Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

Sebastian Gjertsen

Production and long-term bioavailability of organic carbon from cultivated *Saccharina latissima*

Master's thesis in Ocean Resources
Supervisor: Murat V. Ardelan
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Acknowledgement

I would like to express my sincere gratitude to my supervisors, Murat V. Ardelan and Luiza Neves for their invaluable support, guidance, and insightful feedback throughout the course of this study. Their expertise and commitment played a crucial role in shaping this study and refining the methodologies. In particular, I would like to extend special appreciation to Luiza Neves for her exceptional work ethic, dedication, and collaborative approach in overcoming (a lot of) obstacles together.

I would also like to express my gratitude to NTNU and SINTEF for funding sources that provided the necessary resources for this study.

On a more personal note, I am beyond grateful for all the love from my family and their support. I would also like to extend my thanks to Raissa Kehrle, Nathalie Gjertsen and Adrian Hansø for their endorsements and assistance with proofreading and providing valuable perspectives on the work. Their attention to detail and fresh insights greatly contributed to the clarity and quality of this study. In addition to this,

I am truly grateful for the collaborative efforts and contributions of all those involved, as they have significantly enriched this research project.

June 2023, Trondheim

Sebastian Gjertsen

Abstract

As one of the most important primary producers in the ocean, seaweed releases significant amounts of organic carbon during growth and decomposition. However, little is known about the relationship between the biological and chemical processes during the fixating of carbon with seaweed, both in temporal and spatial scales. By increasing the knowledge on these processes, farmers could position their farms in the most favourable conditions to increase carbon sequestration possibilities, on top of economic opportunities already brought through products from the harvested biomass.

This study investigates the production and bioavailability of particulate organic carbon (POC) released from *S. latissima* in two temporal scales: (1) Through a short-term (4 days) mesocosm production experiment, light availability was used to examine how POC impacts the adjacent ecosystem. (2) Through a long-term (188 days) incubation experiment, different temperatures (4°C and 10°C) were used to examine how bioavailable the released POC was on day 0, 5, 10, 15, 30, 60, 94 158 and 188. POC production for light and dark treatments was 303-1384 µg C (g^{-1} DW d^{-1}), and 310-799 µg C (g^{-1} DW d^{-1}), respectively, and revealed strong correlations between the uptake of dissolved organic carbon (DIC), the increase of pH, and the increase of dissolved oxygen (DO). After 188 days of biodegradation, 5.2% of released POC from production experiment were recalcitrant POC (RPOC) in 10°C and 6.5% in 4°C.

The findings support *S. latissima's* importance in contributing organic carbon to the surrounding ecosystem, playing an important part in the biological carbon pump, and acting as a buffer against ocean acidification. Nonetheless, significant knowledge gaps need to be addressed with standardized methods to better understand the contributions of cultivated seaweeds to the recalcitrant carbon pool, and its role in CO_2 sequestration.

Sammendrag

Som en av de mest betydningsfulle primærprodusentene i havet, frigjør tang og tare betydelige mengder organisk karbon under vekst og nedbrytning. Likevel er det lite kunnskap om forholdet mellom de biologiske og kjemiske prosessene under fiksering av karbon med tang og tare. Ved å øke kunnskapen om disse prosessene kan tareoppdrettere optimalisere plasseringen av lokalitetene sine. På den måten vil man få økt mulighetene for karbonbinding, i tillegg øke de økonomiske mulighetene som oppstår gjennom høstet biomasse.

Denne studien undersøker produksjonen og nedbrytningen av partikulært organisk karbon frigjort fra *S. latissima* i to ulike tidsperspektiv: (1) Gjennom et kortvarig (4 dager) mesokosmisk produksjonseksperiment ble tilgjengeligheten av sollys brukt til å undersøke hvordan POC påvirker det nærliggende økosystemet. (2) Gjennom et langvarig (188 dager) inkubasjonseksperiment ble ulike temperaturer (4°C og 10°C) brukt til å undersøke hvor nedbrytbart det frigjorte partikulære organiske karbonet var på dag 0, 5, 10, 15, 30, 60, 94, 158 og 188. Produksjonen av partikulært organisk karbon for lys- og mørkebehandlinger var henholdsvis 303-1384 µg Karbon (g^{-1} tørrvekt d^{-1}) og 310-799 µg Karbon (g^{-1} tørrvekt d^{-1}) og viste sterke korrelasjoner mellom opptaket av oppløst uorganisk karbon, økningen av pH og økningen av oppløst oksygen. Det langvarige nedbrytingseksperimentet viste etter 188 dager at 5.2% av det frigjorte partikulære organiske karbonet fra produksjonseksperimentet var motstandsdyktig ved 10 °C og 6.5% ved 4 °C.

Disse funnene støtter tang og tares betydning når det gjelder å bidra med organisk karbon til det nærliggende økosystemet. I tillegg viser det dens viktige rolle i den biologiske karbonpumpen, samtidig som funnene understreker hvor viktig tang og tare er som en buffer mot havforsuring. Likevel er det betydelige kunnskapshull innenfor feltet, som må utforskes med standardiserte metoder for å bedre forstå deres rolle i CO_2-binding.

Table of Contents

Abbreviations

C	Carbon
CA	Carbonic anhydrase
CCS	Carbon Capture and storage
CDR	Carbon dioxide removal
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate
DIC	Dissolved organic carbon
DO	Dissolved Oxygen
DOC	Dissolved organic carbon
DW	Dry weight
FW	Fresh weight
GHG	Greenhouse gas concentrations
HCl	Hydrochloric acid
HCO_3-	Bicarbonate
L	Liter
LDOC	Labile dissolved organic carbon
LPOC	Labile particulate organic carbon
M	Molar
NPP	Net primary productivity
OM	Organic matter
P g C	Petagram Carbon
POC	Particulate organic carbon
POM/DOM	Particulate/Dissolved organic matter
RDOC	Recalcitrant dissolved organic carbon
RPOC	Recalcitrant particulate organic carbon
SGR	Specific growth rate
SLDOC	Semi-labile dissolved organic carbon
SRDOC	Semi-recalcitrant dissolved organic carbon
T g C	Teragram Carbon
TA	Total Alkalinity
TOC	Total organic carbon
WW	Wet weight

1. Introduction

1.1 Seaweed utilization

There are traces of seaweed (*Proterocladus antiquus*) dating back approximately 1 billion years to the Northern China (Tang et al., 2020), and since the stone age, us humans have been using it for our benefits. We have been harvesting it for domestic purposes, such as food, feed, and insulation (Dillehay et al., 2008; Ainis et al., 2014; Erlandson et al., 2015). In modern time, it has been used for industrial purposes in medicines, gels, cosmetics, bioplastics and fertilizers. With an increasing human population, expected around 9.8 billion people within 2050 (UN, 2023), we might need to start utilizing the seaweed even more. Seaweed is now providing a range of economic prospects while replacing products with higher carbon dioxide (CO_2) emissions (Duarte et al., 2017). Seeing how the population on earth is developing, in combination with our food resources, utilizing the lower trophic levels is required. Comparing the marine and terrestrial trophic levels, we eat two levels higher in the marine, than on land (Duarte et al., 2009). This might be one of the reasons why only 2% of the global food supply is coming from the ocean (Schubel & Thompson, 2019). In parallel to the increasing population, global warming and rising atmospheric greenhouse gas concentrations (GHG) is intensifying. Therefore, a new exploitation-purpose of the seaweed has gotten considerable attention, specifically carbon capture (Buschmann et al., 2017; Sondak et al., 2017; Duarte, Bruhn, et al., 2022).

1.2 Ecosystem services

Along with salt marshes, seagrass, and mangroves (and all other photoautotrophic organisms), seaweed use sunlight, carbon dioxide and water to grow, and in the process, oxygen is produced. Except for seaweed, these coastal ecosystems has been evaluated as functional carbon capturers, due to their own development of organic-rich sediments where sequestration takes place (Macreadie et al., 2019), whereas most seaweed do not have roots, but settle on hard strata with their holdfast (Duarte et al., 2013). Seaweed have therefore been overlooked and assumed to have limited capacity to sequester carbon, but recent studies shows seaweed exceeding all of the coastal ecosystems combined, globally sequestering 173 Tg C yr^{-1} (with a range of 61-268 Tg C yr^{-1}) (Duarte et al., 2013; Krause-Jensen & Duarte, 2016). Duarte, Gattuso, et al. (2022) used models to illustrate that natural macroalgal forests covers an area of 6.06 – 7.22 million km^2, which is comparable both in size and net primary production (NPP) to the Amazon Forest.

They also conclude that the polar regions are one of the areas where the potential of carbon capture is highest.

Norway's natural kelp forests has since the 70's struggled with sea urchins and their grazing on vegetation, removing around 2000 km^2 of kelp forest (Norderhaug & Christie, 2009). But recent studies show a promising comeback. Recovery of the kelp can be explained by several elements, but climate change could be the biggest factor (Christie et al., 2019). Increased temperatures leads to higher invasion of sea urchin's predators, resulting in a healthier ecosystem, both in the north and mid Norway (Norderhaug & Christie, 2013; Krause-Jensen et al., 2020). Considering Norway's potential in seaweed aquaculture, further rising temperatures are not something to be desired for, as extinction of cold water species may become a reality within the 21st century (Raybaud et al., 2013).

1.3 Seaweed aquaculture

Cultivation of seaweed is a fast-growing sector, which has been existing for thousands of years in the Eastern world (Pérez-Lloréns et al., 2020), and now the Western world is trying to catch up. From 2000 until 2019, the global seaweed production increased from 10 595 600 tons to 35 077 600 tons wet weight (WW), growing at about 6.5% $year^{-1}$, with Asia accounting for approximately 97% of it (FAO, 2022). Norway started experimental cultivations and small-scale trials of kelp (Laminariales) over a decade ago, after also having successful trials internationally (Stévant et al., 2017; Forbord, 2020), and has since then grown to be a strong section within Norwegian aquaculture research and production.

In Norway, the main cultivated species are *Saccharina latissima* (Sugar kelp), *Laminaria digitata* (Oarweed/Finger kelp) and *Alaria esculenta* (Winged kelp/Wakame) (Norwegian Directorate of Fisheries, 2023). *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl, et G. W. Saunders (Lane et al., 2006), is a cold water brown algae, distributed on the northern hemisphere. It grows naturally at different depths, ranging from the intertidal zone to as far down as the photic zone (Kain, 1979; Bolton et al., 1983; Steneck et al., 2002). Due to cold and relatively stable temperatures along Norwegian coast, the kelp grows fast and thrives optimally between 10-17°C. This is one of the reasons why up to half of its world's distribution is found exactly here (Moy et al., 2006), and also why the kelp makes a good specie for cultivation in Norway, ranging between an annual harvest of 75-170 tons WW per hectare (Broch et al., 2013).

On a global scale, seaweed aquaculture occupies approximately 0.0004% of the ocean surface (Duarte et al., 2017). However, if the industry would utilise its full potential, seaweed aquaculture could potentially cover 13% of the ocean surface (ca. 48 million km^2) (Froehlich et al., 2019), which could theoretically sequester 72 billion tonnes of CO_2 (Ross et al., 2022). The Norwegian aquaculture industry, on the other hand, has the potential to produce 20 million tons of kelp in 2050 (Olafsen et al., 2012), which would correspond to an annual capture of 4 million tons of CO_2 (Broch et al., 2019). In 2021, the Norwegian seaweed industry produced approximately 250 tons (Norwegian Directorate of Fisheries, 2023), meaning significant upscale of the industry is needed to make any meaningful contributions towards carbon capture or sequestration.

Reid et al. (2013) showed through experiments with *Alaria esculenta* and *S. latissima,* their potential in carbon and nutrient uptake within an integrated multi-trophic aquaculture (IMTA) site. Their findings suggested that *A. esculenta* had approximately twice (1.8 times) the carbon sequestration capacity per WW than *S. latissima*, but the latter specie grows denser (1.5 times) than *A. esculenta*, meaning uptakes are almost balanced out (1.2 times) based on their spatial basis. However, *S. latissima* is one of the preferred species for cultivation in Europe due to ease of production, including controlling its life cycle in the laboratory for induction of sorus and year-round supply for seedling. Despite having considerably high levels of iodine (670-10 000 mg kg^{-1} DW) (Duinker et al., 2020), this can be overcome with blanching of the seaweed, reducing iodine by over 90% (Nielsen et al., 2020; Krook et al., 2023). *A. esculenta* is also popular for human consumption and feed, without the drawbacks of high iodine content, while also having an easier life cycle to control than many other species. A natural step towards increasing the production of these species comes through research and development projects.

In a report from United Nations Environment Programme (2009), the term “Blue Carbon” was established, and has since been used simultaneously with seaweed and carbon capture. Today there are several big projects (Table 1), working towards removing CO_2 using seaweed. Each project has different strategies for production, but one thing they all have in common is carbon capture. Their main purpose is to remove CO_2, and most will either sink it to the ocean floor, letting it naturally degrade and sequester carbon via biochemical processes, or turn harvested biomass into biochar/bio-coal. It is possible that other CDR (Carbon dioxide removal) products are developed in the coming years.

Table 1: Companies/Projects working on carbon capture using seaweed.

Company/Project	Details	Progress	Location
Carbon Kapture	Will turn harvested kelp into biochar (Carbon Kapture, 2023).	2023-	North Donegal, Ireland
Climate Foundation	Marine permaculture: Floating, open-ocean kelp ecosystems, which pumps nutrient rich water from 100m-450m depth up to surface, for seaweed and ecosystem to utilize. Harvest for biofuel, fertilizer, livestock feed, or sink (Climate Foundation, 2023).	Founded in 2007, but is currently in an upscaling trial for offshore production	Nevada, USA
Kelp Blue	Giant underwater farms growing seaweed, harvested for agriculture, pharmaceuticals and textiles (Kelp Blue, 2023).	In a pilot phase for offshore farming within 2029.	Alaska, Namibia, New Zealand
Pull to Refresh	Semi-autonomous surface vessels (ASVs) will tow a wire, which giant kelp bladder will grow on. Through tubes in the wire, the vessel will feed micronutrients to the kelp, which will fall off and sink (Pull To Refresh, 2023).	Not started	California, USA
Running Tide	Evaluating the carbon removal potential of kelp through naturally sinking it using degradable carbon buoys (Running Tide, 2023).	Research and development phase	Portland, USA
Seaweed Carbon Solutions	A joint industry project (JIP) by SINTEF, DNV, Equinor and Lundin Energy. Offshore farming of seaweed, and look into utilization of Bio-coal and storage in the ocean floor (SINTEF, 2023).	Research and development, 2021-2024	Frohavet, Norway
The Southern Ocean Carbon Company	Growing seaweed in the Southern Tasmania to combat climate change, using harvest for biochar, and replacing plastic packaging (The Southern Ocean Carbon Company, 2023).	Research and development, recently acquired by ADEC Innovations	Tasmania, Australia

Before scaling up and commercializing carbon capture with seaweeds, considerable knowledge gaps needs to be addressed (Troell et al., 2022), especially when it comes to actively sinking the seaweed towards the deep oceans (Jones et al., 2022). Mineralization of organic matter (OM) requires cooperation between several functional groups of microbiotas, usually in anoxic conditions due to high metabolic rates. Scientific data involving carbon mineralization and storage is generally lacking on a spatial and temporal scale. Studies have shown that temperature can impact the sulphate reducing bacteria which dominate anoxic conditions, while microorganisms performing hydrolysis and fermentation of organic carbon were not as sensitive to these changes (Weston & Joye, 2005). Accumulation of seaweed is also a factor, as most of it might not even incorporate into the sediments (Hardison et al., 2010), while also changing the underlying environment and communities living there (Brunet et al., 2021).

1.4 Carbon capture

Capturing carbon with seaweed is not a ground-breaking technology that was developed recently, but a natural wonder occurring for 1000 million years, through photosynthesis. The fixation of carbon starts with the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo, EC 4.1.1.39). Through a process called carboxylation, the enzyme catalyses the first steps of photosynthetic carbon reduction and photorespiratory carbon oxidation cycles by merging CO_2 and O_2 together with ribulose-1,5-bisphosphate (RuBP) (Mizohata et al., 2002). Despite this, RuBisCo exhibits low CO_2 compatibility and in general a slow carboxylation turnover rate (k_{cat}^c).

In the ocean, dissolved inorganic carbon (DIC) is available as CO_2 and the ionic forms of DIC, bicarbonate (HCO_3-) and carbonate (CO_3^2-). At a natural pH of around 8 in seawater, HCO_3- makes up about 80% of the carbon species (Figure 1) (Stumm & Morgan, 2012; Pinet, 2019), but while CO_2 can diffuse without any trouble through biological membranes (Korb et al., 1997), other forms of DIC can't without the help of specific carriers (Gutknecht et al., 1977). Therefore, most marine macroalgae have developed several mechanisms to prevail CO_2 constraints and specifically to better utilize the big pool of HCO_3- (Demmig-Adams & Adams Iii, 1992; Larsson & Axelsson, 1999; Giordano et al., 2005).

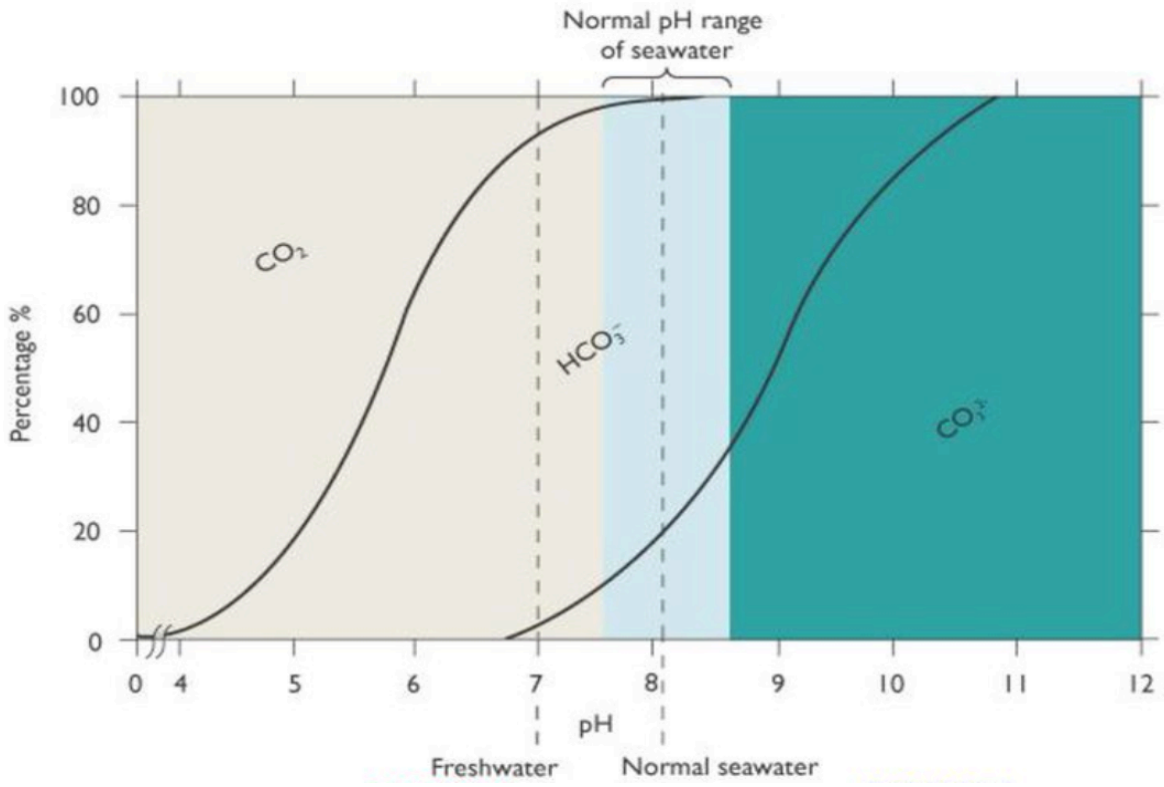

Figure 1: Distribution of inorganic carbon species in water (Pinet, 2019).

Within the cell membrane (Plasmalemma) of *S. Latissima,* utilization of HCO_3^- takes place. Another enzyme, carbonic anhydrase (CA) (EC 4.2.1.1), dehydrates HCO_3^-, converting it into CO_2 (Smith & Bidwell, 1987; Axelsson et al., 2000; Li et al., 2018). CA is essential in the process of speeding up the rate of interconversion between the inorganic carbons and is part of the process called carbon concentrating mechanism (CCM), which has been developed by a broad range of photoautotrophic organisms to aid RuBisCo in effectively fixating carbon. Badger (2003) suggest that CCMs are composed of three useful elements: (1) Influx of CO_2 and/or HCO_3^-, (2) capture of DIC inside the cell (mostly HCO_3^-) and (3) production of CO_2 from the DIC pool around RuBisCo (Mercado et al., 2006).

1.5 Organic carbon

In the ocean we find an enormous pool of OM which has different characteristics and roles in the ecosystem and at a metabolic stage. It serves its purpose as an electron and proton donor, and sometimes nitrogen and phosphorus, for organism growth. In addition, it binds metals, protects against UV-radiation, and reacts with free radicals (West et al., 1999; Morel & Price, 2003; Romera-Castillo & Jaffé, 2015; Lønborg et al., 2020). Total organic matter (TOM) is a broad term including several sub-terms shown in Figure 2. Approximately 50% of TOM is carbon by weight, in the organic form, and it separates into particulate and dissolved organic

matter (POM and DOM). It refers to the entire organic molecule, and includes hydrogen, oxygen, nitrogen and phosphorus (Thurman, 2012). Total organic carbon (TOC) is the quantity of particulate organic carbon (POC) and dissolved organic carbon (DOC), which is terms used to separate the two based on size. DOC can be defined as organic carbon particles that pass through a filter pore size 0.22-0.7 um (GF/F filters), meaning that POC is what stays on the filter (Søndergaard & Middelboe, 1995), while dissolved organic nitrogen and phosphorus (DON & DOP) are subsets that contain nitrogen and phosphorus.

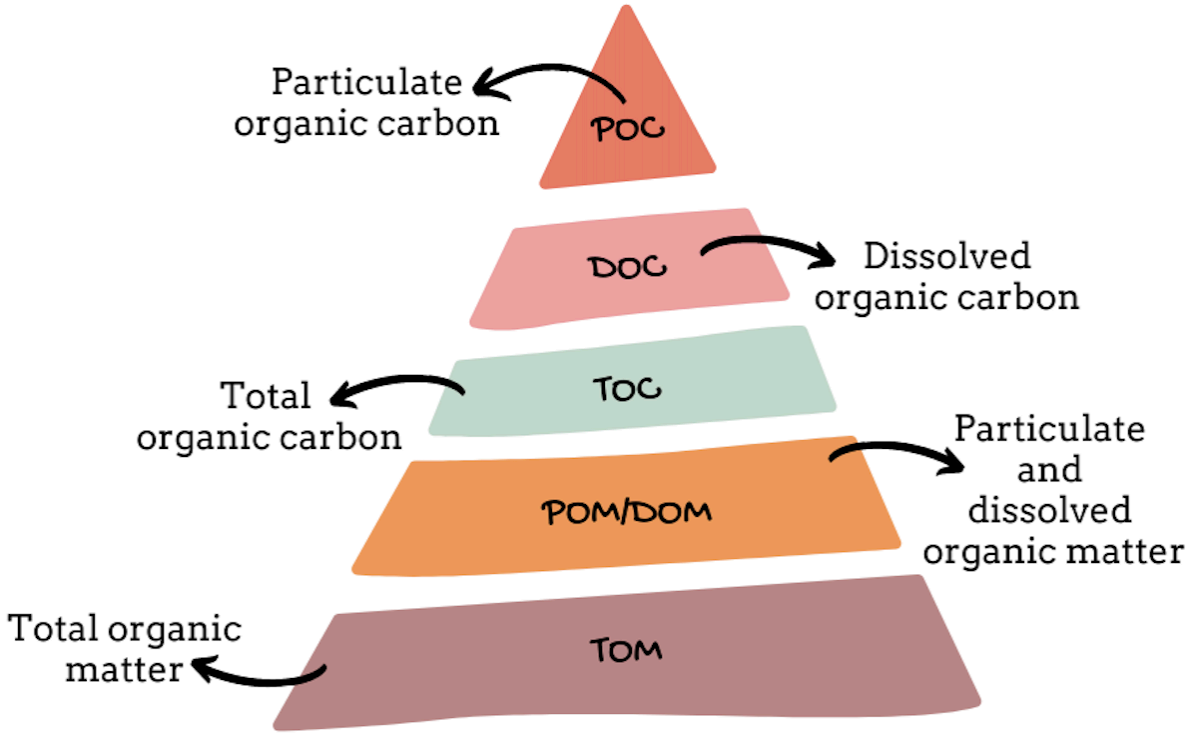

Figure 2: Simplified representation of various terms of organic matter found in the ocean. Illustration: Sebastian Gjertsen

Perdue and Benner (2009) suggest that biomass makes up less than 0.2% of all organic carbon in the ocean, implying that the majority is DOC, POC and sedimentary forms of organic carbon. Approximately 18 Pg C originate from POC, while 662 Pg C comes from DOC, making the pool of DOC ca. 37 times bigger (Menon et al., 2007; Perdue & Benner, 2009; Hansell, 2013). POC and DOC is produced autochthonously as a product of photosynthesis by phytoplankton, seagrass and seaweed in the euphotic zone (0-100m), or through chemosynthetic processes near high tectonic activity (Tunnicliffe, 1991; Carlson et al., 2002; Lønborg et al., 2020). The fixed carbon is released both passively and actively, and then transported to adjacent ecosystems (Weigel & Pfister, 2021). There it is either consumed and metabolized via secondary production and returned to the water column as DIC, or it can be transported via currents and water column

overturn to deeper waters to potentially sequester in sediments (Jiao et al., 2010; Zhang et al., 2017). Though, studies has shown that 44% of sequestered carbon (below 1000m) was reintroduced into the atmosphere after 100 years (Baker et al., 2022).

