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Phosphorus in *Saccharina latissima*

Initial uptake, storage, and possibilities for recycling in IMTA-systems

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Abstract

The potential future phosphorus scarcity is a challenge that can be directly linked to food security, and a range of solutions are needed in order to shift the anthropogenic phosphorus use into renewable and more sustainable forms. One suggestion is to exploit opportunities with recirculation of biological waste from aquaculture production by co-cultivation of kelp species like *Saccharina latissima* in integrated multi-trophic aquaculture (IMTA) systems. The aim of this study was to characterize the initial uptake and storage of phosphorus in *S. latissima*, and assess the recycling potential for phosphorus through the IMTA cultivation of this species.

The initial uptake of dissolved inorganic phosphorus (DIP) in preconditioned *S. latissima* of different nutritional states, was characterized by increasing DIP availability. To explore the incorporation of phosphorus from salmon aquaculture, a cultivation study at Frøya was conducted by deploying seaweed along a gradient downstream from a salmon farm in a period from February to June.

It was found that DIP initial uptake in *S. latissima* was strongly affected by the nutritional state of the kelp, with low DIP uptake levels for the phosphorus depleted group. The acclimatization of this group was found to be specifically harsh as the phosphorus depletion influenced the physiological state of the kelp. The nutritionally saturated group expressed good levels of uptake, with a linear increase in uptake rate (y µg·g$_{DW}^{-1}$L^{-1}hour^{-1}) with increasing treatment concentrations (x µmolP·L^{-1}) expressed as $y = 23.7x + 6.0$. The maximum measured uptake rate was 52.4±6.7 µg·g$_{DW}^{-1}$L^{-1}hour^{-1} for the 2.0 µmolP·L^{-1} treatment.

The internal distribution in tissue content of phosphorus (P) and nitrogen (N) showed highest content near the meristematic tissue in growth phase individuals (April), indicating a prioritization of nutrients to facilitate growth. Biomass yields were significantly higher in the seaweed group integrated to the salmon farm at all sampling days, with a peak yield of 7.01±0.88 kg$_{FW}$·m^{-1} in June. This supported former studies showing increased biomass yield in close proximity to salmon farms.

Biomass yield was promoted as the main component needed to estimate the magnitude of bioremediation and recirculation potential, as nutrient content was determined to be more applicable for evaluating nutritional state and possible limitations.

From the results of this study, IMTA was recognized as a potential opportunity for indirect recirculation of phosphorus by re-introduction to a new value chain. However, in order to optimize both the bioremediatory and recycling potential for phosphorus through IMTA, organisms able to utilize particulate forms of phosphorus waste release should also be included.

Sammendrag

Mulig framtidig fosforknapphet er en utfordring som kan knyttes direkte til framtidens matsikkerhet, og derav behovet for nye løsninger som kan gjøre måten menneskers bruker av fosfor mer gjenvinnbar og bærekraftig. Et alternativ er å nyttegjøre seg av biologisk resirkulering av avfallsstoffer fra akvakulturproduksjon gjennom samdyrking av tarearter som *Saccharina latissima* i et integrert multi-trofisk akvakultursystem (IMTA). Målet med denne studien var å karakterisere initialopptak og lagring av fosfor in *S. latissima*, og vurdere potensialet for fosforresirkulering gjennom IMTA dyrking av denne arten.

Initialopptaket av løst uorganisk fosfor (DIP) i *S. latissima* ble karakterisert ved økende fosforkonsentrasjon for to taregrupper med ulik ernæringstilstand, henholdsvis fosforsultet og -mettet. Det ble også gjennomført en dyrkningsstudie med *S. latissima* utenfor Frøya for å utforske inkorporeringen av fosfor fra lakseoppdrett. Dette ble gjort ved å sette ut dyrkningstau med tare langs en gradient som strakk seg nedstrøms fra oppdrettsanlegget i perioden februar til juni.

Initialopptaket av DIN i *S. latissima* ble vist å være sterkt påvirket av ernæringstilstanden til taren, noe som førte til lavt opptak i den fosforsultede gruppen. Akklimatiseringen av denne gruppen viste seg å være spesielt hard ettersom den fysiologiske tilstanden til taren også ble påvirket. Det ble vist gode opptaksnivå av DIP in den fosformettede gruppen, og den lineære økning i opptakshastighet (y µg·g$_{DW}^{-1}$L^{-1}time^{-1}) ved økende fosforkonsentrasjon (x µmolP·L^{-1}) i mediet ble uttrykt som $y = 23.7x + 6.0$. Den maksimale opptakshastigheten ble målt til 52.4±6.7 µg·g$_{DW}^{-1}$L^{-1}time^{-1} for en mediumskonsentrasjon på 2.0 µmolP·L^{-1}.

Den innvendige fordeling av nitrogen- og fosforinnhold i vev ble vist å være høyest i området nær det meristematiske vevet i vekstfaseindivider (april), som peker mot en prioritering av næringsstoffer for å muliggjøre vekst. Biomasseproduksjonen var signifikant høyere for alle prøvetakingsdagene i taregruppen som var integrert i oppdrettsanlegget, som viste en biomasseproduksjon på 7.01±0.88 kg$_{FW}$·m^{-1} i juni. Dette resultatet støttet tidligere studier som har vist økt biomasseproduksjon i nærhet til anlegg med lakseoppdrett.

Biomasseproduksjon ble fremhevet som en nødvendig komponent for å beregne størrelsesordenen av bioremediering- og resirkuleringspotensial, ettersom næringsinnhold i vev ble ansett å være mer anvendelig for å bedømme ernæringstilstand og mulige begrensninger for tarens fysiologiske tilstand.

Basert på resultatene i denne studien, ble IMTA ansett som en potensiell mulighet for indirekte resirkulering av fosfor ved at det introduseres i en ny verdikjede. For å kunne optimalisere både bioremediering- og resirkuleringspotensialet for fosfor fra IMTA, bør systemet inkludere arter som kan utnytte de partikulære formene av fosfor fra biologisk avfall.

Preface

This thesis has been completed at the Department of Biology at NTNU Trondheim in collaboration with SINTEF Ocean. The work was funded by the Research Council of Norway and Horizon 2020 through affiliation with two projects:

(i) GENIALG (Genetic diversity exploitation for innovative macroalgal biorefinery), an EU-project funded by Horizon 2020. The project aims to develop cost-efficient cultivation systems, processing methods, and a range of products, securing a sustainable use of the entire kelp biomass. The thesis related work have been coordinated with SINTEF Ocean, which also provided numbers for specific parameters collected by EXPOSED Aquaculture Research Centre (Centre for Research based Innovation) funded by the Research Council of Norway (grant number 237790).

(ii) Mind-P (Nutrients in a Circular Bioeconomy: Barriers and Opportunities for Mineral Phosphorus Independence in Norway), a NTNU project funded by the Research Council of Norway (BIONÆR Bionæringsprogram, Project number 268338). Through the use of models combining geographical information systems (GIS) and material flow analysis (MFA), this project aims to identify solutions and develop scenarios for phosphorus independency including the highlighting of consequent barriers, trade-offs, and industrial opportunities.

Table of Contents

List of Tables

List of Figures

Abbreviations

AIC	Akaike information criterion
ANOVA	Analysis of variance
ATP	Adenosine TriPhosphate (Energy carrier in biological processes)
CAM	Cellulose acetate membrane
CI	Confidence interval
DIN	Dissolved Inorganic Nitrogen
DIP	Dissolved Inorganic Phosphorus
DW	Dry weight
FW	Fresh weight
IMTA	Integrated Multi-Trophic Aquaculture
MPD	Model for DIP uptake in phosphorus depleted individuals
NS-EN-ISO6878	Norwegian standard for determination of inorganic phosphorus
NS4745	Norwegian standard for determination of inorganic nitrogen (NO_2/NO_3)
NS9410	Norwegian standard for environmental monitoring of benthic impact from marine fish farms
NTNU	Norwegian University of Science and Technology
PD	Phosphorus depleted group of kelp
POC	Particulate Organic Carbon
PON	Particulate Organic Nitrogen
POP	Particulate Organic Phosphorus
PS	Phosphorus saturated group of kelp
SD	Standard Deviation
SE	Standard Error
SES	Seaweed Energy Solutions
TBS	Trondhjem Biological Station

Chapter 1

Introduction

1.1 Phosphorus

All life forms need a range of different elements and minerals in order for them to function, grow, and reproduce. Phosphorus is an essential nutritional component due to its key role in biochemical processes that are fundamental for all organisms (Westheimer, 1987; Ruttenberg, 2005). It is an important element in structural support, energy transfer (ATP), and genetic material. Due to its high reactivity, phosphorus is rarely or never found as a free element, but is widely distributed in minerals as phosphates (Desmidt et al., 2015). It is fixated in photosynthetic organisms through its simplest form, orthophosphate (PO_4) (Ruttenberg, 2005), and from this it is transferred and used in higher levels of the food web. PO_4 is the main form of inorganic phosphorus that is available for direct uptake from the environment, but in phosphorus depleted areas it has been shown that phytoplankton are able to convert other phosphorus forms into PO_4 to enable uptake (Ruttenberg, 2005).

1.1.1 The Phosphorus Cycle

The Phosphorus Cycle refer to the natural conversion, movement, and distribution of phosphorus forms in an areal and temporal perspective. Ruttenberg (2005) divides the cycle into two main phases; terrestrial and marine, and four main components; (i) tectonic uplift and exposure to phosphorus bearing rocks to forces of weathering, (ii) physical erosion and chemical weathering of rocks producing soils and providing dissolved and particulate phosphorus to rivers, (iii) riverine transport of phosphorus to lakes and the ocean, and (iv) sedimentation of phosphorus associated with organic and mineral matter and burial in sediments. The conversion from inorganic to organic happens through biological fixation and incorporation, while microbial activity influence the rate of incorporation into geological records. Rock-bound forms of phosphorus are converted or released through geo- and biochemical reactions, for example weathering driven by acid reactions from bacterial activity, making phosphorus forms more available for biological

incorporation by photosynthetic organisms (Cosgrove, 1977; Ruttenberg, 2005).

The marine phosphorus cycle is strongly influenced by the terrestrial, as the main input is phosphorus (DIP, DOP, POP) from rivers that supply the ocean through estuarine areas (Lerman et al., 1975; Jahnke, 1992; Ruttenberg, 2005). Fixation of inorganic forms in photosynthetic organisms links the marine phosphorus (P), nitrogen (N), and carbon (C) cycles together. Fixation rates of these nutritional compounds can be limited by their combined availability, and thereby influence physiological processes in the organisms (Ruttenberg, 2005).

1.1.2 Human use and need for phosphorus

The main anthropogenic use of phosphorus is towards food production (Cordell et al., 2009). Phosphorus plays an important role as a macro nutrient in agricultural fertilizer, and as it is crucial for efficient crop production its absence can lead to crop failure (Desmidt et al., 2015). This is the main reason why Cordell et al. (2009) emphasizes the importance of phosphorus security, as it can be directly related to food security. The main phosphorus source for fertilizers are mining of phosphate rock (Cisse and Mrabet, 2004; Cordell et al., 2009), and due to an increase in fertilizer demand in developing countries, the mining of phosphorus is still increasing (Desmidt et al., 2015). With China, Morocco and Western Sahara, and the United States listed as the main producers, the global mining of phosphate rock has increased from 38 million metric tons in 1996 to 270 million metric tons of phosphate rock in 2018 (Steen, 1998; Jasinski, 2015, 2019).

Based on numbers from the US Geological Survey, all available reserves of phosphate rock will be extracted in approximately 250 years if the mining level of 2019 is maintained, and yet the demand has been predicted to increase 50-100% by 2050 (Cordell et al., 2009).

A. Human Impact on the Phosphorus Cycle

The mining of phosphate rock is, directly and indirectly, the main human impact on the global phosphorus cycle (Jahnke, 1992; Ruttenberg, 2005; Cordell et al., 2009). Losses and run-off from accumulated nutrients in agricultural soil, mining activities, and fertilizer production (Cordell et al., 2009), as well as anthropogenic activities like deforestation, and urban and industrial waste, all impact the natural phosphorus cycle (Ruttenberg, 2005). The run-off from anthropogenic activities may lead to increased erosion, and particle and nutrient flux into water masses (rivers and oceans). This can lead to eutrophication, where increased nutrient input stimulate an increase in primary production (Ruttenberg, 2005; Cordell et al., 2009). These algae blooms can promote anoxic or hypoxic conditions in bottom sediments due to higher rates of sedimentation and can even lead to changes in pH, which can be harmful to the local community of organisms (Hongve and Kjensmo, 2018).

B. Drivers and Opportunities for Phosphorus Recycling

As human activities drive an increase in the phosphorus flux, the negative impacts work as drivers towards finding sustainable future solutions for future phosphorus security (Cordell et al., 2009, 2011). These drivers include economic costs of eutrophication and increased phosphorus demand, as well as prospects of phosphorus scarcity from depletion of phosphate rock reserves.

Potential solutions should be assessed according to a range of criteria, such as economic cost, environmental impacts, and technical feasibility, but also have a focus towards ensuring high phosphorus content and bioavailability (Cordell et al., 2009; Desmidt et al., 2015). Several solutions are suggested and have been tested to various extent, and both technological and policy solutions in the supply- and demand-side should be implemented in order to sustain future phosphorus needs (Cordell et al., 2009; Desmidt et al., 2015). Some of the most promising options are in optimization and reduction of losses in food production, including recycling of waste water, and waste from production (food items) and consumption (compost). Other mentioned ways are through more plant based diets (food chain efficiency), recovery from manure/excreta, and precision farming (Cordell et al., 2009, 2011). Also, aquaculture can have comparable opportunities for nutrient recycling as suggested in land based food production.

1.2 Aquaculture: Global aquatic food production

Aquaculture is a collective term for production of aquatic plants and animals in inland, coastal, and marine water bodies (Bostock et al., 2010; FAO, 2018*b*). As the concept of farming in the wet environment, aquaculture is a food production sector with rapid growth in recent decades, from 13 million tonnes in 1990 to 80 million tonnes in 2016 (FAO, 2002, 2018*a*,*b*). From the total aquaculture production in 2016, marine aquaculture accounted for 28.7 million tonnes (FAO, 2018*b*). Even though there are several hundred species produced in aquaculture worldwide today, only a few dominate the market. In 2016, the 20 most produced animal species accounted for 84% of the total production of aquatic animals (FAO, 2018*b*).

As the world population is expected to reach 9.8 billion by 2050 (UN, 2017), increasing demands for fresh water, space, food, and arable land will further increase the pressure on phosphate mining for fertilizer (FAO, 2017). Increases in aquaculture can be important to enhance the resilience in global food production, given that the growth happens in a sustainable way (Brundtland, 1987). Today, feed for certain aquaculture species are dependent on agriculture crops like oilseeds, and increases in the production of these fed species will add to the pressure on agriculture products (FAO, 2017). To enable growth in aquaculture and at the same time reduce the pressure on agriculture production, aspects like variation in species of different trophic levels, synergies between areas of food production, and developments within aquaculture feed manufacturing are needed (FAO, 2014). Future food safety is dependent on climate-smart, efficient, sustainable, and innovative solutions both within aquaculture and agriculture production (FAO, 2017).

As aquaculture has grown, capture levels of fish have stagnated and stabilized in the same period due to over fishing, depletion and bad management of fish stocks (Bostock et al., 2010; FAO, 2018*b*). Thus, the future increase in food supply from the blue sector should come from aquaculture production.

1.2.1 Salmon farming in Norway

The monoculture production of Atlantic salmon (*Salmo salar*) accounts for 4% of the total world-wide production of aquatic animals, with Norway being the largest contributor with a production of 1.2 million tonnes of a total global production of 2.2 million tonnes (Baklien and Steinset, 2018; FAO, 2018*b*). The Norwegian government has established a goal of a five fold value increase in the aquaculture sector by 2050 (Olafsen et al., 2012; Parliament message nr. 16, 2014-2015). This goal of production growth combined with known challenges of monoculture production, like waste release (Folke et al., 1994), give a prospect of increased biological waste release and need for feed resources.

A. Waste release and nutrient water content

The release of biological waste from salmon is thought to have potential negative consequences for ecosystems, both locally and in larger distance from salmon farms (Jansen et al., 2018). The local impact on the seafloor is monitored through the Norwegian standard 9410 (NS9410, 2016), but effects in the water column and further away are difficult to measure. This is because inorganic nutrients, like DIN and DIP, are rapidly diluted in water samples only 20-50 m downstream of fish farms due to rapid uptake in microalgae (Folke et al., 1994; Jansen et al., 2018). Olsen et al. (2014) suggested that a measure of particulate forms of nutrients (incorporated in microalgae tissue) is a better way to detect whether areas have increased inorganic nutrient loads.

Wang et al. (2013) estimated release rates of biological waste products from a Norwegian salmon monoculture, shown in Table 1.1. They showed that only 38% of feed added to the farm was retained as fish biomass, and the balance represented an estimated loss of 57% of the total nitrogen and 76% of the total phosphorus added as feed. Also, it was found that the phosphorus content of faeces was higher than in the feed, indicating low digestibility of phosphorus in the feed. Their results point towards the possibility of faeces being adequate

Table 1.1: Estimated distribution (%) of retention and loss of feed nutrients (nitrogen and phosphorus) in Norwegian salmon monocultures. Losses divided as particulate organic (POP, PON) and dissolved inorganic (DIP, DIN) compounds from biological waste products and feed spill. Retrieved from Wang et al. (2013)

	Retained in fish biomass (%)	Lost to the environment (%)		
		Total	Inorganic	Particulate organic
Phosphorus	24	76	24	44
Nitrogen	43	57	39	15

nutrition for other species in co-cultivation. The addition and loss of phosphorus through feed in salmon aquaculture represent a sinkhole for phosphorus.

Etter et al. (2016) studied the effect of nutrient release from salmon farms in Trøndelag. Their findings showed no significant effect of increased DIN or DIP at stations assumed to be affected by salmon farms, compared to stations assumed to be unaffected by salmon farms. Their results supported the use of particulate forms as a measure of elevated nutrient conditions. Mean$\pm$CI$_{95\%}$ measured water content for DIN was 16.4$\pm$2.9 and 15.4$\pm$5.9 for stations assumed to be affected and not affected by salmon farms, respectively. For DIP, mean$\pm$CI$_{95\%}$ water content was 2.1$\pm$0.4 and 2.3$\pm$0.9 for the assumed affected and not affected stations, respectively.

Water content of DIN and DIP are expected to follow a natural seasonal variations, with higher stable values in winter and nutrient exhaustion in summer after the spring bloom (Skjoldal and Saetre, 2004). Surface water content of DIP rarely exceed values of 1.0 µmol·L^{-1} in areas along the Norwegian coast (Sakshaug, 1994; Erga et al., 2017), but higher natural values can be found in polar areas (2.0 µmol·L^{-1}) and deeper water layers ($\sim$2.5 µmol·L^{-1}) (Breen, 1990; Sakshaug, 1994).

1.2.2 Integrated Multi-Trophic Aquaculture

Integrated Multi-Trophic Aquaculture (IMTA) is a form of aquaculture system aiming to mimic an ecosystem by coupling the production of several species with different trophic levels to increase the total biomass production with the same feed input (Buschmann et al., 2001; Chopin et al., 2001). As illustrated in Figure 1.1, at least two or more species of different feeding modes need to be included and the system has to be within the same water mass to be categorized as IMTA (Chopin et al., 2006). Suitable species should meet the following criteria: enabled controlled

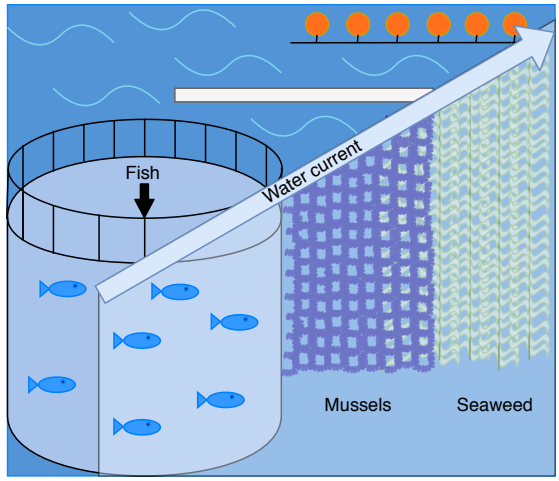


Figure 1.1: Illustrations of an IMTA cultivation system with three species of different trophic level: fish, mussels and seaweed. Position follow the water current from high to low trophic level. Based on illustration from Holdt and Edwards (2014).

reproduction, enhanced growth in the system, low maintenance, and an economically feasible production (Chopin et al., 2001; Ridler et al., 2007).

