CULTIVATION OF SUGAR KELP AS A MARINE MEASURE FOR MITIGATING EUTROPHICATION

Production in large-scale, nutrient removal efficiency, environmental impacts, and economy

Scientific Report from DCE – Danish Centre for Environment and Energy No. 663 2025

AARHUS UNIVERSITY
DCE – DANISH CENTRE FOR ENVIRONMENT AND ENERGY

CULTIVATION OF SUGAR KELP AS A MARINE MEASURE FOR MITIGATING EUTROPHICATION

Production in large-scale, nutrient removal efficiency, environmental impacts, and economy

Scientific Report from DCE – Danish Centre for Environment and Energy No. 663 2025

Annette Bruhn[1]
Teis Boderskov[1]
Mette Møller Nielsen[2]
Peter Søndergaard Schmedes[2]
Daniel Taylor[2]
Signe Høgslund[1]
Line Hermannsen[2]
Johnna Holding[1]
Alina Mostovaya[1]
Freja S. Christiansen[1]
Sidsel G. Pedersen[1]
Sidsel J. Vinbæk[1]
Niels Holst[3]
Sofie Laage Christiansen[1]
Julie L. Ehrenreich[1]

[1] Aarhus University, Department of Ecoscience
[2] DTU Aqua, National Institute of Aquatic Resources, Department of Coastal Ecology
[3] Aarhus University, Department of AgroEcology

AARHUS
UNIVERSITY
DCE – DANISH CENTRE FOR ENVIRONMENT AND ENERGY

Data sheet

Series title and number:	Advisory report from DCE – Danish Centre for Environment and Energy No. 663
Category:	Scientific report
Titel:	Cultivation of sugar kelp as a marine measure for mitigating eutrophication
Subtitle:	Production in large-scale, nutrient removal efficiency, environmental impacts, and economy
Authors(s):	Annette Bruhn[1], Teis Boderskov[1], Mette Møller Nielsen[2], Peter Søndergaard Schmedes[2], Daniel Taylor[2], Signe Høgslund[1], Line Hermannsen[2], Johnna Holding[1], Alina Mostovaya[1], Freja S. Christiansen[1], Sidsel G. Pedersen[1], Sidsel J. Vinbæk[1], Niels Holst[3], Sofie L. Christiansen[1], Julie L. Ehrenreich[1]
Institution(s):	[1] Aarhus University (AU), Department of Ecoscience, [2] DTU Aqua, National Institute of Aquatic Resources, Department of Coastal Ecology, [3] AU, Department of AgroEcology
Publisher:	Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi ©
URL:	http://dce.au.dk
Year of publication:	July 2025
Editing completed:	July 2025
Referee(s):	Michael Bo Rasmussen
Quality assurance, DCE:	Anja Skjoldborg Hansen
External commenting:	Find the comments here:
Financial support:	The National Environmental Protection Agency/
Please cite as:	Bruhn A, Boderskov T, Nielsen MM, Schmedes SS, Taylor D, Høgslund S, Hermannsen L, Holding J. Mostovaya A, Christiansen FS, Pedersen SG, Vinbæk SJ, Holst N, Christiansen SL & Ehrenreich JL. 2025. Cultivation of sugar kelp as a marine measure for mitigating eutrophication - Production in large-scale, nutrient removal efficiency, environmental impacts, and economy. Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi, 84 s. – Scientific report No. 663

Abstract: The cultivation of sugar kelp has been suggested as a marine mitigation measure for the uptake and removal of nutrients from the marine environment. In 2022-2023, sugar kelp was cultivated in the Limfjorden in a 12-hectare experimental facility to document 1) biomass yields, 2) uptake of nitrogen, phosphorus and carbon and 3) effects on the environment by large-scale cultivation. The results show that large-scale cultivation of sugar kelp can remove up to 23 g N m^{-1} line $year^{-1}$, corresponding to an annual nutrient removal potential of 23 kg N ha^{-1} and 0.8 kg P ha^{-1} in a standard cultivation system with 1000 m cultivation line ha^{-1}. Yields and potentials for nitrogen removal in Danish waters vary and are highest in areas with high salinity and nutrient availability. The cost of nutrient removal varies accordingly, with the lowest cost of DKK 2805 kg N^{-1}. Modelling scenarios indicate that a significant upscaling of sugar kelp cultivation is required to achieve any effect on the key environmental indicators for GES: "summer chlorophyll-a" and "light attenuation". At high biomass densities, the environmental effects include 1) a negligible reduction of light to the seabed, 2) altered water flow, 3) reduced sedimentation rates, 4) limited periodic increases in pH and oxygen concentration under, in and near the seaweed cultivation site. Harvesting in April reduces the loss of carbon from the seaweed to the marine environment, and it is unlikely that seaweed cultivation can contribute to carbon sequestration in the inner Danish waters.