1.6 Labile vs recalcitrant carbon

The spectrum of DOC ranges from labile (LDOC), semi-labile (SLDOC), semi-refractory (SRDOC), refractory (RDOC) and ultra-refractory (URDOC) (Table 2) (Jiao et al., 2010; Hansell, 2013). Compared to LDOC (monosaccharides such as mannitol and fucose), seaweed-derived RDOC have a longer residence time in water due to their buildup of humic-like compounds, carbonates, long chain lipids, xylans, sulphated polysaccharides, and phenols, which are less bioavailable for breakdown (Abdullah & Fredriksen, 2004; Wada et al., 2008; Lechtenfeld et al., 2014; Trevathan-Tackett et al., 2015; Powers et al., 2019).

Table 2: Quantitative characteristics of major DOC fractions in the ocean (Hansell, 2013).

DOC fraction	Acronym	Lifetime (years)	Amount (Pg C)
Labile	LDOC	~0.001	< 0.2
Semi-labile	SLDOC	~1.5	6 ± 2
Semi-refractory	SRDOC	~20	14 ± 2
Refractory	RDOC	~16.000	630 ± 32

Amon and Benner (1996) explained how aquatic OM degrades over time based on a humification process (Hatcher & Spiker, 1988) and a size-reactivity model. In the early diagenesis, LDOC are rapidly utilized, while RDOC goes through a humification process. RDOC becomes more resistant due to build-up of humin, which eventually oxidates and creates a formation of humic and fulvic acids, while becoming lighter (molecular weight). The whole process leads to an escape from microbial utilization due to their now low resemblance of a biomolecule. This is also supported by studies, confirming that with decreasing molecular size, comes increasing age and resistance (Walker et al., 2016; Broek et al., 2020). Newer studies and novel analytical approaches also reveal the complexity of the organic matter-world, and shows the vast structural heterogenous pool of oceanic DOM (Seidel et al., 2022).

To date, there are mainly three ways studies address the term “refractory” DOC, but they might not be comparable or complementary, based on where in the world they are used (Baltar et al., 2021). (1) Hansell (2013) characterizes the pool based on its longevity, while (2) Jiao et al.

(2010) and Lechtenfeld et al. (2015) describes how the composition of molecular properties plays a role in prokaryotic enzymes decision in degrading it or not, and lastly (3) the dilution hypothesis; most organic carbon in the ocean are labile, but its concentration is too low for prokaryotic degradation (Jannasch, 1967, 1994; Arrieta et al., 2015). However, the explanation of what makes a compound to be labile or refractory might be more complex than what was previously stated. RDOC in one ecosystem has previously been shown to be LDOC in another (Carlson et al., 2004; Shen & Benner, 2018) and ultraviolet radiation (UV) has also the potential to transform RDOC to LDOC and conversely (Benner & Biddanda, 1998; Mopper et al., 2015). Baltar et al. (2021) suggest six impactful factors affecting DOC reactivity, made up of dilution, UV-radiation, priming, community composition, sorption/aggregation with (in)organic particles and different abiotic factors such as temperature, hydrostatic pressure, pH and O_2. This demonstrates the present knowledge gap among academia, as well as the importance of broadening research surrounding DOC while precisely defining its labels. This study has used definitions based on Hansell (2013), and includes the same terms for POC, e.g., LPOC and RPOC, which can be described as an operational definition.

1.7 Recent findings

Until recently, there were no experiments that looked at POC and DOC from *S. latissima in situ*, and most studies had been conducted on other species, but in the recent years, the interest for the commercialised kelp has increased considerably. A recently published review looking at 382 papers and their methods for monitoring carbon uptake and permanence (Rose & Hemery, 2023), showed that seven were based upon *S. latissima*, but only one looked at POC and DOC (Dolliver & O'Connor, 2022). Out of the 382 papers, seven were based on mesocosm experiments, but none of them covered *S. latissima.* There are however three papers looking at *Saccharina japonica* (same genus as *S. latissima*).

Gao et al. (2021) showed with incubation of *S. japonica* that about 46.8-51.6% of DOC was decomposed after 30 days (labile), while 37.8% of DOC remained after 150 days (refractory) of incubation, leading to high chances of carbon sequestration. Their study also showed lower decomposition rate of bioavailable dissolved organic carbon during January compared to April, and this correlates with earlier studies showing that temperature affects the decomposition rate of bioavailable dissolved organic carbon (BDOC) (Middelboe & Lundsgaard, 2003; Kirchman et al., 2005; Lønborg et al., 2009). The other study done by Li et al. (2022), showed in a long-term experiment that RDOC accounts for approximately 58% of the DOC, and about 85% of

the RDOC were steadily present throughout the whole experiment, while the remaining 15% were converted from LDOC to RDOC through bacterial metabolism. Lastly, Feng et al. (2022) showed with an incubation study more conservative values, where RDOC and RPOC accounted for 1.27% and 0.12% of the initial carbon, respectively.

Even though *S. latissima* and *S. japonica* are kindred, conclusions cannot be drawn from one to the other, but methods and data can be a helpful tool to get a broader picture of the potential the genus has. The current knowledge gap surrounding *S. latissima* and its carbon uptake and release is important to address. Not only will this help farmers to better utilize harvesting, cropping or sinking strategies, but also towards positioning of farms in the most favourable conditions. "Passive" sequestration may occur throughout the growth period at sea before harvest time, an added value contribution, which, if translated to credits or other financial incentives, could become attractive to boost seaweed farming practices. Through mesocosm experiments, where the natural environment is examined under controlled conditions, carbon uptake and degradation can be an important first step in resolving some of the remaining questions. For seaweed it is highly relevant and easily reproducible, and in addition a broad spectre of sample metrics can be conducted (Rose & Hemery, 2023).

1.8 Objectives

The MSc project is part of the project "Seaweed Cultivation as a Climate Positive Solution" by PhD candidate, Luiza Neves. The objective of the study is to perform mesocosm studies to examine how organic carbon released from cultivated *Saccharina latissima* impacts its adjacent ecosystem in short (4 days) and long-term (188 days) perspectives.

Secondary objectives include:

- How does light availability (transparent and dark bags) impact growth rates of *S. latissima,* release of POC, uptake of DIC and abiotic factors
- How temporal changes affect the degradation of particulate organic carbon
- How temperature impacts the degradation of particulate organic carbon
- How determination of POC and DOC further could be developed

2 Material and Methods

2.1 Study sites

SINTEF Ocean seaweed research site:

SINTEF Ocean has a seaweed research site located near Skarvøya, Hitra (63.650430, 8.649162) (Figure 3). The farm is strategically placed in a sheltered area, protected from waves and strong currents, but the location allows natural replacement of water masses coming straight from the north Atlantic. The farm is approximately 100m x 50m and contains of five longlines, from which lines seeded with *S. latissima* were deployed in January 2022, and where 21 individual kelps were collected from in May 2022 for purposes of this study.

Trondheim Biological Station:

The Trondheim Biological Station (TBS) (63.441020, 10.348289) (Figure 3) research facilities are strategically placed on the west side of Trondheim city, not far from other campuses. It has 600m2 of wet lab space and 800 m2 of well-equipped standard laboratories and supply these labs with sea water from pumped in from 80-100m depth. Four of the rooms can be temperature-controlled for long-term temperature-experiments. Just outside the main building, a large outdoor basin (approximately 10m x 20m) is placed, ideal for mesocosm-experiments.

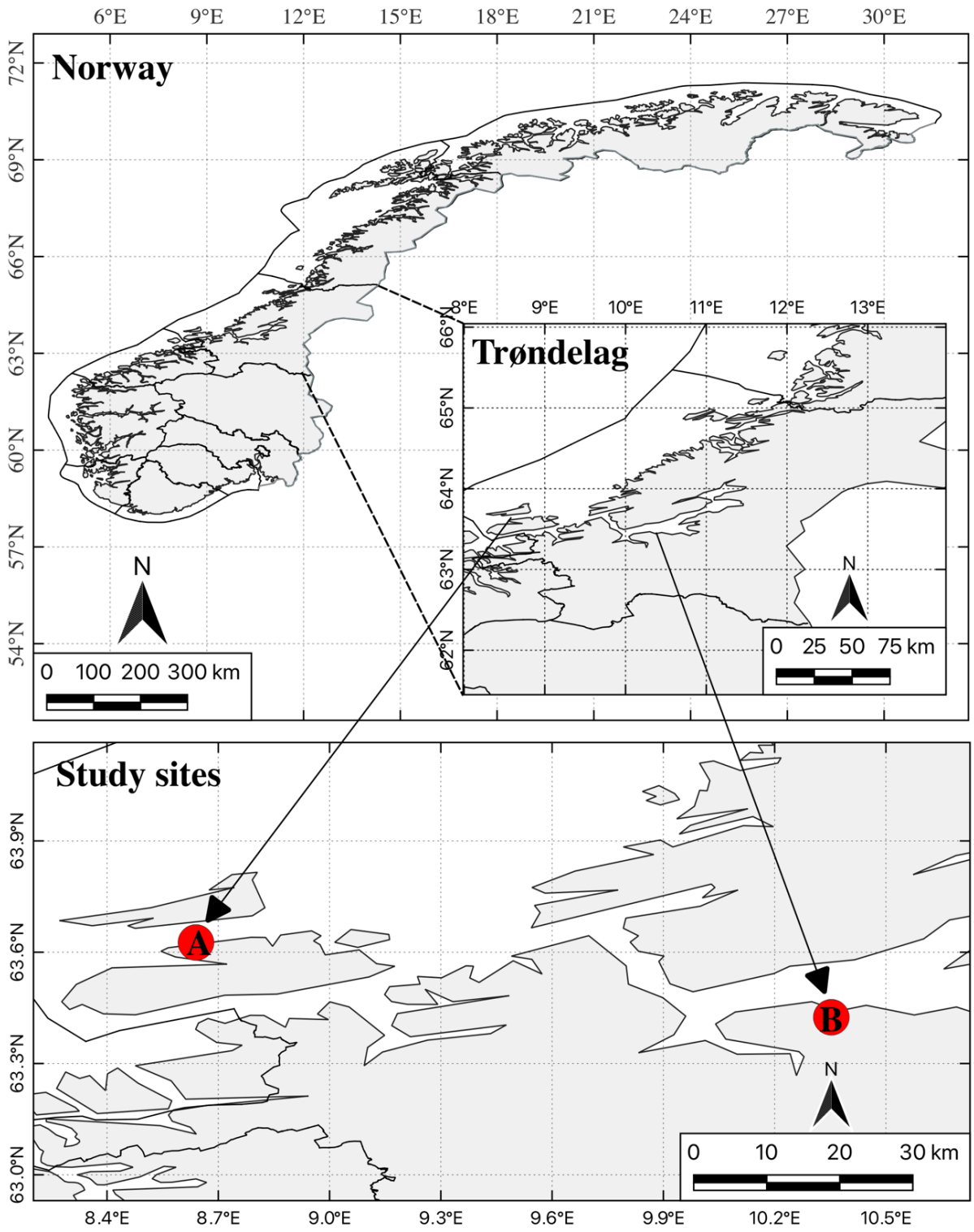

Figure 3: Map of the study sites. (A) SINTEF Ocean Seaweed farm located near Skarvøya, Hitra. (B) Trondheim Biological Station located near Trondheim City. Illustration: Sebastian Gjertsen with QGIS.

2.2 Quality assurance and safety considerations

"A Chemistry Student approached the Master and asked, Master, how do I achieve enlightenment in Chemistry? The Master replied, Wash your glassware." (McCormick, 2006, para. 1).

All equipment used during the experiment (Appendix A) was thoroughly cleaned before, during and after the experiment on all sampling days. All glassware were either combusted at 450°C and/or acid washed and/or machine washed to remove organic materials that might interfere with our results. All non-glassware was only acid and/or machine washed.

Before the experiment and sampling days, equipment was disassembled, and machine washed to remove organic materials. It was then acid washed in hydrochloric acid (37%) (HCl) diluted down to 1M using MQ-water and thereafter rinsed in MQ-water right before usage to ensure cleanliness. All equipment was air-dried before used.

Glass vials (20ml and 40ml) used for storing and analyzing water samples were wrapped into aluminum foil without their caps and then combusted at 450°C for 8 hours in a combustion oven. The caps were placed in a 1M HCl bath for 2 hours, then rinsed thoroughly with MQ-water. The caps were then placed in a semi-closed aluminum foiled container and transferred to a drying machine set to 60°C for 2h, or until they were completely dry. After drying, they were screwed back on the combusted glass vials and stored in aluminum foil inside a sealed container until they were used.

Each of the experiment bags were first washed with a semi-pressure hose to loosen eventual particles stuck during previous storage, and then checked for leakage. They were then washed with MQ-water, acid-washed to remove organic materials with 1M HCl, and lastly again washed with MQ-water.

When working with methods describing acid above, necessary safety measurements were taken each time. The work was always planned and described before started. Laboratory coat, safety goggles, face-shield, chemical-resistant gloves, chemical-resistant boots, and a chemical-resistant apron were used whenever working with acids. Work was done in a fume hood if the task allowed it.

2.3 Experimental preparations

2.3.1 Harvesting and transportation

21 individual plants of *S. latissima* were collected from longlines at SINTEF Ocean's seaweed research site near Skarvøya, Hitra. It was collected by hand and chosen based on size and absence of biofouling. All plants where in similar shape and size. The plants were put into moisturized plastic bags and stored in a cooled (by seawater) and enclosed styrofoam container. It was then transported to Trondheim Biological station by car for approximately 2 hours, then immediately put into an acclimatization tank.

2.3.2 Acclimatization

A pre-cleaned glass tank was used to store and acclimatize the plants for 24 hours before the experiment started. The tank was filled with 105 L of seawater pumped from 80m depth (inlet water), filtered through a plankton mesh (200 µm) to remove large phytoplankton and debris. Plants were allowed to acclimatize to the new water conditions, and kept under constant temperature in a room set to 10°C. An artificial light source with a timer was put over the acclimatization tank during the night, so photosynthesis could continue as close as possible to normal daylight conditions at sea. In addition, air bubbling was added to circulate the water. With the known water volume and number of plants we could determine the amount of carbon released during this period of acclimatization, which may have resulted due to transport stress.

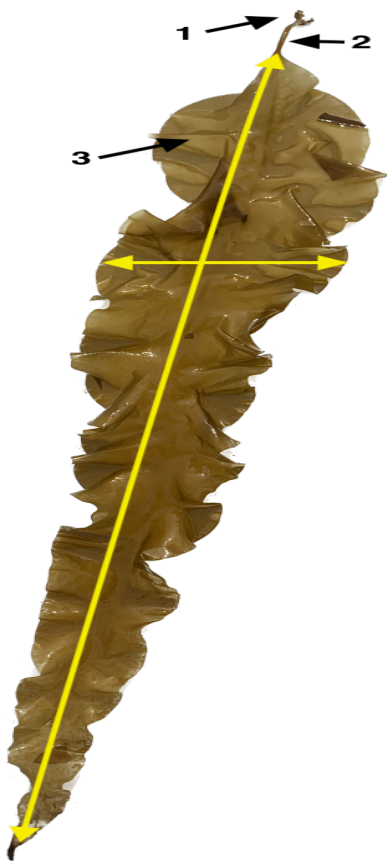

Figure 4: Saccharina latissima measurement spots (in yellow) and location of haptera (1), stem (2) and lamina (3). Illustration: Sebastian Gjertsen

2.3.3 Measurement, weighting, and basin setup

Each plant (N=21) was measured in length (from bottom of stem to tip of the frond) and width (widest part) using a measuring band as shown in Figure 4. The plants were then given a specific label (B1-6, C1-6, D1-6, E1-3) which would follow through the whole experiment. Each of the plants were wet weighed (WW) using a Mettler Toledo weighing scale, calibrated before use. A string was tied to the stem of the plant to allow retrieval from the bag at the time of sampling, and the frond was carefully rolled to enter the mouth of the experiment bags.

The experiment bags were made of polyethylene and could contain 21L. They were rinsed three times with seawater filtrated through a plankton mesh (200 µm) and then filled up to the neck of the bag with filtrated seawater. They were then labelled, and the plants were placed inside of their respectively labelled experiment bag. When the plant was inside of the bag, the string was kept in place between the mouth of the bag and the screw cap. The string was used to easily pull the plant out at a later stage. Each bag (N=42) was topped off with filtered seawater and put into order along a line tied to both ends of the basin.

2.3.4 Monitoring

During the whole experiment (production and long-term) different sensors and monitoring equipment were used to determine abiotic factors.

- **CTD** – Conductivity, temperature, and depth (pressure), is a tool used for determining physical properties in water. A CTD profiler was used both during harvest at the seaweed research site, and in the basin where the production experiment was conducted. The profiler was used both before and after the experiment finished to monitor changes and it was used to chart the distribution and change in temperature, salinity, and density, which can help in understanding the adjacent ecosystem.

- **Optical DO** – Optical Dissolved Oxygen sensor (ProODO) measures the dissolved oxygen in water, using an optical sensor which calculates the lifetime of luminescence, since oxygen affects it (YSI, 2009).
- **pH** – pH meter (WTW pH3110). It was used to monitor and measure hydrogen-ion activity in water, and specifying its acidity shown as pH, before, during and after the experiment was conducted (production experiment and long-term experiment).
- **Temperature** – Was measured before and during both production experiment and long-term experiment, using the pH sensor. A pt1000-sensor from a ferrybox (mounted in the basin) was also used to monitor temperature in the basin throughout the production experiment.
- **ORP** – Oxidation-Reduction Potential (Orion4-star, Thermo Electron), was measured during sampling of experiment bags. It measured the ability of a molecule to oxidize or reduce another molecule (Prasad et al., 2015).

2.4 Production experiment

The production experiment was done over a four-day period in late May, incubating *S. latissima* in individual mesocosm bags, to investigate how released organic carbon impacts the adjacent ecosystem during a short period. The experiment focused on different aspects of the carbon pool and during these days a total of approximately 700 samples were taken. Every sample was done in either duplicates or triplicates. The samples that were collected, but are not a part of this thesis, included samples for DOC extraction for characterization with FT-ICR, isotope ^{13}C and ^{15}N, inorganic nutrients (DIN, DIP), dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOP).

2.4.1 Experiment setup

42 experiment bags (21L Collapsible Polyethylene containers) as shown in Figure 5 were used to house plants and controls in the basin at TBS. 24 bags were light (Transparent), while 18 were dark (experiment bags were put inside two black plastic bags and kept shut with a cable tie). The experiment bags were tied onto a longline approximately one meter apart from each other. Weights were evenly distributed on the longline, so the line of bags would sink approximately 2m down to mimic natural conditions at the sea farm. Three longlines had 12 experiment bags each, while one longline had six experiment bags. Each day while the experiment was ongoing, one longline was taken up and sampled.

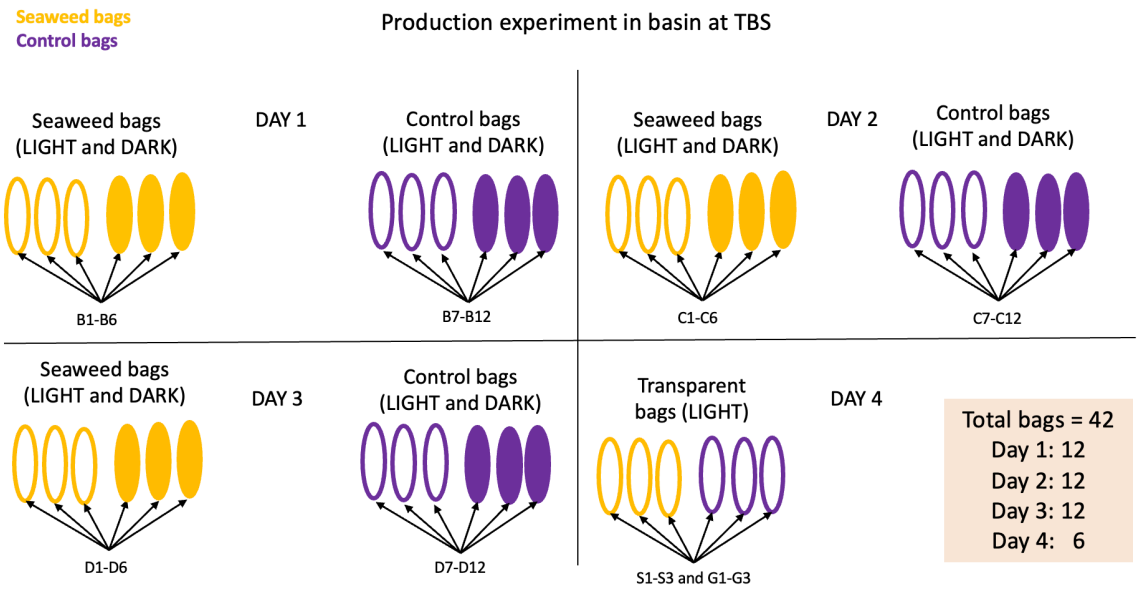

Figure 5: Production experiment in basin at TBS. Illustration: Luiza Neves and Sebastian Gjertsen

2.4.2 Sampling and filtering

Each experiment bag was transported 50m from the basin into the laboratory where sampling and measurements took place (Figure 6). Each seaweed was taken out using the string to easily grab and collect it without causing damage to lamina. The seaweed was then gently shaken for excess water to drop. Bacterial community samples were collected by swabbing the surface tissue of the lamina. The seaweed was then washed in hydrochloric solution (pH 2) for two minutes and rinsed twice for 30 seconds with MQ-water, which was important for kelp isotope sampling afterwards. The seaweed was weighed the same way as before the experiment, but it was rolled and put into an aluminum vessel to not wet the scale. The seaweed was put into a drying oven for 48-72h at 60°C. After drying, the seaweed was milled into homogenous powder, labelled, and stored for later analysis.

Immediately after collecting the seaweed out of the experiment bag, approximately 250ml was taken from the experiment bag to measure temperature (°C), Potential of hydrogen (pH), Dissolved Oxygen (DO) and Oxidation-Reduction Potential (ORP). Triplicate samples were taken from the unfiltered water for TOC-concentration. A 40ml glass vial (pre-combusted at 450°C) was rinsed three times in sample water before being filled up to the neck of the vial. All samples were acidified to pH <2 using 80 µL of 6M HCl. Each vial was systematically labelled and stored dark and in -20°C until further analyses. Duplicate samples were taken from the unfiltered water for community (Phyto- and zooplankton) analysis. A 50ml plastic VWR vial

was rinsed three times in sample water before filled up. Half of the plastic VWR vials got 0.5ml neutral lugol added, while the other half got 0.5ml acidified lugol added. Each vial was labelled and stored in -20°C until further analyses.

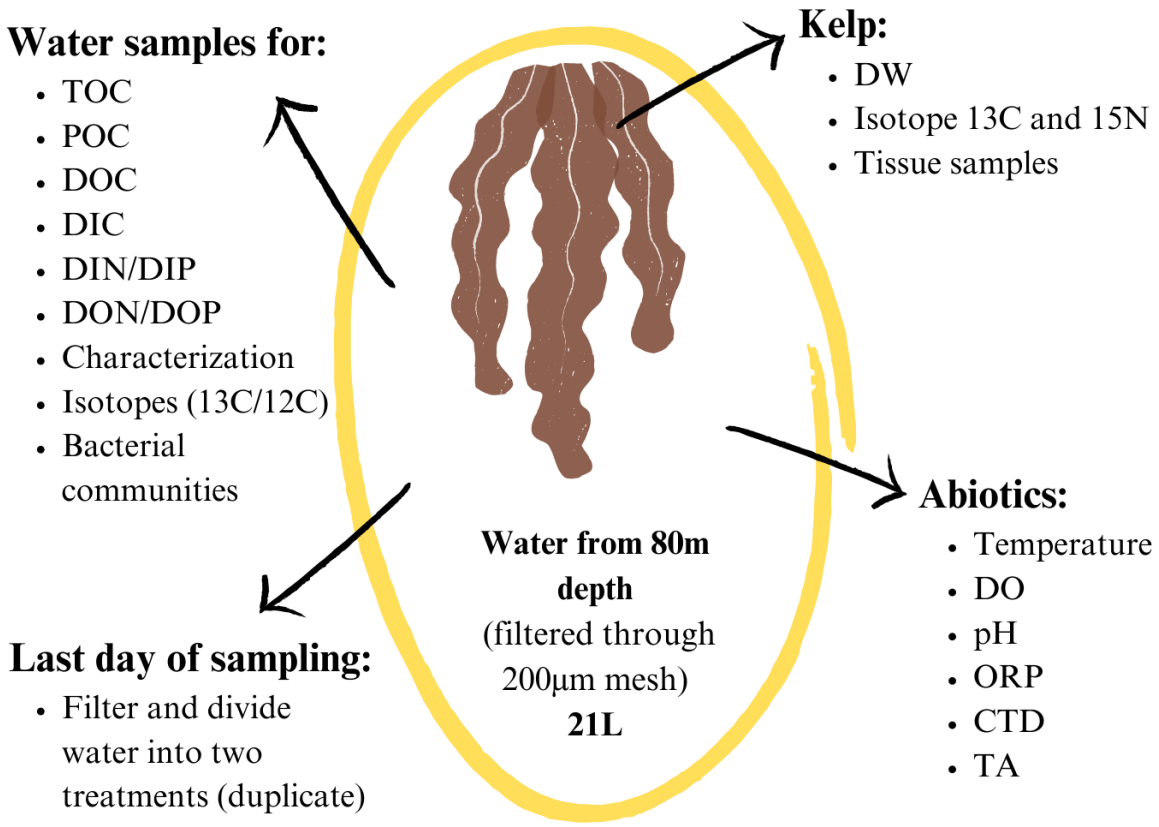

Figure 6: Schematic illustration of all samples and measurements taken from experiment bags before, during and after sampling. Illustration: Luiza Neves and Sebastian Gjertsen

3 liters of water from the experiment bags were measured using a 1000ml beaker and filtered through a filtration unit (Buchner flask) using GF/F filters (0.7 µm). All glassware had previously been acid washed, while GF/F filters had been pre-combusted at 450°C for 8 hours. After 3 liters had passed the GF/F filter, the filter was put into a petri dish, labelled, and then stored in a -20°C until further POC analyses.

Triplicate samples were taken from the filtrated water for determination of DOC-concentration. A 40ml vial (pre-combusted at 450°C) was rinsed three times in sample water before filled up to the neck of vial. All samples were acidified to pH <2 using 80 µL of 6M HCl. Each vial was systematically labelled and stored dark and in -20°C until further analyses. The remaining filtered water was used for inorganic nutrients (nitrate, nitrite, ammonia, and phosphorus) and DOC-characterization following its own methods not relevant for this study.