As species are at different levels of the food chain, they utilize nutrients in different forms and have different nutritional needs, and this variation is exploited in IMTA. For instance, inorganic waste release (DIP, DIN) can be utilized by photosynthetic species like *Saccharina latissima*, and particulate waste (POP, PON) by filter feeders like *Mytilus edulis* (Buschmann et al., 2001; Chopin et al., 2001; Troell et al., 2003). Thus knowledge of the physiology, nutritional needs, and optimalization in regard to abiotic and biotic factors is important for selecting species for IMTA and kelp cultivation in general (Chopin et al., 2001; Harrison and Hurd, 2001). A second aim of IMTA is to reduce potential environmental consequences from fed monocultures by introducing species to extract biological waste from the water column (Troell et al., 2003; Ridler et al., 2007). The bioremediation potential of IMTA can be described as the magnitude of how co-cultivation of extractive species reduce the biological waste load on local ecosystems (Barrington et al., 2009). IMTA represents a concept with potential of being an environmentally sustainable alternative to traditional monoculture, but to be regarded as completely sustainable, economic and social outcomes also have to be beneficial (Ridler et al., 2007).

1.3 Macroalgae

Macroalgae, or seaweed, are autotrophic and multicellular organisms that are the ocean equivalents to land based plants (Hurd et al., 2014). They can be divided into three groups, based on main pigmentation; red (phyla of *Rhodophyceae*), brown (phyla of *Heterokontophyta/ochrophyta*, class of *Phaeophyceae*), and green (phyla of *Chlorophyceae*) (McHugh et al., 2003; Indergaard, 2010). Each of these groups cover a range of different families, genra and species, with wide physiological and morphological variation.

Currently, species of seaweed are harvested and cultivated globally for a range of purposes (Tiwari and Troy, 2015). Industrialized harvesting methods have been discussed to have negative consequences by damaging the seabed and natural habitats through trawling of kelp forest, and cultivation is viewed as the more viable way of production (McHugh et al., 2003; Lorentsen et al., 2010; Stévant et al., 2017). However, only 6% of the production of aquatic plants originated from harvest of natural populations in 2012 (Tiwari and Troy, 2015), with Chile, Norway, and Japan listed as the main contributors. In 2016, China and Indonesia accounted for 86.6% of the total production of aquatic plants, mainly from cultivation (FAO, 2018*b*).

Global seaweed cultivation include a range of species, mostly red and brown macroalgae, which can be produced vegetatively or through a reproductive cycle depending on their physiological capabilities (McHugh et al., 2003; FAO, 2018*b*). For brown seaweeds, like species within the *Laminariaceae* family, a manipulative reproductive cycle is necessary to enable large scale cultivation.

1.3.1 Use of seaweeds

Current uses of seaweed in products include food, animal feed, fertilizers, nutraceuticals, food ingredients, and medicinal and pharmaceutical uses (Tiwari and Troy, 2015). In Asia, the use of seaweed as food can be traced back to ancient times. Specifically for Norway, harvested seaweeds have a history of use as fodder and fertilizer in agriculture and husbandry, as well as for extraction of alginate used as a stabilizer in a variety of products (McHugh et al., 2003; Tiwari and Troy, 2015). For future development, it has been suggested to use seaweeds as raw material for the production of biofuels, as well as expanding the variety of products in current areas of use (Marquez et al., 2015; Tiwari and Troy, 2015).

1.3.2 Norwegian seaweed aquaculture

Norway has ideal conditions for large-scale macroalgae cultivation, with a long coastline, good knowledge of utilizing ocean resources, and a well-established aquaculture industry (Skjermo et al., 2014; Stévant et al., 2017). In 2014 the Norwegian Directory of Fisheries started issuing licenses for species specific cultivation of macroalgae for food and feed uses (Norwegian Directory of Fisheries, 2018). The first year 54 licences were issued, increasing to 406 licenses across 83 sites in 2018 (Norwegian Directory of Fisheries, 2019). However, only 44% of issued licenses were recognized as operational in 2017, resulting in a total production of 149 metric tonnes of kelp. Of the total production 93% was *S. latissima* valued at 355 000 NOK, and the rest was mainly *Alaria esculenta* (9 metric tons valued at 342 000 NOK). These numbers outline a growth in Norwegian kelp cultivation, but it is still categorized as a small scale production.

As *S.latissima* is categorized as a suitable species for large scale kelp cultivation (Kerrison et al., 2015; Schiener et al., 2015; Fossberg et al., 2018), several studies have been conducted within aspects of cultivation feasibility and yield, processing, products and areas of use, chemical composition, physiological- and ecological features (e.g. Handå et al., 2013; Reid et al., 2013; Skjermo et al., 2014; Schiener et al., 2015; Forbord et al., 2018; Fossberg et al., 2018). Methods for this cultivation are described in the protocol by Forbord et al. (2018). Main concerns for future up-scaling and industrialization of Norwegian kelp cultivation include economic profitability, processing techniques, industrial-scale products, area conflicts, tolerance of climate change, as well as potential cultivation risks like epiphytes, disease, genetic interaction with wild stocks, and ecosystem impacts (Stévant et al., 2017).

1.3.3 *Saccharina latissima*

S. latissima, or sugar kelp, is a species of brown algae (class of *Phaeophyceae*, order of *Laminariales*, family of *Laminariaceae*) with a distribution covering large parts of the Northern Hemisphere (Artsdatabanken, 2006; Indergaard, 2010). It is common along the Norwegian coast, and its distribution is in correlation with summer temperatures of $\leq$19 °C. It thrives in the intertidal to littoral zone (maximum depth of 30 m, or light dependent) with low to moderate wave expo-

sure, temperatures (10-15 °C), and on rocky hard or shell substrate (Bolton and Lüning, 1982; Artsdatabanken, 2006). It is characterized by a thin, round, and slick stipe (5-50 cm length and 5-8 mm diameter), and a long lamina with waved edges and often dimpled along the mid section with no midrib (10-30 cm width, 1-3 m length), thus the shape can be quite variable (Rueness, 1998; Indergaard, 2010). The holdfast and stipe are perennial (up to 5 years), but the lamina degenerates from the apex during the late summer/fall and grows back from the growth meristem at the base of the lamina near the stipe during the following late winter/spring. The sugar kelp follows a heteromorph life cycle with free living microscopic gametophytes developing into a sporophyte, which during its second fall/winter develop sporangia with spores that when released develop into gametophytes (female and male for sexual reproduction) (Rueness, 1998; Artsdatabanken, 2006; Fretwell, 2016). Former names include *Laminaria saccharina*, *Fucus saccharinus*, sugar wrack kelp, Devil's apron, *Ulva latissima*, and tangle.

1.3.4 Physiology and nutritional needs

All seaweed use light as a main source of energy through photosynthesis and assimilate inorganic nutrients from the surrounding water masses, and carbon, nitrogen, and phosphorus are the main essential macro elements in algal nutrition (Lobban et al., 1994; Hurd et al., 2014). Rates of physiological processes, like growth, vary among species of different life strategies, and are strongly influenced by a range of abiotic and biotic factors, including nutrient availability, light, temperature, salinity, pH, water movement, and competition and interactions with other organisms. Several of these factors vary geographically and temporally, and thus many seaweed species are well adapted to change as well as their local conditions which can be expressed in their ability to take up, store and utilize nutrients (Harrison and Hurd, 2001; Pedersen et al., 2010).

The acquisition of nutrients is inevitable to enable growth and keep the kelp physiologically functional. This acquisition can be divided into different phases where uptake, assimilation, and incorporation are differentiated: (i) Uptake is the allocation of a given nutrient molecule or ion from the surrounding medium into the cell; (ii) assimilation the conversion of inorganic nutrients to small organic molecule forms in the cell, and (iii) incorporation the production of macro molecules by combination of nutrient-containing organic molecules (Gordillo, 2012; Wiencke and Bischof, 2012). Growth rate is thus dependent on the processes for nutrient acquisition and as it is influenced by their variation, knowledge of these processes are vital when selecting and optimizing the cultivation of seaweed species.

For all factors that influence physiological processes in kelp, there can be established species specific ideal ranges for each factor that will facilitate optimal growth conditions (Harrison and Hurd, 2001; Hurd et al., 2014). In this regard, it is especially important to pinpoint the boundaries for when a factor becomes growth limiting. The limiting nutrient is defined as the nutrient available in the smallest quantity with respect to nutrient requirements (Harrison and Hurd, 2001). Kelp tissue content is used to investigate nutrient limitations, but this can also be

Table 1.2: Measured tissue content of carbon, nitrogen and phosphorus in *S. latissima* from various studies. In Wheeler and Hartwell (1893) and Gordillo et al. (2006) given as % H_3PO_4 g_{DW}^{-1}.

Study by	Phosphorus		Nitrogen		Carbon	
	% of DW	$mg \cdot g_{DW}^{-1}$	% of DW	$mg \cdot g_{DW}^{-1}$	% of DW	$mg \cdot g_{DW}^{-1}$
Fossberg et al. (2018)			1-2		23-28	
Schiener et al. (2015)	0.10-0.56		0.29-2.32		29.6-38.1	
Wang et al. (2014)				16-38		213-285
Gordillo et al. (2006)	0.9-1.3		0.7-1.3		24.0-26.2	
Wheeler and Hartwell (1893)	0.58		1.75			

coupled with knowledge of the life history and strategies for that specific species.

Many macroalgae are able to store nutrients at high nutrient availability, and utilize this for growth when nutrients are limited (Fujita, 1985; Harrison and Hurd, 2001). There is a correlation between storage and growth capacity, so opportunistic macroalgae species often have rapid growth, good nutrient utilization, but relatively low long term storage capacity (Fujita, 1985; Pedersen et al., 2010). This can be reflected in their tissue content in nitrogen (N) limited areas, where low N tissue content (or PON:POP ratio) can imply good utilization of all available N towards growth (Ryther and Dunstan, 1971; Fujita, 1985). Therefore, it is relevant to have knowledge on expected tissue content of different species. Table 1.2 shows expected values for tissue content of C, N, and P in *S. latissima* based on former studies.

Uptake is the main process with direct interaction between the kelp's cells and the external environment, and it is therefore a good process to study to gather knowledge on algal nutrition. This can be estimated experimentally, for example by measuring the removal of a target molecule from the kelp's medium (Harrison and Hurd, 2001).

A. Uptake kinetics

Various nutritional molecules have different uptake mechanisms, where some forms like nitrate (DIN) and phosphate (DIP) require active transport to cross the cellular membrane (Harrison and Hurd, 2001). This way of uptake is expressed as a rectangular hyperbola when uptake rate (V) and substrate concentrations (S) are plotted against each other. This is described by the Michaelis-Menten Equation (Equation 1.1) including the maximum uptake rate (V_{max}) and the half-saturation value (K_S). K_S is the substrate concentration when the uptake rate is half of V_{max}.

$$V = V_{max} \frac{S}{K_S + S} \tag{1.1}$$

The initial slope (α), for V plotted against S, in the rectangular hyperbola is used to compare uptake abilities across species at low nutrient concentrations, where a steep slope indicate high nutrient affinity at low concentrations (Harrison and Hurd, 2001; Hurd et al., 2014). A high V_{max} indicate an ability to take up nutrients at high concentrations. The three kinetic parameters

of uptake, V_{max}, K_S, and α, are used to describe and compare the uptake of different seaweed species as these parameters vary with physical, chemical and biological factors.

B. Phosphorus and nitrogen in *S. latissima*

Nutrient assimilation, uptake and storage in *S. latissima* have been investigated both in field- and laboratory studies (e.g. Chapman et al., 1978; Marinho et al., 2015; Lubsch and Timmermans, 2018; Dahlen, 2018). However, most of these studies have focused on nitrogen, and specific knowledge on DIP uptake and storage is scarce (Pedersen et al., 2010; Lubsch and Timmermans, 2018). Even though nitrogen is regarded as the main driver of increased growth of *S. latissima* in IMTA systems (Wang et al., 2014), knowledge of phosphorus is important when adressing aspects of nutrient recycling, -bioremediation, and -efficiency of cultivated kelp (Harrison and Hurd, 2001).

1.4 Aim of study

The motivation and objective of this thesis was to investigate opportunities for integrated multi-trophic aquaculture (IMTA) regarding phosphorus recycling to prevent future phosphorus scarcity, and increase the knowledge of phosphorus' role as an essential component in macroalgae nutrition.

The aim of this thesis was to characterize the initial uptake and storage of phosphorus in *S. latissima*, and couple this to assess the recycling potential for phosphorus through IMTA-cultivation of *S. latissima*.

The overall aim was divided into executive aims with hypotheses, specified as follows:

1. Describe the initial uptake of phosphorus in *S. latissima*, and how this is affected by the kelp's nutritional state, with the following hypotheses:
   - 1*a*) Phosphorus depleted individuals have more rapid uptake than saturated individuals.
   - 1*b*) Availability of phosphorus and uptake rate are positively correlated.

2. Characterize phosphorus storage and relate this to bioremediation and recycling of phosphorus in an IMTA-system, with the following hypotheses:
   - 2*a*) Internal content of phosphorus is highest near the growth meristem (basal area) and decrease towards the apex.
   - 2*b*) Storage capacity is related to nutrient availability. Individuals cultivated close to salmon pens have higher phosphorus content than individuals cultivated further away.

Hypotheses under aim 1 were investigated through an experimental study where *S. latissima* of two different nutritional states, phosphorus saturated and phosphorus depleted, were exposed to a gradient of increasing phosphorus availability. Hypotheses under aim 2 were investigated through IMTA cultivation of *S. latissima* along a gradient of assumed impact from a fish farm. Evaluations were mainly be focused on water and tissue samples.

Chapter 2

Materials and methods

Methods used in this thesis were divided into three sections: First, the experimental study of initial phosphorus uptake; second, the IMTA cultivation study; and third, chemical- and statistical analyses of both studies.

2.1 Study of initial phosphorus uptake

The experiment was conducted in a climate-regulated room at Trondhjem Biological Station (TBS, Heggdalen, Trondheim) in April 2018. All artificial seawater was produced by a composition of salts dissolved in dH_2O as described in Grasshoff et al. (1999) for a salinity of 35, presented in Table 2.1. Artificial seawater was used in the acclimatization of the phosphorus depleted group and for the study of initial uptake in both the phosphorus saturated and depleted group. The room temperature was kept at 10 °C during all preparations and experimental procedures, and all water used in the experiments was acclimatized at a minimum of 24 hours before use.

Table 2.1: Recipe by concentration ($g{\cdot}L^{-1}$) for each component in artificial seawater for a salinity of 35, as described in Grasshoff et al. (1999).

Compound	Concentration ($g{\cdot}L^{-1}$)	Compound	Concentration ($g{\cdot}L^{-1}$)
NaCl	23.939	$NaHCO_3$	0.196
$MgCl_2$	5.079	KBr	0.098
Na_2SO_4	3.994	H_3BO_3	0.027
$CaCl_2$	1.123	$SrCl_2$	0.024
KCl	0.667	NaF	0.003

2.1.1 Phosphorus saturated and depleted acclimatization

Young individuals of *S. latissima* were provided by Seaweed Energy Solutions (SES). The kelp was measured to fit the size-range of 10-18 cm, and divided between two tanks (∼160 and ∼315 L) for nutritional acclimatization (approximately 200 individuals per tank): Phosphorus depleted (PD) and phosphorus saturated (PS). The two acclimatization tanks were illuminated with a daily cycle light:dark of 16:8 of fluorescent lighting with 40 µmol·PAR·$s^{-1}m^{-2}$, which was close to the natural day length of 15.25:8.75 at that specific time of the year.

The tank (∼315 L) with the saturated group (PS) was supplied with deep sea water (80 m depth) in a flow through system adjusted to ensure sufficient water movement and to ensure continuous nutrient availability. The level of nutrients in deep water was assumed to ensure nutrient saturation (Sverdrup et al., 1942). The acclimatization lasted for 2 days prior to the experiment.

The depleted group (PD) was kept in a tank (∼160 L) with artificial seawater (62.5 L). The water was supplied with nitrogen (16 µmol·$L^{-1}day^{-1}$ equal to 224 µg·$L^{-1}day^{-1}$), and an adjusted f/2-medium (Guillard, 1975). The adjusted f/2-medium was without added nitrogen, phosphorus and silicate. To ensure stable water movement two water pumps (Eheim compactON type 1001.220 FLmax=600 L·$hour^{-1}$, and LifeTech AP1000 FLmax=400L·$hour^{-1}$) were used. Aeration (AM-TOP aquarium air pump CR-30), airtube and airstone (30x15x15 mm, ceramic HOBBY airstones) was used to ensure sufficient gas exchange. The acclimatization lasted for 7 days prior to the experiment.

In both tanks, temperature, salinity (WTW LF330 Conductivity meter, Weilheim), and pH (pH-indicator strips, Merck KGaA, Darmstadt) were measured during the first acclimatization day, and water samples (2 replicates) were collected prior to the addition of kelp. Daily water samples (2 replicates) were collected throughout the acclimatization periods. In the PD-group, daily water samples were collected prior to the daily DIN addition, with exception of samples from day 0 and day 1 which were collected after the daily addition. Analyses of water samples are described in Section 2.3.1.

2.1.2 Experimental setup and procedure

The design of the initial uptake experiment was run for both the PS and PD group. To avoid phosphorus contamination, all equipment used was either disposable or acid-washed during the preparations of the experiment.

The experimental setup consisted of 32 Erlenmayer beakers (5 treatments, 6 replicates, 2 controls) placed on two stirring tables (100 rpm, Orbitron M 850x470 mm, Infors AG, Bottmingen) to ensure water movement. Water movement was important to ensure homogeneous distribution of particles and molecules in the water. A light source (fluorecent lighting, 40 µmol·PAR·$s^{-1}m^{-2}$) was situated behind each stirring table, and all beakers were placed to ensure equal light conditions within each treatment as illustrated in Figure 2.1.

Standard stock solutions were produced for phosphate and nitrate separately in artificial

Table 2.2: Setup for experiment: Concentrations (µmol·L^{-1} and µg·L^{-1}) of increasing phosphorus (P) and equal nitrogen (N) for all treatments and controls. Number of replicates and individuals per replicate are also given. The setup was identical for both acclimatizations: phosphorus depleted (PD) and saturated (PS).

	Treatments					Control	
µmolP·L^{-1}	0.25	0.5	0.75	1.0	2.0	0.25	2.0
µgP·L^{-1}	7.75	15.5	23.25	31	62	7.75	62
µmolN·L^{-1}	8	8	8	8	8	8	8
µgN·L^{-1}	112	112	112	112	112	112	112
Replicates	6	6	6	6	6	1	1
Seaweed individuals per replicate	5	5	5	5	5	0	0

seawater, as well as an adjusted f/2 medium (without phosphate, nitrate and silicate) (Guillard, 1975). The beakers were supplied with a 250 mL solution of artificial seawater containing the adjusted f/2 medium. In each beaker, a specific volume of artificial seawater was removed and substituted for a volume of the nitrate and phosphate stock solutions in correspondence with treatment concentrations given in Table 2.2.

Individuals of *S. latissima* were randomly picked out from the corresponding acclimatization tank and put into piles of five individuals per replicate (30 piles). The experiment started when the piles were added to their designated beakers, and a timer was started.

Figure 2.1: Illustration of experimental setup for determination of uptake rates in *S. latissima* for treatments of increasing phosphorus availability. The picture include replicates for two treatments, as well as one control (without kelp) placed on a stirring table, and the light source situated behind to ensure equal lighting across treatments.

Table 2.3: Sampling regime for water samples collected througout the three hour duration of the experiment. At each time mentioned, all replicates in all treatments were sampled. Controls were sampled at the first (5) and last (180) sampling. The regime was identical for both acclimatizations: phosphorus depleted (PD) and saturated (PS).

	Times (minutes)						
Sampling (4 mL)	5	10	20	30	50	90	180

2.1.3 Sample collection

Water samples

During the 3 hour (180 min) course of the experiment, water samples (4 mL) were collected from each replicate at different times as given in Table 2.3. Stirring tables were stopped during water sampling. All water samples were filtered (25 mm syringe filter with 0.45 µm cellulose acetate membrane (CAM), VWR international, USA) and stored frozen (-20 °C) until analyses. Analyses are described in Section 2.3.1.

Tissue samples

At the end of the experiment all kelp individuals from each replicate were lightly dried off on paper, weighed, and frozen (-20 °C, as batches per treatment) for further analysis. Ten individuals from each acclimatization tank were randomly collected for analysis of initial tissue content. Analyses are described in Section 2.3.2.