Keywords:	Sugar kelp, marine mitigation measures, eutrophication, carbon cycle, hydrology, biomass yield, economy
Illustrations:	The authors and Tinna Christensen
ISBN:	978-87-7156-970-4
ISSN (electronic):	2244-9981
Number of pages:	84

Content

Content **3**

Preface **5**

Sammenfatning **6**

Summary **7**

1 Introduction. Sugar kelp as a marine mitigation measure **8**

2 Materials and Methods **10**

2.1 Cultivation of sugar kelp at 12-hectare scale 10
2.2 Small-scale test cultivation of sugar kelp for modelling 18
2.3 Modelling of sugar kelp growth 21
2.4 Economy of large-scale sugar kelp production 22
2.5 Environmental monitoring at the large-scale cultivation of sugar kelp 22
2.6 Laboratory experiments 25

3 Results **38**

3.1 Sugar kelp production in large scale - Biomass yields, tissue composition and nutrient removal potential 38
3.2 Sugar kelp production in smaller scale for modelling of yield and nutrient removal potential 44
3.3 Economy of large-scale sugar kelp production and nitrogen removal 46
3.4 Sugar kelp growth model 48
3.5 Ecosystem and climate effects of large-scale cultivation of sugar kelp 50

4 Discussion **60**

4.1 Best Available Technology for Cultivation of sugar kelp in Denmark 60
4.2 Cultivation of sugar kelp for nutrient removal 61
4.3 Feasibility of large-scale production of sugar kelp for marine mitigation 64
4.4 Ecosystem effects of large-scale sugar kelp production not relating to GES 65
4.5 Perspectives and knowledge gaps 68

5 Conclusions **70**

6 Acknowledgement **71**

7 References **72**

8 Appendix **82**

Preface

This report summarises the main results from the research project "Development Initiatives for Marine Mitigation Measures" ("Udviklingsinitiativer for marine virkemidler"), which was decided and financed by the "Agreement on the Green Transition of Danish Agriculture" ("Aftale om grøn omstilling af dansk landbrug") from 2021 and implemented via the Danish Environmental Protection Agency (EPA). The project will form the basis for decisions on, and if so, how the marine instruments "Re-establishment of eelgrass" and "Cultivation of seaweed" can be used in the Danish Water Planning to take up nutrients and/or achieve other positive environmental effects that can contribute to a faster achievement of Good Ecological Status in Danish coastal water bodies in accordance with the EU Water Framework Directive. This report constitutes the project background report of the Workpackage 2: "Cultivation of sugar kelp as a tool for marine mitigation". WP2 was led by Aarhus University (Annette Bruhn), and the work was carried out in cooperation between Aarhus University and DTU Aqua. The overall research project was led by DTU Aqua (Karen Timmermann) and carried out in a collaboration between the University of Southern Denmark (SDU), the University of Copenhagen (UCPH), Aarhus University (AU) and DHI. This report is part of the report series "Development initiatives for marine mitigation measures", which includes several background reports with scientific documentation of the project's results, as well as a summary report. The report is peer-reviewed at AU by scientific competent persons, not personally involved in the project. The Danish EPA has had draft reports for commenting, but the choice of methods and conclusions is solely the responsibility of the project group. In agreement with the project group and the Danish EPA this report is in English. All other project reports are in Danish.

This background report summarises upon request from the EPA the following WP2 deliverables:

- Leverance 2.2b. Afrapportering af resultater fra opdræt i Limfjorden
- Leverance 2.3.b. Afrapportering af resultater fra havhaver
- Leverance 2.4: Økosystemeffekter af stor-skala dyrkning af tang.

