110	L₃ = 28.0, p < 0.001	L ₁ = 1.4, p = 0.232	L ₁ = 0.1, p = 0.746	L ₁ = 0.3, p = 0.575	L₁ = 4.2, p = 0.041	L₁ = 5.2, p = 0.023	0.41
n = 100 (-site 7)	L₃ = 23.3, p < 0.001	L ₁ = 1.1, p = 0.300	L ₁ = 0.6, p = 0.454	L ₁ = 0.6, p = 0.440	L ₁ = 0.1, p = 0.800	L ₁ = 2.5, p = 0.112	0.48
Algal epiphytes (g per 100 g lamina) <i>varExp(Current Speed)</i>							
n = 115	L₃ = 11.1, p = 0.011	-	L₁ = 4.1, p = 0.042	L ₁ = 2.5, p = 0.112	L₁ = 8.6, p = 0.003	L₁ = 7.4, p = 0.006	0.37
n = 105 (-site 7)	L₃ = 14.4 p = 0.002	-	L₁ = 10.6, p = 0.001	L ₁ = 0.7, p = 0.414	L ₁ = 0.9, p = 0.339	L₁ = 14.5, p < 0.001	0.34
Encrusting bryozoan cover (cm ² per 100 cm ² lamina) <i>varIdent(Group), varExp(Current speed)</i>							
n = 60	L₃ = 32.7, p < 0.001	-	L₁ = 5.2, p = 0.022	L ₁ = 0.4, p = 0.529	L ₁ = 0.8, p = 0.376	L ₁ = 2.0, p = 0.158	0.28
n = 55 (-site 7)	L₃ = 27.6, p < 0.001	-	L₁ = 4.5, p = 0.034	L ₁ = 0.6, p = 0.448	L ₁ = 0.1, p = 0.801	L ₁ = 1.0, p = 0.314	0.59

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902 **Table 3.** Output of PERMANOVA conducted on the epiphytic community on *Laminaria hyperborea*
 903 stipes collected from farm sites (low effluent load: FF-L, high effluent load: FF-H) and reference
 904 sites (Ref-L, Ref-H) in the Frøya (Ref-L, FF-L) and Smøla (Ref-H, FF-H) archipelagos, Norway, August
 905 2015. The significance of chosen control variables in explaining the variation in the data, and
 906 pairwise comparison of levels within the *a priori* set factor *group* is shown (p-value based on
 907 permutations and adjusted for multiple testing in the former). Bold indicates significance (based
 908 on permutations, $\alpha = 0.05$).

<i>Control variable</i>	<i>Df</i>	<i>SS</i>	<i>Mean SS</i>	<i>F value</i>	<i>R²</i>	<i>P-value</i>
Group	3	3.462	1.154	4.813	0.13	0.001
Kelp age	1	0.881	0.881	3.676	0.03	0.001
Kelp density	1	1.172	1.172	4.888	0.04	0.001
Current speed	1	0.616	0.616	2.570	0.02	0.002
Wave exposure	1	0.325	0.325	1.357	0.01	0.150
Light exposure	1	0.697	0.697	2.905	0.03	0.002
Residuals	84	20.137	0.240		0.74	
Total	92	27.290			1.00	

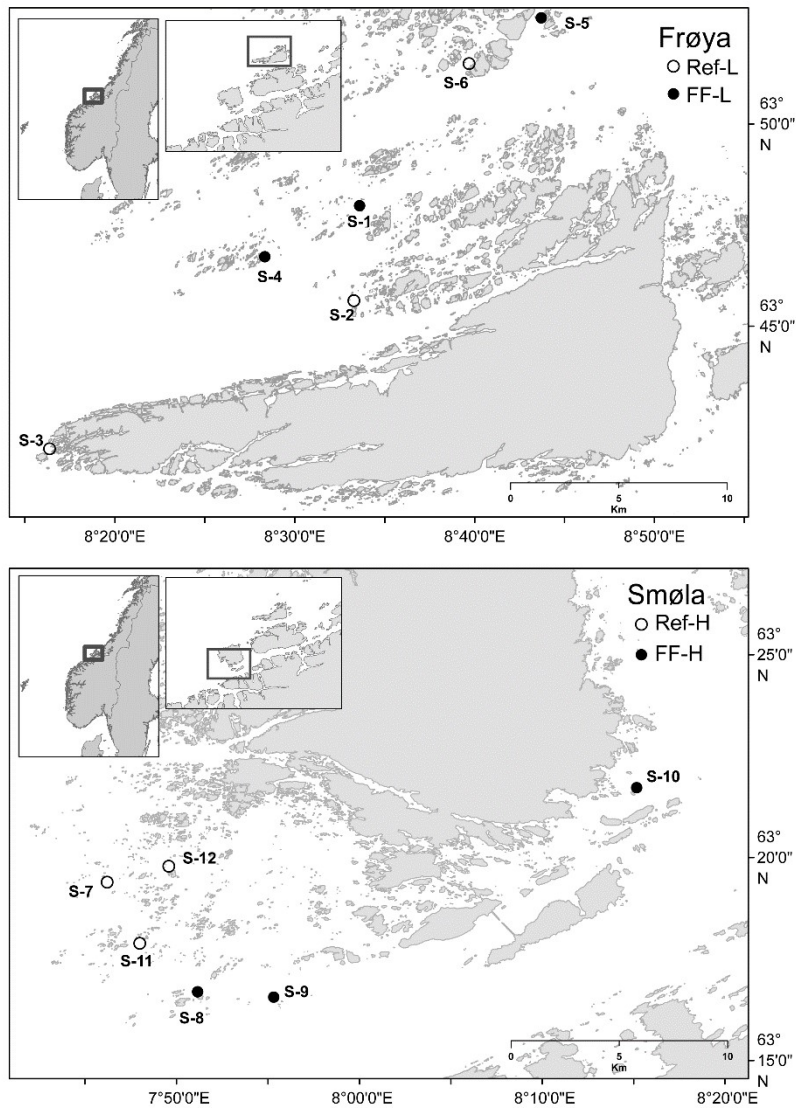
<i>Pairwise test: Group</i>	<i>F value</i>	<i>R²</i>	<i>P-value</i>
FF-L vs. Ref-L	0.803	0.02	0.754
FF-H vs. Ref-H	12.047	0.19	0.006
FF-L vs. FF-H	5.283	0.10	0.008
Ref-L vs. Ref-H	1.584	0.04	0.642

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911 **Table 4:** Results from SIMPER analysis of *Laminaria hyperborea* stipe epiphytes sampled from farm
 912 sites (FF-L, FF-H) and reference sites (Ref-L, Ref-H) in the Frøya and Smøla archipelago, Norway,
 913 August 2015. Cumulative contribution of species up to 90% of the dissimilarities between tested
 914 *group*-pairs is presented. Average biomass (standardized to 10 cm² ungrazed stipe) is shown for
 915 each contributing species in the *groups-pairs*, where bold indicates the highest biomass for each
 916 species. Percent in brackets refers to overall dissimilarity for the respective *group*-pairs (see Supp.
 917 Table 3 for species authorities).