Another liter of water from the experiment bag was filtered through a Durapore Membrane Filter (0.22μm) to capture the bacterial community in the water. The filter was folded with acidified metal tweezers and stored in capsules for further analysis done by SINTEF Ocean.

2.4.3 Calculations

To calculate the specific growth rate (SGR), the following formula was used:

$$\mathrm{SGR}\ (\mathrm{d}^{-1}) = \ln(Wf/W0)/\mathrm{d}$$

Where W0 and Wf was the initial and final DW after sampling during the 4 different days (d) of experiment. W0 was estimated using the mean percentage of all final DW/FW results.

The net release rates (μg g^{-1} DW d^{-1}) of POC were calculated using the following equation:

$$\mathrm{R} = \Delta\mathrm{POC} \times \mathrm{V} \div \mathrm{DW} \div \mathrm{d}$$

Where ΔPOC was the difference between sample and control (μgC/L) during the days (d) of experiment, V was amount of seawater volume (L) and DW was final dry weight (g) (Wada et al., 2007; Xu et al., 2021).

2.5 Long-term biodegradation experiment

A long-term degradation experiment was conducted to monitor the changes to DOC, POC, pH and DO, coupled with changes in bacterial communities and DOC characterization (degree of RDOC) over a period of 188 days. Day 4 of the production experiment was the start (day 0) for the long-term biodegradation experiment. Sampling intervals were selected based on reported labile vs recalcitrant windows in similar studies, to allow for comparison (Wada et al., 2008; Lønborg et al., 2009; Watanabe et al., 2020; Gao et al., 2021).

2.5.1 Experiment setup

Six experiment bags (three transparent seaweed bags and three transparent control bags) from production day 4 were taken from the basin and abiotic factors were first measured: temperature, pH, DO and ORP; before being filtered and sub-divided into a total of 40 bags. Each experiment bag was divided into two different long-term treatments at different temperatures (4°C and 10°C) and each treatment had duplicate bags. The 4°C and 10°C treatment had each 12 bags used for DOC and POC concentration monitoring, four bags for DOC characterization and four bags for bacterial communities as shown in Figure 7 and Appendix A. The experiment bags were also packed into black plastic bags to keep light out

and capped to prevent air exchange. The degradation experiment was conducted in the dark over a period of 188 days, to avoid photosynthesis from planktonic cells.

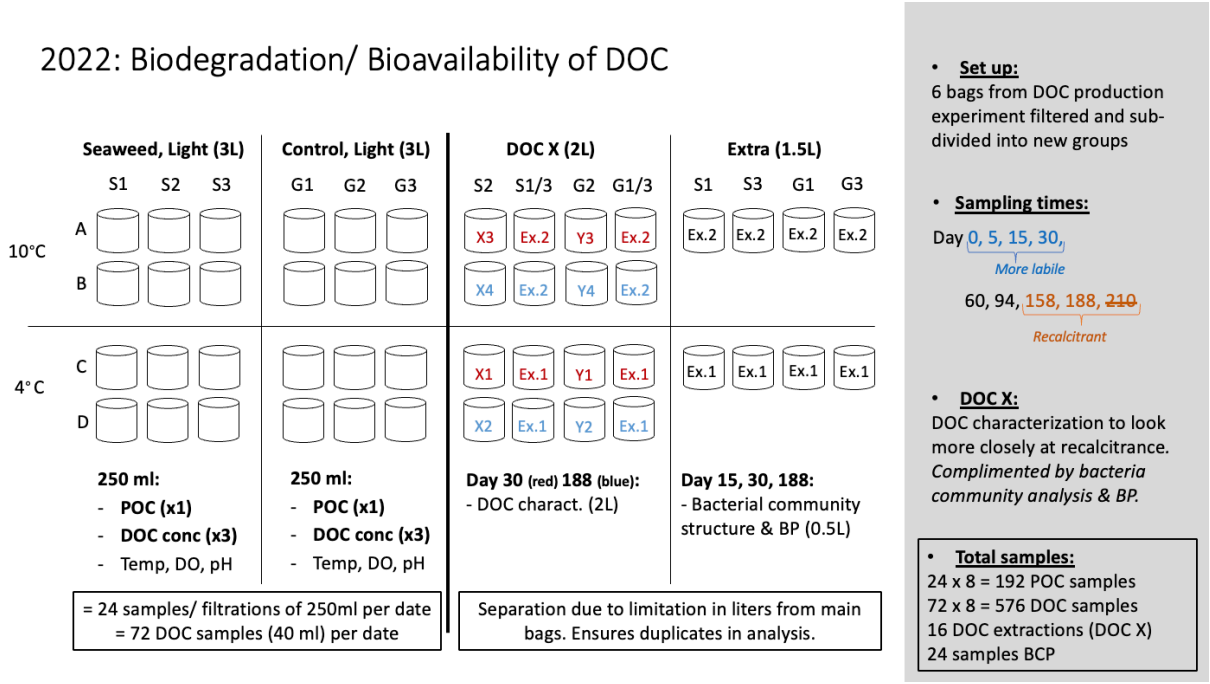

Figure 7: Long-term degradation setup. Seaweed water was labelled with "S", while control water was labelled with "G". Category AB was the duplicate 10°C-treatment(n=12), while CD was the duplicate 4°C-treatment (n=12). "DOC X" (n=8) was water samples for DOC characterization (on day 0, 30 and 188) and "Extra" (n=8) was used for both DOC characterization and bacterial community (on day 0, 15, 30 and 188). In total 40 (N=40) individual bags of water were stored during the long-term degradation experiment. Illustration: Luiza Neves

2.5.2 Sampling and filtering on day 0

From the 21L contained in the experiment bag, 20 liters were filtered through GF/F (0.7 µm) filter (pre-combusted at 450°C for 8 hours) and 1 liter was filtered through GF/C (1.2 µm) filter (pre-combusted at 450°C for 8 hours). Figure 8 shows the flow of procedure from sampling to analyses.

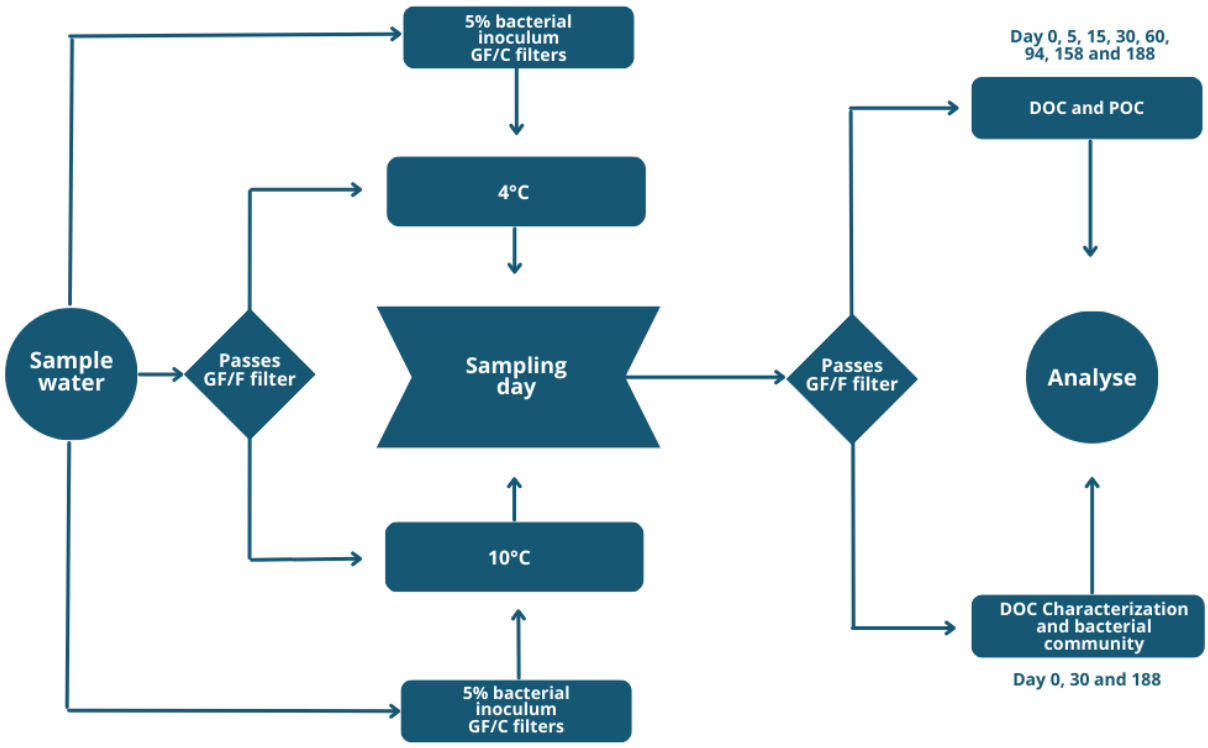

Figure 8: Flow chart of methods for long-term degradation experiment. Illustration: Sebastian Gjertsen

3 liters from the GF/F-filtered water were transferred into the DOC-degradation bag (4 liter Cubitainer linear low/low density polyethylene (LLDPE/LDPE) plastic) and 150ml (5% of 3 liters) of the GF/C-filtered water was added as an inoculum (Lønborg et al., 2009; Gao et al., 2021). This was done to let autotrophic and heterotrophic microorganisms that were < 1.2 µm pass into the water, but autotrophic growth was assumed prevented due to storage in complete darkness. Headspace of 0.85 liter were left in the bag to have sufficient oxygen levels.

For the DOC-characterization setup, filtered GF/F water and 100ml (5% of 2 liters) of inoculum from the GF/C filtered water were transferred to 2.5-liter glass bottles and sealed for storage in the dark until analysis at day 30 at 188. For the bacterial community setup, 8 liters were split into duplicates at 4 liters and filtered through GF/F filters and added 200ml (5% of 4 liter) of inoculum from the GF/C filtered water.

2.5.3 Sub-sampling on days 5, 15, 30, 60, 94, 158, and 188

Sub-samples were done eight times after day 0 to both check the labile and recalcitrant side of the DOC pool. Before each day, glassware, filtration units and other equipment were washed in an acid-bath containing HCl 1M to remove organic materials. Each sampling day consisted of sampling from 12 bags at 4°C and 12 bags from 10°C. At day 15, 30 and 188, extra samples were done for bacterial community and DOC-characterization.

The sampling took place in a laboratory set to 10°C. Since half of the bags were stored in another room at 4°C, they were first collected into 250ml Erlenmeyer flasks and sealed with aluminum**,** but brought in a dark styrofoam container with ice, to keep their temperature. They were transferred to the 10°C-room where abiotic measurements and filtrations were done.

The DOC-concentration bags were turned upside down three times prior to filtration, due to water likely being heterogenous. First, 30ml of water was used to measure temperature, DO and pH. Then 250ml of water was measured using Erlenmeyer flask and filtered through GF/F filters. The filter was gently moved from the filtration unit with an acidified metal tweezer to a petri dish and labelled. The petri dishes were stored in -20°C until further analysis of POC.

Three replicates of the filtered GF/F water were sampled in 40ml glass vials (pre combusted at 450°C for 8 hours), which was rinsed three times in sample water, before filled up to the neck to leave headspace for expansion due to freezing. All samples were acidified to pH <2 using 80 µL of 6M HCl. The glass vials were then labelled and stored in the dark at -20°C until further analyses of DOC concentrations.

2.6 Analyses of samples

2.6.1 DOC analysis

Figure 9: TOC-L analyzer and ASI-L auto sampler (Shimadzu, 2023a).

DOC analyses were performed using a Total Organic Carbon analyzer (Shimadzu, TOC-L CPH, Japan) coupled with an ASI-L auto sampler (Figure 9). The TOC-L analyzer adopts the 680°C combustion catalytic oxidation method, developed by Shimadzu. The machine was coupled with a Non-Dispersive Infra-Red (NDIR) detector, which detects the concertation of CO_2. The machine has a detection limit ranging from 4 µg/L to 30,000 mg/L.

2.6.1.1 Preparations

A test run of samples was conducted prior to beginning the complete experimental analyses. It was performed to decide if sample dilution was required due to the machine's inability to automatically dilute samples during analysis. To validate the test results, spike samples were made with 1 mg/L Glycine (reference material).

Figure 10: Thawing of sample DOC B11-1.

All samples were stored in -20°C and dark until analysis and was brought out to room temperature the day before analysis to thaw, as shown in Figure 10. Two test runs were conducted, separating seaweed samples in one batch and control samples in another batch, to deal with approximately same amounts of analytes each time.

A stock solution made from Potassium hydrogen phthalate (KHP, 1000 mg/L) was used to make standards of different concentrations. A total of seven-point standard curve was used, to get precision and a big enough range to cover both controls (presumably low mgC/L) and seaweed samples (presumably higher mgC/L). The standard concentrations were 0.2 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L, 3 mg/L, 4 mg/L and 5 mg/L, and machine calculations is shown in Appendix B. 6M HCl was used to acidify the standards so bacterial growth and degradation would be excluded.

Certified Reference Material (CRM) was ordered from Hansell's laboratory at the University of Miami. These CRMs contained different values of carbon (surface, mid and deep water), certified by a technically valid procedure, following ISO/IEC 17025. The CRMs were used in different intervals during analysis, to verify data quality and if concentrations are not within ± 10% of the certified values, the analysis would automatically stop.

2.6.1.2 Analysis

The TOC-L analyzer uses three different measurements techniques, TC (total carbon) measurement, TOC (total organic carbon) measurement and NPOC (non-purgeable organic carbon) measurement. TC samples are injected straight onto the catalyst bed, needing no pre-treatment. All sources of carbon are measured, both inorganic and organic. TOC and NPOC samples are acidified to reduce pH (either by machine, or in advance of freezing and storage), and sparged (100 mL/min) for 10 minutes with ultra-pure air as a carrier gas to remove inorganic carbon (IC), before being injected onto the catalyst bed. There on, samples are

combusted (680°C), and the platinum-coated alumina (0.5%) balls breaks down the carbon amalgamations into CO_2, which is then detected by the NDIR detector. The TOC-L analyzer were set to use NPOC measurement (Figure 11). Since working with seawater, where the IC component of the TC is high, meaning that it would lead to significant errors using TOC measurements.

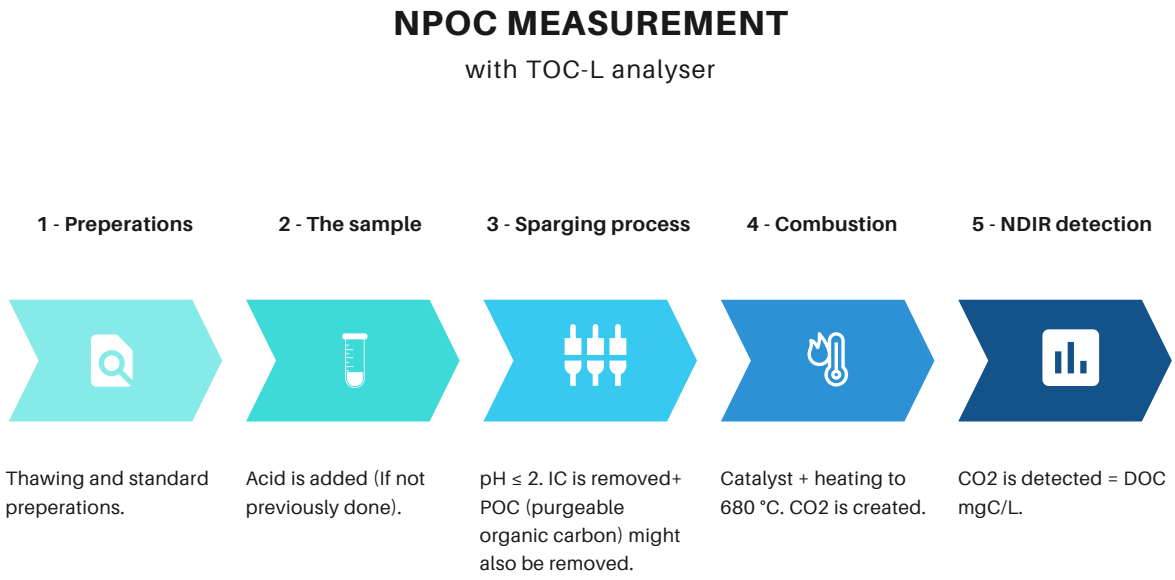

Figure 11: Schematic flow chart of NPOC Measurement using TOC-L analyzer. Illustration: Sebastian Gjertsen

There were several procedures followed each day prior to running samples, including instrument preparation and maintenance, standard and calibration curve preparations, methods, controls, sample load and table. The ASI-L auto sampler had 68 sample spots, and Appendix B provides an example run log-sheet. In between every 10-12 DOC samples, CRMs and CCVs were used to monitor calibrated values and stability specifications (±10%). During and after analysis, results was shown in a sample window as illustrated in Appendix B.

2.6.2 POC analysis

POC analyses were conducted using a Vario EL cube elemental analyzer (Elementar Inc., Vario EL Cube, Germany), performed by a technician from SINTEF Ocean. It is used to analyze Carbon, Hydrogen, Nitrogen, Sulphur (CHNS) and Oxygen, and the CHNS elemental analysis is based on the high temperature combustion and subsequent analysis of the combustion gases (elementar, 2023).

2.6.2.1 Preparation

All equipment used were washed using Ethanol 90%, and the working area was cleaned and wrapped with aluminum foil, to decrease the risk of carbon contamination. To determine µg/C for each filter (GF/F), a small cut-out sample ($1/16^{th}$) from each filter was analyzed.

Each filter was laid on top of a cobber plate, where a tool was used to cut out $1/16^{th}$ of the filter as shown in Figure 12A. Each of the cut-outs were folded twice and then put into a tin foil. The tin foil was thereafter compressed into a tin pellet, using two metal tweezers. The tin pellet was systematically placed inside a storage chamber, and then placed in a drying cabinet and set to 60°C for 24h. A total of 244 filters were prepared for analysis.

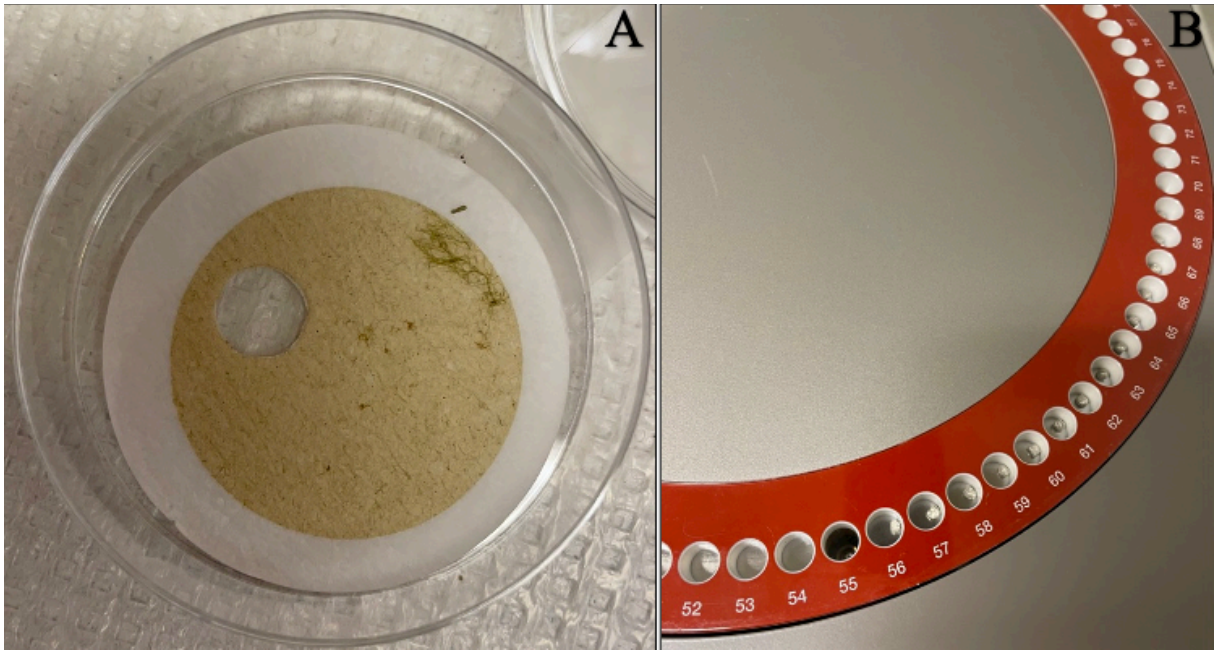

Figure 12: **A)** GF/F filter with 1/16th of it cut out during analysis preparation. **B)** Tin pellets loaded in sample carousel during analysis.

2.6.2.2 Analysis

Prior to beginning the POC analysis, a test run of samples were examined at TBS, using a Vario EL cube elemental analyzer. It was conducted on approximately 30 samples to check the range of µgC/capsule, and to determine which standards to use during analysis. The 30 samples were re-done at SINTEF Ocean lab.

The tin pellets were loaded unto the sample carousel (Figure 12B and Figure 13) where it was automatically weighed and thereafter transferred through a ball valve where atmospheric nitrogen is removed using helium. It is then transferred into the furnace where two different steps are executed, combustion and reduction. Within the combustion tube, the catalytic combustion is carried out at up to 1200 °C and then afterwards the reduction of combustion gases is done in the reduction tube. The gases (N_2, CO_2, H_2O and SO_2) is formed and carried through three trap columns, separating the gases. The gases are then carried towards the detector with a stable pressure and flow, using an electron gas flow controller, and then the gases are detected. A connected computer calculates the element concentration from the detector signal, giving results per capsule (elementar, 2023).

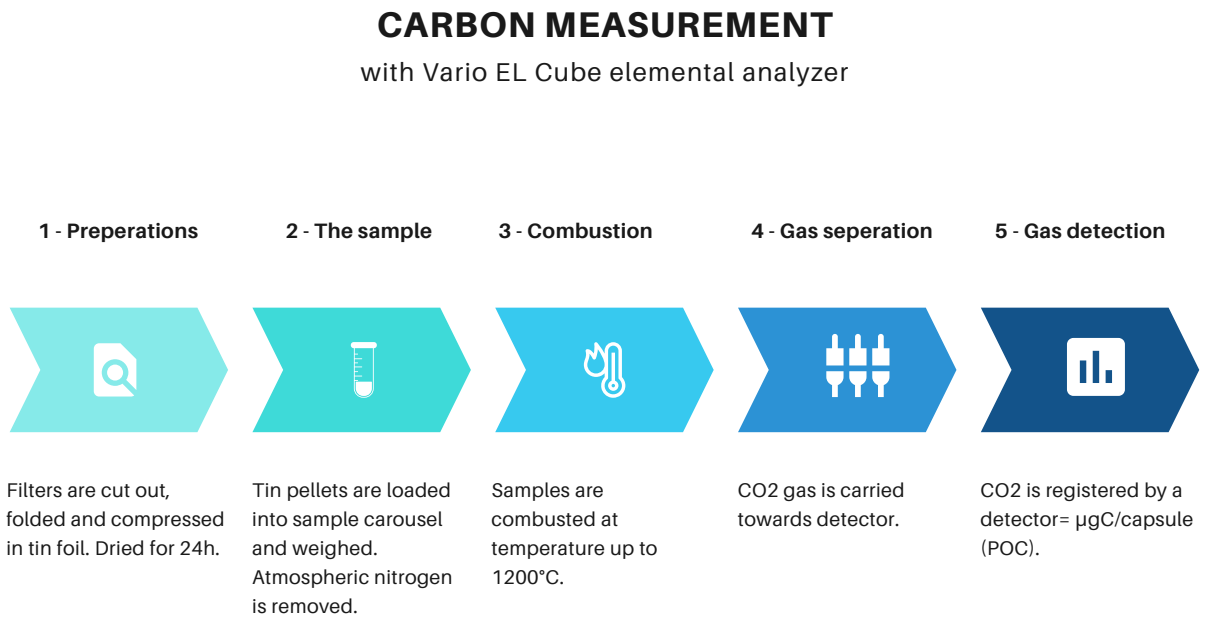

Figure 13: Schematic flow chart of carbon measurement using Vario EL Cube elemental analyzer. Illustration: Sebastian Gjertsen

2.7 Statistics

The data was treated using IBM SPSS Statistics v29 for statistical analysis, and Microsoft Office 365 Excel for graphs. Shapiro-Wilks normality test, together with QQ-plots, was used to determine normal or non-normal distribution of data. A test of homogeneity of variance by Levene was also conducted to determine if equal variance was assumed or not. These two factors and sample size (n) contributed to determine further analysis of the data. Outliers were removed either by recommendation from the technicians' analyzing the samples, and/or from outlier test in SPSS.

Two sample t-tests were used to analyse difference between the means of two groups, while ANOVA was used to determine differences between the means of three or more groups. When ANOVA was used, and normality and homogeneity assumptions (or equal samples sizes) was met, then regular ANOVA was used, with Tukey HSD post hoc criterion. If normality assumptions were met, but not homogeneity assumptions (or equal sample sizes), then Welch ANOVA was used, with Games-Howell post hoc criterion. If normality assumptions were not met, then Kruskal-Wallis ANOVA was used, with Dunn post hoc criterion.

If data violated Sphericity, the epsilon value determined which corrections were used. If epsilon was >.75, then Huynh-Feldt correction was used, and if epsilon <.75, then Greenhouse-Geisser correction was used. An alpha level of .05 for all statistical tests were used, if not specifically stated otherwise.

3 Results

3.1 Production experiment

3.1.1 Growth and POC release rate

The specific growth rates (SGR) for *S. latissima* sampled on day 2, 3 and 4 during the production experiment varied with days (Figure 14). A t-test was conducted to see if growth rate for "Seaweed Light" or "Seaweed Dark" differ between days. "Seaweed Light" showed no statistically significant difference between any of the days, p = >.05. "Seaweed Dark" showed no statistically significant difference between any of the days, p >.05. There was neither any statistically significant difference between "Seaweed Light" and "Seaweed Dark", p = .689.