Sammenfatning

Dyrkning af sukkertang nævnes som et muligt marint virkemiddel til optag og fjernelse af næringsstoffer fra havmiljøet. I 2022-2023 blev sukkertang dyrket i Limfjorden i et forsøgsanlæg på 12 hektar for at dokumentere vækst og optag af kvælstof, fosfor og kulstof ved dyrkning i stor skala. Samtidig blev flere effekter på miljøet undersøgt. Resultaterne viser, at dyrkning af sukkertang i stor skala, og med høst i april for at opnå god kvalitet, årligt kan fjerne op til 23 g N m^{-1} line fra oktober til april, hvor tilgængeligheden af næringsstoffer er højest. Ved anvendelse af et standard dyrkningssystem med 1000 m dyrkningsline ha^{-1}, svarer det til et årligt potentiale for fjernelse af næringsstoffer på 23 kg N ha^{-1} og 0,8 kg P ha^{-1}, og et carbon capture potentiale på 145 kg C ha^{-1}. Resultaterne afspejler et øget produktions- og næringsfjernelsespotentiale, men nedjusterer potentialet for fjernelse af næringsstoffer pr. areal i forhold til tidligere estimater (47,3 kg N ha^{-1} $år^{-1}$). Tidligere potentialer var baseret på antagelser om et dyrkningssystem med en linetæthed på 5000 m ha^{-1}, hvilket – i dette projekt - viste sig ikke at være fordelagtigt. Dette understreger vigtigheden af at teste antagelser i stor skala i flere områder. Udbytte og potentiale for fjernelse af kvælstof er højest i områder med høj saltholdighed og næringstilgængelighed, som i Limfjorden, og lavest i områder med lav salinitet (<10 PSU) og begrænset erfaring med dyrkning af tang. Omkostningerne til fjernelse af næringsstoffer varierer med udbytte og potentialet for fjernelse af kvælstof. De laveste omkostninger ligger på 2805 kr. kg N^{-1}.

Modelscenarier indikerer, at i områder, der er velegnede til dyrkning og næringsfjernelse, kræver det en fordobling af enten de eksisterende dyrkningsområder eller arealeffektiviteten for at opnå en signifikant reduktion i opløst uorganisk kvælstof. Ubetydelige effekter blev opnået for de centrale miljøindikatorer for God Økologisk Status, "sommerklorofyl-a" og "lysdæmpning", muligvis fordi dyrkning af sukkertang og måling af indikatorer ikke sker på samme tid.

Ved dyrkning af sukkertang i stor skala (12 ha) omfatter miljøeffekterne bl.a. en ubetydelig reduktion af lys til havbunden, ændrede strømhastigheder i og omkring tanganlægget, reducerede sedimentationshastigheder under tanganlægget og begrænsede periodiske stigninger i pH- og iltkoncentrationer i dyrkningsområdet. Ved høst senest i april reduceres tab af både volatilt, opløst og partikulært kulstof (VOC, DOC og POC) fra tangen til havmiljøet. Skønt fraktioner af både DOC og POC er langsomt nedbrydelige, er det usandsynligt, at tangdyrkning kan bidrage til en egentlig kulstofsekvestrering (>100 år) i de indre danske farvande. Sukkertang lejret i eller på havbunden kan tværtom stimulere omsætning af langsomt nedbrydeligt kulstof i havbunden (priming effekt).

Der er fortsat behov for omfattende og langsigtede undersøgelser af effektiviteten og miljøeffekterne af dyrkning af sukkertang for at adressere grundlæggende videnshuller, særligt i forbindelse med 1) implementering af mere intensive dyrkningssystemer; 2) reduktion af produktionsomkostninger; indvirkning på og samspil med 3) havets kulstofkredsløb og klimaeffekter i relation til C omsætning og lagring, emissioner af klimaaktive VOC, og 4) biodiversitet. Dette kræver samarbejde med industrien og/eller strategiske infrastrukturinvesteringer for at sikre relevans og reducere omkostninger.

Summary

The cultivation of sugar kelp has been suggested as a marine mitigation measure for the uptake and removal of nutrients from the marine environment. In 2022-2023, sugar kelp was cultivated in the Limfjorden in a 12-hectare experimental facility to document 1) biomass yields, 2) uptake of nitrogen, phosphorus and carbon and 3) effects on the environment by large-scale cultivation. By cultivation of sugar kelp, up to 23 g of N m^{-1} line $year^{-1}$ can be removed in Limfjorden from October to April, where nutrients are most available. This is equivalent to an annual nutrient removal potential of 23 kg of N ha^{-1} and 0.8 kg of P ha^{-1}, and a carbon capture potential of 145 kg C ha^{-1}, using a standard cultivation system with 1000 m of cultivation line ha^{-1}. The result of this project reflects an increase in production yield and nutrient removal efficiency, but a reduction of the area based nutrient removal potential as compared to earlier estimates (47.3 kg N ha^{-1} $year^{-1}$). Earlier potentials were based on extrapolations assuming a different cultivation design with a higher density of cultivation lines (5000 m ha^{-1}), which turned out not to be feasible in this case. This highlights the importance of testing assumptions at scale and in different areas. The highest nutrient removal potentials were found in areas with high salinity and nutrient availability, such as Limfjorden, and the lowest potentials in areas with low salinity (<10 PSU) and limited cultivation experience. The cost of nutrient removal varies accordingly, with the lowest cost resuming 2805 DKK kg N^{-1}.