Farm effect	Cumulative contribution	Average biomass (g)	
<i>FF-L vs Ref-L (91%)</i>			
		FF-L	Ref-L
<i>Phycodrys rubens</i>	0.26	0.643	0.175
<i>Palmaria palmata</i>	0.47	0.031	1.935
Bryozoa	0.62	0.272	0.034
<i>Polysiphonia stricta</i>	0.72	0.051	0.089
<i>Membranoptera alata</i>	0.79	0.130	0.112
<i>Ulva lactuca</i>	0.83	0.069	0.019
<i>Delesseria sanguinea</i>	0.87	0.180	0.000
<i>Ectocarpus</i> spp.	0.91	0.033	0.002
<i>FF-H vs Ref-H (82%)</i>			
		FF-H	Ref-H
Bryozoa	0.61	1.256	0.458
<i>Palmaria palmata</i>	0.69	0.468	0.013
<i>Phycodrys rubens</i>	0.76	0.046	0.061
<i>Membranoptera alata</i>	0.82	0.092	0.048
<i>Ectocarpus</i> spp.	0.87	0.278	0.018
<i>Polysiphonia stricta</i>	0.91	0.021	0.019
<i>FF-L vs FF-H (83%)</i>			
		FF-H	FF-L
Bryozoa	0.47	1.256	0.272
<i>Phycodrys rubens</i>	0.64	0.046	0.643
<i>Palmaria palmata</i>	0.71	0.468	0.031
<i>Membranoptera alata</i>	0.78	0.092	0.130
<i>Ectocarpus</i> spp.	0.83	0.278	0.033
<i>Delesseria sanguinea</i>	0.87	0.000	0.180
<i>Polysiphonia stricta</i>	0.90	0.021	0.051

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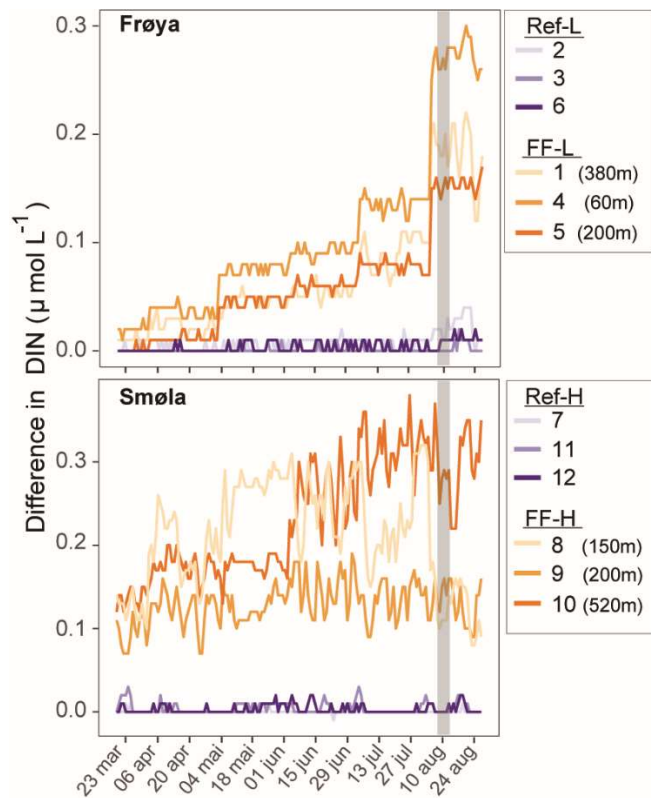


919

920 **Figure 1.** Geographical location of sampling sites in the two study areas; (top) in the Frøya
 921 archipelago and (bottom) in the Smøla archipelago. Norway. *Laminaria hyperborea* plants were
 922 collected along transects at 5 m depth on 3 reference sites (open circles) and 3 salmonid fish
 923 farming sites (closed circles) in each area. FF-L and FF-H denote fish farm with low and high effluent
 924 load at the time of sampling, respectively.