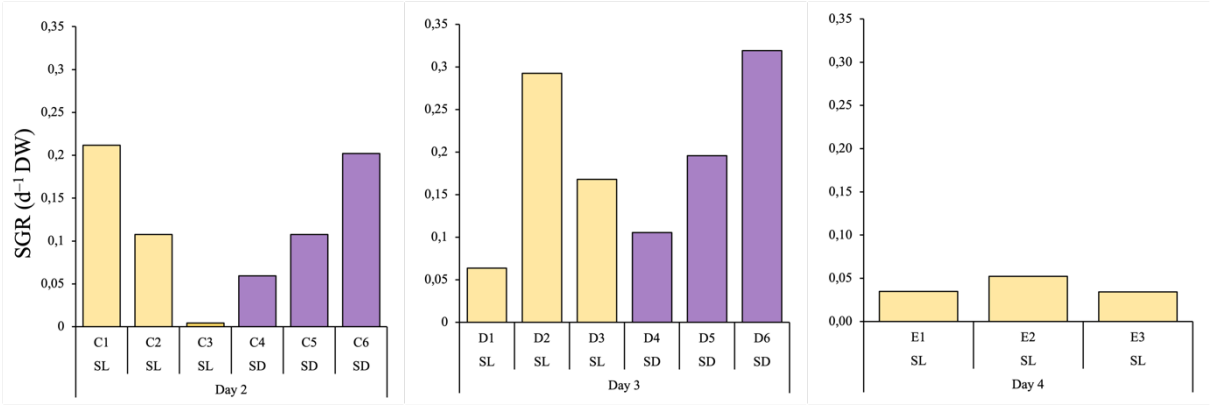

Figure 14: Specific growth rate (SGR) based on dry weight (DW) from day 2, day 3 and day 4. Day 1 is excluded due to minimal growth period. No Seaweed dark bags were sampled on day 4. The x-axis shows the different codes and treatment of each bag. SL=Seaweed Light, SD=Seaweed Dark, CL=Control Light and CD=Control Dark. All graphs show same scale on y-axis for better visual comparison.

Figure 15 shows the mean POC release rates for transparent and dark seaweed bags combined from each day. The POC released from "Seaweed Light" over all days ranged between 303-1384 µg C (g^{-1} DW d^{-1}), while "Seaweed Dark" decreasingly ranged between 310-799 µg C (g^{-1} DW d^{-1}). The highest release rate was obtained during the first day (1383,5 µg C ± 408) by the transparent seaweed bag, 78.10% higher than the next day, but an increasing trend was seen after that.

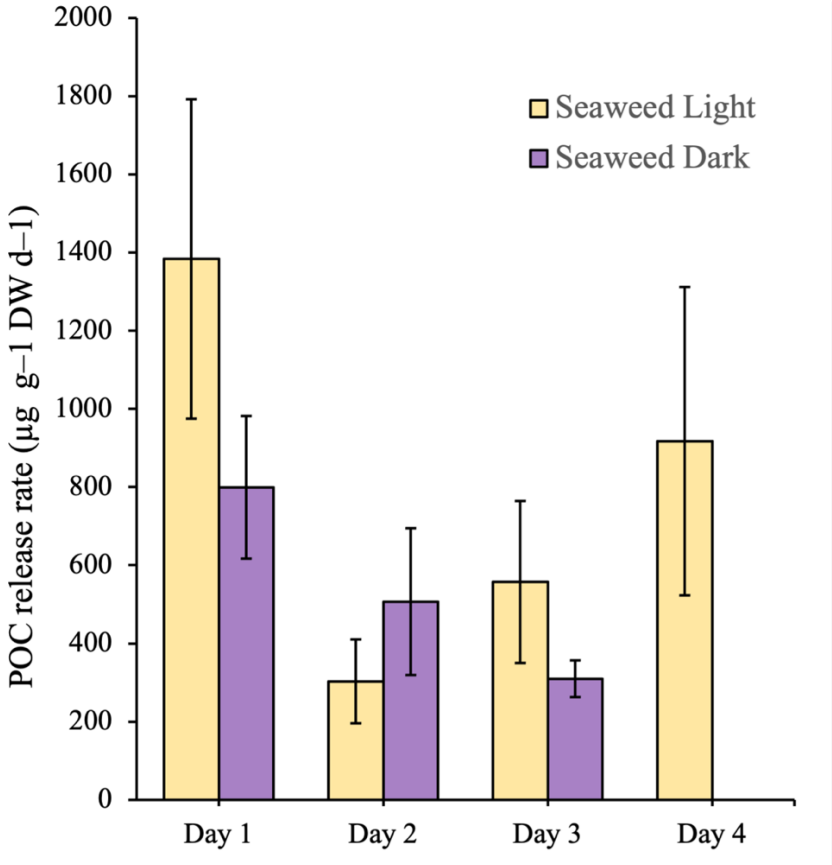

Figure 15: Mean POC release rates (± SD) based on dry weight (DW) for seaweed bags (transparent and dark) on 4 different sampling days. Control POC values was subtracted from the seaweed values.

A one-way ANOVA was performed to compare the release rate between two treatments on different days. The analysis of variance showed that there was a statistically significant difference in mean POC release rate between at least two groups, $F(6, 13) = 7.43$, $p = <.001$.

Post hoc analyses using Tukey HSD criterion for multiple comparisons found that the mean POC release rate was statistically significant different between "Seaweed Light" on day 1 and day 2, $p = .001$, and between day 2 and day 4, $p = 0.25$, but not the other days, $p = >.05$. There was no statistically significant difference between "Seaweed Dark" on any of the days, $p = >.05$, or between "Seaweed Light" and "Seaweed Dark" on their same sampling days, $p = >.05$.

3.1.2 Particulate organic carbon

The mean POC values between transparent and dark treatments is shown in Figure 16. After the first day of sampling, "Seaweed Light" had 184.87 µg POC/L, 7.7% more POC than "Seaweed Dark", while compared to the "Control Light", an 190% increase was measured. The

difference between “Seaweed Dark” and “Control Dark” was 189% (Figure 16A). Day 2 showed equivalent percentage for dark bags (167%), while the transparent bags had an 86% difference (Figure 16B). On day 3 “Seaweed Light” had 338.53 µg POC/L, 87% more than “Seaweed Dark” (Figure 16C). The fourth and last day showed the highest concentration of POC, 805.08 µg C/L, which was 182% higher than “Control Light” (Figure 16D).

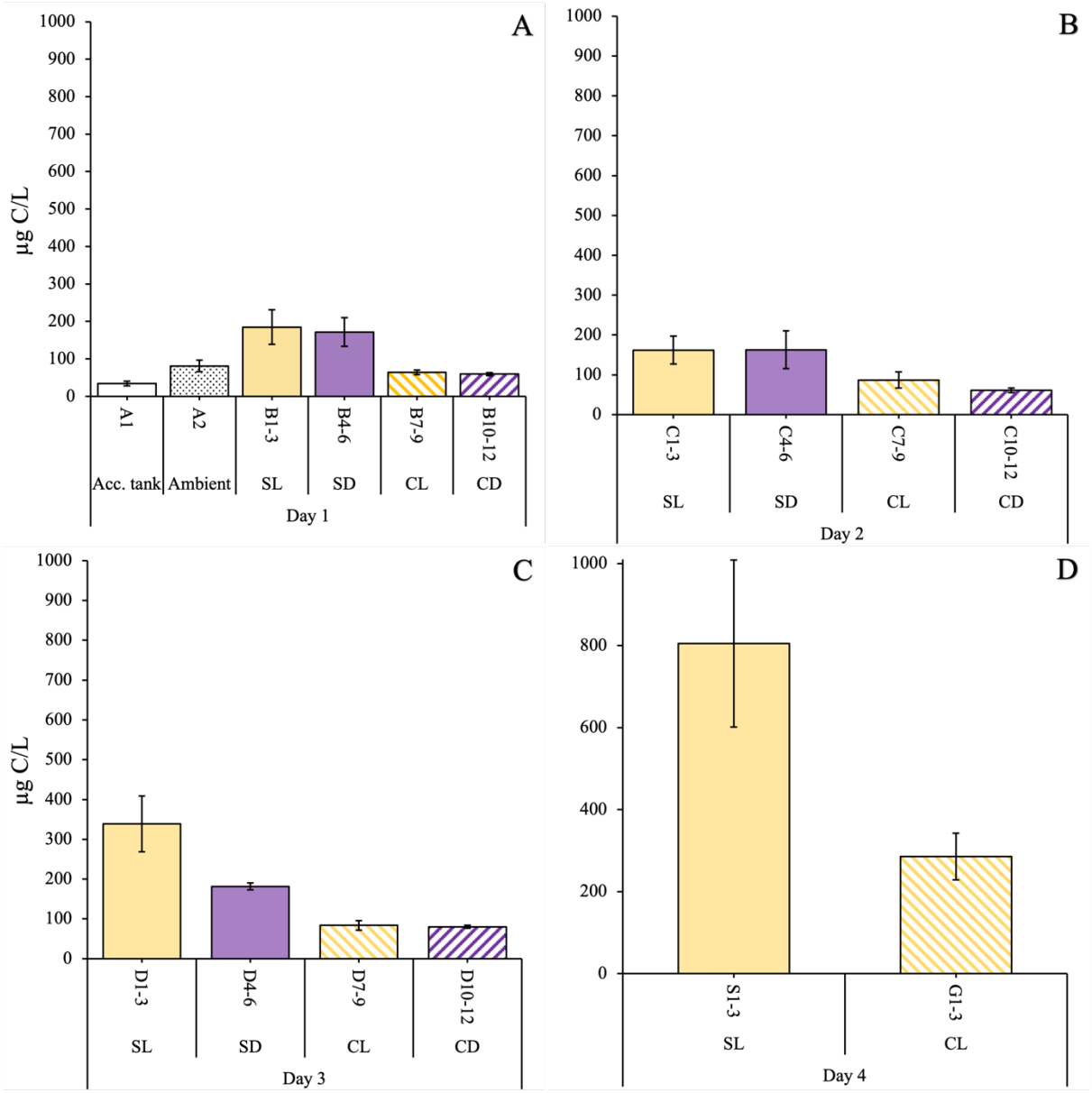

Figure 16: Mean (± SD) concentration (µg C/L) of particulate organic carbon over four different days during the production experiment. The x-axis shows the different codes and treatment of each bag. SL=Seaweed Light, SD=Seaweed Dark, CL=Control Light and CD=Control Dark**.** All graphs show same scale on y-axis for better visual comparison. There were in total 42 bags, but one outlier was removed (N=41). Seaweed Dark (n=8) and Control Dark (n=9) was sampled on day 1, 2 and 3, while Seaweed Light (n=12) and Control Light (n=12) was sampled for all four days. Each bar is the mean of 3 bags. **A)** Day 1 graph also shows POC values from acclimatization tank (n=3) where the value was divided by number of kelp (n=21), and ambient water (n=1).

3.1.2.1 Difference between days

A Kruskal-Wallis one-way ANOVA was performed to check whether the mean distribution of POC values was the same across all days. The analyses of variance showed that there was a statistically significant difference between at least two groups, p = .002.

Post hoc analyses using Dunn criterion for pairwise comparisons of days showed that the mean value of POC was statistically significant different between Day 4 and all other days, p = <.05. There was least difference between Day 1 and Day 2, p = .851.

Several two-sample t-tests were conducted to determine whether "Seaweed Light" was different between different days. In summary, based on the independent two-sample t-tests, there were significant difference observed between "Seaweed Light" on all days, except Day 1 and Day 2, as shown in Table 3.

Table 3: Comparison between Seaweed Light on different days

Seaweed Light comparison

Dependent variable: POC

Test: *Two-sample t test*

	Day 1	Day 2	Day 3	Day 4
Day 1 (M=184.87, SD=46.16)	-			
Day 2 (M=161.84, SD=34.24)	t(4)=.687,p=.530	-		
Day 3 (M = 338.53, SD=70.15)	t(4)=-3.169,p=**.017***	t(4)=-3.898,p=**.018***	-	
Day 4 (M = 805.08, SD = 203.36)	t(4)=-5.151,p=**.007***	t(4)=-5.398,p=**.006***	t(4)=-3.756,p=**.020***	-

*. The mean difference is significant at the 0.05 level.

Another test was conducted to discover if there were significant difference between “Seaweed Light” and “Control Light” throughout the experiment (Figure 17A). In summary, there were significant differences between “Seaweed Light” and “Control Light” during all days, p = <.05.

A two-sample t-test was also conducted to determine whether “Seaweed Dark” was different between days, but no statistically significant difference was found, p = >.05, also visually shown in Figure 17B.

3.1.2.2 Difference between treatments

A one-way Welch ANOVA was performed to compare the effect of four different independent treatments on POC values. The analysis of variance showed that there was a statistically significant difference in mean POC values between at least two groups, F(3, 19.974) = 43.54, p = <.001.

Post hoc analyses using Games-Howell criterion for multiple comparisons (Table 4) found that the mean value of POC was statistically significant different between “Seaweed Light” and “Control Light”, p = .003, and Control Dark, p = <.001. There was also a statistically significant difference between “Seaweed Dark” and “Control Light”, p = .030, and “Control Dark”, p =<.001. There was no statistical difference in mean POC production between “Control Light” and “Control Dark”, p = .061, or between “Seaweed Light” and “Seaweed Dark”, p = .110.

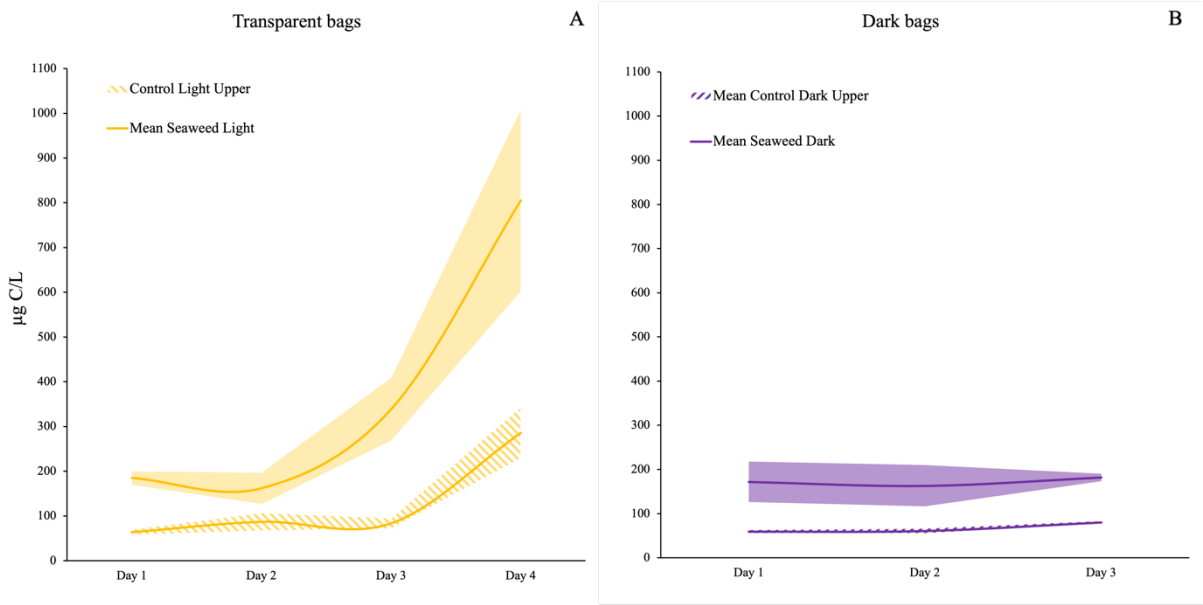

Figure 17: Line chart of mean POC values of POC. **A)** Transparent bags (n=12), with controls(n=12). **B)** Dark bags (n=9), with controls (n=9). Line shows the mean trend value, while coloured area around shows ± SD during the different days.

However, since there was visually a difference between "Seaweed Light" and "Seaweed Dark" on day 3 (Figure 17AB), a two-sample t-test was conducted to determine whether "Seaweed Light" on day 3 was different from "Seaweed Dark" on day 3. The mean value from "Seaweed Light" day 3 (M = 2.52, SD = .09) was significantly different than "Seaweed Dark" day 3 (M = 2.26, SD=.02); t(3) = 4.045, p = .027.

Table 4: Multiple comparisons between Seaweed treatments and control treatments groups using Games-Howell criterion, which uses the mean difference of POC between treatments to calculate significance.

Multiple comparisons

Dependent variable: POC

Test: Games-Howell

	Seaweed Light	Seaweed Dark	Control Light	Control Dark
Seaweed Light	-			
Seaweed Dark	.110	-		
Control Light	.003*	.030*	-	
Control Dark	<.001*	<.001*	.061	-

*. The mean difference is significant at the 0.05 level.

3.1.3 Dissolved inorganic carbon

The DIC values shown in Figure 18 was measured from samples analyzed by the Norwegian Institute for Water Research (NIVA), and the graph shows a comparison of the mean DIC value between transparent and dark bags. There was a 5.5% negative difference in DIC value in "Seaweed Light" from Day 1 to Day 2, and 6.8% from Day 1 to Day 3. Ambient DIC value showed 25.88 mgC/L and is comparable to the mean value of, "Control Light" and "Control Dark" over the span of all three days, 26 mgC/L and 26.01 mgC/L, respectively. "Seaweed Dark" shows a gradually rising trend.

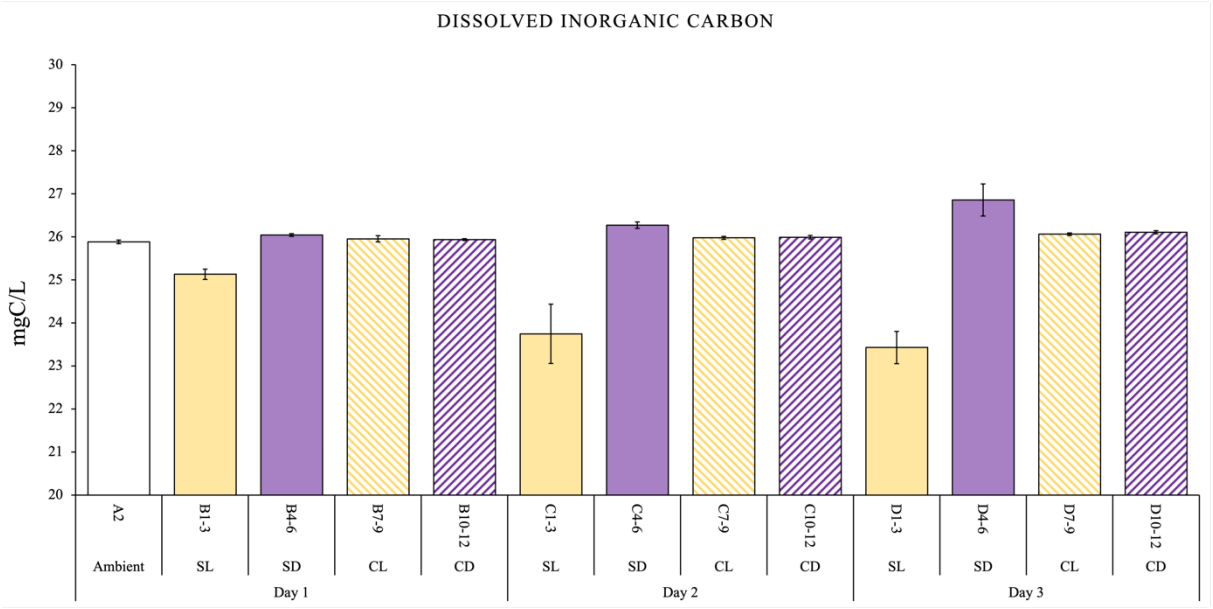

Figure 18: Mean (± SD) concentration (mgC/L) of dissolved inorganic carbon (N=36) over three days during the production experiment. The x-axis shows the different codes and treatment of each bag. SL=Seaweed Light, SD=Seaweed Dark, CL=Control Light and CD=Control Dark. Each bar is the mean of 3 bags, except for ambient (n=1), which was water pumped into the basin from 80m depth.

3.1.3.1 Difference between days and treatments for Seaweed Light and Dark

A one-way ANOVA was performed to compare the days and treatment on DIC values for "Seaweed Light" and "Seaweed Dark". The analysis of variance showed that there was a statistically significant difference in mean DIC values between at least two groups, $F(5,12) = 45.65$, $p = <.001$.

Post hoc analysis using Tukey HSD criterion for multiple comparisons found that the mean value of DIC were statistically significant different between “Seaweed Light” and “Seaweed Dark” on day 2 and 3, p <.001.

3.1.3.2 Difference between days

A one-way ANOVA was conducted to check if days had an effect on the mean “Seaweed Light” DIC values and mean “Seaweed Dark” DIC values. The analysis of variation showed that there was a statistically significant difference in mean DIC values between at least two groups for both treatments, F(2,6) = 11.71, p = .010 (Seaweed Light) and F(2,2.981) = 15.26, p = .025 (Seaweed Dark).

Post hoc analyses using Tukey HSD criterion for multiple comparisons (Table 5) found that the mean value of DIC for “Seaweed Light” was statistically significant different between Day 1 and Day 2, p = .023 and Day 3, p = .009. There was no statistically significant difference between Day 2 and Day 3, p = .689. For “Seaweed Dark” there was a statistically significant difference between Day 1 and Day 3, p = .010 and between Day 2 and Day 3, p = .040, but not between Day 1 and Day 2, p = .462.

Table 5: Multiple comparisons between mean DIC value for Seaweed Light and days, and Seaweed Dark and days, using Tukey HSD criterion which uses the mean difference between them to calculate significance.

Multiple comparisons

Dependent variable: DIC

Test: Tukey HSD

Seaweed Light	Day 1	Day 2	Day 3
Day 1	-		
Day 2	<.023*	-	
Day 3	<.009*	.689	-
Seaweed Dark	**Day 1**	**Day 2**	**Day 3**
Day 1	-		
Day 2	.462	-	
Day 3	.010*	.040*	-

*. The mean difference is significant at the 0.05 level.

3.1.3.3 Difference between treatments

A one-way Welch ANOVA was performed to compare the effect of four independent treatments on DIC values. The analysis of variance showed that there was a statistically significant difference in mean DIC values between at least two groups, F(3,16.001) = 15.46, p = <.001.

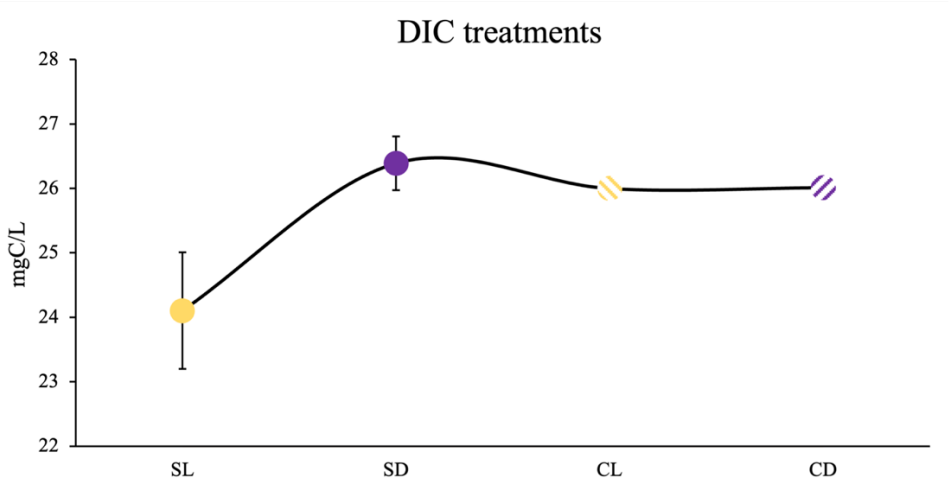

Figure 19: Mean DIC values (± SD) based on four different treatments on all days three days. Each treatment had 9 (n=9) bags each (N=36).

Post hoc analysis using Tukey HSD criterion for multiple comparisons (Table 6) found that the mean value of DIC were statistically significantly different between "Seaweed Light" and all other treatments (Seaweed Dark, Control Light and Control Dark, p = <.001) (Figure 19). There was no statistically significant difference between "Seaweed Dark" and "Control Light" (p = .339) and "Control Dark" (p = .369) or between "Control Light" and "Control Dark", p = 1.000.

Table 6: Multiple comparisons of DIC values between treatment groups using Tukey HSD criterion, which uses the mean difference between treatments to calculate significance.

Multiple comparisons

Dependent variable: DIC

Test: Tukey HSD

	Seaweed Light	Seaweed Dark	Control Light	Control Dark
Seaweed Light	-			
Seaweed Dark	<.001*	-		
Control Light	<.001*	.339	-	
Control Dark	<.001*	.369	1.000	-

*. The mean difference is significant at the 0.05 level

3.1.4 pH

Figure 20 shows a comparison of the mean pH value between transparent and dark bags. “Seaweed Light” shows a positive trend of pH value increasing from Day 1 until Day 4, a total of 8.4% increase, going from the lowest at Day 1 (mean 7.87 pH) to highest at Day 4 (mean 8.53 pH). Both controls and “Seaweed Dark” shows stable values comparable to ambient pH value (7.80 pH) throughout the four consecutive days.