Modelling scenarios indicate that in areas optimal for cultivation and nutrient removal, a doubling of existing cultivation area or areal efficiency is required for achieving a significantly reduced concentration of dissolved inorganic nitrogen. Negligible effects were achieved for the key target environmental indicators for Good Ecological Status, "summer chlorophyll-a" and "light attenuation", potentially due to a mismatch in time of cultivation and indicator monitoring.

For large-scale seaweed cultivation (12 ha), the environmental effects include a) negligible reduction of light to the seabed, b) altered hydrology with reduced overall velocities, increased turbulence, and increased current velocities between and below lines, c) reduced sedimentation rates below the cultivation system, and d) periodic increase in pH and oxygen concentrations in the cultivation area. These effects were limited in space and periodicity, where natural daily and seasonal dynamics more importantly contributed to variability.

Harvest no later than April, will reduce the loss of both volatile, dissolved and particulate carbon (VOC, DOC and POC) from the seaweed to the environment, but while fractions of both DOC and POC are recalcitrant, it is unlikely that C is sequestered (>100 year) in inner Danish waters. In contrast, sugar kelp tissue deposited on/in the sediment can stimulate the degradation of buried recalcitrant carbon (priming effect).

The need remains for further large-scale, and long-term studies of the efficiency and environmental effects of cultivation of sugar kelp to address persisting fundamental knowledge gaps in particular in relation to 1) implementing more area intensive production systems, 2) lowering production costs, impact on and interaction with the 3) marine carbon cycle and climate effects in relation to C burial/sequestration, and emissions of climate active VOC, and 4) biodiversity. This requires cooperation with the industry or strategic infrastructure investments to ensure relevance and reduce costs.

1 Introduction. Sugar kelp as a marine mitigation measure

Cultivation of sugar kelp (*Saccharina latissima*) can be used as a marine measure for the absorption and removal of nutrients from the marine environment, as sugar kelp absorbs nutrients from the surrounding sea water during its growth in the sea (Timmermann et al. 2016). The nutrients are removed from the marine environment when the kelp is harvested. In Denmark, sugar kelp has been cultivated in coastal systems since 2008, by both commercial companies and research institutions. The nutrient removal potential of sugar kelp as a marine mitigation measure in Denmark until 2022 has been summarised including the knowledge and experience published from the Limfjorden, Horsens Fjord and Kattegat (Boderskov et al. 2021a; Boderskov et al. 2022a; Bruhn et al. 2016; Zhang et al. 2022; Marinho et al. 2015), documenting a nutrient removal potential ranging from 0.1 to 16 g nitrogen (N) and 0.01 to 1.6 g phosphorus (P) per m of line per year (Bruhn et al. 2024). This is lower than, but still in range with, data published from the USA and Sweden, where 11-26 g N per meter of line per year and 2-40 g N per m of line per year is reported, respectively. Converted into areal nutrient removal potentials, this is in the range of 19-176 kg N ha^{-1} $year^{-1}$ (Grebe et al. 2021; Visch et al. 2020). Reports from China, the global lead in production of seaweed (Chopin and Tacon 2020; FAO 2022), claim a nutrient removal potential of 600-1,200 kg N ha^{-1} $year^{-1}$ from cultivation of the Japanese kelp, *Saccharina japonica* (Xiao et al. 2017; Zheng et al. 2019). In China, the data are based on large-scale cultivation of optimised kelp cultivars (Hu et al. 2021), whereas in Denmark, Sweden and the USA, the reported spatial nutrient removal potentials are theoretically upscaled from small-scale experiments and seaweed farms and are thus based on assumptions of current cultivation systems and current biomass yields. The cultivation of sugar kelp in Western countries is an evolving technology, and it is therefore to be expected that the technology and yields will be optimised in the coming years with increased biomass and nutrient removal potential as a result. At present, knowledge is lacking about the potential of technology development and its consequences for both biomass and nutrient removal potentials and economy.