2.2 IMTA cultivation study

2.2.1 Study site

The field work was conducted in the period February to June 2018 at the Rataren II (ID: 31959, Salmar farming AS) salmon farm outside Frøya in The Frøy ocean/ Sul fjord (Latitude: 63.782382, Longitude: 8.526350) (BarentsWatch, 2018). The farm consists of 8 cages/pens (illustrated in Figure 2.2) of 150 meter circumference. A second salmon farm, Rataren I (ID: 28636, Salmar farming AS), was situated approximately 400 meters south west of Rataren II (Latitude: 63.780792, Longitude: 8.518067).

2.2.2 Cultivation setup

As illustrated in Figure 2.2, a long line (∼400 meter, 22 mm) with drop ropes (16 mm, 5 meters, 1-6 meters depth, 10 meters apart) was placed -150 meters from the edge of the salmon farm to approximately 300 meters downstream in a North/North-Eastern direction. The long line was anchored by bottom weights (200 kg), and buoys for every second drop rope to keep the line closer to the water surface. Each drop rope, mechanically twisted with seedling string, was

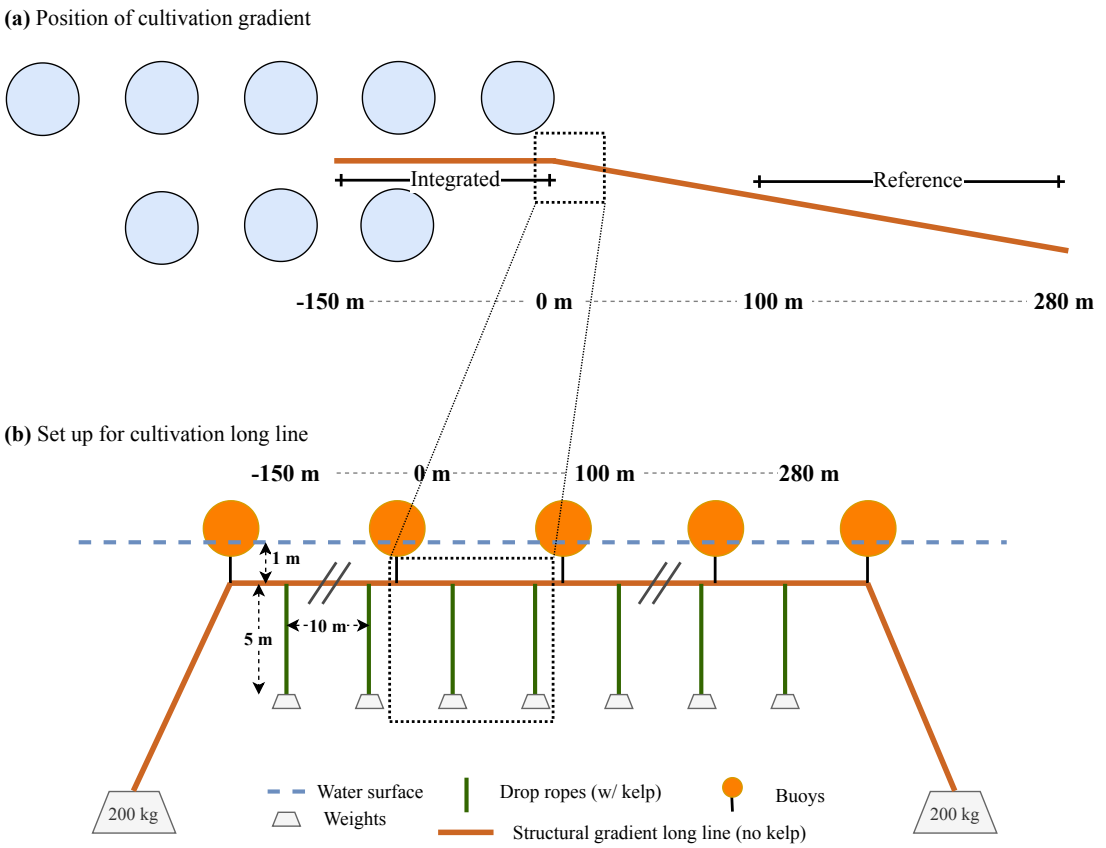


Figure 2.2: Illustration of IMTA cultivation longline for *S. latissima* within and downstream of a salmon farm. Distances indicated are relative to the salmon farm edge. (a) Positioning of the cultivation gradient relative to the salmon farm, with marked areas of the gradient as the integrated (I) and reference (R) group. Circles represent salmon pens, and the brown line the kelp cultivation longline. (b) Set up for cultivation long line with weights, buoys and structural rope for support, and drop ropes for cultivation. Estimated depths, length of rope, and distance between drop ropes are indicated.

attached to the long line and kept vertical in the water column by bottom weights (3-7 kg). The seedling string was produced by the method standardized by SINTEF Ocean (Forbord et al., 2018). Rolls of nylon string (1.2 mm) were sprayed with spores, from a strain of *S. latissima* from Frøya, and incubated in the hatchery for 45 days prior to sea deployment.

For the assessment of biomass yield with different proximity to the salmon farm, the drop ropes were divided into two groups based on the assumption of a rapid nutrient dilution effect downstream, being the Integrated (I) and Reference (R) group. Figure 2.2 illustrate the areas of the long line relevant for the I and R groups. Ropes in the I-group were cultivated within the fish farm by 16 ropes, -150 to 0 meters from the edge of the farm. The R-group was downstream comprising of 10 ropes, 80-160 meters and 280 meters from the farm edge. Due to a boating incident and partial capsize from difficulties with anchoring, ropes from 10-80 and 160-280 meters downstream were excluded from the biomass-analysis.

Table 2.4: Sampling regime for each sampling day for the cultivation period. Including total number of water samples and individuals collected on each sampling date, and whether biomass registration was done.

	Sampling date			
	February 16^{th}	April 23^{rd}	May 29^{th}	June 13^{th}
#Water samples	4	4	4	4
#Individuals	0	30	30	30
Biomass registration	No	Yes	Yes	Yes

Three ropes along the long line were chosen for tissue sampling, with different distances downstream from the salmon farm; -150 m (among the pens), 100 m, and 280 m from the edge of farm.

2.2.3 Data collection & sampling

Water samples

Water samples were collected for nutrient analyses, (1 sample, 1-2m depth, Nansen water sampler) at 4 locations along the gradient, at -150, 0, 100, and 280 meters as shown in Figure 2.2a. This gave a total number of 4 water samples each sampling date, as shown in Table 2.4. The collected water column samples were mixed in a container (1 L), filtered (25 mm syringe filter with 0.45 µm CAM, VWR international, USA) and gathered in sample-bottles (50 mL). Samples were kept cool during transport, and stored (-20 °C) at arrival in Trondheim. Analyses are described in Section 2.3.1.

Tissue samples

Tissue samples were collected from each selected rope, where 10 individuals were chosen haphazardly across the 5 m rope and collected in plastic bags. This gave a total of 30 individuals for each sampling day, as described in Table 2.4. Samples were kept cool during transportation, and frozen (-20 °C) at arrival in Trondheim.

Only samples from April and June were processed and used for the chemical analysis, and they were prepared in the following way: A bag of 10 individuals from a specific rope and date were thawed at room temperature, and 5 undamaged individuals were chosen. For each chosen individual, a scalpel was used to cut out subsamples from three sample areas: apex (A), mid (B), and basal area (C), as illustrated in Figure 2.3. This gave a total of 180 subsamples for analyses of tissue content (2 sampling days, 3 ropes, 5 individuals, 3 sample areas). Samples were stored (-20 °C) until analyses described in Section 2.3.2.

Biomass registration

Fresh weight biomass was registered separately for each rope within I and R groups for each sampling day as described in Table 2.4. During weighing, ropes were lifted into the boat and some water run-off (maximum 30 seconds) was allowed prior to registration. The registration was done either through weighing of the whole rope (5 m) or by weighing 50 cm of harvested biomass from the rope. For the weighing of whole ropes, relevant additional weight from, like rope weight and weighing equipment, was subtracted prior to data processing.

Biomass weight of the ropes were standardized as $kg \cdot m^{-1}$ and used to assess differences in biomass yield along the cultivation gradient. Due to challenges with weighing equipment and differentiating weighing techniques, results from the biomass registration were evaluated with caution.

Data for temperature, salinity, fish biomass and feed

Temperature data, measured at the Rataren II salmon farm during the cultivation period February to June, was retrieved from BarentsWatch (2018). Salmar AS provided data on fish biomass and feed use for both Rataren I and II. Salinity and light intensity were not recorded at site due to equipment malfunction, but EXPOSED Aquaculture Research Centre provided alternate salinity data from a buoy 8 km away at Sula (63.822, 8.382) which were used as reference for expected values of the Sul fjord area.

2.3 Analyses

2.3.1 Water sample analysis of DIP and DIN

All water samples were thawed at room temperature. Analysis were done photometrically on an autoanalyser (Flow Solution IV System, O.I. Analytical) following the Norwegian standards

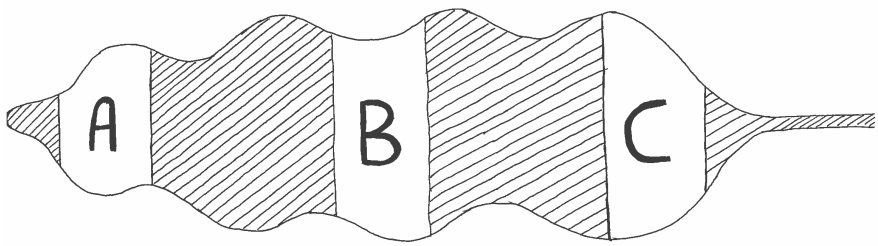


Figure 2.3: Illustrations of the stipe and lamina of a *S. latissima* individual. Sample areas of the lamina collected for tissue content analysis are indicated: A, B, and C. All etched areas, including the stipe, were not analysed.

NS4745 and NS-EN-ISO6878 for determination of DIN and DIP, respectively (NS4745, 1991; NS-EN ISO 6878, 2004). Measured values were given as water content ($\mu g \cdot L^{-1}$) estimated from a standard curve, with a margin of error of $\pm 2 \mu g \cdot L^{-1}$ for the autoanalyser.

2.3.2 Tissue samples

Freeze drying

All samples, both bulk samples from the uptake experiment and subsamples from field cultivated individuals were freeze dried and homogenized in the following way. All samples were placed in separate size-approriate plastic containers, flash frozen (-80 °C, minimum of 1 hour), and freeze dried (-40 °C for 48 hours, Hetosicc CD 13-2). Freeze dried samples were kept in the dark in closed bags with silica gel in room temperature to avoid rehydration. Samples were homogenized to a fine grained powder by pestel and mortar, and stored with silica gel (-20 °C) preliminary to chemical analyses.

Analysis of internal phosphorus content

Internal phosphorus content (POP) was determined by a method for determining particulate phosphorus (Koroleff, 1976). A preliminary test was performed to establish the needed sample concentration to fit the detection range of the autoanalyser, which was determined to be approximately 0.5 mg_{DW} of kelp powder. Samples were weighed out (0.5 mg, exact weight recorded, Mettler Toledo UMT2) onto coverglasses, transferred to separate marked plastic bottles, and stored (-20 °C). To enable photomeric determination, solutions were added to convert POP to DIP (PO_4); destilled H_2O (11 mL), oxidising reagent (2mL, 50g $K_2S_2O_8$ L^{-1} dH_2O) and acid (0.1 mL, 4M H_2SO_4). Samples were autoclaved (120 °C, 30 minutes) before analysis by the NS-EN-ISO6878 method on an autoanalyser (Flow Solution IV System, O.I. Analytical).

Estimates of phosphorus tissue content was given as $\mu gP \cdot L^{-1}$ from the autoanalyser analysis. The conversion from measured phosphorus content (MC, $\mu gP \cdot L^{-1}$) to tissue content (TC, $\mu gP \cdot mg_{DW}^{-1}$) was done by sample solution volume (SSV, 0.0131 L) and weight of sample (WS, mg_{DW}) as descibed in Equation 2.1.

$$\frac{MC(\mu gP \cdot L^{-1}) \cdot SSV(L)}{WS(mg_{DW})} = TC_P(\mu gP \cdot mg_{DW}^{-1}) \tag{2.1}$$

Analysis of nitrogen and carbon tissue content by CN-analysis

Samples of approximately 2 mg freeze dried kelp powder were weighed (Mettler Toledo UMT2) into tin capsules (5x9 mm, exact weight recorded). Capsules were wrapped into balls on a carbon free metallic plate stored (-20 °C) in separate wells (in 96-well plates). 24 hours prior to analysis, all samples were dried (60 °C) and determination of carbon and nitrogen was done by use of acetanilide as standard on an elemental analyser (Elementar vario EL cube, Elementar Americas Inc., New York).

Analysis of tissue carbon and nitrogen content was given as a measure of µg per sample, and was converted to tissue content (TC, µg· mg_{DW}^{-1}) by the weight of sample (WS, mg_{DW}), as described in Equation 2.2.

$$\frac{\mu gC}{WS(mg_{DW})} = TC_C(\mu gC \cdot mg_{DW}^{-1}) \tag{2.2}$$

2.3.3 Calculations

Fresh weight/dry weight conversion

Dry weight of replicate individuals from the uptake studies was estimated based on a factor (∼10% dry matter content, F_{DW}) established for individuals of equal stage to individuals used in this study (Etter et al., 2017 [Unpublished data]). By this, the dry weight could be estimated from registered fresh weight biomass (g, FW_5) for both 5 individuals (g, DW_5) and per individual (g, DW_1) for each replicate as described in Equation 2.3

$$\frac{gFW_5}{5\,individuals} \cdot F_{DW} = \frac{gDW_5}{5\,individuals} = gDW_1 \tag{2.3}$$

Conversion to % of dry weight

Tissue content values were given as µgP· mg_{DW}^{-1}, but in order to make the results comparable with other studies these values were converted to % of dry weigh (%DW) as given in Equation 2.4.

$$\frac{TC(\mu g \cdot mg_{DW}^{-1}) \cdot 1000(\mu g \cdot mg^{-1})}{WS(mg_{DW})} = \%DW \tag{2.4}$$

Ratios

Tissue ratios of C, N and P were determined as described in Equation 2.5. Ratios (C:P and N:P) were used to establish the estimated C:N:P ratio in tissue.

$$\frac{TC_N(\mu g \cdot mg_{DW}^{-1})}{TC_P(\mu g \cdot mg_{DW}^{-1})} = N : P\ ratio \tag{2.5}$$

2.4 Statistical analyses

All statistical analyses were performed either by the use of Excel (Version 1904, Microsoft Office 365 ProPlus) or R-studios (Version 1.1.463), with a level of significance of $p<0.05$ throughout.

Data was fitted with linear regression or linear mixed-effect models to get estimates of change. Model choice was based on AIC, as well as evaluation of the residual distribution, signficance level of parameters and the adjusted R^2.

Differences among groups were investigated by ANOVA, T-test or comparison of $CI_{95\%}$.

2.4.1 Study of initial uptake

A. Inspection of data

The water content of N and P for all treatments in both groups were plotted against time for visual inspection. In the saturated group, two very high values of nitrogen (180 min control replicate 1, and 30 min 0.25 µmolP·L^{-1} replicate 5) were excluded from further analysis, as they diverged strongly from corresponding replicates and adjacent measures in time. Possible explanations are contamination or technical defects during analysis. Also, one value at 30 minutes in the 2.0 µmol·L^{-1} treatment was excluded as the value was very low, probably due to a mix up with a sample of distilled water.

When treatments in the saturated group were plotted separately, the same pattern of a possible sigmoid curve was present (Figures A.2 in Appendix A.2). However, due to the uneven temporal distribution in measurements, a linear regression was chosen and used for the proceeding analyses. This was done in order to establish the highest initial uptake in relation to the time of exposure.

In the depleted group, the inspection of the data did not indicate the same sigmoid pattern. Data points for water content of DIP (in the 2.0 µmolP·L^{-1} treatment) and nitrogen for all treatments at the 5 minute sampling showed several values higher than what would be expected according to treatments (Table 2.2). Replicate 4 was excluded from further analysis, due to high values and higher mean FW of individuals in this replicate assumed to effect water content in a larger degree than other replicates. Linear regression was used to further establish maximum initial uptake in relation to time of exposure.

B. Uptake: Linear regression and two-way ANOVA

Data for DIP and DIN water content were fitted to a linear model separately per acclimatization to establish removal rate of DIP and DIN from the medium. Three different versions of the data was fitted, with different time-intervals of the experiment: 0-50 (mod_{50min}), 0-90 (mod_{90min}), and 0-180 minutes (mod_{180min}). This was done to establish the time frame of maximum initial uptake for different nutritional states. AIC-model tests, residual distribution (∼normal distribution), slope steepness, and comparison of adj.R^2 and p-values were used to choose the best model. All chosen models had a residual distribution ∼N, exceptions are described in the text. The residual distribution of all chosen models are given in Table A.1 in Appendix A.4.

For DIP uptake in the saturated group the model from 0 to 90 minutes was selected, mod_{90}, including treatment, time and their interaction as explanatory variables. This implied that DIP water content decreased with increasing time, and that the rate of DIP removal was different among treatments. The model had an adj.R^2=0.90 and a significant effect ($p<0.05$) of all variables and levels (except change in removal rate between treatment 0.25 and 0.5 µmolP·L^{-1} with a p=0.07). It was also the model estimating the highest rate of removal of all tested models. A two-way ANOVA established treatment as the main explanatory variable, and was used to assess differences among treatments.

For DIN uptake in the saturated group, mod_{90} was also selected as the best model including treatment (factor) and time as explanatory variables. This implied that DIN water content decreased with increasing time, but the rate of DIN removal was equal across treatments. The model had an adj.R^2=0.59 and a significant effect ($p<0.05$) of time, and varied effect of treatment levels. It had the highest removal rate of all tested models. A two-way ANOVA established time as the main explanatory variable, and was used to assess differences among treatments.

For DIP uptake in the depleted group, two models including 0 to 180 minutes were considered as they had the highest removal rates, and best explanatory degree (R^2). The first model (MPD_{180ma}) included treatment, time and their interaction as explanatory variables, implying a decrease in DIP water content with increasing time and different removal rates across treatments. This model had an adj.R^2=0.88, and a significant effect ($p<0.05$) of time for the 0.75, 1.0 and 2.0 µmolP·L^{-1} treatment levels. The only significant difference ($p<0.05$) between treatments was between the highest (2.0 µmolP·L^{-1}) and lowest (0.25 µmolP·L^{-1}) treatments. The second model (MPD_{180mb}) included treatment and time as explanatory variables, implying a decrease in DIP water content with increasing time and equal removal rate across treatments. This model had an adj.R^2=0.88, and a significant effect ($p<0.05$) of all variables and levels. Due to statistical significance, MPD_{180mb} was chosen for establishing estimates for the rate of uptake. However, the second model MPD_{180ma} was used in the evaluation of differences in uptake rate with increasing phosphorus availability. This, because the MPD_{180ma} was regarded as more biologically relevant despite the effects not being statistically significant. A two-way ANOVA established treatment as the main explanatory variable in both models, and was used to assess differences among treatments.

Also for DIN uptake in the depleted group, the model including 0 to 180 minutes was selected, mod_{180m}, including treatment and time as explanatory variables. This implied that DIN water content decreased with increasing time, but the rate of DIN removal was equal across treatments. The model had an adj.R^2=0.58 and a significant effect ($p<0.05$) of time, and varied effect of treatment. A two-way ANOVA established treatment as the main explanatory variable, and was used to assess differences among treatments. The residual distribution was a bit skewed, but the model was still regarded as the best option and enabled comparison with the other models. Also, there was a lot of variation in the data regarded to be due to biological factors that should not affect model choice.

C. Tissue content

To explore differences in mean tissue content between initial samples and treatments within and among acclimatizations, comparison of $CI_{95\%}$ was used.

2.4.2 Study of nutrient recycling in IMTA

A. Inspection of data

Biomass ($kg_{FW} \cdot m^{-1}$) for each sampling day was plotted against distance from the farm edge for visual inspection. No observations were excluded. Linear regression was used to establish changes in growth at different distances from the farm edge for different sampling days.

No observations from results of water samples or tissue samples were excluded in the further statistical analyses.