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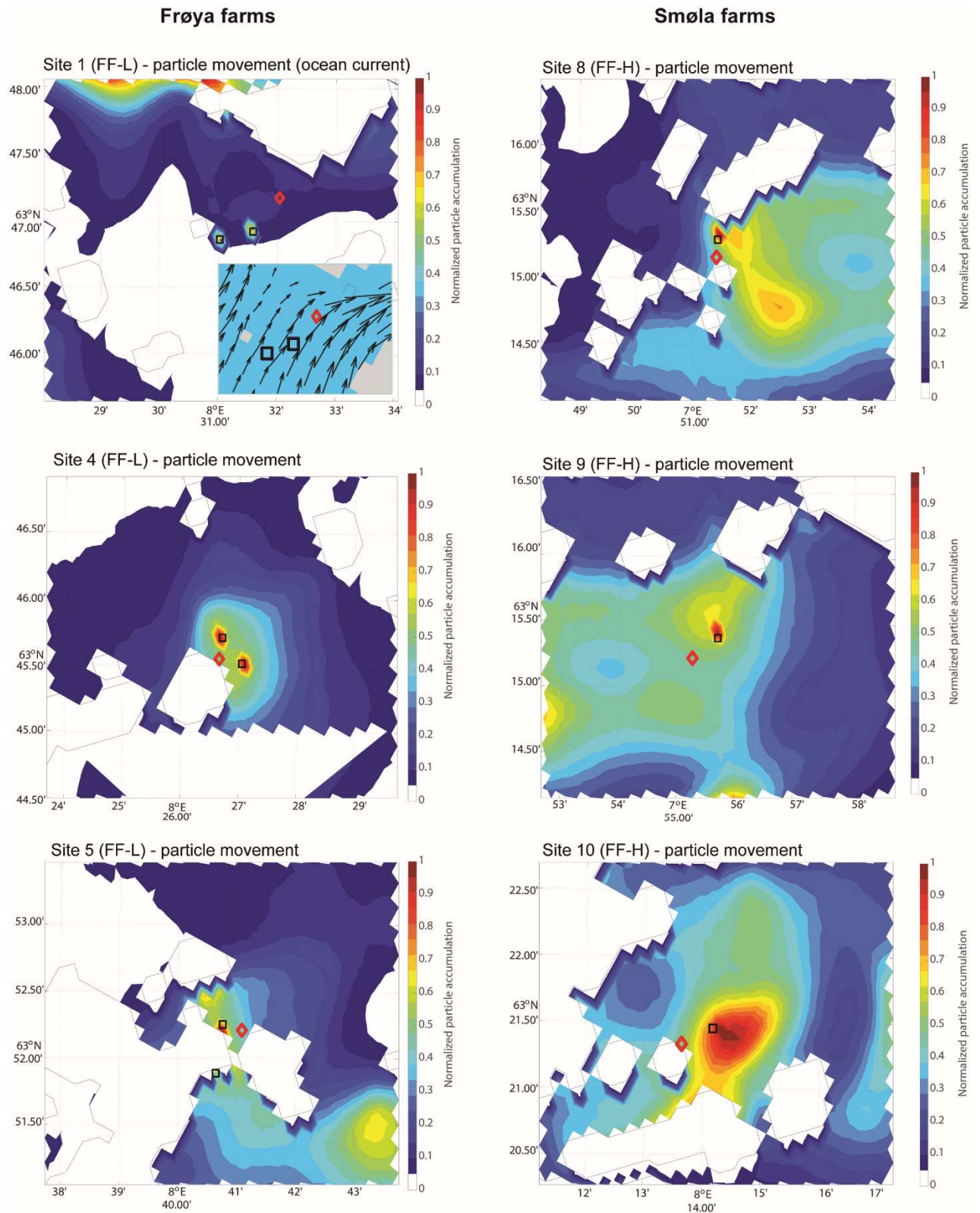
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928 **Figure 2.** Predicted effect of fish farm effluents (low levels: FF-L. high levels: FF-H) on background
 929 dissolved inorganic nitrogen concentration (DIN; $\mu\text{mol l}^{-1}$) in the upper 5 m of the water column at
 930 3 farm sites (FF-L) and 3 reference sites (Ref-L) located in the Frøya archipelago (top), and at 3 farm
 931 sites (FF-H) and 3 reference sites (Ref-H) located in the Smøla archipelago (bottom), Norway. The
 932 difference in predicted DIN concentrations is between two model simulations (NORWECOM) run
 933 for 4.5 months prior to sampling; one simulation conducted with, and one simulation without, the
 934 presence of the study farms. The grey bar marks the sampling time. For farm sites, the distance to
 935 the closest farm is included in parenthesis to show the lack of impact-gradient in the study area.

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Figure 3. Predicted effluent plume and movement of particles released from fish farms (low
 939 levels: FF-L, high levels: FF-H) at 3 farm sites (FF-L) located in the Frøya archipelago (left), and at 3
 940 farm sites (FF-H) located in the Smøla archipelago (right), Norway. Sampling sites for the
 941 collection of *Laminaria hyperborea* are shown as red diamonds, and fish farms as black squares.

942 For Site 1, modeled ocean currents are also presented (inset), as the resolution of the particle
 943 model did not capture small skerries north of the sampling site in this specific area, resulting in
 944 an unrealistic prediction of particle movement.

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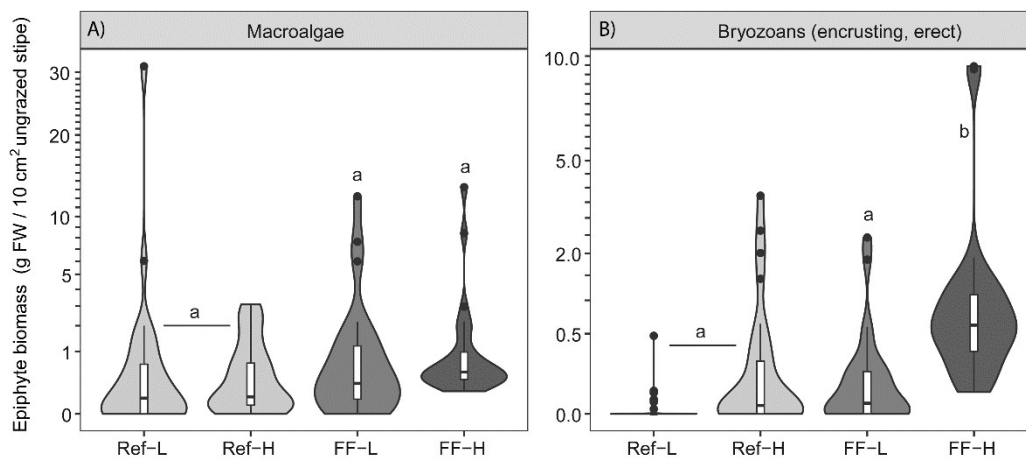
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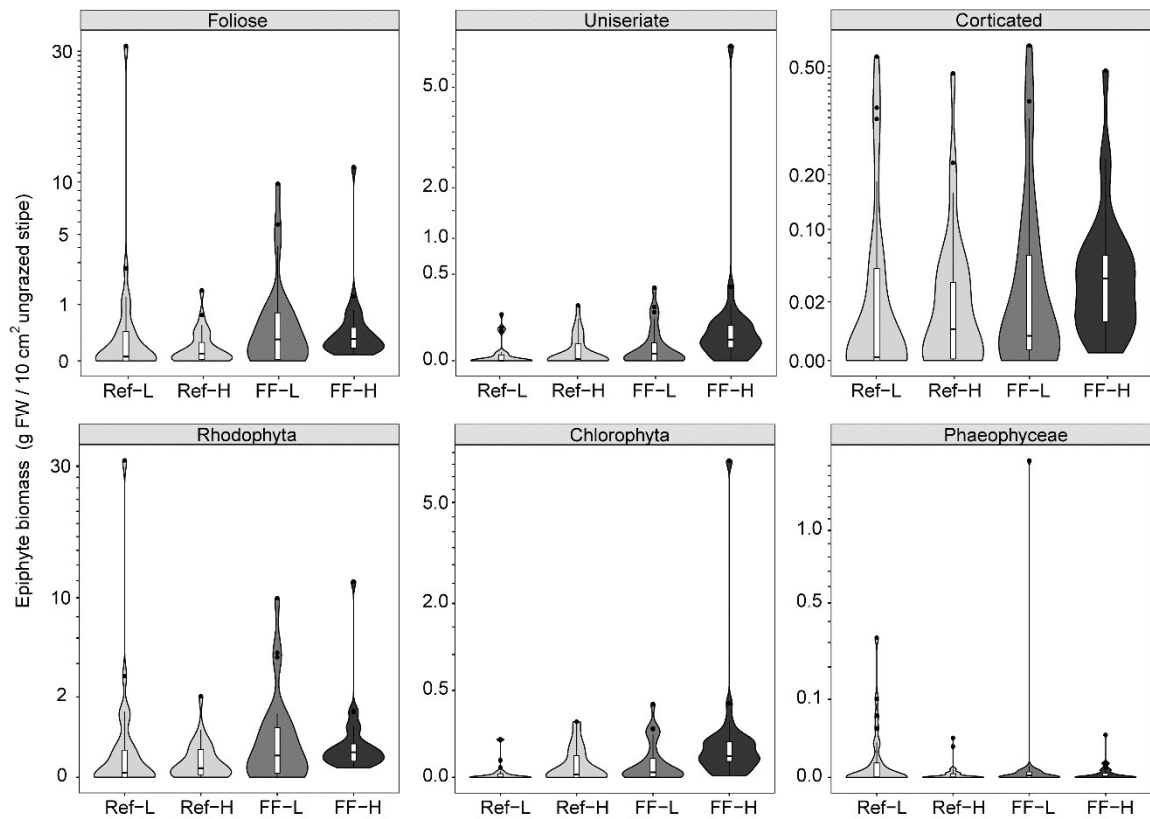