Other than the nutrient uptake potential, limited knowledge exists on other effects of large-scale cultivation of sugar kelp on the marine environment. Many environmental effects and ecosystem services are described for natural populations of kelp, such as increase of biodiversity (Steneck et al. 2002; Fragkopoulou et al. 2022), storage of carbon (Krause-Jensen and Duarte 2016), and dampening of wave energy (Elsmore et al. 2023). Major differences exist however, between natural kelp populations and cultivated kelps, both regarding the position of the biomass in the water column and the seasonality, where cultivated kelps are located in the surface and harvested regularly, thus not constituting a permanent habitat. This also translates into differences in environmental impact between natural populations and cultivated kelp, where the documented positive impact on biodiversity of kelp forests is not documented in kelp farms (Forbes et al. 2022). The effects on local hydrology and sedimentation patterns have been documented for mussel cultivation on longlines, where within-farm current attenuation produced by the mussel rope drag was documented, compensated by flow acceleration and downwelling beneath the ropes and around farm (Mascorda-Cabre et al. 2024). The effect on Carbon burial and ultimately, sequestration, is being discussed with

arguments ranging from significant carbon sequestration (Froehlich et al. 2019) to limited and varying impact (Pessarrodona et al. 2024) and questioning of the ethics of the deliberate sinking of seaweed to the ocean floor (Ricart et al. 2022), and large dependency on locality, sediment type and hydrological regime (Duarte et al, in press). Also in Denmark, the use of cultivation of sugar kelp as a measure for marine mitigation by taking up and removing N and P from the marine environment, as well as the potential climate effects of cultivating sugar kelp have so far been assessed on the basis of small-scale experiments. The results can therefore not be expected to reflect the realistic impact of a large-scale sugar kelp production. Thus, consistent knowledge gaps exist concerning the effects of large-scale cultivation on the marine environment – in particular regarding hydrology, sedimentation patterns, biodiversity and impact on the carbon cycle.

In the research project, Udviklingsinitiativer for marine virkemidler, a sugar kelp cultivation experiment in a scale of 12 hectares was carried out during one single cultivation season at an independent research facility in the Limfjorden. Tests were carried out for comparison between different cultivation systems, corresponding to a total of 12 hectares with full production. The experiment was designed and carried out for testing and documenting 1) state-of-the-art methods of cultivation technology for optimised growth and biomass yields, 2) effects on the local marine environment (shadow effect, hydrology, sedimentation of organic matter, pelagic chlorophyll a concentrations, pH), 3) effects on carbon cycle and climate (uptake of CO_2, release and degradation of dissolved and particulate carbon, release of greenhouse gases) and 4) cost-effectiveness of sugar kelp production.

2 Materials and Methods

The experimental work was based on one year of large-scale cultivation of sugar kelp in a 12-hectare longline cultivation site in the Limfjorden. While this production size is considered as being 'large-scale' sugar kelp cultivation in Denmark, taking up 2/3 of a typical mussel cultivation area, it only meets the definition of 'small-medium' scale (0-50 × 200 m lines) and not 'large-scale' (> 50 × 200 m lines) as defined by the Scottish government (Marine_Scotland 2017) and used by Campbell et al. (2019).

At the 12-hectare cultivation site in Limfjorden, impact of cultivation design on biomass yield and nutrient uptake potential was investigated (section 2.1). In addition, small-scale cultivation of the same batch of sugar kelp was carried out at seventeen locations in inner Danish waters, to provide data on yield and nutrient uptake potential in waters differing in salinity and nutrient status, for the modelling of nutrient uptake potential (section 2.2). The data on growth and composition of the sugar kelp in large and small scale served as input for the development of a simplistic growth model of sugar kelp (section 2.3). The cost of large-scale sugar kelp production was calculated based on the large-scale cultivation experiment (section 2.4), and here also the environmental impact was assessed with focus on hydrology, light, sedimentation, and nitrogen and carbon cycles (section 2.5). Finally, laboratory experiments were conducted using primarily biomass, sediment and water from the cultivation site in the Limfjorden, to provide in depth knowledge on the impact on the pelagic and benthic carbon cycling of seaweed cultivation, including climate gas emissions (section 2.6).

2.1 Cultivation of sugar kelp at 12-hectare scale

2.1.1 Cultivation site and cultivation system design

The cultivation site was located in Sallingsund, Limfjorden, Denmark (fig.1). Prior to this project, the cultivation site was dedicated to cultivation of blue mussels with a concession license of 18 hectares (ha). A cultivation license for seaweed was granted by the Danish Coastal Authorities for cultivation of seaweeds in 12 ha of the southwestern part of the original concession (fig. 1. Table 1).