B. Biomass yield in IMTA: linear regression and group comparison

Differences between the I and R group at different field days were established by a Welch two-sample t-test. Linear regression was applied to biomass yield as a function of rope distance from the fish farm edge, in order to evaluate differences in biomass yield across the cultivation gradient. Four models were fitted, one for each field day (all R^2 <0.15) and one separately for the I group in June 13^{th} (R^2=0.67).

C. Variation in tissue content: Linear mixed-effect models and nested two-way ANOVA

The analyses of tissue content of phosphorus, nitrogen and carbon were investigated separately and as ratios. Differences among group means were considered by an ANOVA and $CI_{95\%}$ comparison.

Variation in tissue content was modelled by a linear mixed effect model to account for nesting in the data, and evaluate effects of internal distribution of content and differences between months and distance from the fish farm. Models for were fitted for each month separately to simplify the model and avoid three-way interactions. All chosen models included a nesting of individual and sample area ($1|Individual/SampleArea$) as a random effect, resulting in a low residual variance meaning that most of the natural/random variation in the data was accounted for in the model. The models all had a residual distribution ∼N (Table B.2 in Appendix B.4).

The model for variation in phosphorus content included sample area and farm distance as fixed effects in April, and sample area, farm distance, and their interaction in June. Effects of all factors were significant in April. In June all factors were significant, but there was not a significant difference in the farm distance effect on the A and B sample areas (t=1.33). The models in April and June had an adj.R^2-value of 0.75 and 0.73, respectively.

The model for variation in nitrogen content included sample area as a fixed effect in April, and sample area, farm distance, and their interaction in June. In April there was thus no effect of farm distance, but significant effects of all sample area levels. In June there was a significant effect off farm distance, but the effect did not differentiate between the A and B sample areas (t=1.39). Also, there was not a significant difference in content between sample area A and C. The models in April and June had an adj.R^2-value of 0.36 and 0.57, respectively.

The model for variation in carbon content included sample area and farm distance as fixed effects in April, and sample area, farm distance, and their interaction in June. All effects and levels were significant in both months. Even though the differences were statistically significant, they were of such low magnitude that their biological relevance was included in the evaluation of these results. The models in April and June had an adj.R^2-value of 0.42 and 0.62, respectively.

Further comparisons of mean$\pm CI_{95\%}$ were used to establish differences in each month between and within sample areas, and between and within different distances from the farm. This was also done for the tissue ratio PON:POP.

Chapter 3

Results

3.1 Initial nutrient uptake of DIP

3.1.1 Acclimatization to phosphorus depletion and saturation

Phosphorus saturated acclimatization (PS)

During the 2 day acclimatization, the saturated group had stable mean±SE levels of available phosphorus (DIP) and nitrate (DIN) of 19.75±0.55 and 136.08±0.96 µg·L^{-1}, respectively. As shown in Table 3.1, the levels (with kelp) did not differentiate ($CI_{95\%}$ overlap) from samples collected prior to the addition of kelp to the tank (no kelp). Temperature, salinity and pH corresponded to expected values.

Phosphorus depleted acclimatization (PD)

As expressed in Table 3.1 the level of DIP and DIN prior to the kelp addition to the depleted tank was 9.69±0.04 and 13.20±2.91, respectively. The level of DIP or DIN before kelp addition was significantly (no $CI_{95\%}$ overlap) different from the mean levels during the acclimatization period.

Across the 7 day acclimatization, the mean±SE level of DIP was determined to 2.36±0.21 µgP·L^{-1} implying a low availability of DIP. The measured mean level of DIN (with kelp) express a residue content of 17.92 ±3.00 µgN·L^{-1} in the tank prior to the daily addition of DIN. However, results from the day 0 and 1 samples were evaluated separately. The day 1 sample was collected right after the DIN addition, showing a content of 260.99±2.21 µgN·L^{-1}. Differences between the DIN residue content and the daily addition of DIN, express a large daily uptake.

Temperature and pH corresponded to expected values. However, measured salinity for the PD group was 30.6 (Table 3.1), diverging from the expected value of 35 from Grasshoff et al. (1999).

Table 3.1: Temperature, salinity, pH, and nutrient content for acclimatization of the PS and PD group: Measured values for temperature, salinity and pH on day 0 are expressed, as well as mean±SE measured nutrient content in the tanks from daily water samples. Nutrient content (no kelp) are from samples collected prior to the addition of kelp to each acclimatization tank on day 0.
$_1$Measured mean residue of DIN in tank before the daily DIN addition.

	Fixed parameters			Nutrient content			
				Phosphate (µg·L^{-1})		Nitrate (µg·L^{-1})	
	Salinity	Temperature (°C)	pH	No kelp	With kelp	No kelp	With kelp
PS	34.5	7.5	7.0	20.15±0.10	19.75±0.55	135.64±0.77	136.08±0.96
PD	30.6	10.7	7.0	9.69±0.04	2.36±0.21	13.20±2.91$_1$	17.92 ±3.00$_1$

3.1.2 DIP uptake

Removal of DIP from the water, as an estimate for uptake rate, is shown in Figure 3.1a and 3.1b for the PS and PD group, respectively.

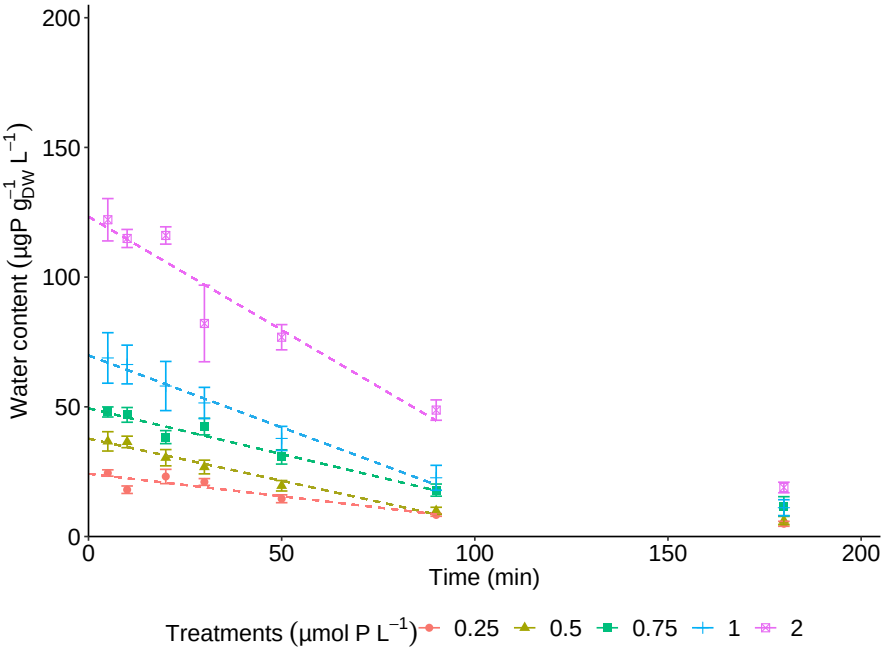
Phosphorus saturated group (PS)

When plotted separately, with DIP water content against time, all treatments showed a similar pattern of uptake that could indicate a sigmoid curve in initial uptake response. However, the removal of DIP from water is expressed as a linear model due to the distribution in data points making the fitting of a sigmoid curve less reliable. Plots per treatment can be investigated in Figure A.2 in Appendix A.2.

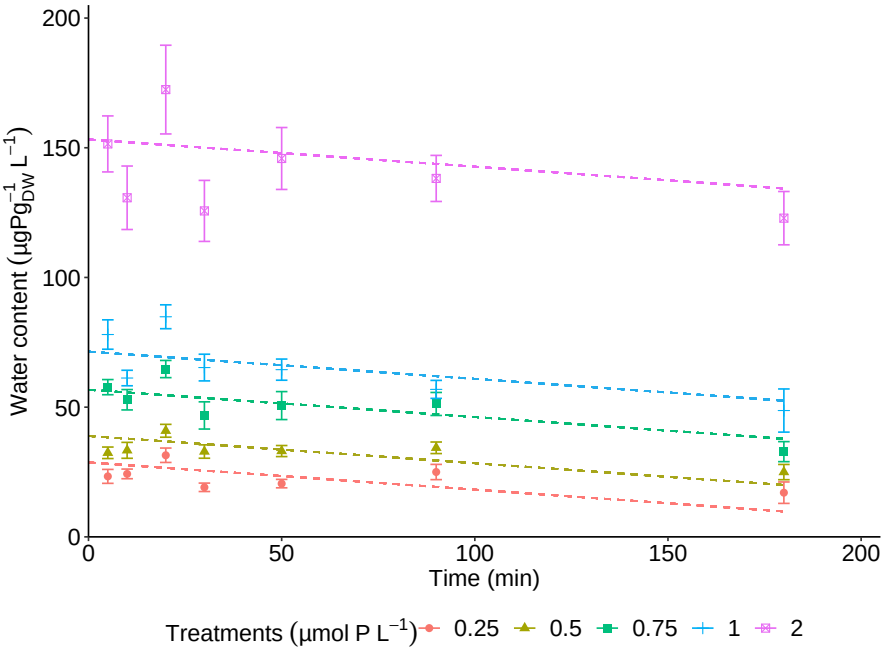
DIP uptake was significant ($p<0.05$) in all treatments, with the model adjusted $R^2=0.90$. The rate of uptake, within 90 minutes, increased with increasing phosphate availability from a rate of 10.42±1.52 to 52.42±6.65 µg·g$_{DW}^{-1}$L^{-1}hour^{-1} for the treatments with the lowest (0.25 µmolP·L^{-1}) to the highest (2.0 µmolP·L^{-1}) phosphate availability, respectively. For the uptake rates, there were no significant difference between treatment 0.25 and 0.5 ($p=0.07$), 0.75 and 0.5 ($p=0.74$). Uptake rates for all treatments are expressed in Table 3.2.

Table 3.2: Estimated initial uptake rate±SE (µg·g$_{DW}^{-1}$L^{-1}hour^{-1}) of DIP in treatments of increasing phosphorus concentrations. Estimates are given for phosphorus saturated (PS) *S. latissima* during 90 minutes of uptake, and phosphorus depleted (PD) *S. latissima* during 180 minutes of uptake. Significance level of slopes expressed as *($p<0.05$).

Treatments	Uptake rate (µgP·g$_{DW}^{-1}$L^{-1}hour^{-1}) per treatment				
µmolP L^{-1}	0.25	0.5	0.75	1.0	2.0
µgP L^{-1}	7.75	15.5	23.25	31	62
PS_{90min}	10.42±1.52*	19.48±2.15*	21.14±2.24*	33.27±5.8*	52.42±6.65*
PD_{180min}					
MPD_{180mb}	6.31±1.13*	6.31±1.13*	6.31±1.13*	6.31±1.13*	6.31±1.13*
MPD_{180ma}	2.41±2.46	3.14±3.47	7.88±3.47*	9.04±3.47*	9.63±3.64*



(a) Phosphorus saturated group (PS)



(b) Phosphorus depleted group (PD)

Figure 3.1: Phosphorus removal expressed as mean±SE DIP water content for each treatment at different times during the experiment. Fitted slopes indicate the estimated rate of DIP removal for *S. latissima* in (a) the saturated group (PS), for the first 90 minutes of the experiment, and (b) the phosphorus depleted group (PD), for all 180 minutes of the experiment.

Phosphorus depleted group (PD)

When plotting DIP water content against time for each treatment in the PD group, no similar pattern as described for the PS group was recognized. The rate of uptake was estimated by the best fitting linear model, MPD_{180mb} (adj.R^2=0.88), showing no significant difference in uptake rate between treatments. This gave an estimated DIP uptake rate of 6.31±1.13 $\mu gP \cdot g_{DW}^{-1} L^{-1} hour^{-1}$ across the 3 hours of the experiment. The alternate model, MPD_{180ma}, included a difference in uptake rate among treatments that was not statistically significant. However, estimates from this model was regarded as more biologically relevant and corresponded better with the measured uptake rates. It was included in the assessment of how uptake rates vary with increased phosphorus availability (Figure 3.2). Uptake rates for all treatments from both models (MPD_{180ma} and MPD_{180mb}) are included in Table 3.2.

Uptake with increasing DIP availability

A model was fitted with uptake rate as a function of increasing DIP availability for both acclimatization groups (Figure 3.2). This gave estimated values for the initial uptake rate and the change in uptake rate as availability increases, as given in Table 3.3. The estimates for the PS group were significant ($p<0.05$, R^2=0.98), with a change in uptake rate of 23.7 per increase of 1.0 $\mu molP \cdot L^{-1}$. For the PD group (based on slopes from the model MPD_{180ma}), the estimated change in slope of 4.2 per increase of 1.0 $\mu molP \cdot L^{-1}$ was not significant (p=0.07), but had a R^2=0.67.

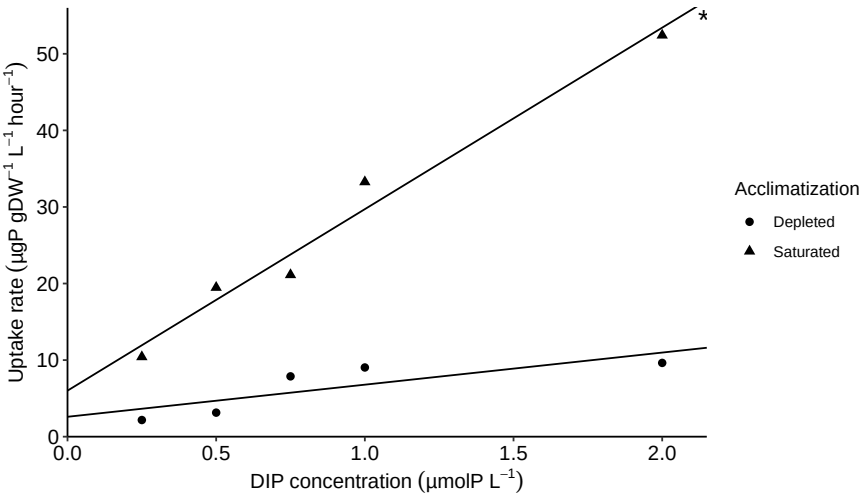


Figure 3.2: Estimated uptake rates plotted against treatment concentrations of increasing DIP availability, for both acclimatizations: Phosphorus saturated and phosphorus depleted *S. latissima*. Slopes fitted are uptake rate as a function of increasing DIP concentrations. Significant linear correlation is indicated by *($p<0.05$).

Table 3.3: Estimates from uptake rate (y) as a function ($y \sim ax + b$) of treatments of increasing DIP availability (x). Expressed as estimates of the change in DIP uptake rate per increase of 1.0 µmolP·L^{-1} (a), and initial uptake rate (b). Significant estimates expressed as *(p<0.05).

Acclimatizations	Change in uptake rate	Initial uptake rate	Degree of fit (R^2)
Saturated	23.70±1.95*	6.01±2.11*	0.976
Depleted	4.19±1.95	2.60±2.11	0.667

3.1.3 Nitrogen uptake

Removal of DIN from the water, as an estimate for uptake rate, is shown in Figure 3.3a and 3.3b for the PS and PD group, respectively.

Phosphorus saturated group

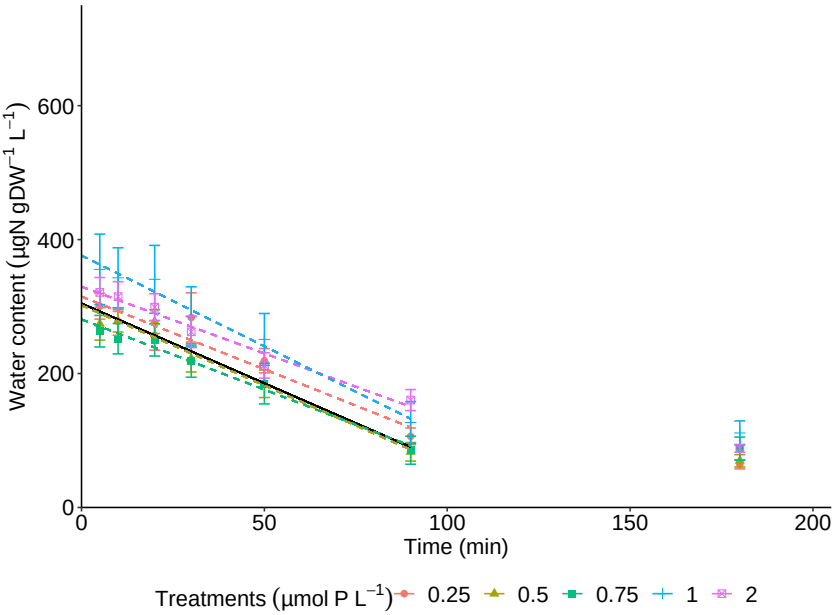
In the saturated group, DIN uptake did not differ significantly across treatments of increasing DIP availability, with an estimated uptake rate of 80.01±4.87 µgN·$g_{DW}^{-1}L^{-1}hour^{-1}$ in the first 90 minutes of the experiment. Model intercepts varied with a mean±CI of 292.3±24.3 across treatments with treatment 1.0µmolP·L^{-1} diverging significantly from the rest (p<0.05), visually described in Figure 3.3a. For inspection of details in Figure 3.3a see Figure A.1 in Appendix A.1.

Phosphorus depleted group

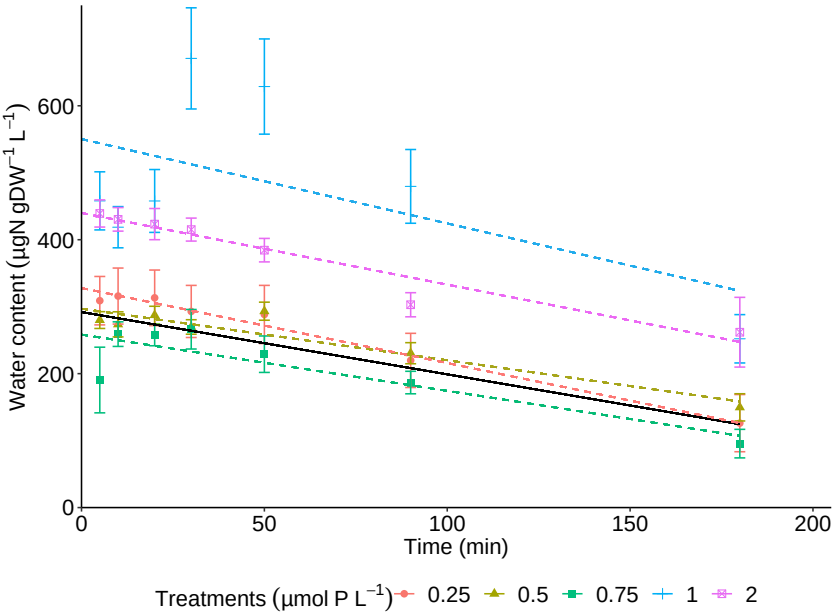
Uptake of DIN in the saturated group, did not differ significantly across treatments, giving an estimated rate of 60.53±7.10 µgN·$g_{DW}^{-1}L^{-1}hour^{-1}$ for the entire duration of the experiment. Intercepts varied significantly between treatments, with the exception of the two lowest (0.25 and 0.5 µmolP·L^{-1}, p=0.59). As shown in Table 3.4, there was no large difference in uptake between the PS and PD groups as their $CI_{95\%}$ overlap.

Table 3.4: Estimated initial uptake rate±SE (µg·$g_{DW}^{-1}L^{-1}hour^{-1}$) of DIN across all treatments of increasing DIP availability. Estimates are given for phosphorus saturated (PS) *S. latissima* during 90 minutes of uptake, and phosphorus depleted (PD) *S. latissima* during 180 minutes of uptake. Significance level of slope expressed as *(p<0.05).

Treatments	Uptake rate (µg·$g_{DW}^{-1}L^{-1}hour^{-1}$) for all treatments
PS_{90min}	80.66±4.89***
PD_{180min}	60.53±7.10***



(a) Phosphorus saturated group (PS)



(b) Phosphorus depleted group (PD)

Figure 3.3: Nitrogen removal expressed as mean±SE DIN water content for each treatment at different times during the experiment. Fitted slopes indicate the estimated rate of DIN removal for *S. latissima* in (a) the PS group, for the first 90 minutes of the experiment, and (b) the PD group, for all 180 minutes of the experiment. Coloured dashed lines are estimated slopes for each treatment individually, black line is the significant estimated rate of removal across all treatments.

Table 3.5: Water content as mean±SE of DIP and DIN at start and end of experiment for all treatments and controls. Given for both acclimatizations: Phosphorus saturated (PS) and depleted (PD).