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952 **Figure 4.** Epiphytic biomass (g FW standardized to 10 cm² ungrazed stipe) on stipes of *Laminaria*
 953 *hyperborea* collected from 5 m depth at farm sites (low effluent levels: FF-L, high effluent levels:
 954 FF-H) and reference sites (Ref-L, Ref-H) in the Frøya (Ref-L, FF-L) and Smøla (Ref-H, FF-H)
 955 archipelagos, Norway. Epiphytes are split in macroalgae (A) and bryozoans (B), the latter including
 956 bush-like species (erect) and macroalgae >40 % covered by encrusting species. Each *group* consists
 957 of samples from 3 sites. each with 9-10 thalli. Note that the y-axes are on different square root
 958 scales to allow high values to be displayed without hiding the distribution of low values, where
 959 most of the data lie. The distribution of the data within each *group* is shown by violin plots (scaled
 960 to have constant maximum widths); overlaid are boxplots which show the median, upper and
 961 lower quartiles (boxes), as well as the spread of the data within 1.5x the interquartile range (tails).
 962 Points represent observations outside this range. Different letters denote significant difference
 963 (Dunnett's post-hoc, $\alpha = 0.05$).

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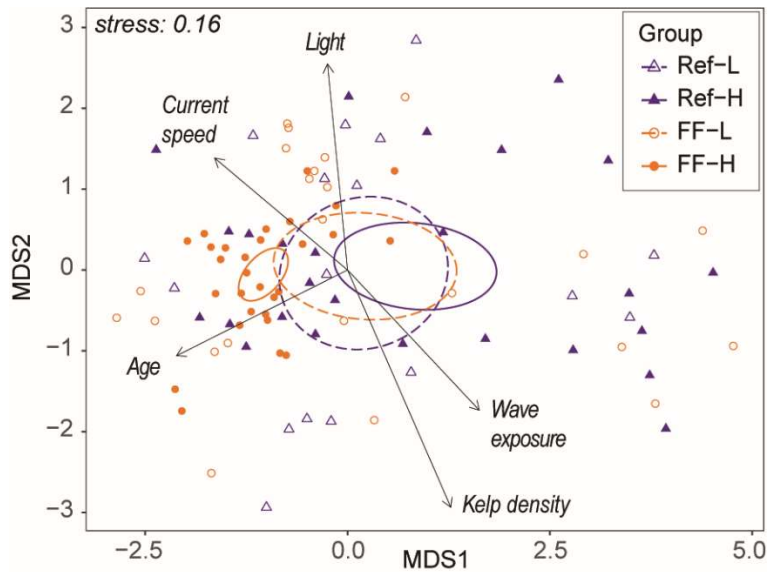
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967 **Figure 5.** Biomass of macroalgal epiphytes (g FW standardized to 10 cm² ungrazed stipe) present
 968 on stipes of *Laminaria hyperborea* at farm sites (low effluent levels: FF-L, high effluent levels: FF-
 969 H) and reference sites (Ref-L, Ref-H) in the Frøya (Ref-L, FF-L) and Smøla (Ref-H, FF-H) archipelagos,
 970 Norway. Epiphytes are split by morphological type (top row), or by taxonomic *group* (bottom row).
 971 Note that the y-axes are on different square root scales, to allow high values to be displayed
 972 without hiding the distribution of low values. For plot explanation see Figure 4.