Table 1. Coordinates for the seaweed cultivation site at Sallingsund (Decimal degrees, Datum WGS84).

Corner	Degrees East	Degrees North
North	8.9159	56.7876
East	8.919433	56.786733
South	8.914667	56.782133
West	8.910867	56.78305

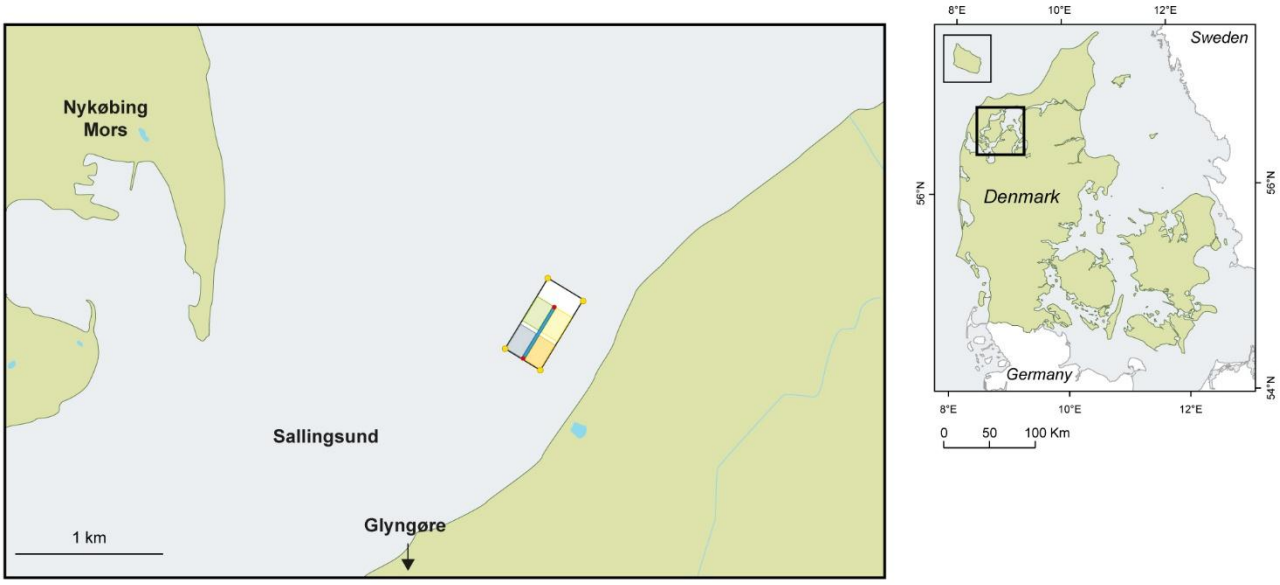

Figure 1. Location of the seaweed cultivation site in Sallingsund, Limfjorden, Denmark. The colored areas were all used for seaweed deployment, whereas the white area was in use for cultivation of blue mussels. This area was not part of the project. The differently colored rectangles indicate the distribution of different cultivation designs of the cultivation lines. The two red dots indicate 2 data logger stations deployed in the area for continuous logging of light, salinity and temperature.

In the cultivation site, the seaweed longlines were arranged in four sections (Seaweed Northeast (SNE), Seaweed Northwest (SNW), Seaweed Southeast (SSE) and Seaweed Southwest (SSW) (fig. 2). Each section contained 7 longlines of around 150 m. Each longline was anchored to the seabed with a drill anchor at each end and fitted with a concrete block (20 l) for every 5-10 m, and a buoy (18 l) for every 10 m to secure correct buoyancy of the longline system. To test optimisation of deployment of seeded seaweed lines on longline system, three deployment designs were prepared: In two sections (SNW and SSE), each longline was fitted with one single horizontal seaweed line hanging approximately 75 cm below the surface (fig. 3a). In section SSW, each longline was fitted with two horizontal seaweed lines approximately 75 and 125 cm below the surface (fig. 3b), and in section SNE, the seaweed lines were arranged as 'loops' hanging app. 0.5 m from the longline and 2.5 m down, equivalent to between 1.3 and 3.8 m depth (fig. 3.c). Between the SSE and SSW sections was an empty longline with data loggers.