Acclim.		Treatments					Controls	
	µmolP L^{-1}	0.25	0.5	0.75	1.0	2.0	0.25	2.0
	µgP L^{-1}	7.75	15.5	23.25	31	62	7.75	62
	DIP							
PS	5 min	12.7±0.5	17.73±1.1	24.6±1.2	31.6±2.1	60.4±2.8	10.8±0.6	50.4±4.0
Phosphorus	180 min	2.6±0.7	2.9±0.7	6.1±2.2	5.0±1.2	9.5±1.2	7.8±0.1	51.8±2.5
saturated	DIN							
	5 min	154.7±8.6	133.2±8.1	133.1±6.4	163.1±10.8	158.3±8.5	132.6±1.1	145.7±0.6
	180 min	33.8±4.6	33.4±2.6	46.7±11.3	51.0±6.7	44.5±4.8	127.1	129.2±3.0
	DIP							
PD	5 min	11.9±0.6	18.9±1.2	29.8±2.1	38.1±1.7	70.8±4.9	11.7±1.5	57.1±7.3
Phosphorus	180 min	9.0±2.4	14.5±1.6	16.9±2.3	24.6±4.9	58.3±6.8	12.5±1.6	55.0±6.9
depleted	DIN							
	5 min	156.6±7.0	163.0±4.3	91.0±18.1	221.4±10.9	212.8±29.6	126.7±7.5	128.8±5.6
	180 min	67.2±25.3	86.2±10.3	51.0±13.8	123.2±17.1	122.3±20.8	133.8±8.2	132.7±12.2

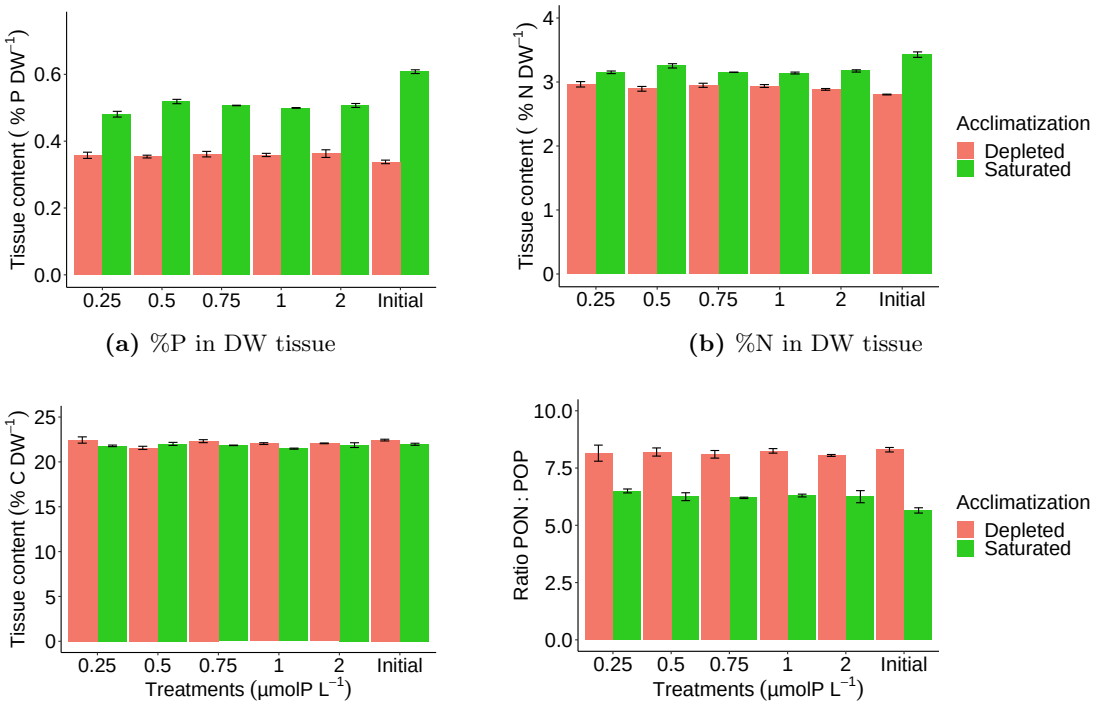
3.1.4 Start values and residue in treatments

Mean content of DIP and DIN at the first (5 min) and final (180 min) measurements from the experiments can be viewed in Table 3.5. Starting content of DIP did not differentiate drastically from expected values for the corresponding treatment. This evaluation take the error margin of ±2 µg·L^{-1} of the autoanalyser into account, as well as the values for the controls. Values for 180 min in the PS group show a very low residue level of DIP in most treatments, as well as quite low values for DIN. In comparison, residue values in the PD group are a lot higher for DIP and DIN.

3.1.5 Tissue content: Saturated and depleted kelp

Mean tissue content (% of DW) and tissue PON:POP ratio for both acclimatizations are shown in Figure 3.4. For both acclimatizations, nitrogen (N) and phosphorus (P) content differed significantly, $CI_{95\%}$ did not overlap, between treatments and initial content. The N and P content was different between the acclimatization groups on a statistically significant level ($p<0.05$), with no overlaps in $CI_{95\%}$. However, the difference was largest for P content, as shown in Figure 3.4a and 3.4b. From Figure 3.4d show that the difference between the initial and treatments in the PD group was not detectible as a change in PON:POP ratio. The ratio was significantly lower in the PS group, and different between the initial content and treatments.

As visualized in Figure 3.4c, carbon content did not differentiate significantly between acclimatizations or between treatments and initial content.



(a) %P in DW tissue

(b) %N in DW tissue

(c) %C in DW tissue

(d) Ratio N:P in DW tissue

Figure 3.4: Mean±CI$_{95\%}$ tissue content as % per g$_{DW}$ of (a) phosphorus, (b) nitrogen, and (c) carbon, for the phosphorus saturated and phosphorus depleted group. (d) mean±CI$_{95\%}$ tissue ratio N:P for both groups, based on % per g$_{DW}$. No overlap in CI$_{95\%}$ indicate significant differences between groups and treatment levels.

3.2 Potential for phosphorus recycling in IMTA

3.2.1 Parameters at the site

Temperature, salmon biomass and feed use at Rataren I and II during the cultivation period, as well as salinity from Sula, are given in Table 3.6.

3.2.2 Seaweed growth

Cultivated *S. latissima* showed good growth with a clear difference between the ropes cultivated among the pens (I group) and ropes cultivated downstream (R group, 100-300 m). A Welch two-sample t-test determined the difference to be significant ($p<0.05$) between all groups. Measured biomass for the cultivation period are given in Table 3.7.

There was large variation in growth within the I group, indicating a pattern of increased growth for ropes closer to the downstream edge than ropes situated in amongst salmon pens, shown in Figure 3.5. A linear regression (R^2=0.67) estimated an increased biomass yield of

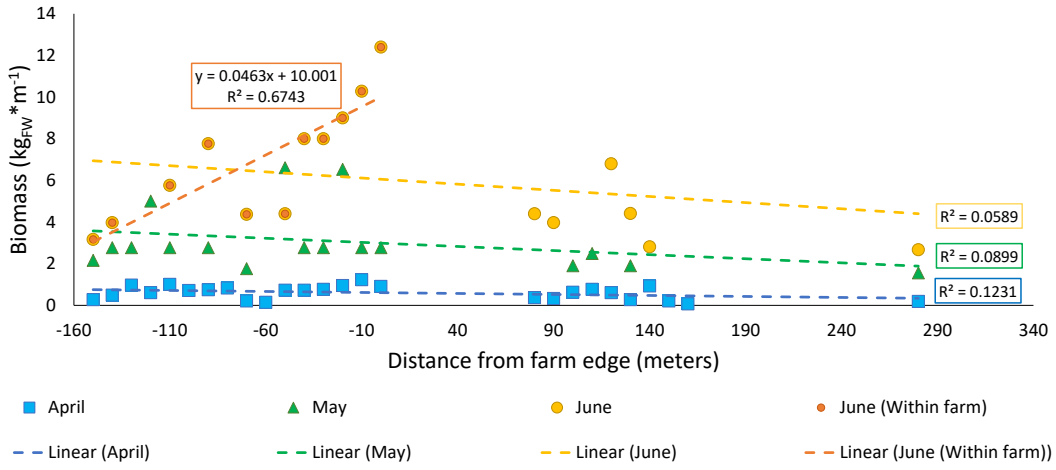


Figure 3.5: Rope biomass yield ($kg_{FW} \cdot m^{-1}$) at different distances from the farm edge for three sampling days: April 23^{rd}, May 29^{th}, and June 13^{th}. Slopes for estimation of change in biomass yield across the gradient, with their respective R^2, are included. Data points for the I group (within farm) in June are highlighted in orange, with a separate fitted linear regression where the estimated function and R^2 are included.

0.046 $kg_{FW} \cdot m^{-1}$ for each meter a rope was situated closer to the farm edge, giving a span from 3.1 $kg_{FW} \cdot m^{-1}$ at $rope_{-150m}$ to 10.5 $kg_{FW} \cdot m^{-1}$ at $rope_{0m}$ (farm edge). Also, $rope_{-150m}$ has similar growth to $ropes_{90\ to\ 140m}$.

Table 3.6: Temperature, salinity, salmon biomass, and feed use during the cultivation period: Salinity at Sula expressed as the monthly mean±SE, water temperature (°C) for Rataren II expressed as monthly mean±SE based on weekly measures. Biomass as tons (determined at the start of each month), and feed use as tons $month^{-1}$, for each of the two salmon farms.

Month	Temp. (°C)	Salinity (ppt)	Rataren II		Rataren I	
			Biomass (tons)	Feed use (tons)	Biomass (tons)	Feed use (tons)
February	5.93±0.27	33.18±0.01	3393	466	3887	504
March	5.10±0.07	32.37±0.05	3738	563	4266	509
April	5.33±0.23	30.01±0.06	4164	578	4648	421
May	7.43±0.46	30.24±0.01	4659	887	3231	570
June	9.13±0.17	29.06±0.03	5649	809	3698	610

Table 3.7: Rope biomass yield ($kg_{FW} \cdot m^{-1}$) in *S. latissima* as mean±SE along the gradient, for the integrated (I, among the pens) and reference (R, downstream from the farm) group. Significant differences between the I and R groups for each month are marked by *($p<0.05$), and p-values are given.

	Biomass ($kg_{FW} \cdot m^{-1}$)		p-value for difference between groups
	I	R	
April 23^{rd}	0.71±0.08*	0.44±0.09*	0.03
May 29^{th}	3.40±0.44*	1.96±0.19*	0.01
June 13^{th}	7.01±0.88*	4.18±0.61*	0.02

Table 3.8: Nutrient availability ($\mu g \cdot L^{-1}$) at different sampling days during the IMTA cultivation period. Given as mean±SE for all ropes within each sampling day.

	Nutrient availability ($\mu g \cdot L^{-1}$)	
	DIP	DIN
February 16^{th}	14.8±1.0	59.0±7.3
April 23^{rd}	7.2±2.4	14.4±2.6
May 29^{th}	8.8±0.7	23.6±3.1
June 13^{th}	8.6±1.0	29.8±3.8

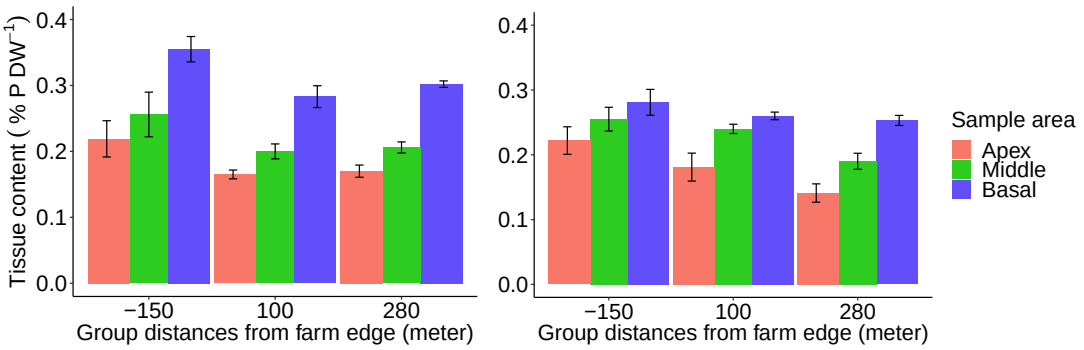
3.2.3 Nutrient availability

Measured water content of nitrate/nitrite and phosphate are given in Table 3.8 as means±SE across all ropes for each sampling day. Statistical significance and differences between groups could not be estimated as there were only one observation per sampling position for each sampling day. All measurements can be visually inspected in Figure B.1 in Appendix B.1.

3.2.4 Seaweed tissue content

Phosphorus content

There was a clear difference in phosphorus tissue content among the sampling areas as shown in Figure 3.6a. The content was highest in the basal area and decreased towards the apex, which was a pattern in both months independent of rope distance from the farm edge. In April, within each rope, there were significant differences ($p<0.05$) between the basal sample area (C) and each of the other sample areas: Middle (B) and apex (A). The difference between B and A differed, and was only significant at $rope_{100m}$ and $rope_{280m}$. However, the $rope_{-150m}$ had a wider $CI_{95\%}$, indicating larger individual variation. Across all ropes, independant of sample



(a) April **(b)** June

Figure 3.6: Variation and distribution in phosphorus tissue content in *S. latissima*, given as mean±$CI_{95\%}$ for different sample areas of the lamina and at different distances from the fish farm edge.

Table 3.9: How much of the variation in phosphorus tissue content was due to different explanatory variables in April and June. Given as proportion of variance (%), based on a nested two-way ANOVA analysis.

	April	June
	Variance proportion (%)	Variance proportion (%)
Distance from farm (m)	15.6	22.5
Sample area	72.7	53.1
Sample area:individual	3.8	11.2
Individual	11.5	12.9
Residuals	0.2	0.2

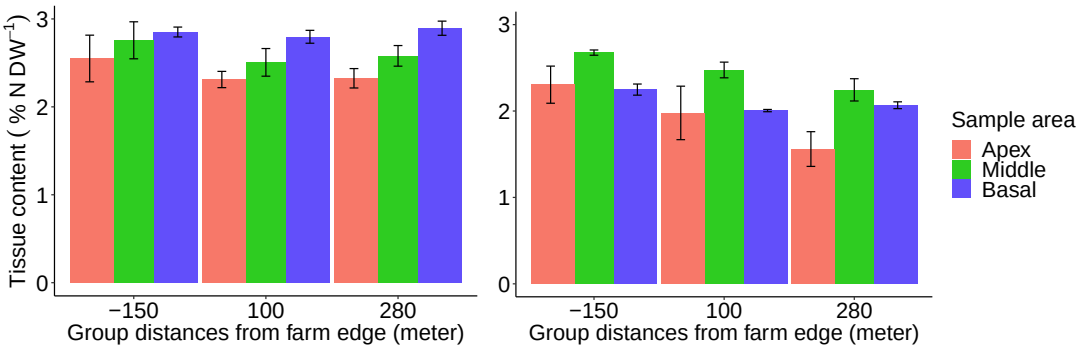
area, there was a significant difference ($p < 0.05$) between $rope_{-150m}$ and the two other ropes, while the difference between $rope_{100m}$ and $rope_{280m}$ was not significant. The difference between sample areas within and among ropes was not as apparent in June (Figure 3.6b). Sample area C was significantly different from sample area A, but only for B in $rope_{100m}$ and $rope_{280m}$. The difference between A and B was statistically similar to April. Ropes did not differentiate significantly in C content, nor in B content between $rope_{100m}$ and $rope_{280m}$.

A variance analysis (nested two-way ANOVA) indicated sample area as the main factor explaining the variation in phosphorus tissue content. In April and June sample area was accountable for 72.7 and 53.1 % of the variance, while distance from farm explained 15.6 and 22.5 %, respectively. Proportions of all variables are presented in Table 3.9, including the effect of individual variation, and the varying effect between months of the interaction between sample area and individual.

To assess the effect of change in phosphorus content at different distances from the farm edge, a linear mixed effect model was fitted. In April, the model estimated a change of -1.3 $\mu gP \cdot g_{DW}^{-1} meter^{-1}$ (0.00013 $\%P \cdot DW^{-1} meter^{-1}$) in content downstream the gradient, which was equal for all sample areas. In June the estimated change varied between sample areas. The apex and middle section had similar changes (difference not significant, t-value=1.32) of -1.9 and -1.5 $\mu gP \cdot g_{DW}^{-1} meter^{-1}$ (0.00019 $\%P \cdot DW^{-1} meter^{-1}$ and 0.00015 $\%P \cdot DW^{-1} meter^{-1}$), respectively, while only -0.6 $\mu gP \cdot g_{DW}^{-1} meter^{-1}$ (0.00006 $\%P \cdot DW^{-1} meter^{-1}$) for the basal area.

Nitrogen content

In April (Figure 3.7a), there was no significant effect of distance from farm on nitrogen tissue content, but content did vary across sample areas with the highest values in the basal area (C), decreasing towards the apex (A). However, as shown in Figure 3.7a, this difference was not significant between most groups as $CI_{95\%}$ overlapped. In June, nitrogen tissue content decreased with increasing distance downstream from the farm edge in the B and A areas, while the decrease in the C area was not significant (t=1.2). The B sample area had the highest nitrogen content values in June, and the A and C area had lower and more similar content values as given in Figure 3.7b.



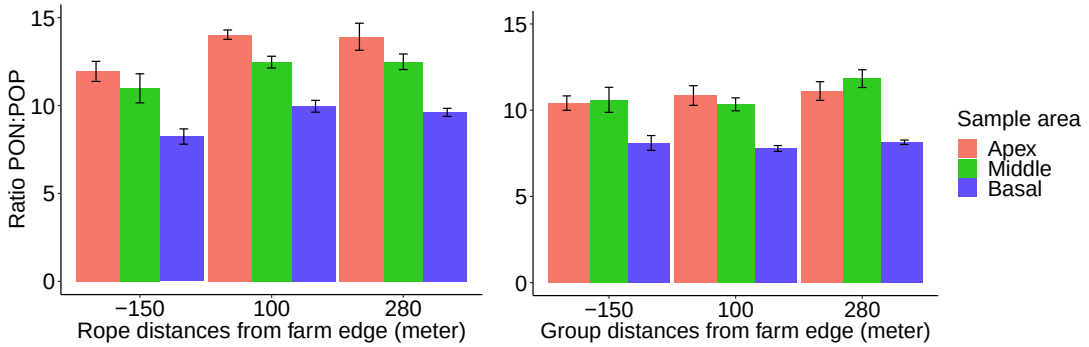
(a) April **(b)** June

Figure 3.7: Variation and distribution in nitrogen tissue content in *S. latissima*, given as mean±$CI_{95\%}$ for different sample areas of the lamina and at different distances from the fish farm edge.

Carbon content & ratios

Carbon content had less variation among the months, ropes and sample areas, with few significant differences among groups. The A area tended to have the highest, and the C area the lowest with a significant difference between them in April ($rope_{100}$) and June ($rope_{100}$ and $rope_{280}$). All differences in carbon content can be viewed in Figure B.2 in Appendix B.2.

The nitrogen and phosphorus tissue content ratio was investigated, and are shown in Figure 3.8. The ratios showed significant differences between the basal area (C) and the other two areas (A and B) in both months. There was little variation between ropes for each sample area, but some difference between months. Sample area B and A did not vary significantly in June, but did in April ($rope_{100}$ and barely in rope $_{280}$).



(a) April **(b)** June

Figure 3.8: Variation and distribution of PON:POP ratio in tissue of *S. latissima*, given as mean±$CI_{95\%}$ for different sample areas of the lamina and at different distances from the fish farm edge.

Chapter 4

Discussion

This study showed that the phosphorus uptake in *S. latissima* was strongly affected by their physiological state, and that initial phosphorus uptake increases with increasing phosphorus availability. There was also an internal tissue content distribution prioritizing phosphorus near the meristem to facilitate growth. These results supported other studies suggesting that IMTA kelp growth and nutrient utilization depend on the distance they are situated from the farm, and further promoting the use of kelp like *S. latissima* for phosphorus recycling through IMTA.

Phosphorus depleted individuals did not have more rapid uptake than phosphorus saturated individuals, possibly due to the lack of tolerance for phosphorus drained medium over a period of several days. The uptake in the nutritionally saturated group of *S. latissima* had a strong linear increase in rate with increasing availability, pointing towards their ability to utilize increased nutrient pulses. This increase was non-significantly insinuated in the phosphorus depleted group.

Phosphorus storage was suggested to be higher closer to the salmon farm, but this may have been due to limitations in other factors and need to be investigated further. Kelp biomass yield was higher closer to the salmon farm, but decreased as the gradient moved in among farm pens.