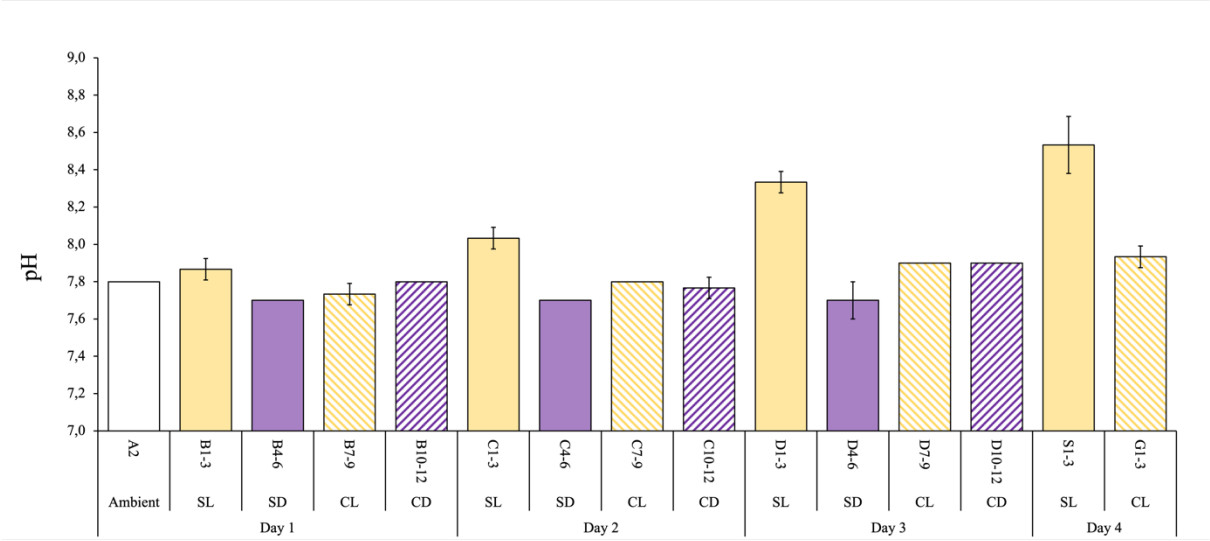

Figure 20: Mean (± SD) value of pH (N=42) over four days during the production experiment. The x-axis shows the different codes and treatment of each bag. SL=Seaweed Light, SD=Seaweed Dark, CL=Control Light and CD=Control Dark. There was in total 42 bags, Seaweed Dark (n=9) and Control Dark (n=9) was sampled on day 1,2 and 3, while Seaweed Light (n=12) and Control Light (n=12) was sampled for all four days. Each bar is the mean of 3 bags, except for ambient (n=1), which was water pumped into the basin from 80m depth.

3.1.4.1 Difference between days and treatment for Seaweed Light and Dark

A Mann-Whitney U test was conducted to determine if “Seaweed Light” was different than “Seaweed Dark” on different days. Results showed that the mean pH between “Seaweed Light” and “Seaweed Dark” on day 2 was significantly different, $Z = -2.121$, $p = .034$. There was also a statistically significant difference on day 3, $Z = -1.993$, $p = .046$.

3.1.4.2 Difference between days

A Kruskal-Wallis one-way ANOVA was performed to check if the distribution of pH was the same across all days. The analyses of variance showed that there was a statistically significant difference between at least two groups, $p = .002$.

Post hoc analyses using Dunn criterion for pairwise comparisons of days (Table 7) showed that the mean value of pH was statistically significant different between Day 1 and Day 3, p = .025, and Day 4, p = <.001 and between Day 2 and Day 4, p = .004. Seaweed Light on Day 1 was statistically significantly different from Day 3, p = .035 and Day 4, p = .003. "Seaweed Light" was also statistically significantly different between Day 2 and Day 4, p = .046.

Table 7: Pairwise comparison of mean pH values(N=42) from each sampling day (n=4). The significance level is set to <.05.

Pairwise comparison

Dependent variable: DIC

Test: Dunn's test

	Day 1	Day 2	Day 3	Day 4
Day 1	-			
Day 2	.469	-		
Day 3	.025*	.130	-	
Day 4	<.001*	.004*	.098	-

Each row tests the null hypothesis that the Day X and Day Y distributions are the same.

Asymptotic significances (2-sided tests) are displayed. *. The mean difference is significant at the 0.05 level

3.1.4.3 Difference between treatments

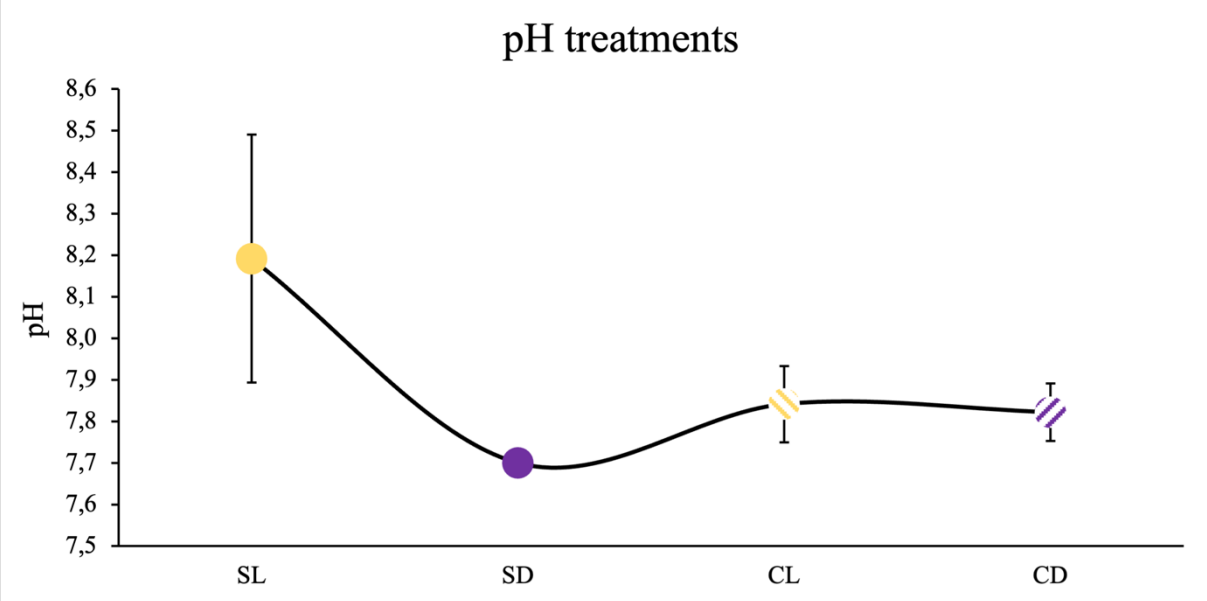

Figure 21: Mean pH values (± SD) based on four different treatments on all days four days. Seaweed Light (n=12) and Control Light (n=12) was measured on all four days, while Seaweed Dark (n=9) and Control Dark (n=9) only was measured for three days.

A Kruskal-Wallis one-way ANOVA was performed to check if the distribution of pH was the same across categories of treatment. The analysis of variance showed that there was statistically significant difference between at least two groups, p = <.001.

Post hoc analyses using Dunn criterion for pairwise comparisons of treatments (Table 8) found that the mean value of pH was statistically significantly different between "Seaweed Light" and "Seaweed Dark", p = <.001 and "Control Light", p = .006 and "Control Dark", p = .004. "Seaweed Dark" was statistically significantly different from "Control Light", p = .008, and "Control Dark", p = .030 (Figure 21).

Table 8: Pairwise comparison of mean pH values (N=42) from each treatment (n=4). The significance level is set to <.05.

Pairwise comparison

Dependent variable: DIC

Test: Dunn's test

	Seaweed Light	Seaweed Dark	Control Light	Control Dark
Seaweed Light	-			
Seaweed Dark	<.001*	-		
Control Light	<.006*	.008*	-	
Control Dark	<.004*	.030*	.747	-

Each row tests the null hypothesis that the Treatment X and Treatment Y distributions are the same.

Asymptotic significances (2-sided tests) are displayed. *. The mean difference is significant at the 0.05 level

3.1.5 Temperature

The temperature shown in Figure 22 was measured from the experiment water when bags were sampled on its respective day. The temperature on Day 1 was stable at a mean 13.6 °C. Day 2 ranged between 12.5 °C and 13 °C, while Day 3 temperature kept decreasing until 11.8 °C. Day 4 showed a mean temperature at 12.5°C.

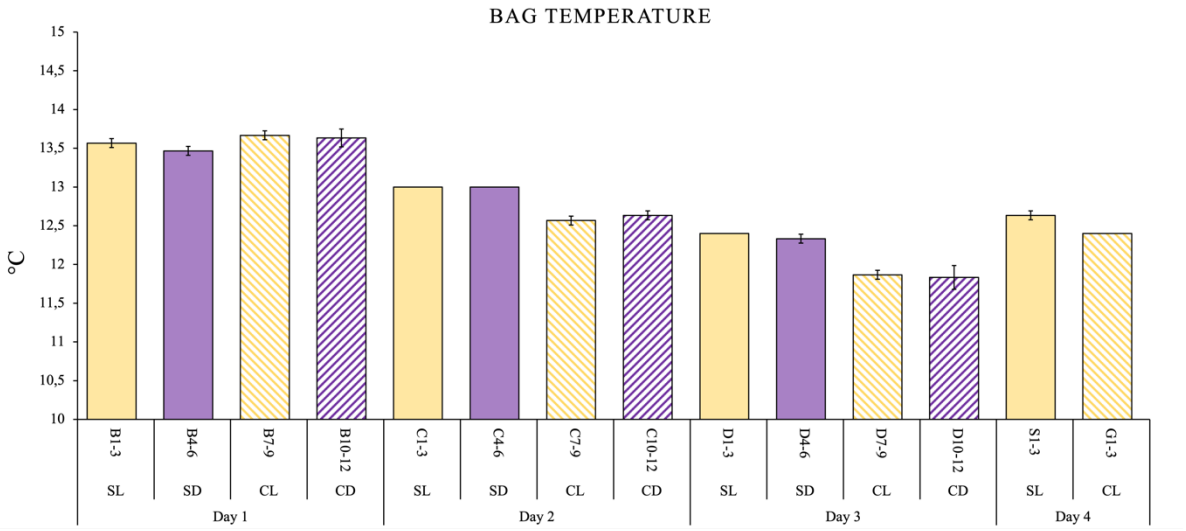

Figure 22: Mean (± SD) value of temperature (N=42) over four days during the production experiment. The x-axis shows the different codes and treatment of each bag. SL=Seaweed Light, SD=Seaweed Dark, CL=Control Light and CD=Control Dark. There was in total 42 bags, Seaweed Dark (n=9) and Control Dark (n=9) was sampled on day 1,2 and 3, while Seaweed Light (n=12) and Control Light (n=12) was sampled for all four days. Each bar is the mean of 3 bags, except for ambient (n=1), which was water pumped into the basin from 80m depth.

A Kruskal-Wallis one-way ANOVA was performed to check if the value of temperature was the same across all days. The analysis of variance showed that there was a statistically significant difference between at least two days, p = <.001.

Post hoc analyses using Dunn criterion for pairwise comparison of treatments found that the mean value of temperature was statistically significantly different between Day 1 and Day3, p = <.001, and Day 4, p = .006. There was also a statistically significant difference between Day 2 and Day 3, p = .005. There was shown no difference between Day 1 and Day 2, p = .055, Day 2 and Day 4, p = 1.000 or between Day 3 and Day 4, p = .734. Another Kruskal-Wallis one-way ANOVA was conducted to look at temperature and treatment, but no statistically significant difference was found, p = .684.

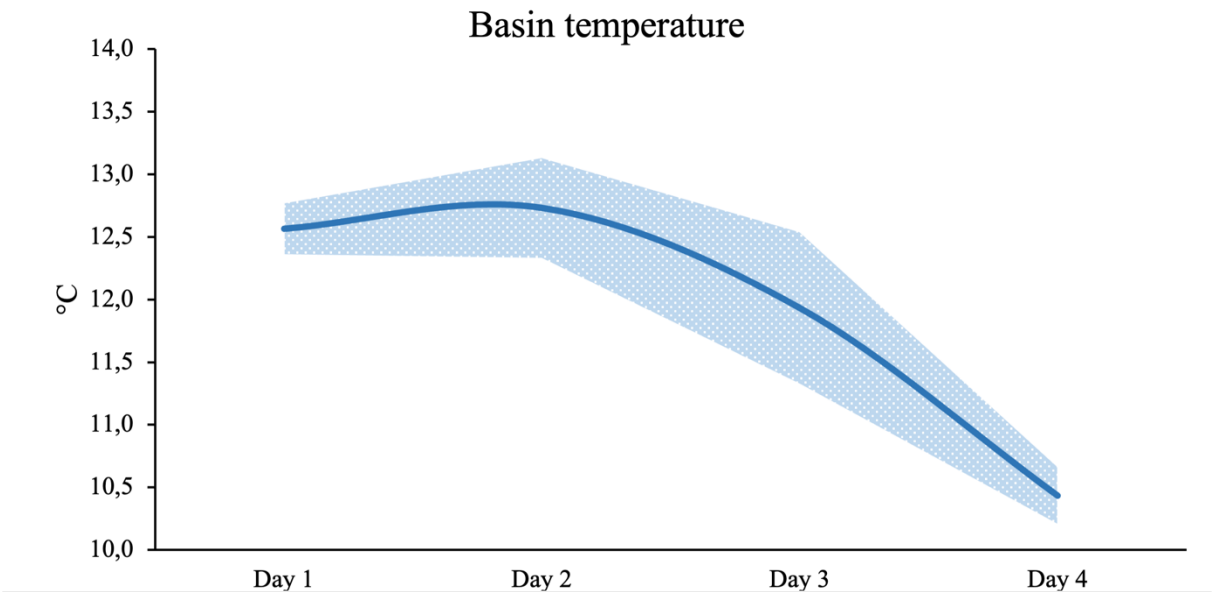

Figure 23: Mean temperature of basin water from ferrybox (Pt1000-sensor) during four days of experiment. Line shows mean temperature from three different measurements each day, while coloured area around shows ± SD during the four days.

Figure 23 shows the basin temperature during the experiment. The measurement was conducted with a ferrybox established in the basin next to experiment bags. The graph shows the mean value of three measurements during the day. There was a mean 0.1°C increase in temperature from Day 1 to Day 2, before it decreased steadily towards day 4, to a mean 10.4 °C.

3.1.6 Dissolved Oxygen

The dissolved oxygen shown in Figure 24 was measured from the experiment water when bags was sampled on its respective day. The Seaweed Light treatments is distinguishable from the rest of the treatments, increasing in DO during the experiment, ranging from 119% L to 167% L.

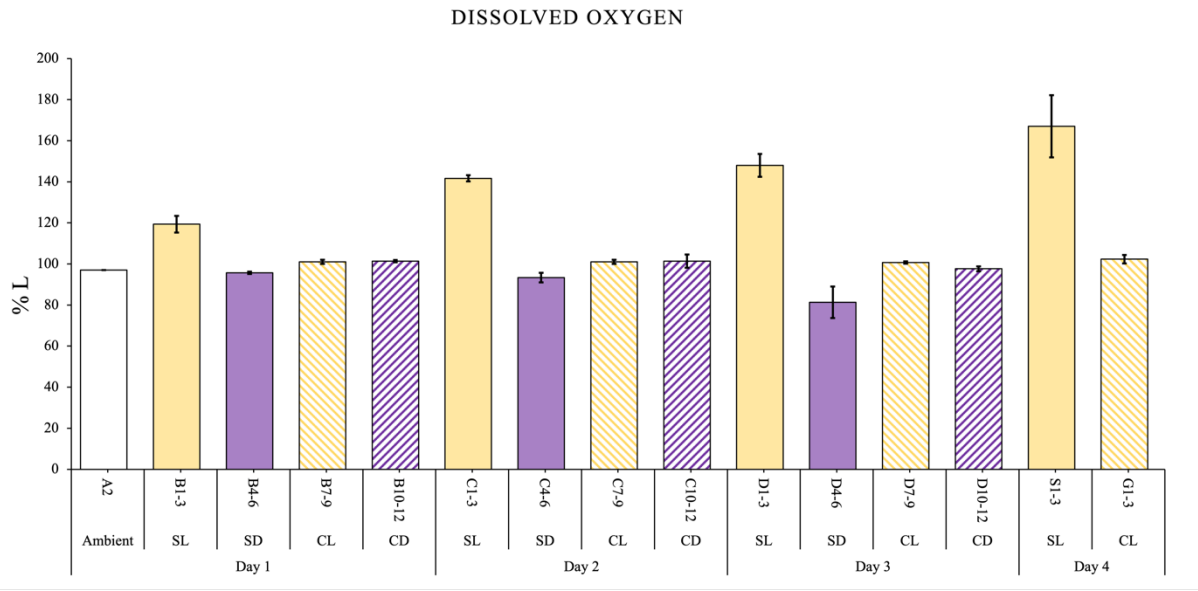

Figure 24: Mean (± SD) value of dissolved oxygen (N=40) over four days during the production experiment. The x-axis shows the different codes and treatment of each bag. SL=Seaweed Light, SD=Seaweed Dark, CL=Control Light and CD=Control Dark. There was in total 42 bags, but 2 measurements were removed. Seaweed Dark (n=8) and Control Dark (n=8) was sampled on day 1,2 and 3, while Seaweed Light (n=12) and Control Light (n=12) was sampled for all four days. Each bar is the mean of 3 bags, except for ambient (n=1), which was water pumped into the basin from 80m depth.

3.1.6.1 Difference between days and treatmensts for Seaweed Light and Dark

A Mann-Whitney U test was conducted to determine if "Seaweed Light" was different than "Seaweed Dark" on its respective sampling day. Results showed that the mean DO between "Seaweed Light" and "Seaweed Dark" on day 1 was significantly different, $Z = -1.993$, $p = .046$. There was also a statistically significant difference on day 2, $Z = -1.993$, $p = .046$, but not on day 3, $Z = -1.964$, $p = .050$.

3.1.6.2 Difference between treatments

A one-way Welch ANOVA was performed to check if the distribution of DO was the same across categories of treatments. The analyses of variance showed that there was a statistically significant difference between at least two groups, $F(3,15.948) = 28.59$, $p = .<001$.

Post hoc analyses using Games-Howell criterion for multiple comparisons found that the mean value of DO was statistically different between "Seaweed Light" and all other treatments, $p = <.001$. "Seaweed Dark" was also statistically significant different from "Control Light", $p = .004$ and "Control Dark", $p = .012$. "Control Light" and "Control Dark" was not statistically significant from each other, $p = .149$.

3.1.7 Total alkalinity

The TA values shown in Figure 25 was measured by NIVA, analyzing it from the same samples DIC was measured from. The values for Seaweed Light and Seaweed Dark decreases on Day 3, while controls remain stable all three days.

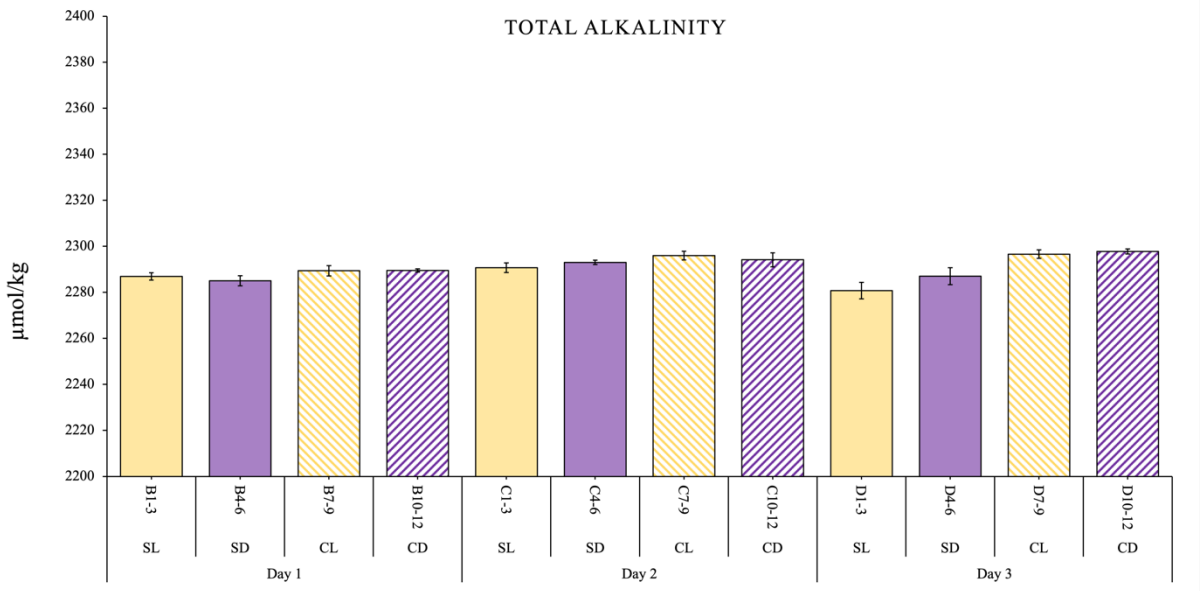

Figure 25: Mean (± SD) concentration (µmol/kg) of Total Alkalinity (N=33) over three days during the production experiment. The x-axis shows the different codes and treatment of each bag. SL=Seaweed Light, SD=Seaweed Dark, CL=Control Light and CD=Control Dark.

3.1.7.1 Difference between days and treatmenst

A two-sample t-test was conducted to determine whether "Seaweed Light" on day 3 was different from "Seaweed Dark" on day 3. The mean value from "Seaweed Light" day 3 (M = 2280.77, SD = 3.56) was not significantly different than "Seaweed Dark" day 3 (M = 2287.02, SD=3.65); t(3) = -1.890, p = .155.

3.1.7.2 Difference between days

A one-way Welch ANOVA was performed to check if the value of alkalinity was the same across the days. The analysis of variance showed that there was statistically significant difference between at least two days, F(2,18.418)=14.35 p = <.001. Post hoc analyses using Tukey HSD criterion for multiple comparisons found that the mean value of TA was statistically significantly different between Day 1 and Day 2, p = .016, but no difference between the other days, p = >.050.

3.1.7.3 Difference between treatments

A one-way ANOVA was further performed to check if the value of alkalinity was the same across the categories of treatment. The analysis of variance showed that there was a statistically significant difference between at least two treatments, $F(3,29)=6.79$, $p = .001$. Post hoc analyses using Tukey HSD criterion for multiple comparison found that the mean value of alkalinity was statistically significantly different between "Seaweed Light" and "Control Light", $p = .007$ and "Control Dark", $p = .008$, but no difference was found between "Seaweed Light" and "Seaweed Dark", $p = .743$. "Seaweed Dark" was statistically significant different from "Control Light", $p = .038$ and "Control Dark", $p = .046$. "Control Light" and "Control dark" had no difference between them, $p = 1.000$.

3.1.8 Production experiment correlations

Figure 26A shows the correlation between DIC and DO, which had a high negative correlation ($R^2 = 0.9589$). Figure 26B shows the correlation between pH and DO, which had a high positive correlation ($R^2 = 0.8536$).

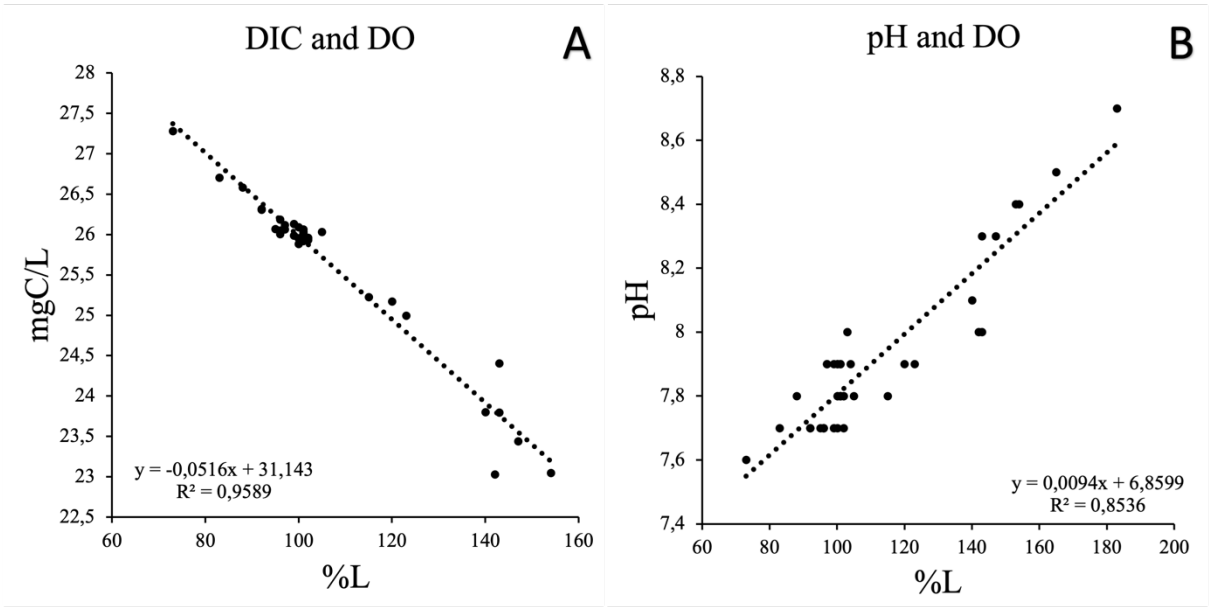

Figure 26: A) Shows the correlation between Dissolved inorganic carbon and dissolved oxygen during three days of experiment (n=36). **B)** Shows the correlation between pH and dissolved oxygen during four days of experiment (n=42).

Table 9 shows the correlation between all measured values through the production experiment. Even though some correlations were statistically significantly different, their correlation coefficient were low, e.g., DO and POC, $p = .009$ had a correlation coeffect at .406. In addition,

"Seaweed Light" (pH and DIC) from all 3 days was checked for correlations, and results showed a significant correlation, p = .012 (R^2= -0.784), as shown in Figure 27.

Table 9: Shows the correlations between all values that were measured through the production experiment.