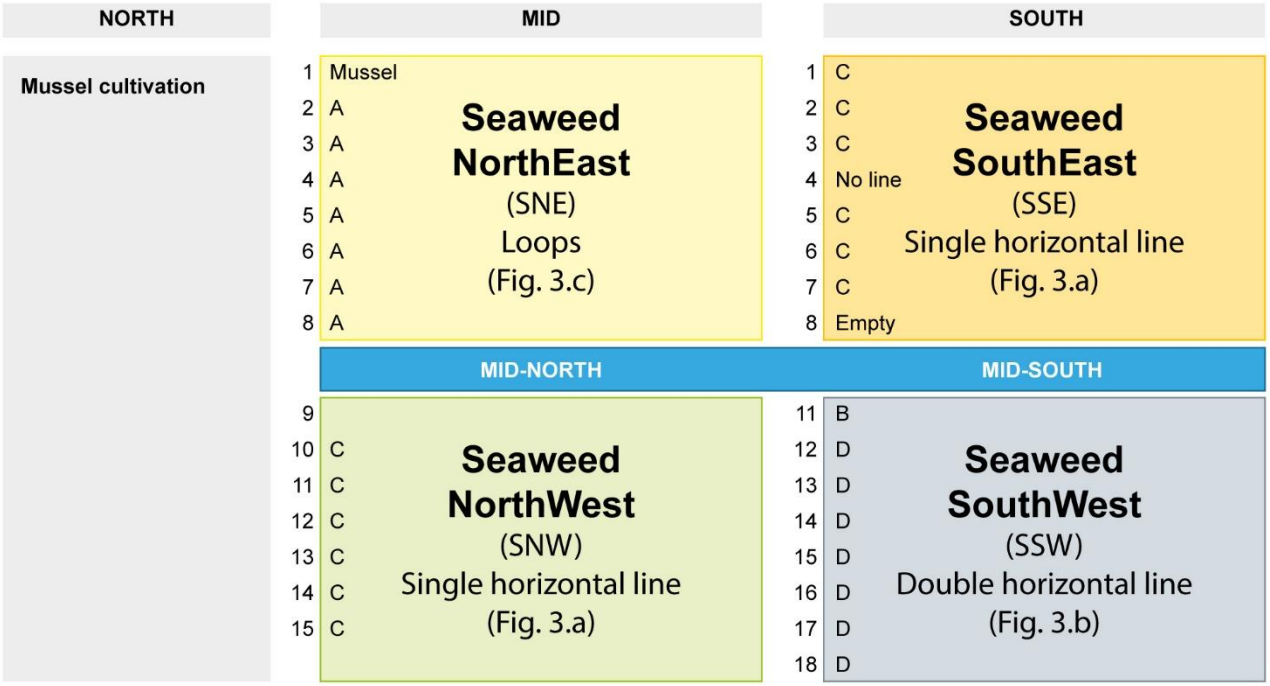

Figure 2. Distribution of the cultivation lines in the seaweed cultivation area in Sallingsund (see fig.1). Numbers and letters A, B, C and D indicate the ID of the specific longlines. 'Mussel' indicates that one longline was used for mussels, 'No line' indicates absence of longline. 'Empty' indicates that no seeded line was deployed on the longline. SNE: Seaweed Northeast. SSE: Seaweed Southeast. SNW: Seaweed Northwest. SSW: Seaweed Southwest.