4.1 Initial uptake in *S. latissima*

4.1.1 Uptake rates of phosphorus saturated (PS) vs. depleted (PD) individuals

Factors like temperature, salinity, light, nutrients, and water movement are all required at a certain level for kelp to function and grow optimally (Harrison and Hurd, 2001; Hurd et al., 2014). Thus the nutritional state of an individual will influence their ability for nutrient uptake and execution of physiological processes. In this study, water samples of nutrients from the acclimatization tanks displayed good nutrient availability for the phosphorus saturated (PS) group, and low DIP as well as high DIN for the phosphorus depleted (PD) group. Also, the PON:POP tissue ratio implied differentiating states between the two groups. The PD group

was subjected to a phosphorus drainage that shifted them into a sub-optimal nutritional and physiological state, and it was expected that their DIP uptake would be more rapid when re-introduced to phosphorus rich media through replenishment of their depleted phosphorus reserves. This would correspond to a suggested expectation of surge uptake for individuals of macro- and microalgae with depleted nutrient reserves (Harrison and Hurd, 2001). However, the hypothesis 1a was contradicted by the current results, as nutritionally saturated individuals had higher and a more definite pattern of DIP uptake.

The uptake of DIP in the PS group was high during the first 90 minutes for all treatments, and decreased onwards to the 3 hour mark. All available DIP and almost all DIN was extracted from the medium within the duration of the experiment. For PD individuals, the uptake rates were only significant for the complete 3 hour experimental period, but rates did not differ significantly among treatments and did not exceed any of the rates for the PS group.

However, observations indicated that the depleted group did not handle the acclimatization well, and their physiological state at the time of the experiment made them almost unable to utilize nutrients from their medium within the given time frame. Possibly these individuals may have been able to acclimize back to more of a normal state had the experiment duration been longer. Another possible negative effect from the acclimation was the low measured salinity content (30.6) which diverged from the expected value (35) for the artificial seawater (Grasshoff et al., 1999). However, *S. latissima* is reckoned as one of the species in the *Laminariaceae* family that are tolerant to wider ranges of temperature and salinity, but younger individuals may still be more sensitive (Druehl, 1967; Karsten, 2012).

S. latissima is known to be a fast growing species, with the capacity to utilize higher nutrient availability towards growth (Barrington et al., 2009; Kerrison et al., 2015; Schiener et al., 2015). The current results suggest that *S. latissima* did not handle phosphorus absence for several days, which could be a disadvantageous trait in nature. However, this can possibly be explained evolutionary by environmental adaption and the geographical distribution of sugar kelp in coastal temperate waters. These waters are known for high productivity, driven by its relatively high and stable nutrient availability during winter and early spring (Skjoldal and Saetre, 2004), in addition to nitrogen being regarded as the main limiting nutrient (Howarth, 1988; Howarth and Marino, 2006). Additionally, individuals used in this study were of a size and physiological stage that naturally are present during seasons of high nutrient availability, and it might not be of any function to have large phosphorus reserves or high tolerance of DIP absence.

Pattern of initial DIP uptake

The observed pattern in DIP removal for all treatments of the saturated group, was regarded to possibly fit a sigmoid curve. Even though uptake rates were estimated by a linear model in this study, this pattern can suggest a three phase mechanism for initial DIP uptake in *S. latissima*: (i) recognition and enzyme activation of active uptake and/or stabilization in medium; (ii) increase to max initial uptake rate (dependent on nutrient availability); (iii) saturation, decrease in

uptake rate, osmotic- and/or medium-regulated limit of uptake. This hypothesis should be tested in a similar system with more rapid sampling throughout the experimental period.

The estimated rates of uptake in the saturated group add to the knowledge of how *S. latissima* utilize increased availability of DIP in pulse events, but the actual mechanism and storage ability cannot be established by this study. It will require more research to establish how *S. latissima* utilize pulse increases in available nutrients for increased growth. There was an observed increase in DIP water content from 90 to 180 minutes in the study, implying that the storage ability might not be large and differentiate among individuals during pulses of increased nutrient availability. A longer study period and/or more frequent sampling could possibly be needed to discover and understand the mechanisms and variation in uptake ability, storage capacity and incorporation rate of phosphorus in *S. latissima*.

DIN uptake not dependent on DIP availability

Results of DIN uptake rates showed no significant differences across treatments of DIP availability in either acclimatization group. The uptake rate was higher in the saturated than the depleted group, possibly implying that also DIN initial uptake ability might be affected by the physiological state. However, the $CI_{95\%}$ in DIN uptake overlapped between the PS and PD groups.

During the PD acclimatization, water samples showed that the kelp extracted almost all daily supply of DIN. Even though DIN limitation can influence DIP uptake (Hurd et al., 2014), these results suggested that DIP limitation did not influence DIN uptake during the study. Also, the measured differences in tissue content between the PS and PD groups, may suggest that PD group individuals had grown during the acclimatization (data not recorded). This because the PD group had extracted most of its supplied DIN during the acclimatization, and with slightly lower tissue N and a lot lower tissue P compared to the the PS group. This imply that PD *S. latissima* were able to utilize the supplied DIN towards growth, thereby diluting their phosphorus tissue content.

Another aspect related to the DIN uptake was that all treatments of the depleted group had higher starting values of DIN than expected (most extreme in the 1.0 $\mu g \cdot L^{-1}$ treatment $\sim$3 times higher than the expected values). This can imply stress responses to handling and/or that the physiological state of the PD individuals resulted in a lack of ability to keep and/or incorporate DIN taken up during the acclimatization when reintroduced to DIP-rich medium. Further, this may suggest that DIN incorporation depend on a certain level of available phosphorus in the tissue. However, this cannot be established by the current results.

4.1.2 DIP uptake rate depend on DIP availability

From the theory of uptake kinetics by active transport over the cellular membrane, uptake rates of DIP at increasing substrate concentrations should fit a rectangular hyperbola as predicted by the Michaelis-Menten equation (Equation 1.1) (Harrison and Hurd, 2001). This have been

suggested to be applicable to other macroalgal species for uptake of both DIN and DIP (e.g. Rosenberg and Ramus, 1984; Friedlander and Dawes, 1985). The uptake rate is thus predicted to have a linear increase before reaching V_{max} at a saturating concentration. The results of this study indicate a clear linear correlation (R^2=0.976) between the DIP uptake rate and treatment concentration, supporting hypothesis 1b and the specified theory of uptake kinetics. Even though the V_{max} for DIP could not be established within the applied range of concentrations, the aim of this study was mainly to explore the maximum initial uptake for concentrations relevant for IMTA.

Harrison and Hurd (2001) state that some species can have a linear increase in uptake rate even when availability levels increase beyond what is normal. This further support the results of this study, as the treatment with the highest DIP load had an availability double of what is expected from high natural values (Sakshaug, 1994; Erga et al., 2017). These findings suggest that the V_{max} for DIP in *S. latissima* first become apparent for DIP concentrations higher than the levels seaweeds are expected to be exposed to in nature.

Figure 3.2 showed the linear relationship between increasing DIP uptake rate with increasing medium concentration of DIP, in both the PS and PD groups. However, for the PD group this was based on estimated slopes varying among treatments (MPD_{180ma} in Table 3.2), which were not statistically significant. This pattern is still biologically interesting, as it may imply that the effect of increased DIP availability can be applicable for different physiological states.

4.2 Phosphorus storage capacity and bioremediation potential from kelp IMTA

4.2.1 Internal distribution in content

In this study a significant internal variation in phosphorus tissue content was found between the basal (C), middle (B), and apex (A) areas of the lamina in *S. latissima*. Mean phosphorus content implied a pattern across all ropes in both April and June supporting hypothesis 2a, with the highest phosphorus tissue content in the basal area (C) and the lowest at the apex (A). In April, there was a significantly higher phosphorus content in all C areas, while in June this was only significant for one rope. The C area is also the area of the growth meristem, implying an internal transport and prioritization of phosphorus in this area in order to facilitate growth or other physiological functions. This is in agreement with the knowledge that phosphorus, for instance as a component in ATP, is vital for physiological functions relating to growth (Westheimer, 1987; Hurd et al., 2014). The results are also in agreement with knowledge that kelp species like *S. latissima* show great individual and seasonal variation in tissue components (Black, 1948, 1950), as well as an internal variation in the lamina (Black, 1954).

For the nitrogen tissue content there was a significant internal variation, but less of an unanimous pattern. In April, the pattern was similar to the phosphorus content, with the

highest mean in the C and lowest in the A area. However, differences among sample areas were generally not significant. In June, the middle area (B) had the highest and the A and C areas largely overlapped in content. This could imply that nitrogen is more important in growth phase individuals (April), as June individuals are facing a season of epiphytes, low nutrient availability, and low rates of tissue generation (Skjoldal and Saetre, 2004; Artsdatabanken, 2006; Førde et al., 2016). Thus, specific nitrogen allocation to the basal area was not prioritized in June individuals. This is in accordance with former findings establishing nitrogen as the main growth promoter for *S. latissima* in IMTA systems (Wang et al., 2014).

When tissue content is compared to the biomass yield in the corresponding months, it can be argued that the prioritization of phosphorus and nitrogen to the basal area is stronger in April individuals as they have a lot of tissue generation in store towards June.

4.2.2 Tissue content and farm cultivation proximity

The farm distance account for 15.6 and 22.5 % of the variation in phosphorus tissue content in April and June, respectively (Table 3.9), suggesting a link between tissue content and farm distance. When regarding the ANOVA between all ropes, no significant differences between the two downstream ropes$_{100m,\ 280m}$ were established. However, differences were significant between these ropes and the upstream rope$_{-150m}$, implying an effect on the tissue content upstream that was not present downstream. When coupled with the biomass data for June 13^{th} (Figure 3.5), the similarity in biomass between rope$_{-150m}$ and rope$_{100m}$, in addition to a higher biomass yield at rope$_{0m}$, suggest that there were other or additional limitations for the biomass yield in ropes cultivated in among salmon pens. All fish farms consist of a lot of equipment as well as having a lot of particulate matter in the water from faeces and feed spill (Wang et al., 2012, 2013; Klebert et al., 2013). This can affect light availability and water movement within the farm, which by affecting rope$_{-150m}$ resulted in limitations leading to higher tissue content of P without enabling any further increase in biomass.

This study did not include measures of tissue content from ropes at the farm edge (rope$_{0m}$). However, given that rope$_{0m}$ was not exposed to limitations applicable to rope$_{-150m}$, it can be assumed that the tissue content at rope$_{0m}$ would be more similar to the downstream ropes. Thus, the downstream tissue content may be examples of the ideal content and internal distribution of phosphorus at optimal utilization of available nutrients, when other factors are equal. This, in accordance with literature stating that tissue content can be a measure of the nutrient state of the kelp, and that opportunistic species, like *S. latissima*, will have lower PON:POP ratios as most of all available nitrogen can be used towards growth when no other factors are limiting (Harrison and Hurd, 2001). Thus, an ideal PON:POP ratio for *S. latissima* could be used to assess whether other conditions are limiting the ability to utilize available nutrient in a cultivation area. Nevertheless, more knowledge on the PON:POP ratio in seaweeds is needed to further support these suggestions.

Based on the results of variation in phosphorus tissue content, there was an effect of farm

distance that supports hypothesis 2b. However, due to the mentioned discrepancies, it is not definite how relevant this estimated effect is. Knowledge of internal variation in tissue content closer to the farm edge than 100 m is needed to further establish whether cultivation distance from the fish farm affect internal tissue content. This should be taken into account when assessing storage capacity and bioremediation potential. For nitrogen and carbon tissue content, there were no significant effect of farm distance in April or June. The measures in nitrogen tissue content differentiated between the months, and was regarded as the natural variation in tissue content at different age and time of year.

It is suggested that varied nutrient availability between cultivation ropes is mainly expressed by differences in biomass yield, and only slightly in the tissue content. As the downstream ropes are assumed to be more nutrient limited, they are thus expected to have lower biomass yield than the ropes cultivated closer to the farm edge.

4.2.3 Farm proximity effect biomass yield

The biomass yield differentiated along the cultivation gradient. Mean biomass ($kg_{FW} \cdot m^{-1}$) was significantly higher in the integrated (I) compared to the reference (R) group for all sampling days. This is in accordance with other studies supporting the correlation of higher biomass yield in close proximity to salmon farms (Sanderson et al., 2012; Broch et al., 2013; Marinho et al., 2015).

There was shown a linear increase towards the farm edge in the I group in June (Figure 3.5). As mentioned in the preceding section, this variation was probably due to other factors limiting growth in among the pens. Thus, this study add to the knowledge of IMTA cultivation by addressing a possible pattern of biomass yield for kelp cultivated in among farm pens. Cultivation far in among pens gave a low biomass yield, possibly due limitations in physical factors. Limitations can probably be related to light and/or water movement patterns. Light scarcity can be due to farm equipment shading or higher particulate matter in the water column. Water movement can be changed and/or reduced within the farm due to equipment and bio-fouling on cages, and may further influence the distribution of particulate matter and nutrients within the farm system and indirectly affecting the availability of nutrients and light (Klebert et al., 2013). However, effects of these limitations have to be investigated in future studies. Kelp cultivation among pens can also be regarded as obstructive for everyday tasks for fish farmers. Therefore, it is advised to position kelp cultivation fields close downstream to optimize growth and bioremediation potential, but minimize effects on and from fish farm activities.

4.2.4 Assessing the bioremediation and recycling potential of phosphorus in IMTA

In regard of presented arguments, it is suggested that differences in phosphorus content between ropes cannot be used solely to assess the bioremediation and recycling potential of *S. latissima*. This is further supported by the seasonal and geographic variation in tissue content (Wheeler

and Hartwell, 1893; Black, 1948; Schiener et al., 2015). It is rather advised to use biomass yields together with phosphorus tissue contents in *S. latissima* to get an estimated magnitude of phosphorus uptake for a given crop of kelp. This is also supported by other studies evaluating bioremediation from kelp IMTA and the possibilities of upscaling the production to industrial scale (Broch et al., 2013; Fossberg et al., 2018).

Other suggested solutions of re-circulation to support future phosphorus scarcity are directed towards transformation into forms that can be used directly in fertilizers (e.g. from waste waters) (Cordell et al., 2009). The suggested method of using IMTA kelp cultivation is an indirect form of recycling, focusing on retaining more of the nutrients used through feed in fish production in the value chain. In addition, there are prospects of novel products from cultivated kelp biomass, and this is needed in order to make industrial scale kelp production feasible (Stévant et al., 2017). Some potential products are biofuels and feed, but their feasibility depend on whether product properties can meet marked demands as well as being cost effective. Kelp based feed components can be important in decreasing the pressure on agriculture crops in a growing aquaculture industry (Olsen, 2011). This can also be an opportunity to increase the proportion of ocean-resources utilized for ocean-farming of fish.

Challenges with POP for bioremediation purposes

For the sake of bioremediation, suggested solutions have to result in positive changes for the local ecosystem/environment. It has been established that kelp species like *S. latissima* have increased biomass yield when cultivated in IMTA systems (Sanderson et al., 2012). However, Wang et al. (2013) estimated the main effluent of phosphorus from salmon farms to be POP, which are forms of phosphorus that can not be utilized by photosynthetic organisms. Therefore to optimize the bioremediation potential for phosphorus in IMTA systems, it is recommended to look at systems that can also include filter feeders or other organisms able to utilize the particulate fraction (POP and PON) of biological waste products from fish (Barrington et al., 2009). Such species also have respiratory waste products that further can increase the nutrient availability for kelp, and possibly increase kelp yield. If this is not applied, the IMTA cultivation of kelp is rather regarded as recycling of nutrients than bioremediation.

4.3 Challenges and limitations

4.3.1 Experimental study

Replicates and control samples

In the uptake study, the stock solutions used to adjust the nutrient contents of all treatments and the PD acclimatization tank were not sampled, adding to the insecurity of actual nutrient availability in treatments. Also, only one control replicate was included for treatments with highest and lowest DIP availability. Future similar studies are advised to include control repli-

cates for all treatments, as this can add to the certainty in assessing potential nutrient leakages from kelp at the start of experiment.

Acclimatization

The depleted group did not tolerate the acclimatization well. However, they did extract almost all supplied DIN, raising the question of whether the addition of DIN was large enough and if other parameters of the acclimatization should have been different. It might be that a total DIP depletion is not the ideal way to go, and that this acclimatization should have included some addition of phosphorus to ensure better ability to cope. Other factors, like salinity, might also influence the state of the kelp, and should have been adjusted as the measured salinity in the PD tank diverged from the expected value of the artificial seawater that was used, with 30.6 against 35. This may have had an additional effect on the kelp, even though *S. latissima* are known to be quite tolerant to variations in salinity (Druehl, 1967; Karsten, 2012).

Adjustments in the study design (longer time, adjustment of the depleted acclimatization) could be necessary to characterize the uptake in a nutritional depleted group in a better way.

4.3.2 Field work

Field studies are impacted by different external factors (ecosystems, weather, seasonality, competing species), and other unpredictable challenges that affect the recorded data. This adds to the insecurity when predicting trends and interpreting results. This study faced challenges influencing both the methodology of how data was collected, but also experienced loss of data resulting in a lower number of observations for the registration of biomass. Some unpredictable incidents are not necessary to account for, but good standardization of field sampling methods as well as contingency plans for specific type of events can be crucial and beneficiary to ensure data quality.

One of the preliminary planned ropes for sample collection, at the farm edge (0 m), had to be discarded due to a boating accident. In retrospect, samples from this rope could have been a determining factor in the interpretation of the results of internal variation in tissue content. However, the reason for this was that the results of the biomass yield differentiated from our expectations, which changed the importance of having collected tissue samples from the farm edge. Ideally, such events should have been accounted for, by sampling a nearby unaffected rope instead.

Tissue sampling techniques

The sample preparation for exploring the within-variation in individual tissue content for kelp cultivated in IMTA systems should have been done in a manner facilitating calculation of actual mean content per individual. This study has characterized the large internal variation in tissue content, but as shown in Figure 2.3 several sections of the lamina were not included in the analyses. For future studies, it is advisable to design sectioning methods to include all areas

of the lamina to enable comparisons between the mean content between sections, as well as calculating the individual mean. Such sectioning may also be implemented to aid the combined record of body mass (FW and/or DW) and surface area (cm^2) per individual/section, as well as simplify the comparison of these type of results with other studies.

Another issue with the tissue sample preparation was during defrosting, as a lot of liquid was excreted from the tissue and was not included in the further analysis. This could potentially inflict the measured content of the samples, as the liquid released from the kelp can contain nutrients that would normally be present in the tissue. Therefore, measured values may not be equal to actual total tissue content. However, all samples were processed in the same manner, so it is assumed that the differences recorded are still applicable, but that the real tissue content may be higher. A solution to this would have been to section all samples in the field or at arrival in Trondheim, and freezing them separately.

When comparing uptake rates between individuals and species of kelp, units are standardized either by body mass (FW or DW) or surface area (Lobban et al., 1994; Harrison and Hurd, 2001; Hurd et al., 2014), and the variation of whats being used in different studies complicate the comparability between studies. Lubsch and Timmermans (2018) suggest that surface area is the best estimate to use, as there are known to be large variation in measuring FW biomass, and seasonal and individual variation in the DW proportion (Handå et al., 2013; Schiener et al., 2015). Still, many analysing techniques are performed on a dry weight basis, so this measure will have to be incorporated in some way. For future work on species like *S. latissima* it can be important to collect data for both body mass and surface area, or put in the effort towards establishing a conversion factor to facilitate better comparison between studies choosing different parameters of reference.

Water sampling techniques

There were not collected replicate water samples during any of the field days, partly due to the work load during each trip. This is not recommended, as replicate samples would give a more certain estimates of water nutrient content.

4.4 Future work and prospects

Further work with IMTA cultivation of kelp should focus on parameters for optimization, as well as how tissue components affect potential products from the biomass. It would also be interesting to compare the internal distribution in content for kelp cultivated at the farm edge with reference individuals collected from non-IMTA wild populations. This, to evaluate whether there is higher tissue content of nitrogen and phosphorus in cultivated individuals compared to wild types, or if there is only a difference in growth. Further knowledge of determining factors for kelp growth are important to establish the best recommendations for optimal kelp IMTA.

Another aspect of optimizing IMTA is making the mentioned inclusion of organisms able to

utilize the particulate fraction of biological waste release feasible. More research and knowledge is needed to enable this, and it can be important for regarding IMTA as a better alternative for phosphorus recycling.