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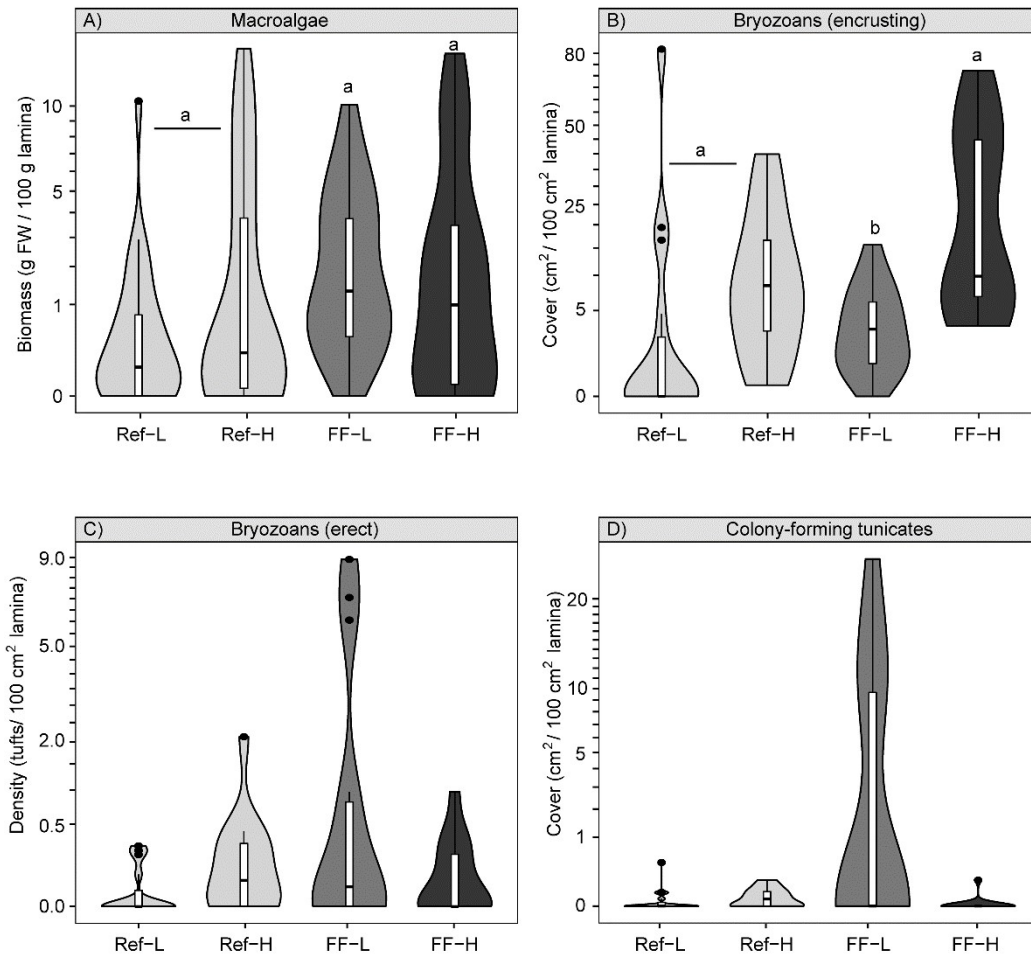


974

975 **Figure 6.** NMDS of the epiphytic community on stipes of *Laminaria hyperborea* collected from 5 m
 976 depth at farm sites (low effluent levels: FF-L, high effluent levels: FF-H) and reference sites (Ref-L,
 977 Ref-H) in the Frøya (Ref-L, FF-L) and Smøla (Ref-H, FF-H) archipelagos, Norway. Each *group*
 978 consisted of 3 sites. The NMDS was based on Bray-Curtis similarity matrix on square-root
 979 transformed biomass (g FW standardized to 10 cm² ungrazed stipe) community data. Overlaid are
 980 vectors of significant environmental variables (including site 7, see statistical analysis), and 95%
 981 confidence ellipses around *group* centroids.

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985 **Figure 7.** Biomass of algal and invertebrate epiphytes present on *Laminaria hyperborea* laminae at
 986 farm sites (low effluent levels: FF-L, high effluent levels: FF-H) and reference sites (Ref-L, Ref-H) in
 987 the Frøya (Ref-L, FF-L) and Smøla (Ref-H, FF-H) archipelagos, Norway. (A) Algal epiphytes (3 sites
 988 per *group*, each with 9-10 thalli) is in g FW per 100 g lamina weight; (B, D) Encrusting bryozoans
 989 and colony-forming tunicates (3 sites per *group*, each with 5 thalli) is in cm² per 100 cm² lamina
 990 surface area; (C) Erect bryozoans (3 sites per *group*, each with 5 thalli) is in number of tufts per 100
 991 cm² lamina surface area. Note that the y-axes are on different square root scales to allow high
 992 values to be displayed without hiding the distribution of low values. Different letters denote
 993 significant difference (Dunnnett's post-hoc, $\alpha = 0.05$). For plot explanation see Figure 4.

994 **Supplementary information**

995 **Supplementary Table 1.** Mean and standard deviation (SD) of *Laminaria hyperborea* stipe length,
 996 ungrazed stipe surface area (SA) and lamina weight used to standardize epiphytic response
 997 variables, and the control variables used in the statistical analyses.

Group	Stipe length (cm)	Ungrazed SA (cm ²)	Lamina weight (g)	Kelp age	Kelp density (ind m ⁻²)	Wave exposure index	Mean current speed (m s ⁻¹)	Light exposure
<i>Ref-L</i>	96 (30)	98 (97)	626 (385)	6 (3)	9 (8)	81 511 (69 732)	0,01 (0,02)	0,02 (0,04)
<i>FF-L</i>	76 (25)	98 (113)	756 (360)	5 (1)	3 (3)	150 964 (112 948)	0,04 (0,04)	-0,06 (0,06)
<i>Ref-H</i>	65 (21)	42 (43)	559 (259)	5 (2)	7 (7)	379 474 (277 787)	0,03 (0,03)	-0,04 (0,07)
<i>FF-H</i>	57 (13)	130 (119)	530 (254)	6 (1)	5 (2)	79 598 (53 607)	0,04 (0,01)	-0,07 (0,06)

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1002 **Supplementary Table 2.** Variance inflation factors (VIF) for the control variables used in statistical
 1003 models to assess the impact of fish farm effluents on the epiphytic community of *Laminaria*
 1004 *hyperborea*.

Control variable	VIF
Kelp age	1.30
Kelp density	3.17
Current speed	2.57
Wave exposure	1.34
Light exposure	1.60

1005

1006

1007 **Supplementary Table 3.** Species list of macroalgae found growing epiphytically on stipes of
 1008 *Laminaria hyperborea* in the Frøya and Smøla archipelagos, Norway, August 2015. Morphological
 1009 group is a rough grouping of the species to examine whether there were trends relating to fish
 1010 farming.