	POC	DIC	pH	Temp	DO	TA
POC	-					
DIC	.400	-				
pH	<.001**	<.001**	-			
Temp	.196	.023*	.021*	-		
DO	.009**	<.001**	<.001**	.553	-	
TA	.002**	.005**	.888	.008**	.048*	-

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

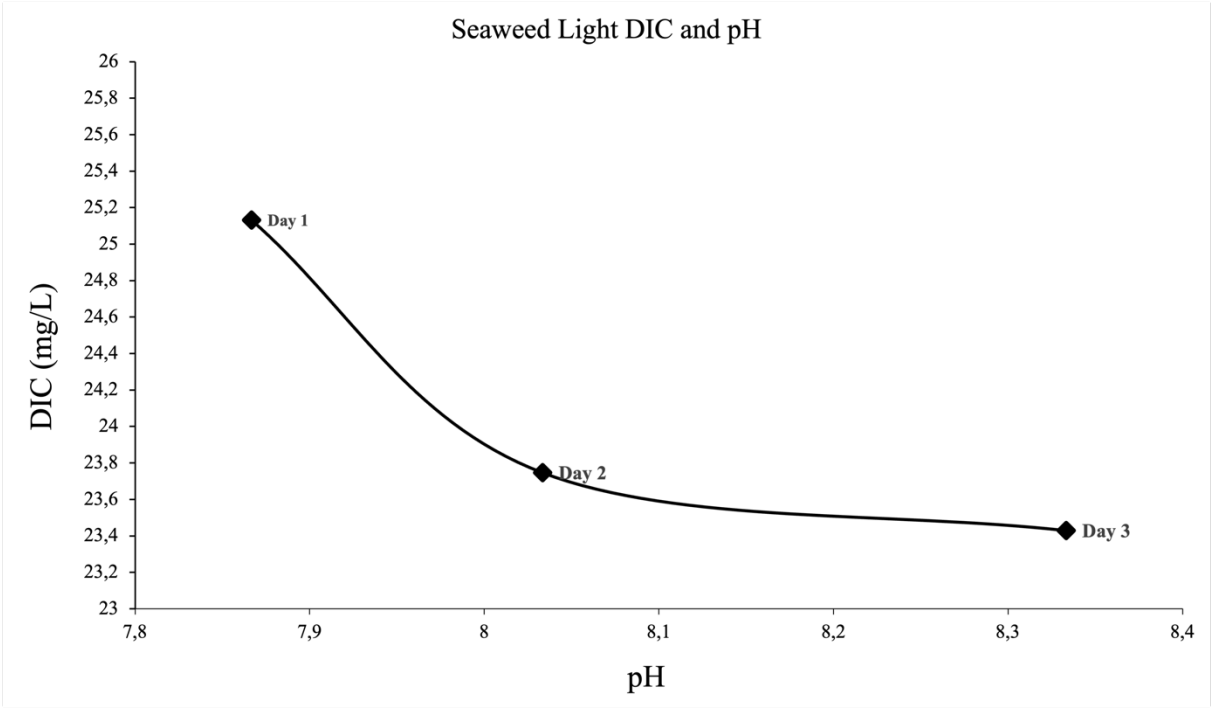

Figure 27: Relationship between dissolved organic carbon (DIC) and pH for Seaweed Light during the production experiment.

3.2 Long-term biodegradation experiment

3.2.1 Particulate organic carbon

Figure 28 shows the mean POC value of all bags from 10°C and 4°C treatments (duplicate values in each of 3 replicas). Graphs A Seaweed 10°C increases early (day 15) before decreasing steadily towards day 188. Graph B and C Seaweed 10°C show a similar trend, where POC values increase early (Day 30-60) and decreases towards Day 94, before increasing again at day 94. Graph D 4°C Seaweed increases until day 60, before rapidly decreasing. Graph E and F 4°C Seaweed shows a similar trend, but graph E has higher values.

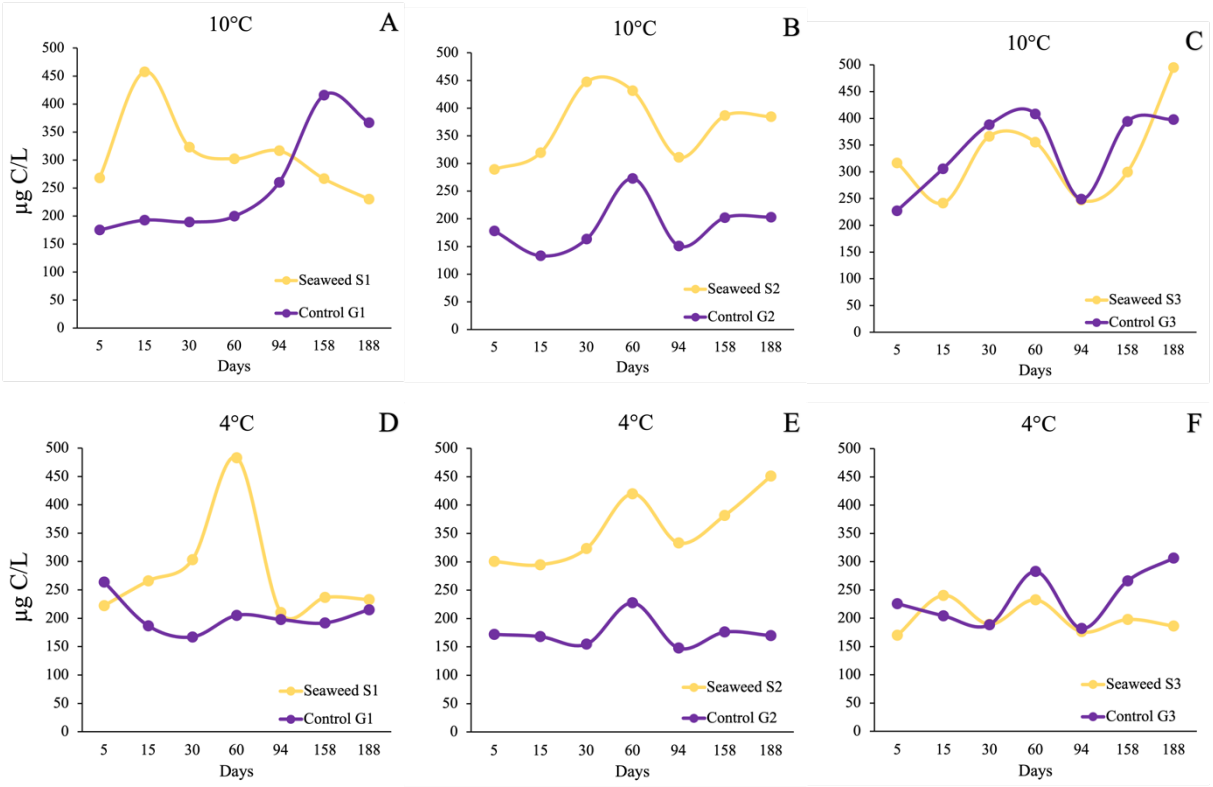

Figure 28: Temporal change of POC value in two different temperatures throughout seven sampling days. **ABC)** Each graph shows the mean POC values for 10°C-treatment and its development of Seaweed (n=2) (10°C S1, S2, S3) and Control (n=2) (10°C G1, G2, G3) during 188 days. **DEF)** Each graph shows the mean POC values for 4°C-treatment and its development of Seaweed (n=2) (4°C S1, S2, S3) and Control (n=2) (4°C G1, G2, G3) during 188 days. All graphs (N=24) have same scale to better visualize differences.

Figure 29 shows the % remaining POC at day 188 of the long-term degradation experiment for both treatments. RPOC is assumed to be fraction of POC stable after 188 days. “RPOC 10°C” accounted for 5.18% while “RPOC 4°C” accounted for 6.51%. A two-sample t-test was conducted to determine whether “RPOC 10°C” was different from “RPOC 4°C”. The mean value from “RPOC 10°C” (M = 47.50, SD = 164.86) was not significantly different than “RPOC 4°C” (M = 59.69, SD=203.81); t(4) = -.081, p = .940.

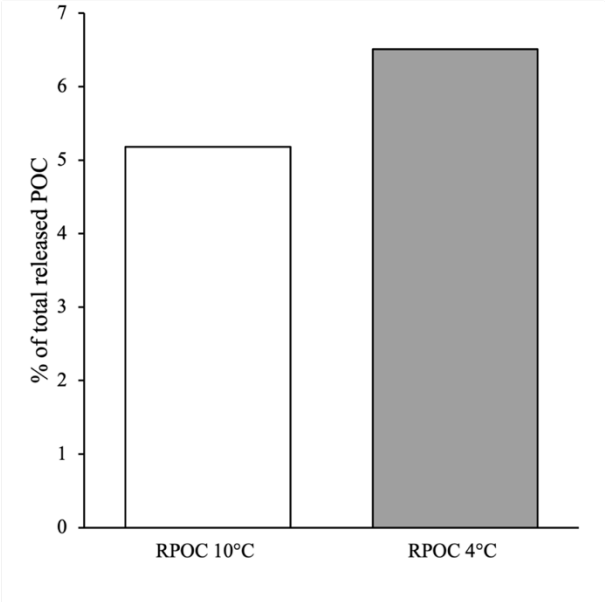

Figure 29: Mean % of RPOC remaining after 188 days of long-term degradation experiment. Controls were deducted from seaweed water to calculate RPOC values.

A two-way repeated ANOVA was performed to analyze the effect of temperature (4°C and 10°C) and time (5, 15, 30, 60, 94, 158 and 188 days) on POC concentration. The repeated analyses of variance showed that there was a statistically significant interaction between the effects of temperature and time on mean POC values, F(3.592, 10.777) = 6.56, p = <.001. (or F(3.592, 10.777) = 2.13, p = .029).

Table 10 shows a pairwise comparison of the mean POC values based on the combination of days and treatment. The comparison found that the mean POC value of “Seaweed 10°C” was statistically significantly different from “Control 10°C” on three different days, p = <.05 while “Seaweed 4°C” was statistically significantly different from “Control 4°C” on two different days, p = <.05. The pairwise comparison found no statistically significant difference between “Seaweed 10°C” and “Seaweed 4°C” on any days, p = >.05.

Table 10: Pairwise comparison of mean POC difference between treatments based on days. The tables show four different treatments, and their comparison during 188 days.

Days	Treatment 1	Treatment 2	Sig.
Day 5	Seaweed 10°C	Control 10°C	.009*
Day 15	Seaweed 10°C	Control 10°C	.020*
Day 30	Seaweed 10°C	Control 10°C	.013*
Day 60	Seaweed 10°C	Control 10°C	.269
Day 94	Seaweed 10°C	Control 10°C	.050
Day 158	Seaweed 10°C	Control 10°C	.756
Day 188	Seaweed 10°C	Control 10°C	.908

Days	Treatment 3	Treatment 4	Sig.
Day 5	Seaweed 4°C	Control 4°C	.804
Day 15	Seaweed 4°C	Control 4°C	.060
Day 30	Seaweed 4°C	Control 4°C	.010*
Day 60	Seaweed 4°C	Control 4°C	.029*
Day 94	Seaweed 4°C	Control 4°C	.067
Day 158	Seaweed 4°C	Control 4°C	.248
Day 188	Seaweed 4°C	Control 4°C	.487

*. The mean difference is significant at the .05 level.

Figure 30 shows the temporal change of mean POC value of all seaweed and controls in the two different temperatures. All treatments showed approximately the same trend throughout the 188 days.

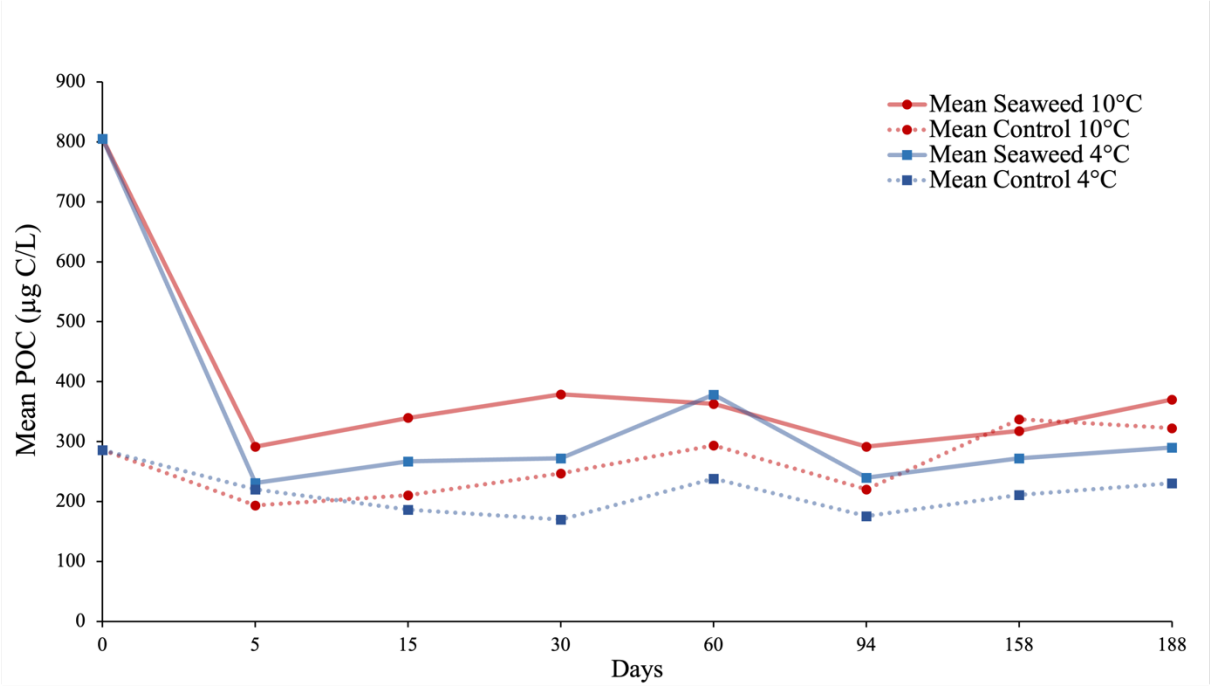

Figure 30: Temporal change of mean POC values during eight sampling points. Day 0 was Day 4 of the production experiment. Values are means from combined bags within each treatment.

3.2.2 pH

Figure 31 shows the mean pH values of all bags from 10°C and 4°C treatments (duplicate values in each of 3 replicas). Seaweed 10°C (graphs ABC) shows an early increase in pH at Day 15, before steadily decreasing until day 188. Control 10°C (graphs ABC) also shows an early increase in pH at day 15 but continues more stable until day 94, before it decreases. Seaweed and control 4°C (graphs DEF) shows an increase in pH until day 60-94, before decreasing until day 188.

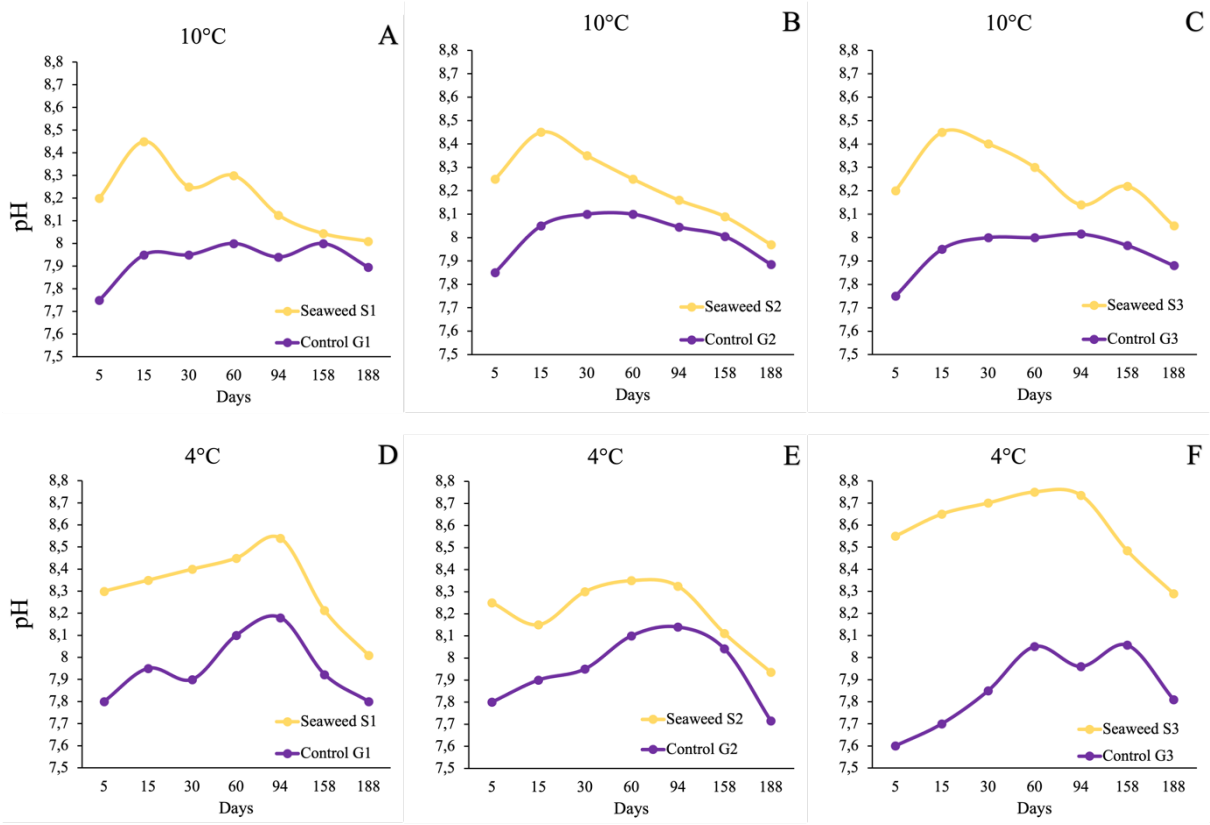

Figure 31: Temporal change of pH in two different temperatures throughout seven sampling days. **ABC)** Each graph shows the mean pH values for 10°C-treatment and its development of Seaweed (n=2) (10°C S1, S2, S3) and Control (n=2) (10°C G1, G2, G3) during 188 days. **DEF)** Each graph shows the mean pH values for 4°C-treatment and its development of Seaweed (n=2) (4°C S1, S2, S3) and Control (n=2) (4°C G1, G2, G3) during 188 days. All graphs (N=24) have same scale to better visualize differences.

3.2.2.1 Difference between days and treatments

A two-way repeated ANOVA was performed to analyze the effect of temperature (4°C and 10°C) and time (5, 15, 30, 60, 94, 158 and 188 days) on pH values. The repeated analyses of variance showed that there was a statistically significant interaction between the effect of temperature and time on mean pH values, $F(3.757,11.271) = 11.15$, $p = <.001$.

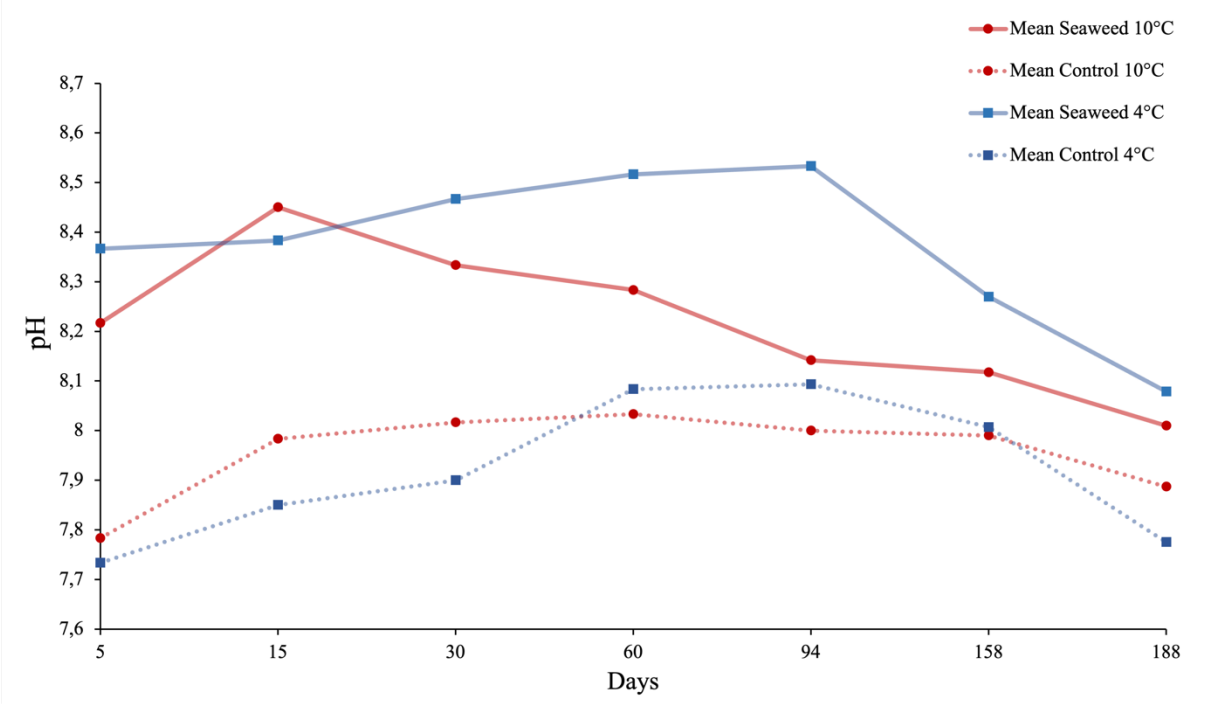

Figure 32: Temporal change of mean pH values during eight sampling points. Day 0 was Day 4 of the production experiment. Values are means from combined bags (n=6) within each treatment (N=24).

Table 11 shows a pairwise comparison of the mean pH values based on the combination of days and treatment. The comparison found that the mean pH value of "Seaweed 10°C" was statistically significantly different from "Control 10°C" until Day 60, p = <.05. From Day 94 until Day 188 no statistically significant difference was found, p >.05. "Seaweed 4°C" was statistically significantly different from "Control 4°C" on all days, p = <.05. "Seaweed 10°C" and "Seaweed 4°C" were statistically significantly different on Day 60, p = 0.30, and Day 94, p = <.001, but not on other days, p = >.05.

Table 11: Pairwise comparison of mean pH difference between treatments based on days. The tables show four different treatments, and their comparison during 188 days.

Days	Treatment 1	Treatment 2	Sig.
Day 5	Seaweed 10°C	Control 10°C	<.001*
Day 15	Seaweed 10°C	Control 10°C	<.001*
Day 30	Seaweed 10°C	Control 10°C	.007*
Day 60	Seaweed 10°C	Control 10°C	.020*
Day 94	Seaweed 10°C	Control 10°C	.097
Day 158	Seaweed 10°C	Control 10°C	.120
Day 188	Seaweed 10°C	Control 10°C	.117

Days	Treatment 3	Treatment 4	Sig.
Day 5	Seaweed 4°C	Control 4°C	<.001*
Day 15	Seaweed 4°C	Control 4°C	<.001*
Day 30	Seaweed 4°C	Control 4°C	<.001*
Day 60	Seaweed 4°C	Control 4°C	<.001*
Day 94	Seaweed 4°C	Control 4°C	<.001*
Day 158	Seaweed 4°C	Control 4°C	.003*
Day 188	Seaweed 4°C	Control 4°C	<.001*

Days	Treatment 1	Treatment 3	Sig.
Day 5	Seaweed 10°C	Seaweed 4°C	.095
Day 15	Seaweed 10°C	Seaweed 4°C	.555
Day 30	Seaweed 10°C	Seaweed 4°C	.227
Day 60	Seaweed 10°C	Seaweed 4°C	.030*
Day 94	Seaweed 10°C	Seaweed 4°C	<.001*
Day 158	Seaweed 10°C	Seaweed 4°C	.067
Day 188	Seaweed 10°C	Seaweed 4°C	.387

*. The mean difference is significant at the .05 level.

3.2.3 Dissolved Oxygen

Figure 33 shows the mean DO values of all seaweed and control bags from 10°C and 4°C treatments (duplicate values in each of 3 replicas). Both temperatures (graphs AB) show similar trends with increasing DO until Day 94, before decreasing until Day 188.

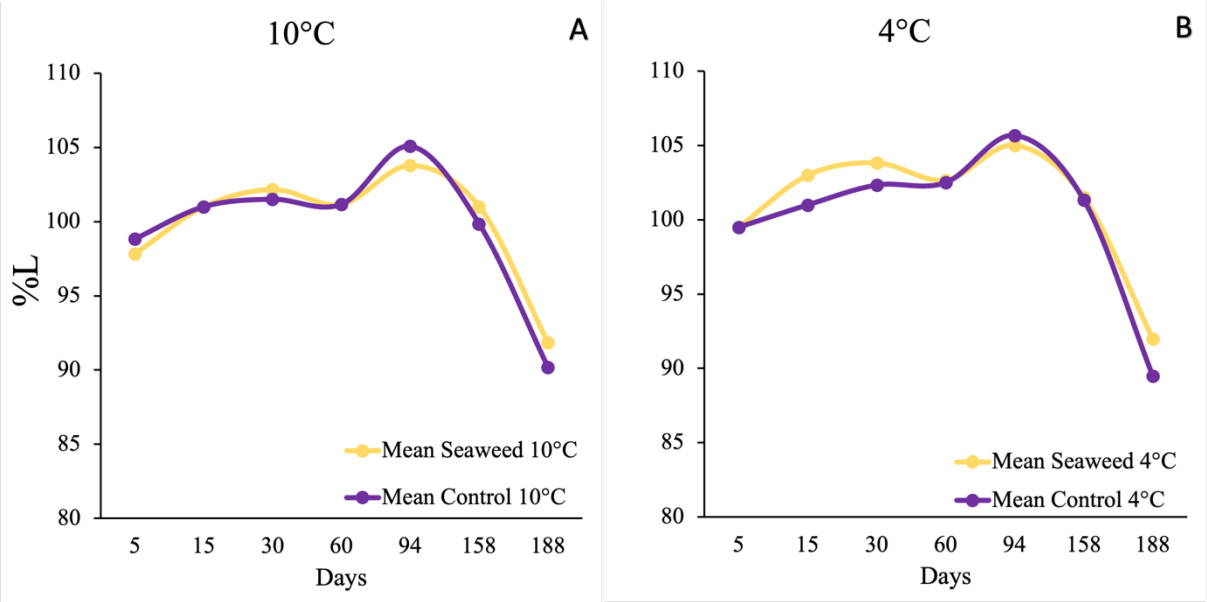

Figure 33: Mean values of dissolved oxygen during 7 sampling points. **A)** Yellow line shows mean POC values from seaweed bags (n=6) from 10°C treatment, while dashed line is control (n=6). **B)** Yellow line shows mean POC values from seaweed bags (n=6) from 4°C treatment, while dashed line is control (n=6).

3.2.3.1 Difference between days and treatments

A two-way repeated ANOVA was conducted to analyze the effect of temperature (4°C and 10°C) and time (5, 15, 30, 60, 94, 158 and 188 days) on DO concentration. The repeated analyses of variance showed that there were no statistically significant interaction between the effects of temperature and time on DO values, $F(6,18) = 1.62$, $p = .072$.

3.2.4 Long-term experiment correlations

Spearman's rho correlation showed statistically significant correlation between pH and DO (Controls 10°C), $p = .001$, pH and DO (Seaweed 4°C), $p = .019$ and pH and DO (Control 4°C), $p = <.001$. No statistically significant correlation was found for "Seaweed 10°C". All correlation coefficients were $< \pm .500$.