This study of initial uptake used nitrate, which is the source of DIN that is most common in ocean systems (Skjoldal and Saetre, 2004). Due to salmon respiration, ammonia is released in large quantities from salmon farm systems, making this an available DIN source for IMTA cultivated kelp. It is recommended to repeat the experiment of initial uptake to include ammonia, in order to investigate how the DIP uptake responds to a different source of DIN, which can be more applicable to an IMTA situation.

Chapter 5

Conclusions

Initial DIP uptake in *S. latissima* was strongly affected by the kelp's nutritional state, where phosphorus depleted individuals did not display any DIP surge uptake and performed lower uptake rates. Phosphorus saturated individuals expressed good initial DIP uptake, and had a significant linear increase in uptake with increasing concentrations of DIP.

The internal tissue distribution of nitrogen (N) and phosphorus (P) showed highest content of both nutrients close to meristematic tissue in growth phase individuals (April). This prioritization of nutrients was thought to facilitate increased biomass yield when and where nutrients were in high availability.

Results regarding variation in nutrient content at different proximity to the fish farm were non-conclusive, but suggested that nutrient content is more applicable for evaluating the nutritional state of kelp and whether other factors may be limiting. The results support former studies suggesting that IMTA kelp biomass yield is higher in closer proximity to a fish farm. Thus, biomass yield was determined as the main variable affecting the magnitude of phosphorus re-circulation and bioremediation from kelp cultivation. However, it is recommended to keep cultivation systems for kelp downstream of the fish farm, and not among salmon pens.

This thesis support the use of *S. latissima* as an IMTA candidate, and recognize IMTA with kelp as an opportunity of re-introducing phosphorus back into the value chain. This given that applicable and cost effective kelp products are made feasible. Further, it is recommended to gather more knowledge on how filter feeders can be incorporated into IMTA systems. If this is achieved, IMTA systems will express higher bioremediary potential, as well as recycling capacity for phosphorus.

References

Artsdatabanken (2006), 'Artsdatabankens faktaark: Sukkertare (*Saccharina latissima*)', www.artsdatabanken.no. nr. 5 ISSN 1504-9140.

Baklien, T. and Steinset, T. (2018), 'Akvakultur: år, endelige tall (2017)', Statistisk sentralbyrå (SSB). Accessed May 5th at https://www.ssb.no/fiskeoppdrett.

BarentsWatch (2018), 'Nedlasting av fiskehelsedata', Accessed on March 27^{th} at https://www.barentswatch.no/nedlasting/fishhealth/lice?locality=31959.

Barrington, K., Chopin, T., Robinson, S. et al. (2009), 'Integrated multi-trophic aquaculture (imta) in marine temperate waters', *Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper* **529**, 7–46.

Black, W. (1948), 'The seasonal variation in chemical constitution of some of the sub-littoral seaweeds common to scotland. part iii. *Laminaria saccharina* and *Saccorhiza bulbosa*', *Journal of the Society of Chemical Industry* **67**(5), 172–176.

Black, W. (1954), 'Concentration gradients and their significance in *Laminaria saccharina* (l.) lamour.', *Journal of the Marine Biological Association of the United Kingdom* **33**(1), 49–60.

Black, W. á. (1950), 'The seasonal variation in weight and chemical composition of the common british *Laminariaceae*', *Journal of the Marine Biological Association of the United Kingdom* **29**(1), 45–72.

Bolton, J. and Lüning, K. (1982), 'Optimal growth and maximal survival temperatures of atlantic *Laminaria* species (*Phaeophyta*) in culture', *Marine Biology* **66**(1), 89–94.

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I. et al. (2010), 'Aquaculture: global status and trends', *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**(1554), 2897–2912.

Breen, O. (1990), 'Oseanografi', *Gyldendal norsk forlag, Oslo* p. 179.

Broch, O. J., Ellingsen, I. H., Forbord, S., Wang, X., Volent, Z., Alver, M. O., Handå, A., Andresen, K., Slagstad, D., Reitan, K. I. et al. (2013), 'Modelling the cultivation and biore-

mediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in norway', *Aquaculture Environment Interactions* **4**(2), 187–206.

Brundtland, G. H. (1987), 'Our common future', *World Commission for Environment and Development* .

Buschmann, A. H., Troell, M. and Kautsky, N. (2001), 'Integrated algal farming: a review', *Cahiers de Biologie marine* **42**(1), 83–90.

Chapman, A., Markham, J. and Lüning, K. (1978), 'Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina (Phaeophyta)* in culture 1, 2', *Journal of phycology* **14**(2), 195–198.

Chopin, T., Buschmann, A. H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G. P., Zertuche-González, J. A., Yarish, C. and Neefus, C. (2001), 'Integrating seaweeds into marine aquaculture systems: a key toward sustainability', *Journal of Phycology* **37**(6), 975–986.

Chopin, T. et al. (2006), 'Integrated multi-trophic aquaculture'.

Cisse, L. and Mrabet, T. (2004), 'World phosphate production: Overview and prospects', *Phosphorus Research Bulletin* **15**, 21–25.

Cordell, D., Drangert, J.-O. and White, S. (2009), 'The story of phosphorus: Global food security and food for thought', *Global Environmental Change* **19**(2), 292–305.

Cordell, D., Rosemarin, A., Schröder, J. J. and Smit, A. L. (2011), 'Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options', *Chemosphere* **84**(6), 747–758.

Cosgrove, D. J. (1977), Microbial transformations in the phosphorus cycle, *in* 'Advances in microbial ecology', Springer, pp. 95–134.

Dahlen, V. R. (2018), Characterization of the initial ammonium uptake in *Saccharina latissima* in an imta system, Master's thesis, Norwegian University of Science and Technology, Trondheim.

Desmidt, E., Ghyselbrecht, K., Zhang, Y., Pinoy, L., der Bruggen, B. V., Verstraete, W., Rabaey, K. and Meesschaert, B. (2015), 'Global phosphorus scarcity and full-scale p-recovery techniques: a review', *Critical Reviews in Environmental Science and Technology* **45**(4), 336–384.

Druehl, L. D. (1967), 'Distribution of two species of *Laminaria* as related to some environmental factors1', *Journal of Phycology* **3**(2), 103–108.
URL: *https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1529-8817.1967.tb04641.x*

Erga, S. R., Haugen, S. B., Bratbak, G., Egge, J. K., Heldal, M., Mork, K. A. and Norland, S. (2017), 'Seasonal variations in c: N: Si: Ca: P: Mg: S: K: Fe relationships of seston from norwegian coastal water: Impact of extreme offshore forcing during winter-spring 2010', *Marine chemistry* **196**, 1–12.

Etter, S. A., Andresen, K., Øystein Leiknes, Wang, X. and Olsen, Y. (2016), Miljødokumentasjon trøndelag - utslipp og vurdering av miljøvirkninger av næringssalter tilført fra oppdrett i trøndelag regionen i 2014, Technical report, Institutt for biologi, Norges teknisk-naturvitenskapelige universitet (NTNU), Trondhjem biologiske stasjon. NTNU internrapport.

FAO (2002), The state of world fisheries and aquaculture (sofia) 2002, Technical report, Food and Agricultural Organization of the United Nations, Fisheries Department, Rome.

FAO (2017), The future of food and agriculture - trends and challenges, Technical report, Food and Agricultural Organization of the United Nations, Rome.

FAO (2018*a*), '2018 the state of world fisheries and aquaculture (sofia)', Food and Agricultural Organization of the United Nations [Online]. Accessed on May 7th on http://www.fao.org/state-of-fisheries-aquaculture.

FAO (2018*b*), The state of world fisheries and aquaculture (sofia) 2018 - meeting the sustainable development goals, Technical report, Food and Agricultural Organization of the United Nations, Rome.

FAO, I. (2014), 'Building a common vision for sustainable food and agriculture', *Principles and approaches, The High Level Panel of Experts on Food Security and Nutrition June, Report P* pp. 1–47.

Folke, C., Kautsky, N. and Troell, M. (1994), 'The costs of eutrophication from salmon farming: implications for policy', *Journal of environmental management* **40**(2), 173–182.

Forbord, S., Steinhovden, K. B., Rød, K. K., Handå, A. and Skjermo, J. (2018), 'Cultivation protocol for *Saccharina latissima*', *Protocols for Macroalgae Research, 1st Edn, eds B. Charrier, T. Wichard, and CRK Reddy (Boca Raton, FL* pp. 37–59.

Førde, H., Forbord, S., Handå, A., Fossberg, J., Arff, J., Johnsen, G. and Reitan, K. I. (2016), 'Development of bryozoan fouling on cultivated kelp (*Saccharina latissima*) in norway', *Journal of applied phycology* **28**(2), 1225–1234.

Fossberg, J., Forbord, S., Broch, O. J., Malzahn, A. M., Jansen, H., Handå, A., Førde, H., Bergvik, M., Fleddum, A. L. and Skjermo, J. (2018), 'The potential for upscaling kelp (*Saccharina latissima*) cultivation in salmon-driven integrated multi-trophic aquaculture (imta)', *Frontiers in Marine Science* **5**.

Fretwell, K. (2016), 'Sugar kelp, sugar wrack kelp - *Saccharina latissima*, formerly *Laminaria saccharina*', Biodiversity of the Central Coast. Accessed on March 28th at https://www.centralcoastbiodiversity.org/sugar-kelp-bull-saccharina-latissima.html.

Friedlander, M. and Dawes, C. J. (1985), 'In situ uptake kinetics of ammonium and phosphate and chemical composition of the red seaweed *GRACILARIA TIKVAHIAE* 1', *Journal of phycology* **21**(3), 448–453.

Fujita, R. M. (1985), 'The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae', *Journal of Experimental Marine Biology and Ecology* **92**(2-3), 283–301.

Gordillo, F. J. (2012), Environment and algal nutrition, *in* 'Seaweed biology', Springer, pp. 67–86.

Gordillo, F. J., Aguilera, J. and Jiménez, C. (2006), 'The response of nutrient assimilation and biochemical composition of arctic seaweeds to a nutrient input in summer', *Journal of Experimental Botany* **57**(11), 2661–2671.

Grasshoff, K., Kremling, K., Ehrhardt, M. and Anderson, L. G. (1999), *Methods of Seawater Analysis*, 3 edn, Wiley-VCG, Weinheim. completely rev. and extended ed.

Guillard, R. R. (1975), Culture of phytoplankton for feeding marine invertebrates, *in* 'Culture of marine invertebrate animals', Springer, pp. 29–60.

Handå, A., Forbord, S., Wang, X., Broch, O. J., Dahle, S. W., Størseth, T. R., Reitan, K. I., Olsen, Y. and Skjermo, J. (2013), 'Seasonal-and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in norway', *Aquaculture* **414**, 191–201.

Harrison, P. J. and Hurd, C. L. (2001), 'Nutrient physiology of seaweeds: application of concepts to aquaculture', *Cahiers de biologie marine* **42**(1-2), 71–82.

Holdt, S. L. and Edwards, M. D. (2014), 'Cost-effective imta: a comparison of the production efficiencies of mussels and seaweed', *Journal of applied phycology* **26**(2), 933–945.

Hongve, D. and Kjensmo, J. (2018), 'Eutrofiering', Store Norske Leksikon. Accessed May 7th 2019 at https://snl.no/eutrofiering.

Howarth, R. W. (1988), 'Nutrient limitation of net primary production in marine ecosystems', *Annual review of ecology and systematics* **19**(1), 89–110.

Howarth, R. W. and Marino, R. (2006), 'Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades', *Limnology and Oceanography* **51**(1part2), 364–376.

Hurd, C. L., Harrison, P. J., Bischof, K. and Lobban, C. S. (2014), *Seaweed Ecology and Physiology*, 2 edn, Cambridge University Press, Cambridge. DOI: 10.1017/CBO9781139192637.

Indergaard, M. (2010), 'Tang og tare-i hovedsak norske brunalger: Forekomster, forskning og anvendelse'.

Jahnke, R. A. (1992), 14 the phosphorus cycle, *in* S. S. Butcher, R. J. Charlson, G. H. Orians and G. V. Wolfe, eds, 'Global Biogeochemical Cycles', 3 edn, Vol. 50, Elsevier, The address of the publisher, chapter 14, pp. 301–315. In the International Geophysics series.

Jansen, H. M., Broch, O. J., Bannister, R., Cranford, P., Handå, A., Husa, V., Jiang, Z., Strohmeier, T. and Strand, Ø. (2018), 'Spatio-temporal dynamics in the dissolved nutrient waste plume from norwegian salmon cage aquaculture', *Aquaculture Environment Interactions* **10**, 385–399.

Jasinski, S. (2015), Mineral yearbook: Phosphate rock, Technical report, U.S. Geological Survey. Advance release at http://www.usgs.gov/centers/nmic/phosphate-rock-statistics-and-information.

Jasinski, S. (2019), Mineral commodity summaries: Phosphate rock, Technical report, U.S. Geological Survey. http://www.usgs.gov/centers/nmic/phosphate-rock-statistics-and-information.

Karsten, U. (2012), Seaweed acclimation to salinity and desiccation stress, *in* 'Seaweed biology', Springer, pp. 87–107.

Kerrison, P. D., Stanley, M. S., Edwards, M. D., Black, K. D. and Hughes, A. D. (2015), 'The cultivation of european kelp for bioenergy: site and species selection', *Biomass and bioenergy* **80**, 229–242.

Klebert, P., Lader, P., Gansel, L. and Oppedal, F. (2013), 'Hydrodynamic interactions on net panel and aquaculture fish cages: A review', *Ocean Engineering* **58**, 260–274.

Koroleff, F. (1976), Determination of total phosphorus, *in* K. Grasshoff, ed., 'Methods of Seawater Analysis', Verlag Chemie, Weinheim, Denmark, pp. 123–125.

Lerman, A., Mackenzie, F. and Garrels, R. (1975), 'Modeling of geochemical cycles: Phosphorus as an example', *Geol. Soc. Am. Mem* **142**, 205–218.

Lobban, C. S., Harrison, P. J. and Harrison, P. J. (1994), *Seaweed ecology and physiology*, Cambridge University Press.

Lorentsen, S.-H., Sjøtun, K. and Grémillet, D. (2010), 'Multi-trophic consequences of kelp harvest', *Biological Conservation* **143**(9), 2054–2062.

Lubsch, A. and Timmermans, K. (2018), 'Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding n: P dynamics in *Ulva lactuca* (*Chlorophyta*)', *Journal of phycology* **54**(2), 215–223.

Marinho, G. S., Holdt, S. L., Birkeland, M. J. and Angelidaki, I. (2015), 'Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in danish waters', *Journal of applied phycology* **27**(5), 1963–1973.

Marquez, G. P. B., Santiañez, W. J. E., Trono Jr., G. C., de la Rama, S. R. B., Takeuchi, H. and Hasegawa, T. (2015), Seaweeds: a sustainabile fuel source, *in* 'Seaweed Sustainability', Elsevier, chapter 16, pp. 421–458.

McHugh, D. J. et al. (2003), 'A guide to the seaweed industry'.

Norwegian Directory of Fisheries (2018), Statistikk for akvakultur 2017 - foreløpige tall, Technical report, Norwegian Directory of Fisheries, Bergen, Norway. Conducted by the department of statistics.

Norwegian Directory of Fisheries (2019), 'Alger', Norwegian Directory of Fisheries [Online]. Accessed on May 13th at https://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Alger.

NS-EN ISO 6878 (2004), Vannundersøkelse - bestemmelse av fosfor - spektrometrisk metode med ammoniummolybdat, Technical Report 1, Standard Norge. Alternative title: Water quality - Determination of phosphorus - Ammonium molybdate spectrometric method, ICS 13.060.50 - Undersøkelse av kjemikaler i vann.

NS4745 (1991), Vannundersøkelse - bestemmelse av summen av nitritt- og nitrat-nitrogen, Technical Report 2, Standard Norge. Alternative title: Water analysis - Determination of the sum of nitrite nitrogen and nitrate nitrogen, ICS 13.060.50 - Undersøkelse av kjemikaler i vann.

NS9410 (2016), Miljøovervåking av bunnpåvirkning fra marine akvakulturanlegg, Technical report, Standard Norge. Alternative title: Environmental monitoring of benthic impact from marine fish farms, ICS 13.020.40;65.150.

Olafsen, T., Winther, U., Olsen, Y. and Skjermo, J. (2012), Verdiskaping basert på produktive hav i 2050, Technical report, Det Kongelige Norske Videnskabers Selskab (DKNVS) and Norges Tekniske Vitenskapsakademi (NTVA). [Norwegian].

Olsen, Y. (2011), 'Resources for fish feed in future mariculture', *Aquaculture Environment Interactions* **1**(3), 187–200.

Olsen, Y., Reinertsen, H., Sommer, U. and Vadstein, O. (2014), 'Responses of biological and chemical components in north east atlantic coastal water to experimental nitrogen and phosphorus addition–a full scale ecosystem study and its relevance for management', *Science of the Total Environment* **473**, 262–274.

Parliament message nr. 16 (2014-2015), 'Forutsigbar og miljømessig bærekraftig vekst i norsk lakse- og ørretoppdrett'. p.15.

Pedersen, M. F., Borum, J. and Fotel, F. L. (2010), 'Phosphorus dynamics and limitation of fast- and slow-growing temperate seaweeds in oslofjord, norway', *Marine Ecology Progress Series* **399**, 103–115.

Reid, G., Chopin, T., Robinson, S., Azevedo, P., Quinton, M. and Belyea, E. (2013), 'Weight ratios of the kelps, *Alaria esculenta* and *Saccharina latissima*, required to sequester dissolved inorganic nutrients and supply oxygen for atlantic salmon, *Salmo salar*, in integrated multi-trophic aquaculture systems', *Aquaculture* **408**, 34–46.

Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S., Page, F., Reid, G., Szemerda, M., Sewuster, J. et al. (2007), 'Integrated multi- trophic aquaculture (imta): A potential strategic choice for farmers', *Aquaculture Economics & Management* **11**(1), 99–110.

Rosenberg, G. and Ramus, J. (1984), 'Uptake of inorganic nitrogen and seaweed surface area: volume ratios', *Aquatic Botany* **19**(1-2), 65–72.

Rueness, J. (1998), *Alger I farger. En felthåndbok om kystens makroalger*, Almater Forlag.

Ruttenberg, K. C. (2005), Biogeochemistry: The global phosphorus cycle, *in* W. H. Schlesinger, ed., 'Treatise on Geochemistry', Vol. 8, Elsevier, Pergamon, Oxford, chapter 13, pp. 201–213.

Ryther, J. H. and Dunstan, W. M. (1971), 'Nitrogen, phosphorus, and eutrophication in the coastal marine environment', *Science* **171**(3975), 1008–1013.

Sakshaug, E. (1994), *Økosystem barentshavet*, Universitetsforl.

Sanderson, J., Dring, M., Davidson, K. and Kelly, M. (2012), 'Culture, yield and bioremediation potential of *Palmaria palmata* (linnaeus) weber & mohr and *Saccharina latissima* (linnaeus) ce lane, c. mayes, druehl & gw saunders adjacent to fish farm cages in northwest scotland', *Aquaculture* **354**, 128–135.

Schiener, P., Black, K. D., Stanley, M. S. and Green, D. H. (2015), 'The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*', *Journal of Applied Phycology* **27**(1), 363–373.

Skjermo, J., Aasen, I. M., Arff, J., Broch, O. J., Carvajal, A. K., Christie, H. C., Forbord, S., Olsen, Y., Reitan, K. I., Rustad, T. et al. (2014), 'A new norwegian bioeconomy based on cultivation and processing of seaweeds: Opportunities and r&d needs'.

Skjoldal, H. R. and Saetre, R. (2004), *The Norwegian sea ecosystem*, Akademika Pub.

Steen, I. (1998), 'Phosphorus availability in the 21st century: management of a non-renewable resource', *Phosphorus Potassium* **217**, 25–31.

Stévant, P., Rebours, C. and Chapman, A. (2017), 'Seaweed aquaculture in norway: recent industrial developments and future perspectives', *Aquaculture International* **25**(4), 1373–1390.

Sverdrup, H. U., Johnson, M. W., Fleming, R. H. et al. (1942), *The Oceans: Their physics, chemistry, and general biology*, Vol. 7, Prentice-Hall New York.

Tiwari, B. K. and Troy, D. J. (2015), Seaweed sustainability–food and nonfood applications, *in* 'Seaweed Sustainability', Elsevier, chapter 1, pp. 1–6.

Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A., Kautsky, N. and Yarish, C. (2003), 'Integrated mariculture: asking the right questions', *Aquaculture* **226**(1-4), 69–90.

UN (2017), World population prospects: The 2017 revision, Technical report, United Nations, Department of Economic and Social Affairs, Population Division. https://www.un.org/development/desa/publications/world-population-prospects-the-2017-revision.html.