Species	Authority	Morphological group
Phaeophyceae		
<i>Alaria esculenta</i>	(Linnaeus) Greville	Other
<i>Chaetopteris plumosa</i>	(Lyngbye) Kützing	Corticated
<i>Desmarestia aculeata</i>	(Linnaeus) J.V.Lamouroux	Other
<i>Desmarestia viridis</i>	(O.F.Müller) J.V.Lamouroux	Other
<i>Desmarestia</i> spp.	J.V.Lamouroux	Other
<i>Dictyota dichotoma</i>	(Hudson) J.V.Lamouroux	Foliose
<i>Ectocarpus</i> spp.	Lyngbye	Uniseriate
<i>Hincksia</i> spp.	J.E.Gray	Uniseriate
<i>Laminaria hyperborea</i>	(Gunnerus) Foslie	Other
Laminariales indet.		Other
<i>Litosiphon laminariae</i>	(Lyngbye) Harvey	Other
<i>Mesogloia vermiculata</i>	(Smith) S.F.Gray	Other
<i>Pogotrichium filiforme</i>	Reinke	Uniseriate
<i>Pylaiella littoralis</i>	(Linnaeus) Kjellman	Uniseriate
<i>Pylaiella</i> spp.	Bory	Uniseriate
<i>Scytosiphon lomentaria</i>	(Lyngbye) Link	Other
<i>Sphacelaria cirrosa</i>	(Roth) C. Agardh	Corticated
<i>Sphacelaria plumula</i>	Zanardini	Corticated
<i>Sphacelaria rigidula</i>	Kützing	Corticated
<i>Sphacelaria</i> spp.	Lyngbye	Corticated
Chlorophyta		
<i>Acrosiphonia arcta</i>	(Dillwyn) Gain	Uniseriate
<i>Bryopsis plumosa</i>	(Hudson) C.Agardh	Uniseriate
<i>Chaetomorpha ligustica</i>	(Kützing) Kützing	Uniseriate
<i>Chaetomorpha melagonium</i>	(F.Weber & D.Mohr) Kützing	Uniseriate
<i>Chaetomorpha</i> spp.	Kützing	Uniseriate
<i>Cladophora rupestris</i>	(Linnaeus) Kützing	Uniseriate
<i>Cladophora</i> spp.	Kützing	Uniseriate
<i>Codium fragile</i>	(Suringar) Hariot	Other
<i>Derbesia marina</i>	(Lyngbye) Solier	Uniseriate
<i>Rhizoclonium</i> spp.	Kützing	Uniseriate
<i>Spongomorpha aeruginosa</i>	(Linnaeus) Hoek	Uniseriate
<i>Ulva lactuca</i>	Linnaeus	Foliose
<i>Ulva prolifera</i>	O.F. Müller	Foliose
<i>Ulva</i> spp.	Linnaeus	Foliose
Rhodophyta		
<i>Aglaothamnion tenuissimum</i>	(Bonnemaison) Feldmann-Mazoyer	Uniseriate
<i>Antithamnion nipponicum</i>	Yamada & Inagaki	Uniseriate
<i>Apoglossum ruscifolium</i>	(Turner) J.Agardh	Foliose
<i>Bonnemaisonia hamifera</i>	Hariot	Uniseriate
<i>Callithamnion corymbosum</i>	(Smith) Lyngbye	Uniseriate
<i>Callithamnion tetragonum</i>	(Withering) S.F.Gray	Uniseriate

<i>Ceramium virgatum</i>	Roth	Corticated
<i>Ceramium</i> spp.	Roth	Corticated
<i>Corallina officinalis</i>	Linnaeus	Other
<i>Cryptopleura ramosa</i>	(Hudson) L.Newton	Foliose
<i>Dasysiphonia japonica</i>	Yendo	Corticated
<i>Delesseria sanguinea</i>	(Hudson) J.V.Lamouroux	Foliose
Delesseriaceae spp.	Bory	Foliose
<i>Euthora cristata</i>	(C.Agardh) J.Agardh	Foliose
<i>Haraldiophyllum bonnemaisonii</i>	(Kylin) A.D.Zinova	Foliose
<i>Lomentaria clavellosa</i>	(Lightfoot ex Turner) Gaillon	Other
<i>Membranoptera alata</i>	(Hudson) Stackhouse	Foliose
<i>Metacallophyllis laciniata</i>	(Hudson) A.Vergés & L.Le Gall	Foliose
<i>Nitophyllum punctatum</i>	(Stackhouse) Greville	Foliose
<i>Palmaria palmata</i>	(Linnaeus) F.Weber & D.Mohr	Foliose
<i>Phycodrys rubens</i>	(Linnaeus) Batters	Foliose
<i>Polysiphonia brodiei</i>	(Dillwyn) Sprengel	Corticated
<i>Polysiphonia elongata</i>	(Hudson) Sprengel	Corticated
<i>Polysiphonia fibrillosa</i>	(Dillwyn) Sprengel	Corticated
<i>Polysiphonia stricta</i>	(Mertens ex Dillwyn) Greville	Corticated
<i>Symphocladia parasitica</i>	(Hudson) Savoie & G.W.Saunders	Corticated
<i>Ptilota gunneri</i>	P.C.Silva, Maggs & L.M.Irvine	Corticated
<i>Rhodochorton purpureum</i>	(Lightfoot) Rosenvinge	Uniseriate
<i>Rhodomela lycopodioides</i>	(Linnaeus) C.Agardh	Corticated
<i>Rhodomela</i> sp.	C.Agardh	Corticated
<i>Scagelia pylaisaei</i>	(Montagne) M.J.Wynne	Uniseriate
<i>Spermothamnion repens</i>	(Dillwyn) Magnus	Uniseriate
<i>Spermothamnion</i> sp.	Areschoug	Uniseriate

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1013 **Supplementary Table 4.** Results of vector fitting to community data. Significance (based on 999
 1014 permutations) and explained variation (r^2) are shown for control variables and the *a-priori* factor
 1015 *Group* fitted to the 2-dimensional ordination space (Fig. 5) of epiphytic biomass on *Laminaria*
 1016 *hyperborea* stipes collected from 12 sites in the Frøya and Smøla archipelagos, Norway. Each level
 1017 of *Group* consisted of samples from 3 sites, each with 9-10 thalli. Italics show the results when the
 1018 site with high wave-exposure (in Ref-H) was excluded; bold indicates significance ($\alpha = 0.05$).