3.3 Dissolved organic carbon

3.3.1 NPOC pre-tests

To determine whether samples needed to be diluted before starting to analyse bigger batches, comparisons of manually diluted and undiluted samples were conducted with an OK calibration curve (R^2: 1.0000). Figure 34A shows "Seaweed Light D1" from day 3 of the production experiment. The diluted sample was not at it expected range (25%), but at 12.6%, while Figure33B shows "Seaweed Dark D4" from day 3. The diluted sample was not at it expected range (25%), but at 10.9%. "Seaweed Light D1" had a mean 1.06mg/L, while "Seaweed Dark D4" had a mean 2.16mg/L. Figure 34C shows the comparison of "Control Light D8" and "Control Dark D11" from day 3, where "Control Dark" showed the highest value of the two.

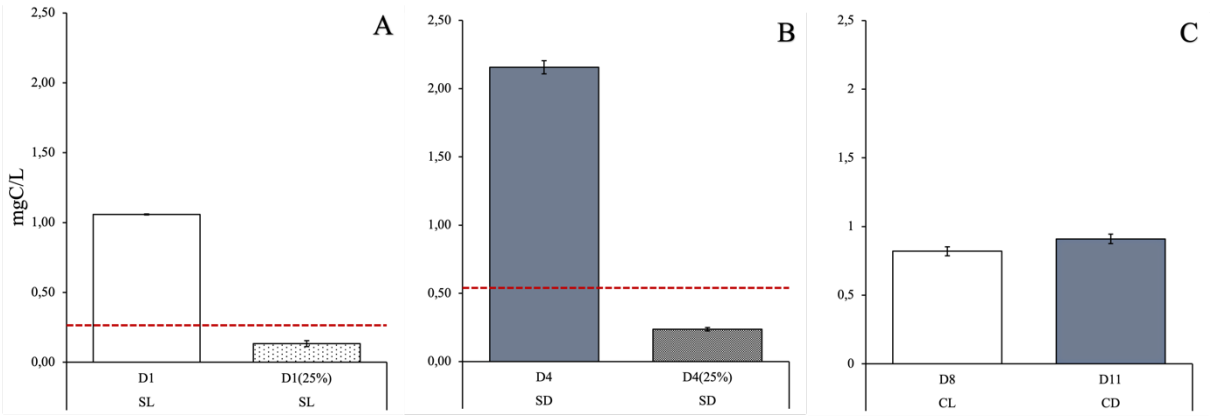

Figure 34: Mean concentrations (± SD) of DOC samples from day 3 of production experiment. Each sample (n=1) had up to 3 injections. The x-axis shows the label for each bag. SL = Seaweed Light, SD = Seaweed Dark, CL = Control Light, CD = Control Dark. **A)** Comparison of D1 undiluted and diluted down to 25%. Red striped line shows the actual 25% limit of undiluted sample. **B)** Comparison of D4 undiluted and diluted down to 25%. Red striped line shows the actual 25% limit of undiluted sample. **C)** Comparison of Control Light (D8) and Control Dark (D11) samples.

3.3.2 NPOC Controls

Figure 35ABCD shows the mean concentration from DOC controls (Control Light and Control Dark) during the production experiment. The calibration curve was OK (R^2: 0.9734), but samples show relatively high SD. No statistical analyses were conducted since Figure 35E shows the trend of how "REF-S" developed throughout the analyses. "REF-S1" was analysed at 3 different occasions during the analyses run, while "REF-S2" were analysed 2 times (Appendix B). All samples were outside their expected limit (±10%). "REF-S1(1)" started the closest to its expected value but after that, the value decreased for "REF-S1(2&3)". "REF-S2(1)" had the lowest value, before increasing (REF-S2(2)).

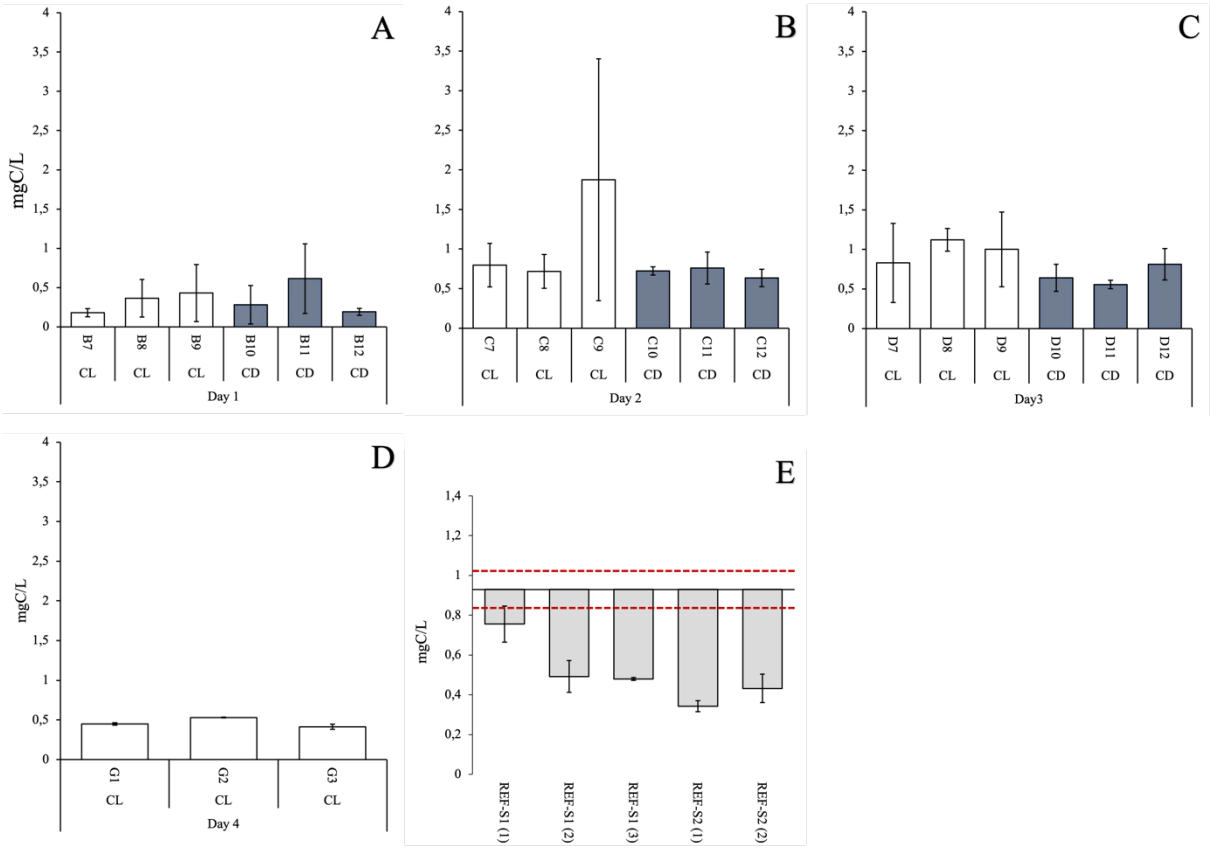

Figure 35: ABCD) Mean concentrations (± SD) of DOC controls from day 1-4 of the production experiment. Each sample (n=2) had up to 3 injections. The x-axis shows the label for each bag. CL = Control Light, CD = Control Dark. **E)** Mean concentration (± SD) of certified reference material, REF-S1 and REF-S2 (DOC from surface water). Brackets show which sequence sample was analysed. X-axis starts at expected REF-S value on y-axis (0.929574 mgC/L), and red striped line shows ± 10% of deviation.

3.3.3 CRM, ICV, CCV and spike tests

After several batches of DOC samples were analysed, and values gave misleading and confusing results, tests were conducted to determine whether it was something wrong with samples or the TOC-L analyzer. Figure 36AB shows CRM-tests conducted with a sampels which was suspected to be old. The run was done with the same calibration curve error (R^2: 0.8943), where results were decreasing drastically for each sample. Figure 36CD shows the CRM-test conducted at a later stage, with a new batch of samples. The run was done with an OK calibration curve (R^2: 0.9994). "REF-S" and "REF-M" results were far away from expected values, and they were also violating ± 10% deviation (red striped line), having deviated negatively with 20.10% and 21.01%, respectively. Figure 36E had the same calibration curve as CD, but the ICV was inside ± 10 % deviation. Figure 36F shows "CCV" and "spike" (glycine) test, with an OK calibration curve (R^2: 0.9978). "CCV" was stable at expected value

(2.5mgC/L), while "spike" started at 1.435 mgC/L (43.45% over expected value of 1 mgC/L) but decreased slightly over the next two samples.

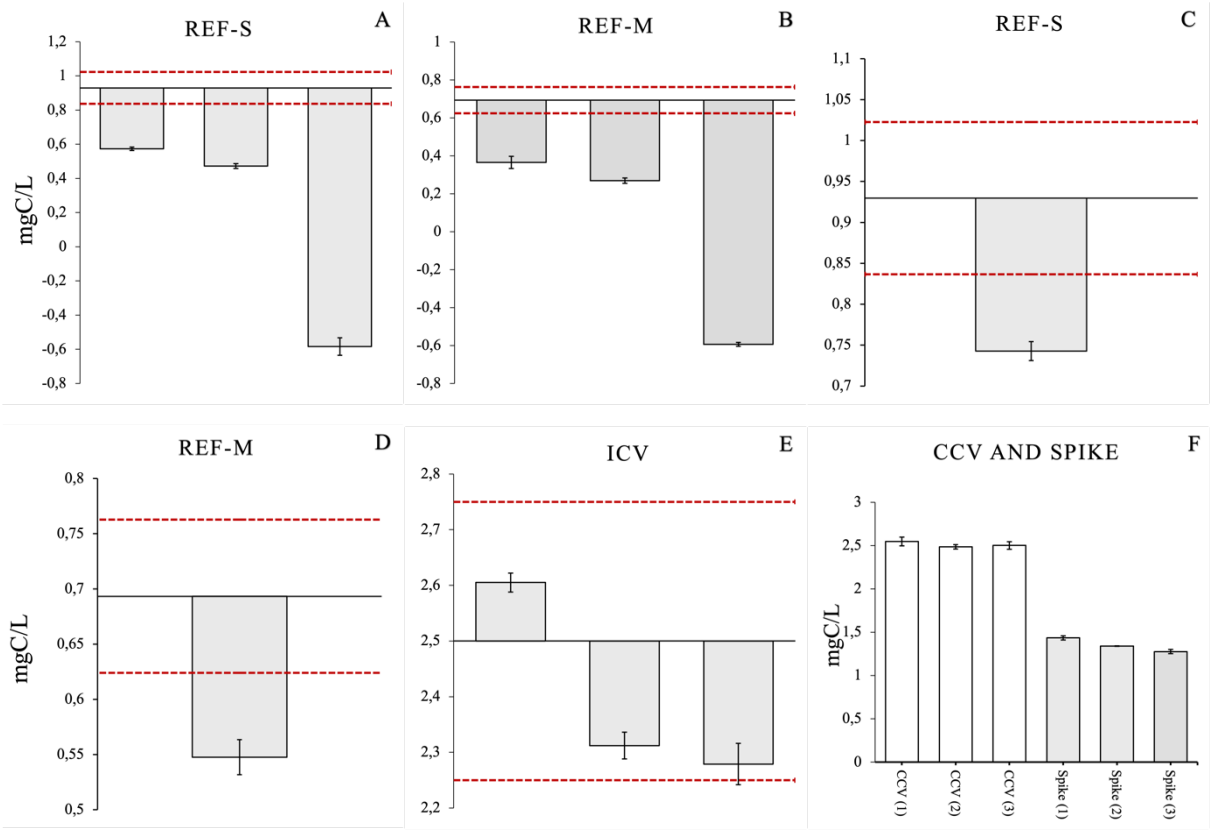

Figure 36: AB) Mean concentration (± SD) of ("old") certified reference material (CRM) (DOC from surface and mid water). **CD)** Mean concentration (± SD) of ("new") certified reference material (CRM) (DOC from surface and mid water). All bars were from same sample, and each bar had up to five injections, where two were excluded. X-axis starts at expected REF-S value on y-axis (0,929574 mgC/L) and the same for REF-M value at y-axis (0,693377 mgC/L). Red striped line shows ± 10% of deviation. **E)** Mean concentration (±SD) of internal calibration verification 2.5mg (ICV). All bars were from same sample, and each bar had up to five injections, where two were excluded. Red striped line shows ± 10% deviation. F) Mean concentration (± SD) of continuous calibration verification 2.5mg (CCV) and glycine 1mg (Spike).

3.3.4 Comparison of NPOC with MQ vs SW matrix

The CRM's contained SW matrix, while ICV, CCV and spikes contained MQ-water, therefore a test to determine differences of the matrixes were conducted. Figure 37 shows the comparison of NPOC in solutions with MQ vs SW matrix. The visual difference between SW and MQ matrix is minimal, so was their R^2 values (SW = 0.9993 vs MQ = 0.995). An independent t-test was conducted to compare the different matrixes, but no statistically significant difference was found, p = .453.

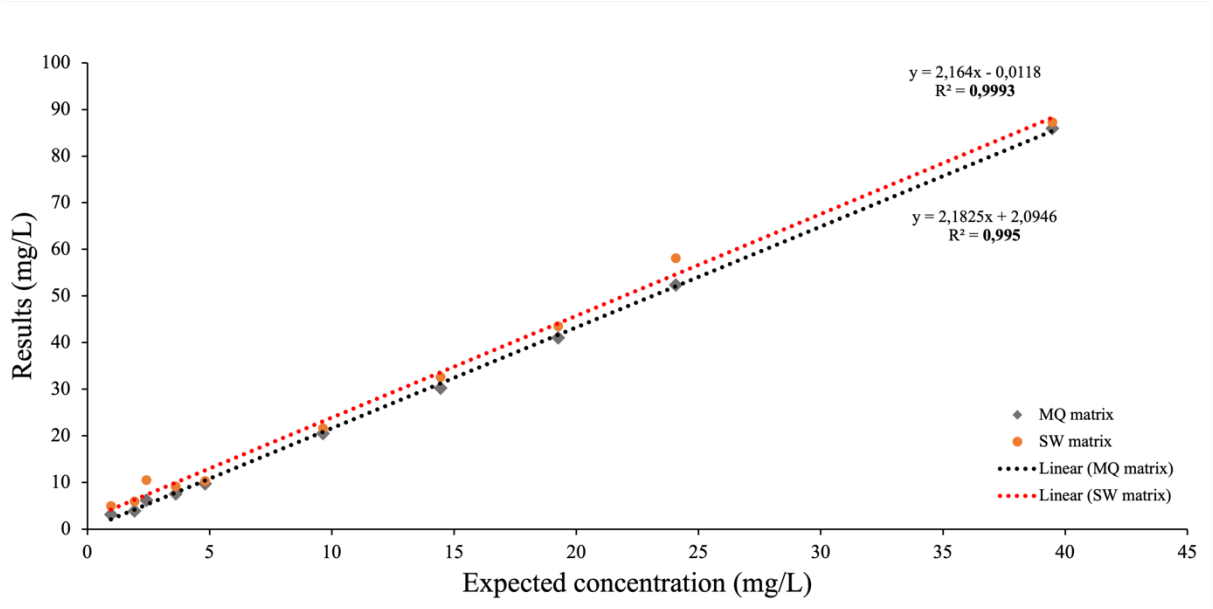

Figure 37: Comparison of concentration and linear trendline of Non-Purgeable Organic Carbon (NPOC) in Milli-Q water and NPOC in Seawater. All samples were acidified to pH ≤ 2 before analyses.

4 Discussion

4.1 Production experiment

To better understand the importance of *S. latissima* here in Norway, or generally seeing the value of seaweed around the world, the development of state-of-the-art experimental methods is needed, to better compare and quantify the crucial role *S. latissima* has in our ecosystem. Not the least, to be able to determine how big the potential is for carbon capture and storage (CCS), experiments need to be conducted in small scales before brought into the industry where the three ground pillars of sustainability enters and demands decisions based on scientific data, and not gut feelings (Duarte, Bruhn, et al., 2022) .

In the present study, a mesocosm experiment was performed over four days to better understand the behaviour between *S. latissima* and its environment. Growth and production of seaweeds is determined by biotic and abiotic factors including herbivory, inorganic nitrogen, irradiance, temperature, salinity, pH and CO_2 concentrations (Paine et al., 2021). The relationship between these variables can determine whether CCS with seaweed is effective or not.

During late spring in Norway, ambient nitrate concentrations is not at its highest, which is a key growth factor for *S. latissima.* In this period, the kelp mainly relies on intracellular nitrogen components, which can vary greatly between plants (Jevne et al., 2020). In a period as short as four days in the end of May, SGR between the different treatments did not show any significant difference, even though the strain has the potential to grow 1-2 cm day^{-1}, with an average in March-May being 0.75 cm day^{-1} (Nielsen et al., 2014) under the right circumstances. Water temperature in the basin was continuously decreasing throughout the days, showing significant temperature changes in bags which overall could be a contributor to lower growth rates for the kelp that was sampled on the last day.

4.1.1 Light vs dark

In mid-Norway the main production period for *S. latissima* lasts from January to May/June, with an additional growth period possible with early deployment in September/October. In such a short season, the seaweed goes through periods with reduced amounts of light and up to days of continuous light. The further north production gets, the less light during winter months. This impacts growth, uptake of key nutrients and DIC and release of organic material (Nielsen et al., 2014; Bruhn et al., 2016; Martins et al., 2022). With the two treatments, transparent and dark bags, there were a clear difference in most cases. Transparent bags let the process of photosynthesis develop and take advantage of sunlight, inorganic carbon and water, while the

dark atmosphere prevented it. The transparent bags containing seaweed showed a significant decrease of DIC (Figure 18) compared to other treatments, meaning *S. latissima* effectively used the inorganic carbon through photosynthesis while greatly producing oxygen (Figure 24, mean 144% ± 19.6% SD), but there were no indications of increased microbial respiration. The significant reduction of DIC is also supported by Watanabe et al. (2020), which suggested that macroalgal beds could serve as CO_2-sinks.

The seaweed dark treatments showed the opposite trend, microbial respiration increased the DIC minimally (Santos et al., 2021), but enough to see significant difference between days, where day 3 stood out. Most of the CO_2 is then converted to carbonic acid through hydration processes, which dissociates into bicarbonate and carbonate, making the water acidic, meaning a decrease in pH (Pinet, 2019). The decrease can be seen on DO levels as well, where the small significant decreasing trend in oxygen levels (Figure 24) for dark seaweed treatments mean that bacterial degradation is happening while they are respiring (Robinson, 2019). This data could suggest that the organic carbon is more recalcitrant than labile. If the organic carbon would have been labile, the oxygen drop would most likely be faster and bigger, due to higher bacterial degradation, but this is hard to state without a look into DOC, where the biggest potential of recalcitrant carbon lays.

Another factor contributing to tell the same story is pH values (Figure 20). In a natural seaweed ecosystem, or dense farms, pH increases during daytime when plants are photosynthesizing (Middelboe & Hansen, 2007; Krause-Jensen et al., 2015; Duarte et al., 2017) taking up CO_2 which regulates the acidity of the seawater (Pinet, 2019). The same trend was shown in the transparent treatments containing seaweed, significantly different from all other treatments. There was a small pH and DO increase in transparent control treatments most likely due to phytoplankton activity, but so minimal that it didn't show any significant difference from control dark treatments. Dark seaweed treatments showed a statistical lower pH value throughout the 3 days, compared to all other treatments, which supports the fact that there was an increase in DIC in the same treatments. There was a statistical correlation between pH and DIC (Table 9), but the correlation coefficient was not high enough to conclude the relationship in dark seaweed treatments.

The relationship between DIC, pH and TA is as well interesting to investigate. Changes in pH (and pCO_2) remain smaller in well buffered seawater with a high TA:DIC ratio. Even though DIC is decreasing in transparent seaweed treatments, due to photosynthesis, TA did not

increase. A slight increase would be expected since removal of CO_2 leads to a higher pH, which again can lead to dissociation of single charged HCO_3^- into double charged CO_3^{2-}, which due to its double negative charge increases TA. However, this is not visible in any of the transparent seaweed treatments, which underlines how effective *S.latissima* was during photosynthesis. It was showing a strong correlation between the uptake of DIC and increase of pH (Figure 27). This compensates the TA increase by converting HCO_3^- into CO_2 (by Carbonic anhydrase), making TA decrease slightly on day 3 (Figure 25). This results in in a lower TA:DIC ratio, which again increases pH change (Zeebe & Wolf-Gladrow, 2001; Middelburg et al., 2020). This is also supported by Axelsson and Uusitalo (1988), which have shown TA decrease through cation generation, likely a result from proton addition to seawater (Stepien et al., 2016).

4.1.2 Particulate organic carbon

Generally, *S. latissima* contains more carbon during summer and autumn months, than the spring months (Black, 1950; Mann, 1973), resulting in an increased release of carbon into the adjacent water during warmer periods (Nielsen et al., 2014). Growth of the kelp occurs during photosynthesis, when the inorganic carbon is fixed into organic carbon to build carbon skeletons (Paine et al., 2021), and in the same process organic carbon is released through several mechanisms into the ecosystem.

One theory suggests that organic carbon is released passively during cell growth and lysis, or actively due to exudation of photosynthates (Bjørrisen, 1988; Morán & Estrada, 2002). Another theory suggests that organic carbon is released into adjacent ecosystems due to excessive carbon relative to the available nutrients, meaning organic carbon released should increase with nutrient depletion, and vice versa (Weigel & Pfister, 2021). These processes are often overlooked when suggesting carbon fixation and sequestration with seaweeds, but they are important factors to investigate for potential species in both temporal and spatial scales. Unfortunately, to contribute with such knowledge, analyses of DOC would need to be conducted to calculate NPP, and thereby the % of released carbon based on NPP.

Looking at the POC values in Figure 16, an increasing rate is seen throughout the production experiment, with POC accumulating especially on day 3 and 4. However, the POC release rates (Figure 15) shows another story of the trend, which is varying over the span of 4 days. Day 1 showed a high release rate based on DW, but the DW data for all seaweed (transparent and dark treatments) from day 1 could be inaccurate. When kelp was dried the first day, it was approximately 20h longer in the oven than other days, most likely resulting in a lower DW. If

DW would have been higher, release rates would decrease on the respective day. Taking that into account, results might also indicate a higher stress factor during the first day, due to handling of the kelp, but POC values from the acclimatization tank did not support that theory (Figure 14A). The POC values from the acclimatization tank (per kelp) showed low values, meaning either low stress release, or unrepresentative water samples from the top of the tank. However, earlier studies have shown that removing the holdfast from its substrate, introducing kelp into new conditions or even desiccation can lead to an increased release rate of organic carbon, and thereby skew results (Moebus & Johnson, 1974; Paine et al., 2021). Day 2, 3 and 4 showed increasing POC release each day, which is backed up with uptake of DIC in the respective bags. Seaweed dark treatment showed a decreasing POC release trend, meaning both ineffective photosynthesis and increased microbial degradation of the organic carbon.

To date there are not many POC-release studies conducted on comparable species. Feng et al. (2022) conducted an experiment with *S. japonica,* which showed approximately 10x higher POC values. On the other hand, they used tissue samples which were cut into pieces, most likely resulting in excessive release of POC due to stress, and temperatures far exceeding ours. Wada et al. (2007) showed with *Ecklonia cava* values that might be comparable, ranging from approximately 100-400 µg g^{-1} DW d^{-1}, during different seasons, compared to the obtained results of approximately 300-1400 µg g^{-1} DW d^{-1}. Another study, done on *Pyropia haitanensis* showed more comparable values, ranging from 600-1700 µg g^{-1} DW d^{-1}, and their highest release rate occurred under the condition with the lowest nitrogen and phosphorus concentrations, indicating excessive carbon relative to nutrients (Xu et al., 2021). The lack of comparable data stresses the importance of further research needed to understand and quantify the release of POC, both at a temporal and spatial scales.

4.2 Long-term biodegradation experiment

Previous studies have shown that average released proportions of organic carbon as compared to total carbon varies to some extent. Chen et al. (2020) showed with 11 different seaweeds that mean DOC and POC released into the ecosystem was 14.39% and 9.02%, respectively. Dolliver and O'Connor (2022) showed with *S. latissima* that approximately 43% (33-58%) of carbon mass was lost through exudation during two different cultivation periods.

Released organic carbon into the adjacent ecosystem is an important food source fueling microorganisms (Ogawa et al., 1999; Ogawa et al., 2001; Trevathan-Tackett et al., 2020; Brunet et al., 2021; Li et al., 2022). As described in section 1.5, most of the carbon quickly degrades within the first few hours and days (LDOC and LPOC), while some parts manage to persist for a bit longer (SLDOC and SLPOC), but a fraction can survive for decades (RDOC and RPOC) (Hansell, 2013). The released carbon will then travel with water masses, but POC and DOC will react differently to the same export conditions. Transport of POC is related closely to its size and buoyancy, and might even settle in nearby benthic environments, while DOC will freely move with tides and currents (Hyndes et al., 2014). It is therefore necessary to look closely into how the organic carbon reacts in different environments, especially when the global ocean temperatures is on a rise (Cheng et al., 2019; Filbee-Dexter et al., 2022).

The long-term experiment showed that temperature (10°C and 4°C) had no impact on POC values throughout the 188 days. Lønborg et al. (2009) had a similar outcome with three different temperatures (8°C, 14°C and 18°C), suggesting the amounts of mineralized POC was temperature independent, even though low temperatures have been shown to alter mineralization rates by bacterial communities (Nedwell, 1999). One factor that might have contributed to no difference was the temperature fluctuations in the 4°C-room. The room temperature was set by a Dixell Cool mate (XLR100-series), which was showing indications of big variations at times, it was observed ranging between 4°C and 6°C, meaning a 50% increase in temperature.