Wang, X., Andresen, K., Handå, A., Jensen, B., Reitan, K. I. and Olsen, Y. (2013), 'Chemical composition and release rate of waste discharge from an atlantic salmon farm with an evaluation of imta feasibility', *Aquaculture environment interactions* **4**(2), 147–162.

Wang, X., Broch, O. J., Forbord, S., Handå, A., Skjermo, J., Reitan, K. I., Vadstein, O. and Olsen, Y. (2014), 'Assimilation of inorganic nutrients from salmon (*Salmo salar*) farming by the macroalgae (*Saccharina latissima*) in an exposed coastal environment: implications for integrated multi-trophic aquaculture', *Journal of applied phycology* **26**(4), 1869–1878.

Wang, X. X., Olsen, L. M., Reitan, K. I. and Olsen, Y. (2012), 'Discharge of nutrient wastes from salmon farms: Environmental effects, and potential for integrated multi-trophic aquaculture', *Aquaculture Environment Interactions* **2**(3), 267–283.

Westheimer, F. H. (1987), 'Why nature chose phosphates', *Science* **235**(4793), 1173–1178.

Wheeler, H. J. and Hartwell, B. L. (1893), *Sea-weeds: Their Agricultural Value, and the Chemical Composition of Certain Species*, Agricultural Experiment Station of the Rhode Island College of Agriculture ....

Wiencke, C. and Bischof, K. (2012), 'Seaweed biology', *Ecological studies* **219**.

Appendix A

Appendix for Laboratory study of initial uptake

A.1 More detailed plot of DIN uptake in PS kelp

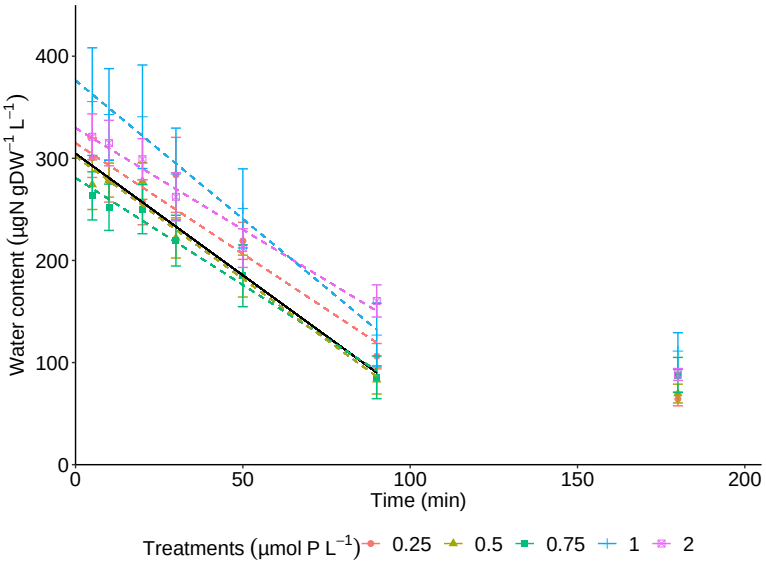
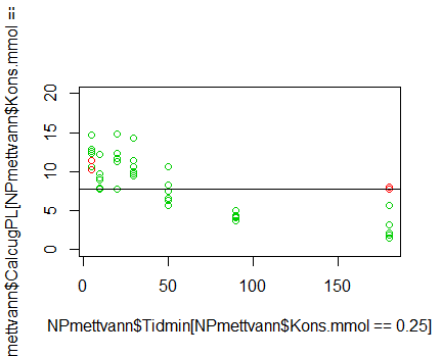
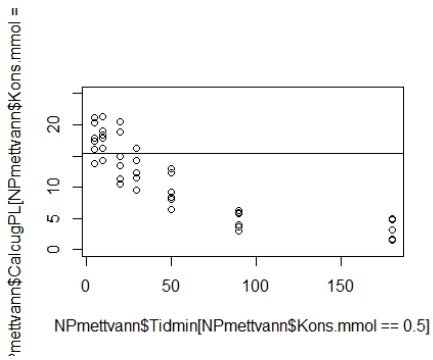


Figure A.1: Mean±SE phosphate water content and rates of uptake for saturated [other scale] *S. latissima*

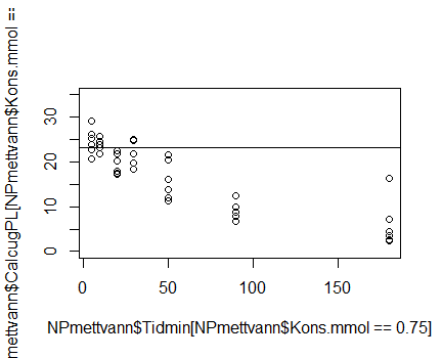
A.2 Visual inspection of water content in the PS group experiment



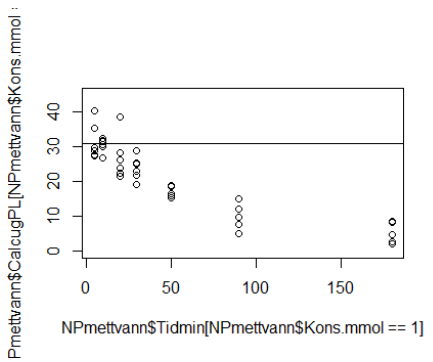
(a) DIP water content treatment 0.25 µmol·L^{-1} in PS group



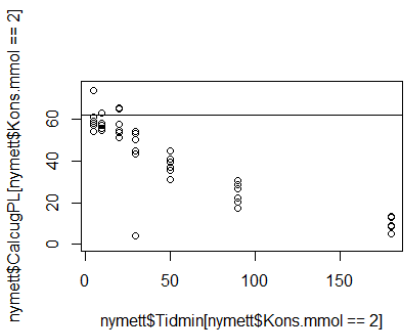
(b) DIP water content treatment 0.5 µmol·L^{-1} in PS group



(c) DIP water content treatment 0.75 µmol·L^{-1} in PS group



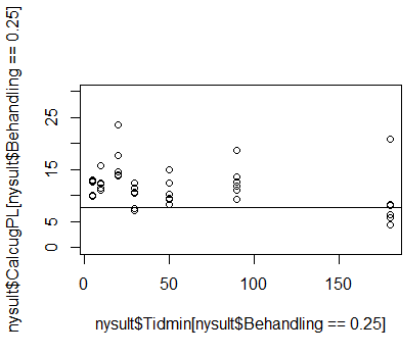
(d) DIP water content treatment 1.0 µmol·L^{-1} in PS group



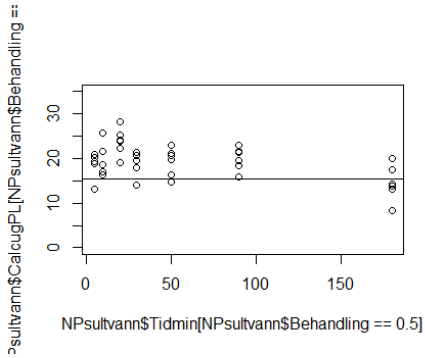
(e) DIP water content treatment 2.0 µmol·L^{-1} in PS group

Figure A.2: Measured water content of DIP for all treatments in the PS group. Expected water content in each treatment at the start is indicated by a horizontal line.

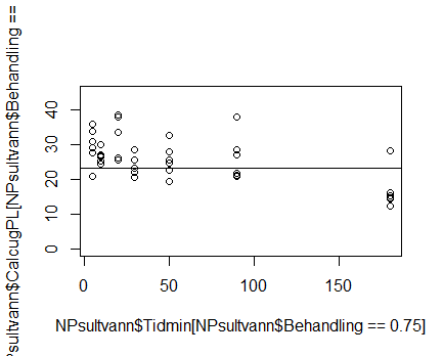
A.3 Visual inspection of water content in the PD group experiment



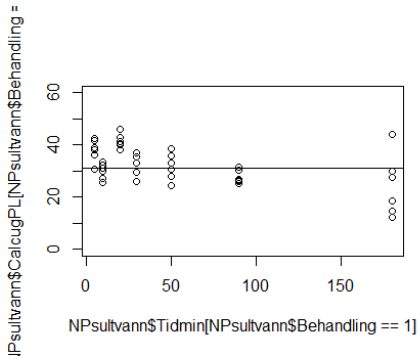
(a) DIP water content treatment 0.25 µmol·L^{-1} in PD group



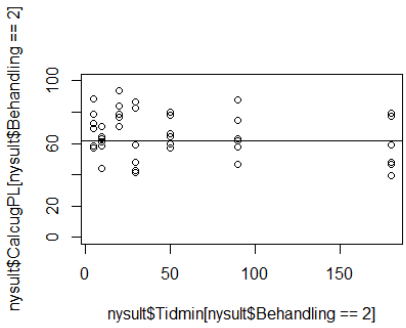
(b) DIP water content treatment 0.5 µmol·L^{-1} in PD group



(c) DIP water content treatment 0.75 µmol·L^{-1} in PD group



(d) DIP water content treatment 1.0 µmol·L^{-1} in PD group



(e) DIP water content treatment 2.0 µmol·L^{-1} in PD group

Figure A.3: Measured water content of DIP for all treatments in the PD group. Expected water content in each treatment at the start is indicated by a horizontal line.

A.4 Residual distributions for linear models estimating DIP and DIN removal rates in the PS and PD group

Table A.1: Residual distribution of models

Model	Residuals				
	min	1Q	median	3Q	max
PS_{DIP}	-32	-5	0	5	43
PD_{DIP} MPD_{180b}	-62	-7	-1	7	80
PD_{DIP} MPD_{180a}	-64	-7	-1	7	78
PS_{DIN}	-192	-40	5	37	190
PD_{DIN}	-228	-45	-15	42	392
PS_{90min}	10.42±1.52**	19.48±2.15***	21.14±2.24***	33.27±5.8***	52.42±6.65***
PD_{180min} -inter r2=0.88	6.31±1.13***	6.31±1.13***	6.31±1.13***	6.31±1.13***	6.31±1.13***
PD_{180min} p.treat	2.42±1.15*	3.14±1.06**	7.88±1.77***	9.04±2.25***	8.18±5.01
R2 per treat	0.08	0.16	0.31	0.27	0.04
PD_{180min} +inter r2=0.89	2.41±2.46	3.14±3.47	7.88±3.47**	9.04±3.47***	9.63±3.64***

A.5 Model estimates per treatment in the PD group

Table A.2: Uptake rates±SE of DIP in phosphorus depleted (PD) *S. latissima* estimated slopes for each separate treatment for 180 minutes. Significance level of slope expressed as *(p<0.05).

Treatments	Uptake rate ($\mu g \cdot g_{DW}^{-1} L^{-1} hour^{-1}$) per treatment				
µmolP L^{-1}	0.25	0.5	0.75	1.0	2.0
µgP L^{-1}	7.75	15.5	23.25	31	62
PD_{180min} p.treat	2.42±1.15*	3.14±1.06*	7.88±1.77*	9.04±2.25*	8.18±5.01
R2 per treat	0.08	0.16	0.31	0.27	0.04

A.6 Mean tissue content in the PS and PD group

Table A.3: Mean±CI(95%) tissue content ($\mu g \cdot g_{DW}^{-1}$) of carbon (C), nitrogen (N) and phosphorus (P) of nutritionally saturated and phosphorus depleted *S. latissima*

Element	Saturated		Depleted	
	Initial	Treatments	Initial	Treatments
Phosphorus	6 080±56	5 025±69	3 380±53	3 589±34
Nitrogen	34 277±523	31 737±279	28 040±75	29 254±240
Carbon	219 653±1418	217 970±1295	224 311±1195	220 838±2121

A.7 Estimated uptake rates per minute for the PS and PD groups

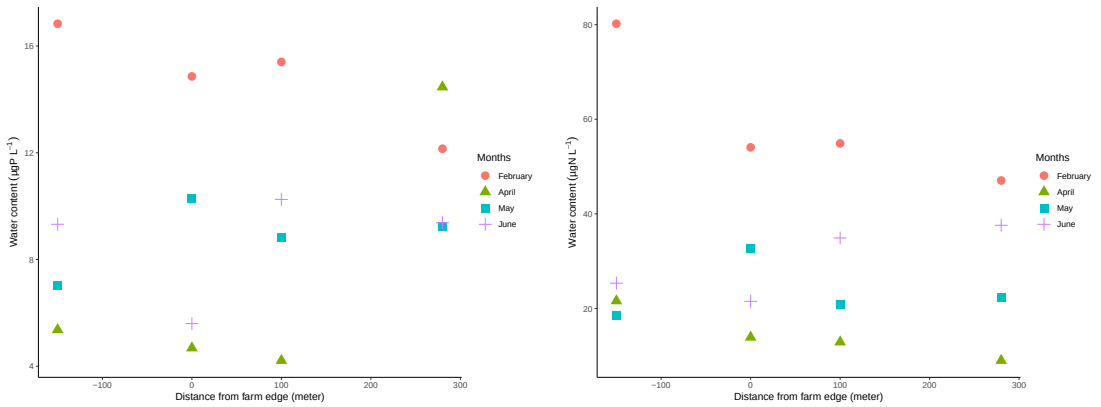
Table A.4: Uptake rates±SE of DIP ($\mu g \cdot g_{DW}^{-1} L^{-1} min^{-1}$) in phosphorus saturated (PS) and depleted (PD) *S. latissima*. Estimated slopes per treatment for PS is for 90 minutes, and for PD 180 minutes.

Treatments	Uptake rate ($\mu g \cdot g_{DW}^{-1} L^{-1} min^{-1}$) per treatment				
µmolP L^{-1}	0.25	0.5	0.75	1.0	2.0
µgP L^{-1}	7.75	15.5	23.25	31	62
PS	0.17±0.03	0.32±0.04	0.35±0.04	0.55±0.10	0.87±0.11
PD	0.04±0.02	0.05±0.02	0.13±0.03	0.15±0.04	0.14±0.04

Appendix B

Appendix for Field study of IMTA cultivation of *S. latissima*

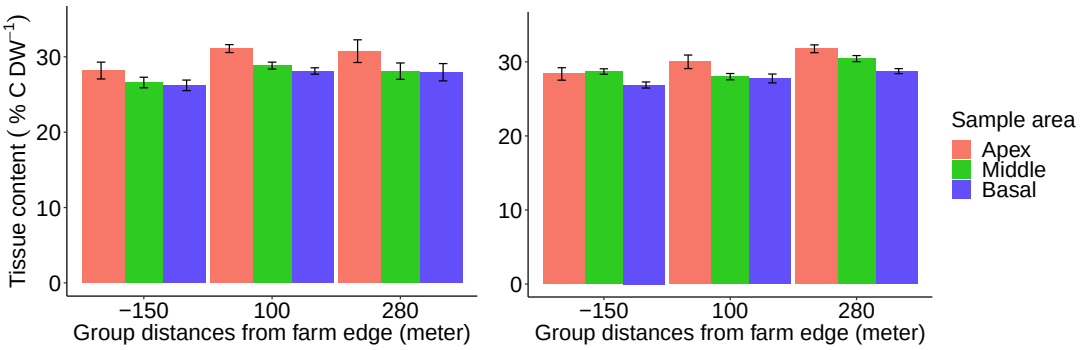
B.1 Water content of DIN and DIP from field samples



(a) Phosphate **(b)** Nitrate/nitrite

Figure B.1: Measured phosphate and nitrite water content in different distances from salmon farm

B.2 Carbon content from field samples



(a) April **(b)** June

Figure B.2: Variation and distribution in carbon tissue content in *S. latissima*, given as mean±$CI_{95\%}$ for different sample areas of the lamina and at different distances from the fish farm edge.

B.3 Results of nested two-way ANOVA, with variance estimates

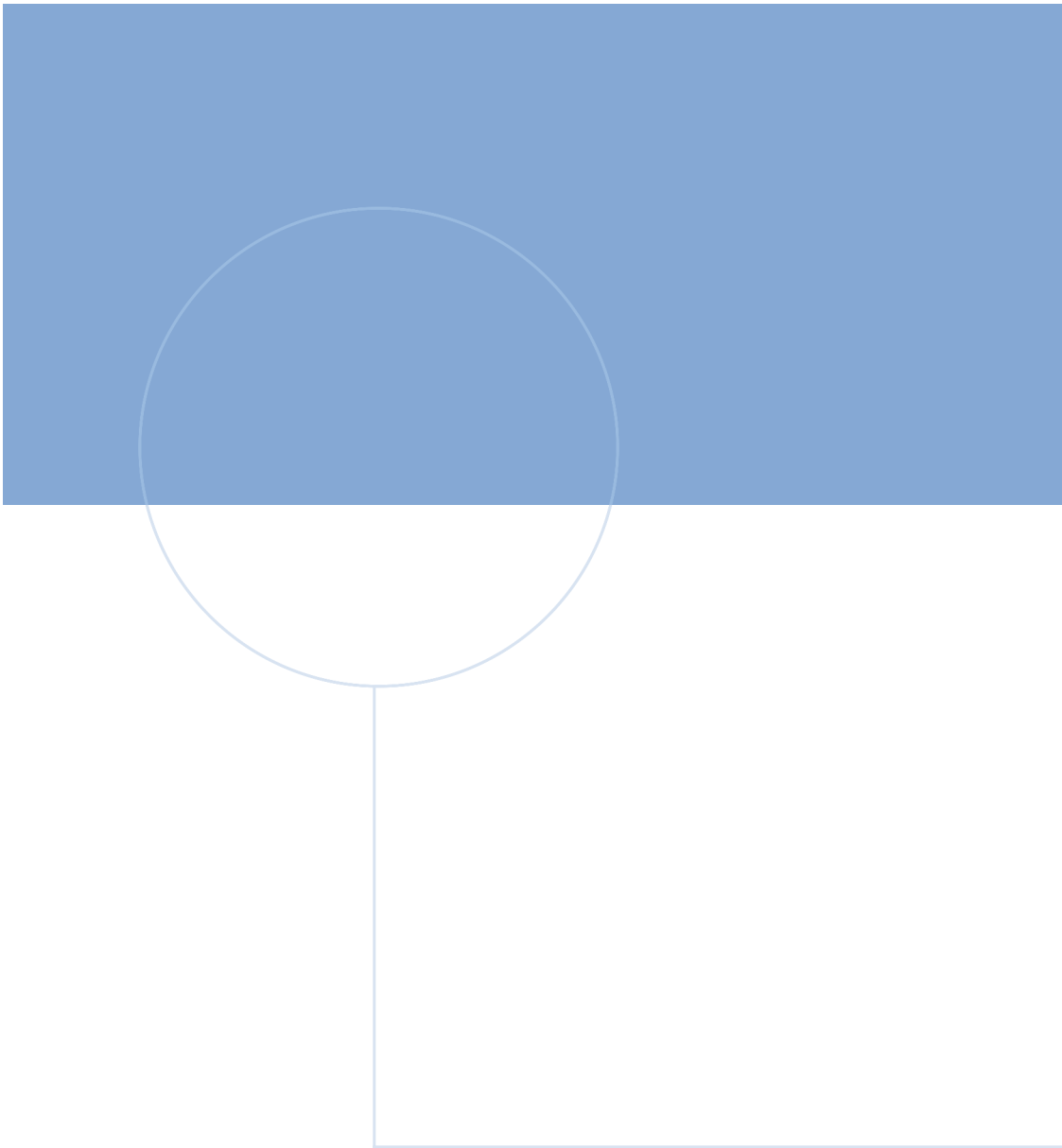
Table B.1: Portion of explanatory variables for phosphorus tissue content as % of variance

	April		June	
	Variance	proportion (%)	Variance	proportion (%)
Distance from farm (m)	93140	15.6	72861	22.5
Sample area	432477	72.7	171683	53.1
Sample area:individual	22349	3.8	36327	11.2
Individual	68308	11.5	41874	12.9
Residuals	1259	0.2	712	0.2

B.4 Residual distributions for models looking at differences in tissue content along the IMTA gradient

Table B.2: Residual distribution of models

Element	Month	Residuals				
	min	1Q	median	3Q	max	
P	April	-1.63197	-0.40631	-0.02634	0.44233	1.49251
P	June	-2.2547	-0.3959	0.0275	0.3620	2.4143
N	April	-2.19357	-0.39041	-0.00917	0.33547	2.60621
N	June	-1.74530	-0.49280	-0.00925	0.53327	1.78483
C	April	-1.83894	-0.42119	-0.01078	0.39448	2.23194
C	June	-2.43746	-0.39460	0.06021	0.31127	2.40117



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