Model info	Control variable					
	Group	Kelp age	Kelp density	Current speed	Wave exposure	Light exposure
$n = 95$	$r^2 = 0.11,$ $p = 0.005$	$r^2 = 0.14,$ $p = 0.003$	$r^2 = 0.27,$ $p = 0.001$	$r^2 = 0.12,$ $p = 0.007$	$r^2 = 0.15,$ $p = 0.001$	$r^2 = 0.17,$ $p = 0.001$
$n = 86$ (-site 7)	$r^2 = 0.08,$ $p = 0.043$	$r^2 = 0.13,$ $p = 0.002$	$r^2 = 0.13,$ $p = 0.003$	$r^2 = 0.02,$ $p = 0.356$	$r^2 < 0.01,$ $p = 0.945$	$r^2 = 0.10,$ $p = 0.016$

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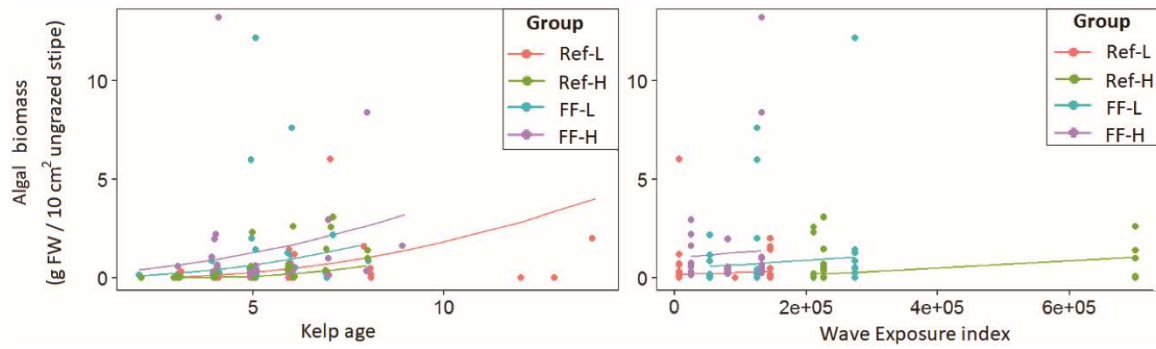
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1025 **Supplementary Table 5.** Results of PERMDISP on the epiphyte community of *Laminaria hyperborea*
 1026 stipes collected from 5 m depth at farm sites (low effluent levels: FF-L, high effluent levels: FF-H)
 1027 and reference sites (Ref-L, Ref-H) in the Frøya (Ref-L, FF-L) and Smøla (Ref-H, FF-H) archipelagos,
 1028 Norway. The degree of dispersion (i.e. average distance to centroid) is shown for each level within
 1029 *Group* for the epiphytic macroalgae community, and for the epiphyte community including
 1030 bryozoans. Each level of *Group* consisted of samples from 3 sites, each with 9-10 thalli.

Community	Group			
	Ref-L	Ref-H	FF-L	FF-H
<i>Macroalgae</i>	1.370	1.326	1.219	0.931
<i>Macroalgae + bryozoans</i>	1.230	1.336	1.407	0.652

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1033 **Supplementary Figure 1.** Model predictions (lines) for kelp age (left) and wave exposure (right)
 1034 over collected data (points) of algal epiphytes on stipes of *Laminaria hyperborea* at farm sites (low
 1035 levels: FF-L, high levels: FF-H) and reference sites (Ref-L, Ref-H) in the Frøya (Ref-L, FF-L) and Smøla
 1036 (Ref-H, FF-H) archipelagos, Norway. The points have been jittered to reduce overplotting.

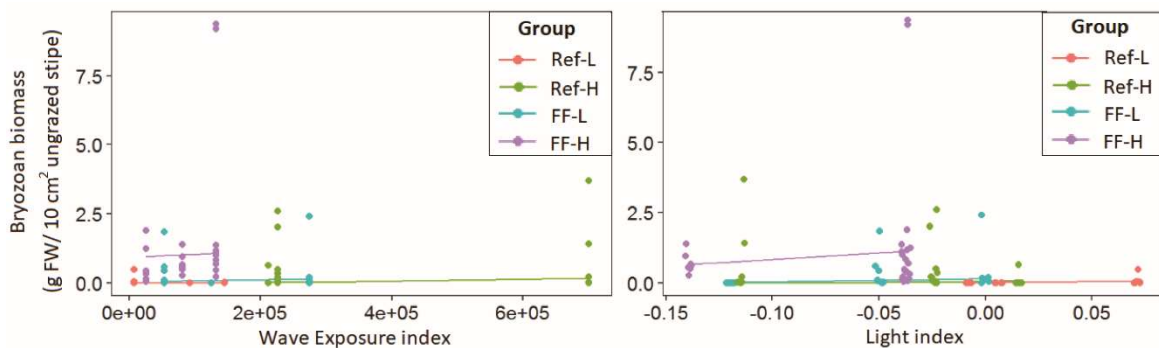
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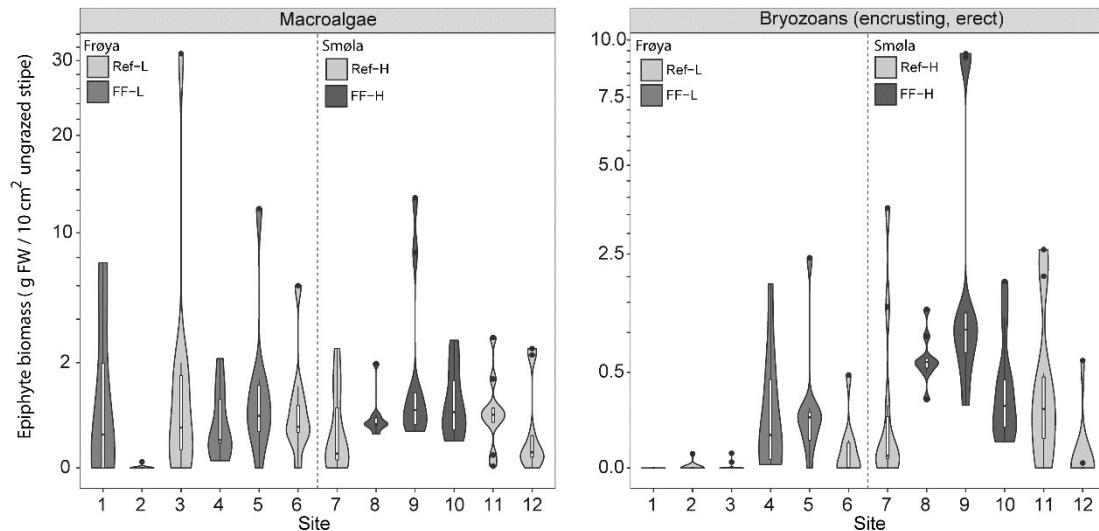
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1043 **Supplementary Figure 2.** Model predictions (lines) for wave exposure (left) and light (right) over
 1044 collected data (points) of the biomass of bryozoan epiphytes present on stipes of *Laminaria*
 1045 *hyperborea* at farm sites (low levels: FF-L, high levels: FF-H) and reference sites (Ref-L, Ref-H) in the
 1046 Frøya (Ref-L, FF-L) and Smøla (Ref-H, FF-H) archipelagos, Norway. The points have been jittered to
 1047 reduce overplotting.