4.2.1 Temporal change on particulate organic carbon

As seen in Figure 28, individual POC values fluctuates during the long-term experiment, even though the mean values (Figure 30) were more or less stable at the same rate for all treatments. The results obtained in the long-term biodegradation experiment showed that there was a small difference in RPOC between the two treatments. For the 10°C-treatment, 5.2% of the total POC was RPOC, while for the 4°C-treatment, 6.5% of total POC was RPOC after 188 days (Figure

29). Temperature might play a role, but these differences do not show any clear trend. Feng et al. (2022) showed with *S. japonica* that 0.12% RPOC remained after 200 days, while Ishii et al. (2021) had approximately 20-30% RPOC left after 200 days (*U. ohnoi* and *U. pinnatifida*). Their calculations were based on %RPOC out of total carbon released, meaning our result is not comparable, since DOC values would be needed to obtain that data. However, it certainly shows that %RPOC would decrease relative to total carbon released.

There was not much distinguishing the seaweed water with controls, meaning that after filtering the water during day 0 of the long-term experiment, it eliminated almost all POC. The water was filtered on day 0 following relevant methods to compare results (Watanabe et al., 2020; Gao et al., 2021), but in retrospect it inhibited the value of temporal changes in POC. The values range between 291-379 µgC/L (± 37 µg) for Seaweed 10°C and 231-378 µgC/L (± 49 µg) for Seaweed 4°C, which is comparable to values shown by Feng et al. (2022), after an incubation period of 120 days (396.33 ug ± 4.8 µg). From day 120 until 210 their values were stable, suggesting RPOC. However, their initial POC values during day 0-120 was much higher. They did not filter their water pre incubation, meaning an initial high POC value in their bags from the start. There is also no mentioning of which filter was used for POC determination, meaning it could be smaller than 0.7µm, leading to an increase in particles (Kawasaki et al., 2011). They also had an excised piece of the lamina inside of their bags, most likely leading to an excessive amount of carbon released due to stress.

The fluctuation in POC values (Figure 28) could be related to several factors. Coagulation of DOC into POC could most likely be an explanation, but further investigation into DOC values would be needed to conclude anything. However, the decline in POC is most likely underestimated due to coagulation. Another factor is sample heterogeneity, resulting in varying amounts of POC values throughout the experiment. Although, this is unlikely because the experiment bags were shaken prior to sampling. However, another element that could be considered is the GF/F filter that was analysed for POC. Several studies have shown variations in POC values due to the sampling methods (Moran et al., 1999) or even GF/F filters (Novak et al., 2018).

Vacuum filtration, as used in this study, can lead to POC being pulled through the filters in varied intervals, leading to fluctuating results in experiments (Gardner et al., 2003; Cetinić et al., 2012). Furthermore, $1/16^{th}$ of the filter was cut out, analysed and quantified to determine total volume; this might result in an unrepresentable amount, even though in probability theory,

the law of large numbers would correct it and should thus be close to the true value (Hsu & Robbins, 1947). However, working with such a small analyte (<500 µg C) even with adequate precautions, could lead to these uncertainties. Still, a more interesting explanation for these observations could be the relationship between autotrophic and heterotrophic microorganisms.

As explained in the method section 2.5.2, inoculum was added to have a natural culture of both autotrophic and heterotrophic organisms inside the bags (Lønborg et al., 2009; Gao et al., 2021). Li et al. (2022) used bigger filters to add inoculum during their experiment, while Watanabe et al. (2020) did not add inoculum, due to their understanding of a significant fraction of bacteria would naturally pass GF/F-filters. Yet, the latter study suggest DOC to be <0.2µm (Bauer & Bianchi, 2011), going against the mainstream of DOC and POC-researchers (Turnewitsch et al., 2007; Hansell, 2013; Halewood et al., 2022). Regardless of adding or not adding inoculum, all papers agree and assume autotrophic bacteria not to grow, since bags were stored in the dark. Still, results might indicate otherwise.

In environments where light is limited or absent (benthic zone), certain autotrophic bacteria have developed several metabolic pathways to utilize inorganic carbon (Hügler & Sievert, 2011). One would assume that these bacterial communities are not part of the epipelagic zone, where photoautotrophic organisms utilize sunlight. However, Prazeres and Renema (2019) suggest that in coastal waters, light might not penetrate deeper than 50m, while in the open ocean it might reach down to 200m. Our sample water used during the experiment was taken from 80m, in the coastal water outside TBS, meaning it could potentially include autotrophic bacteria using the well-known Calvin-Benson cycle (Benson et al., 1950), or one of the primary pathways employed by autotrophs in the absence of light, the reductive tricarboxylic acid (rTCA) cycle (Evans et al., 1966). These cycles in combination with heterotrophic degradation could explain why POC and pH is changing.

Within the carbon cycle, heterotrophic microorganisms break down and consume complex organic compounds (LPOC) releasing CO_2 as a by-product through respiration. As expected, this would result in a lower POC values during the experiment, but also a lower pH. On the other hand, if autotrophic microorganisms utilize the released CO_2 from heterotrophic respiration, synthesizing organic carbon, it could release DOC which coagulate and forms POC. This would lead to higher POC values, and higher pH readings, which is shown throughout the experiment. However, as stated earlier, DO values does not have the same trend. Without any additional data about DOC and bacterial community, it is hard to conclude anything specific.

Yet it shows the complexity of these ecosystems, where different metabolic strategies is what keeps the ecosystem functioning.

It can be assumed that temperature impacts the rate of degradation based on the pH trend in the two temperature treatments. There was no significant difference between the two except between day 60-94, yet their trend was visually different. Seaweed 4°C-treatment increased steadily before decreasing at day 94, while seaweed 10°C-treatment decreased after day 15. DO on the other hand did not follow the same changing trend as POC and pH. The oxygen levels showed a slight increase for all treatments until day 158, before it decreases rapidly (Figure 33). The microbial activity was likely too low to have an impact on oxygen levels because it is expected to decrease during heterotrophic respiration. The sudden decrease on day 188 can most likely be explained by an uncalibrated sensor, which was detected after the sampling had been done. Based on both pH and POC values from the same day, and the general trend of oxygen throughout the experiment, there was no certain indications that would result in the decrease.

4.3 Dissolved organic carbon

Recalcitrant carbon is where the big potential in carbon storage lays. Potentially released directly from the lamina as RDOC, or degraded from POC into RDOC, resistant to further degradation and eventually ending up deep into the deep abyss for thousands of years (Carlson et al., 1994; Paine et al., 2021). A variety of studies is suggesting 14-62% of NPP is released as DOC, proving how big the range of release is based on variables like ecosystems and species (Abdullah & Fredriksen, 2004; Wada et al., 2007; Reed et al., 2015).

Watanabe et al. (2020) showed a promising 56%-78% of macroalgal DOC being RDOC, Gao et al. (2021) concluded with 37.8 %, while Li et al. (2022) found 33%-58%, all three indicating relative high potentials for brown seaweeds (*S. japonica and Saragassum horneri)*. On the other hand, Feng et al. (2022) had only 1.27% left after their long-term degradation experiment with *S. japonica*. Ishii et al. (2021) looked into approximately 20 different species from the shallow coastal zone, but for green (*U. ohnoi*) and brown (*U. pinnatifida*) seaweed, the RDOC fraction was 3.5% and 11.9%, respectively. None of the mentioned studies were conducted using *S. latissima,* but Dolliver and O'Connor (2022) estimated based on Krause-Jensen and Duarte (2016) methods, that approximately 33% of the organic carbon released from *S.latissma* was recalcitrant, equivalent to 12kg sequestered organic carbon per 100m kelp longline. This thesis was built around these studies, trying to provide a cutting-edge method for DOC quantification

in the northern hemisphere with *S. latissima*. Unfortunately, instrumental issues meant that DOC results could not be analyzed in time.

4.3.1 Limitations and challenges

Based on previous research, expected range of DOC values were approximately 3.2-65 mg/L (Gao et al., 2021; Feng et al., 2022; Li et al., 2022). Therefore, to fit every sample into an accurate and OK calibration curve, dilution of samples was planned. However, the machine had a malfunction and could not automatically perform dilutions, and thereby a manual dilution test was conducted. The test used a perfect calibration curve, but test values were not. The diluted samples were supposed to be equivalent to 25% of original sample; both were in reality closer to 10%. This could be caused by heterogeneous samples, if samples were not shaken after freezing, but then one would expect a bigger deviation between the two diluted samples. An explanation could therefore be calculation error when diluting samples, but further analyses showed that the instrument might be the cause for error.

When comparing the undiluted samples (Figure 34) seaweed dark treatment (D4) had DOC values 104% higher than Seaweed Light treatment (D1), which could be explained due to decomposition in the dark treatment. However, Seaweed Light treatment had a higher POC release rate, higher POC accumulation, higher DO value and lower DIC levels compared to Seaweed Dark treatment. Therefore, one could assume DOC to have the same trend as POC in the respective treatment. Either decomposition may have been strong on day 3, or the TOC-L analyzer was precise, but not accurate.

During analyses of the control batch from the production experiment, CRMs (REF-S1 and S2) showed that the machine was not accurate, even though the calibration curve was OK and with a new batch of CRMs. The first REF-S was outside its 10% deviation range, meaning that the machine should have stopped the analyses, but it was kept going to check the trend. From then CRMs were far outside their range, which can be explained by the further away a sample is from the calibration curve, the less accurate the analyses will be, but not even one of the CRMs were accurate. In addition, precision of the machine was reduced with high SD, and when comparing results with the previous dilution test, D8 and D11 showed 31% and 48% value difference, respectively.

During several of these analytical sample trials conducted over many months, DOC results seemed inaccurate based on expected values for DOC, CRMs, CCVs, ICVs and spike solutions. This can have several explanations, but results from testing indicated systematic issues when

the calibration curve was OK, contraindicating that something was wrong with the machine, but rather the preparation of samples (stock solution, samples, spikes and CRMs). Testing also ruled out any difference between seawater and MQ-water matrixes. Though when the calibration curve was inaccurate, samples showed fluctuating values lower than the expected range and even negative values, emphasizing machine error. On the other hand, several recommended daily maintenance tasks were not done, which could potentially disrupt values.

Halewood et al. (2022) has recently documented a best practice for analysis of DOC in seawater, using TOC analyzers (TOC-L and TOC-V). Their Appendix D states 7 daily tasks to be done and checked before analysis, however during our sample run, 2 out of 7 tasks were done. 3 of their recommended tasks were to (1) clean the injection slider due to build-up of salts, but also (2) replacing the perchlorate trap and (3) halide scrubber, indicating high degradation of parts and the importance of quality assurance when working with a small analyte. Their protocol also included weekly and monthly tasks, which was not done. This was not because of ignorance, but rather the lack of knowledge within the scientific world; showing the importance of sharing knowledge.

Their best practice document goes through every step, from sample collection to analytical quality limits. One of many suggested requirements for documentation is incorporating "(…) details of problems or trouble-shooting that occurred with sampling or analysis" (Halewood et al., 2022, Documentation), highlighting the importance of sharing knowledge, even if it did not lead to novel results. One could argue that you learn best from your own mistakes, and therefore one should experience them, but within the biogeochemical world, 700 samples worth of mistakes could be costly.

4.3.1.1 What went wrong?

Peltzer and Brewer (1993) explained what it takes to study DOC:

> The proper analysis of DOC in marine samples requires more than just the 'right' catalyst and the 'right' operating conditions. It requires painstaking attention to detail from the beginning of sample collection all the way through to the last steps of the analysis and data interpretation. (Conclusions and recommendations)

Throughout the production experiment (4 days), approximately 900L of water was filtered. During the first days, filtration units were rinsed in unfiltered sample water, both the funnel and inside the flask, meaning contaminating the DOC sample. From midway through day 2, filtration equipment was rinsed using MQ-water. This would most likely result in elevated DOC values for the respective samples (A1-2, B1-12, and C1-6), even though all equipment used was acid washed each day to remove OM. Although, the GF/F filters is another factor causing uncertainty. About 3-4 μM DOC per liter of filtered ocean water could potentially end up on the GF/F filter during filtration (Abdel-Moati, 1990; Turnewitsch et al., 2007). Novak et al. (2018) therefore estimates that 50% of the reported POC concentrations from ultra-oligotrophic waters originates from DOC, but uncertainties do not stop there.

There are several standards stating different methods for storage of samples. The International Organization for Standardization (2021) states that DOC samples (applicable to all water types) should be stored in the dark at 3 ± 2°C (unacidified) and analysed within 48h, or alternatively, stored in the dark at 3 ± 2°C (acidified) and analysed within 8d. While the European Committee for Standardization (1997) states that samples could be stored in -15°C to -20°C for several weeks. Due to uncertainties for storage handling, samples were acidified and stored in refrigerated temperature (4°C) for approximately two weeks, before freezed at -20°C.

Studies have shown that up to 35% of DOC could potentially decline within 27 days if stored in 4°C (Nachimuthu et al., 2020), so minimal impact is expected from that timeframe. However, some samples were not properly prepared for storage in the freezer, and as a result of water expanding during freezing, some of them broke, while several septas (membrane on the cap) were seen expanded, almost rapturing. Halewood et al. (2022) shows that DOC potentially could extrude through the cap threads, resulting in diluted DOC concentration due to freezing. Nevertheless, they concluded through several test that frozen samples could safely be stored for many years without having any significant decline in DOC values.

4.4 Future work and perspectives

POC and DOC release from seaweed and its importance within the carbon cycle is an understudied section (Paine et al., 2021). However, methods used to quantify its importance and understand the broader picture is lacking and may contribute to the still existing knowledge gap. The mentioned limitations throughout this experiment highlight the possibility for improvement. To date, different techniques, terminology and methods are being used within the scientific world (Rose & Hemery, 2023), even though their aim is the same. Within these methods, DOC can range between 0.2-1 µm (Druffel et al., 1992; Knap et al., 1996; Bauer & Bianchi, 2011), while POC has varied historically between 0.45-5 µm (Gardner et al., 2003; Kharbush et al., 2020). Even though the most standardised way to separate the two is using 0.7 µm GF/F filters (Carlson et al., 1998), the International Organization for Standardization (2021) has their own suggestion to determine DOC (0.45 µm), and one could argue and ask questions on why not ISO-standards are not followed.

This calls for a method which can be used across the globe, both within phytoplankton and seaweed world, to continue to build up knowledge and close the gap that currently exist. It is therefore suggested that future research on DOC follows Halewood et al. (2022) best practice protocol on determination of DOC in seawater using HTC analysis, written by Dennis Hansell from the university of Miami (Organic biogeochemistry lab) and Craig Carlson from the University of California Santa Barbara (Microbial Oceanography lab). The protocol also states important and highly necessary steps to reduce human and product (GF/F) errors in POC determination.

5 Conclusion

As carbon capture with seaweed becomes an increasingly central mitigation tactic against climate change, it is important to investigate the intricate dynamics of seaweed-mediated carbon capture. Elucidating the relationship between these biogeochemical processes will lead to valuable insights of the overall effectiveness and sustainability of using seaweed as a tactic. Moreover, it will build upon the few relevant studies conducted to date.

By testing the effect of light availability on the production of organic carbon, this study established that there was a significant difference in POC release, pH and DIC between the treatments. During photosynthesis seaweeds effectively decreased DIC, by converting HCO_3^- into CO_2, which increases pH and DO. Additionally compensating for TA increase, highlighting the effect seaweed has as a buffer. Although, no significant difference was found in plant growth rates between treatments. POC release was between 303-1384 µgC (g^{-1} DW d^{-1}), and 310-799 µgC (g^{-1} DW d^{-1}) in light and dark treatments, respectively. Furthermore, it was observed that bacterial respiration exerted a pronounced impact on DIC levels in the dark treatments, leading to a significantly lower pH. However, DO levels did not decrease remarkedly, indicating the release of recalcitrant organic carbon. In addition to this, the study showed that the effect of different temperatures did not significantly impact bacterial degradation of POC. After 188 days, colder temperature (4°C) had a slightly bigger pool of RPOC than the warmer temperature (10°C) accounting for 6.5% and 5.2%, respectively. It was shown that colder temperature plays a key role in microorganisms degradation rate, resulting in a higher pH, but also a slower decrease trend. Temporal analyses concluded fluctuating POC levels, indicating DOC coagulation. Further investigation into DOC levels is needed.

Additional research into seaweed and POC is warranted to expand the understanding of release rates, and broaden the comprehension of how temperature influences the degradation rates of semi-labile and recalcitrant organic matter, while examining the coagulation of DOC into POC. Last, but not least, it is imperative to standardize and quality control DOC and POC determination methods.

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7 Appendix A: **Supplementary method**

Table A.1: Equipment used during experiment that needed cleaning.

Name	**Size**	**Cleaned**
Beakers	200-800ml	Acid washed HCl 1M combusted at 450°C 8h
Erlenmeyer Flasks	250ml	Acid washed HCl 1M
Glass pipettes	100ml	Acid washed HCl 1M
Glass vials and caps	20-40ml	Acid washed HCl 1M (caps) and combusted at 450°C 8h (glass vials)
Burette	250ml?	Acid washed HCl 1M
Volumetric Flasks	100-250ml	Acid washed HCl 1M
Graduated cylinder	100-250ml	Acid washed HCl 1M
Funnels		Acid washed HCl 1M
Buchner Flask (Filtration flask) and filtration funnel	1-2L	Acid washed HCl 1M
Metal tweezer		Acid washed HCl 1M
Spatula		Acid washed HCl 1M
Filters	GF/F (0.7 µm) GF/C (1.2 µm)	Combusted at 450°C 8h
Experiment bags	4-21L	Acid washed HCl 1M

biodegradation experiment.

Previously label	New label	DOC-degradation	DOC-degradation	DOC-characterisation and bacterial communities	DOC-characterisation and bacterial communities
		4°C	10°C	4°C	10°C
E1	S1	C, D	A, B	Extra1	Extra2
E2	S2	C, D	A, B	X1=D30 X2=D150	X3=D30, X4=D150
E3	S3	C, D	A, B	Extra1	Extra2
E4	G1	C, D	A, B	Extra 1	Extra2
E5	G2	C, D	A, B	Y1=D30 Y2=D150	Y3=D30 Y4=D40
E6	G3	C, D	A, B	Extra1	Extra2

8 Appendix B: DOC and POC analyses

Table B.1: Example of ASI-L auto sampler run log-sheet.

Row	Description
1-5	MQ water
6-13	Standards for calibration curve
14	MQ water
15	CRM
16-25	DOC Samples
17	CCV (Continuing Calibration Verification)
18	MQ water
19-28	DOC samples
28+	Follows same structure as above

Standard calculations:

The standards are calculated by the following equation:

$$mg\frac{DOC}{L} = \frac{(ASTD)}{m}$$

Where:

ASTD = area of the standard

m = slope of the regression line

DOC calculations:

The DOC values are calculated by the following equation:

$$mg\frac{DOC}{L} = \frac{(As - \|y\|)}{m}$$

Where:

As = area of the sample

‖y‖ = absolute value of the y intercept

m = slope of the regression line

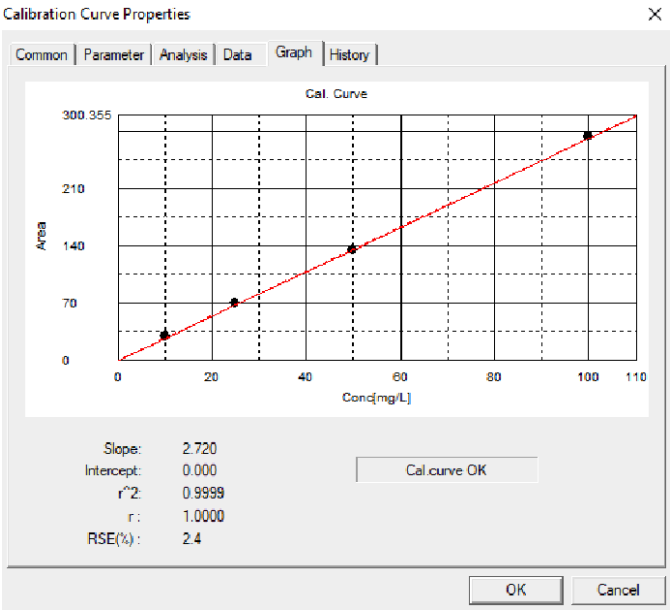


Figure B.1: Viewing calibration curves during analyses (Shimadzu, 2023b). The calibration curve example is shown as OK (R^2: 0.9999), and further analyses can therefore be conducted. Relative standard error (RSE) over 25% are subject to high sampling error and might therefore be used with caution.

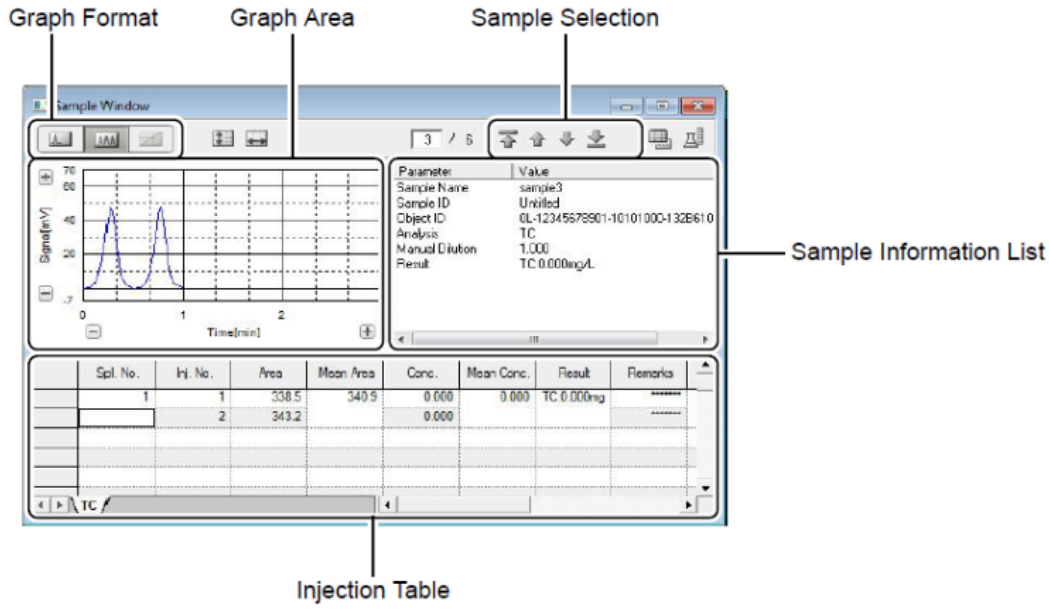
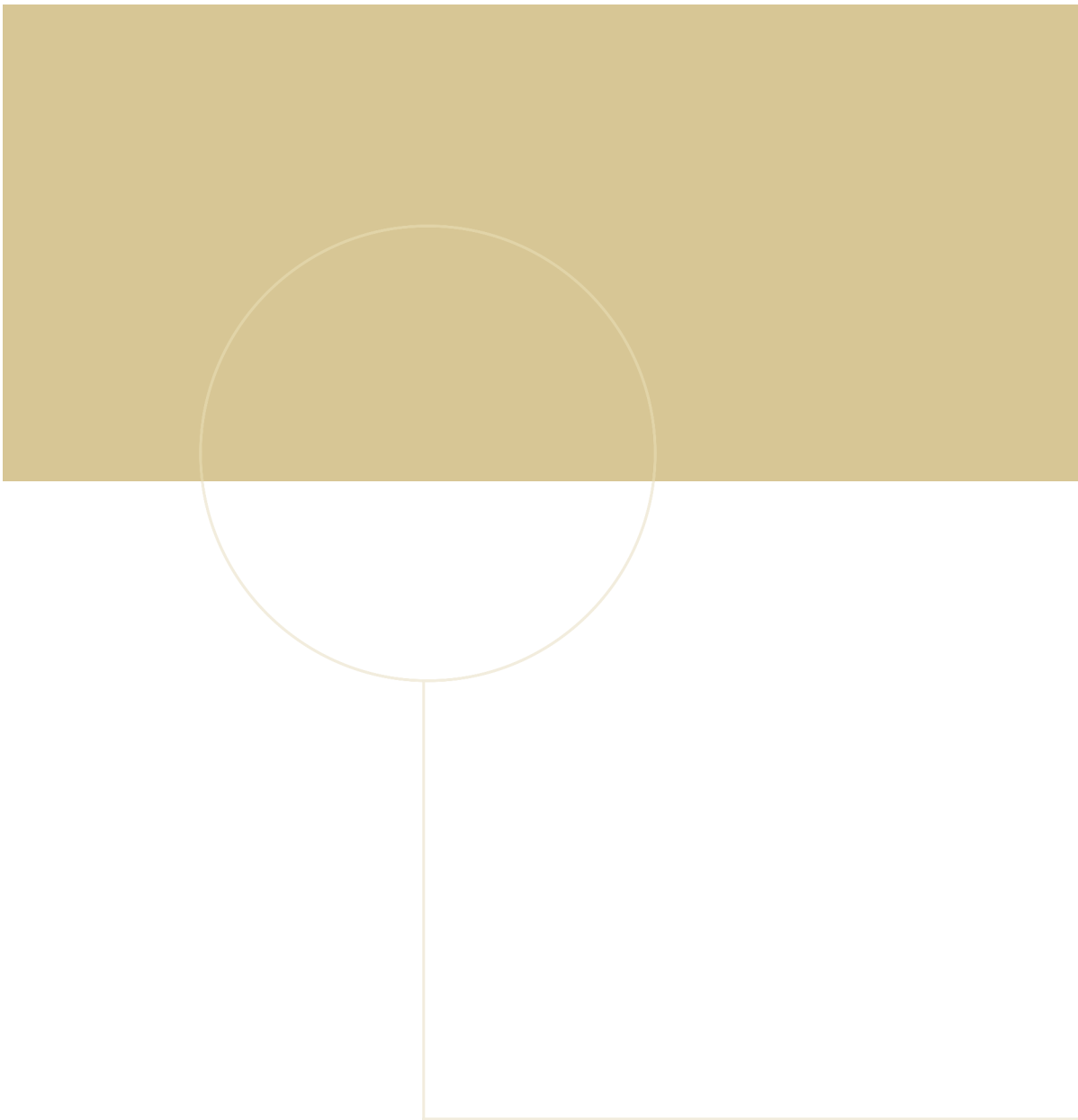


Figure B.2: Viewing sample results during analyses with five different information points (Shimadzu, 2023b). "Graph Format" was switched between current peaks, all peaks and calibration curves, to manually quality assure that values are within certified values. Within the "Graph Area", peaks from the injection results were drawn in real time, and the "Injection Table" shows number of injections, concentrations, and results.



NTNU

Norwegian University of
Science and Technology