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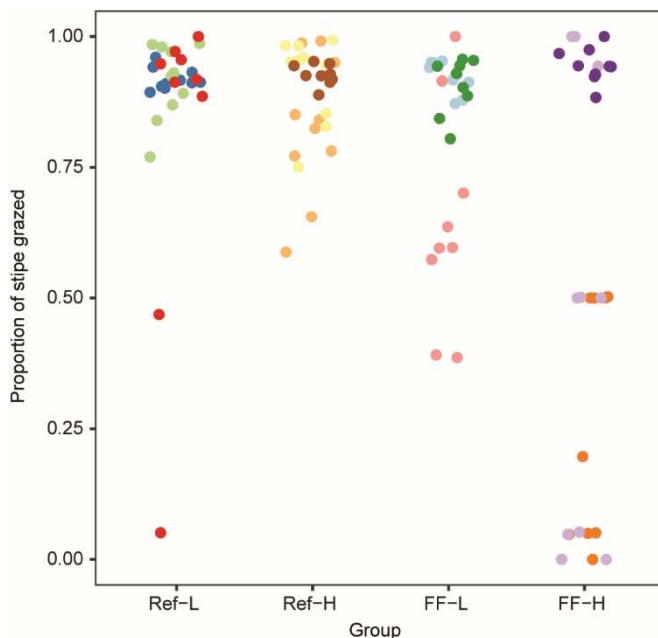


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1050 **Supplementary Figure 3.** Biomass of epiphytes present on stipes of *Laminaria hyperborea* at farm sites (low levels: FF-L, high levels: FF-H) and reference sites (Ref-L, Ref-H) in the Frøya (Ref-L, FF-L)
 1051 and Smøla (Ref-H, FF-H) archipelagos, Norway. The two areas are separated by a vertical dashed
 1052 line (left-hand side = FF-L and Ref-L, right = FF-H and Ref-H). Each site consists of 9-10 thalli. Note
 1053 that the y-axis is on a square root scale, to allow high values to be displayed without hiding the
 1054 distribution of low values, where most of the data lie. For plot explanation see Figure 4.
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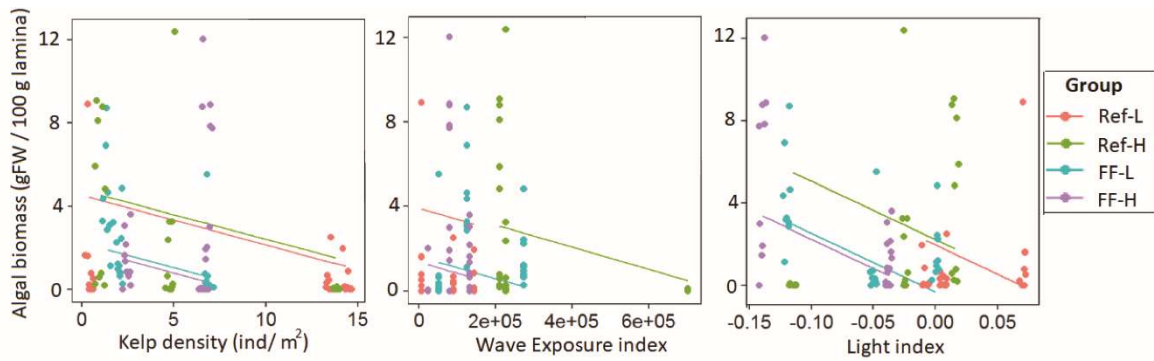
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1059 **Supplementary Figure 4.** Urchin grazing on stipes of *Laminaria hyperborea* at farm sites (low levels:
 1060 FF-L, high levels: FF-H) and reference sites (Ref-L, Ref-H) in the Frøya (Ref-L, FF-L) and Smøla (Ref-
 1061 H, FF-H) archipelagos, Norway. Different colors represent the 12 sites. The points have been
 1062 jittered to reduce overplotting.



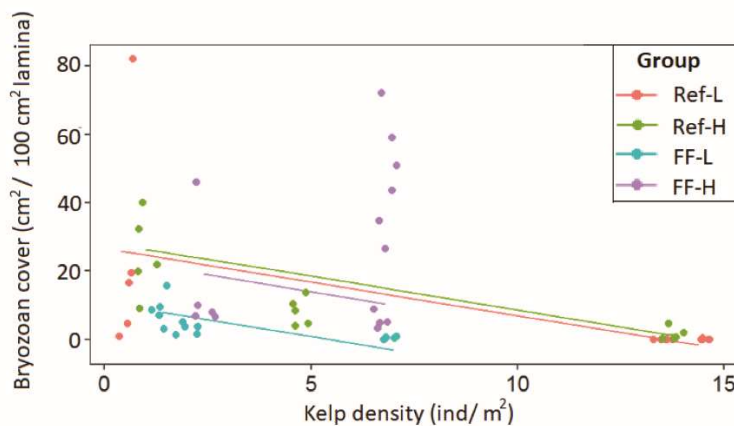
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1064 **Supplementary Figure 5.** Model predictions (lines) for kelp density (left), wave exposure (middle)
 1065 and light (right) over collected data (points) of algal epiphytes on laminas of *Laminaria hyperborea*
 1066 at farm sites (low levels: FF-L, high levels: FF-H) and reference sites (Ref-L, Ref-H) in the Frøya (Ref-
 1067 L, FF-L) and Smøla (Ref-H, FF-H) archipelagos, Norway. The points have been jittered to reduce
 1068 overplotting.

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1073 **Supplementary Figure 6.** Model predictions for kelp density (lines) over collected data (points) of
 1074 encrusting bryozoan cover on laminas of *Laminaria hyperborea* at farm sites (low levels: FF-L, high
 1075 levels: FF-H) and reference sites (Ref-L, Ref-H) in the Frøya (Ref-L, FF-L) and Smøla (Ref-H, FF-H)
 1076 archipelagos, Norway. The points have been jittered to reduce overplotting.

1 **High-intensity finfish farming and kelp forests at dispersive locations. II.**
2 **Resilience in macrofauna communities in *Laminaria hyperborea* holdfasts**
3 **exposed to farm emissions**

4

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