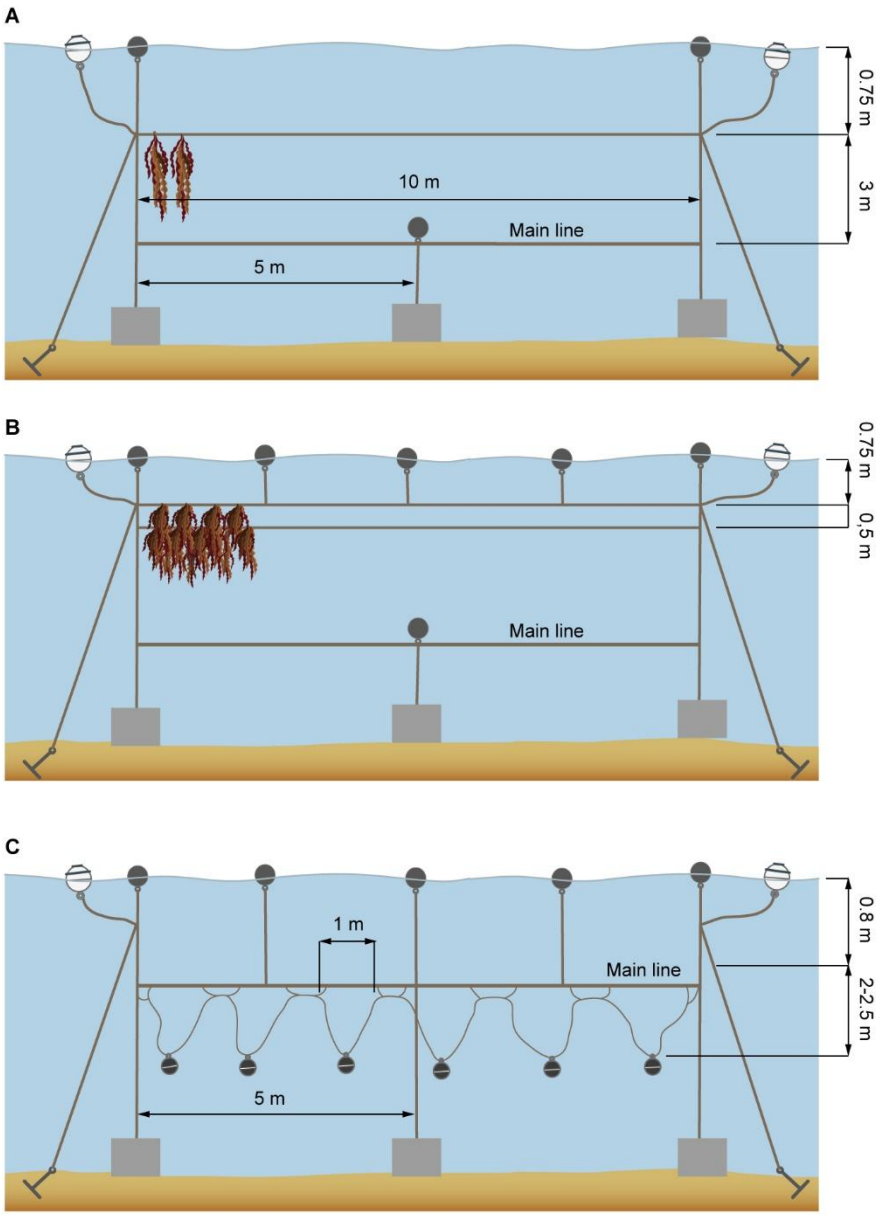

Figure 3. Illustration of the three different cultivation designs used. A: single horizontal lines (areas SNW and SSE). B: Double horizontal lines (area SSW). C: loops (SNE).

2.1.2 Production of seeded lines

To reduce the risk of seeding failure, two independent hatcheries were installed at DTU Aqua, Nykøbing Mors from July to October 2022. Hatchery 1 was installed in an existing climate room, and Hatchery 2 was installed in a 40-foot cooling container rented for this purpose. In both hatcheries, the seeded lines were produced according to (Boderskov et al. (2021a); (Boderskov et al. 2021b; Boderskov et al. 2022a).

In both hatchery systems, filtered seawater (0.2 µm) from DTU's seawater intake was used. The salinity ranged between 29 and 31 ppt during the seeding and nursing period.

2.1.3 Lines, tubes and nets for seeding

Lines of the type "cross-braided nylon 4 mm" were won around 63 mm PVC pipe of 1 m length. Thus, each pipe had approx. 47 meters of line per pipe. After winding the line onto the pipes, pipes and lines (rolls) were soaked and

rinsed in fresh water for one week, including several water changes. Upon rinsing, a white residue from the spinning of the lines was observed in the water, and the rinsing was therefore repeated until this was no longer observed. Finally, the rolls were drained and let to dry before seeding.

2.1.4 Hatchery 1. Existing Climate Room

Two recirculating water systems each consisting of a reservoir tank, plus two or three tanks with dimensions of 20 x 125 x 250 cm (total volume of 1250 and 1750 L, respectively) were used for seeding the lines. Water movement in each tank was secured by the constant recirculation of seawater through water pipes at the bottom, with an overflow directing the water back to the reservoir tank. Each tank was illuminated by 4 × Nordlys LED lamps (44W) with shades to reduce irradiance level during the first 2 weeks followed by full illumination during the rest of the nursery period.

2.1.5 Hatchery 2. Cooling Container

A 40-foot cooling container was rented for the purpose and placed on the pier at DTU-Aqua, Nykøbing Mors. The cooling container was furnished with 9 tanks of 350 x 60 x 18 cm in transparent plastic (Polyethylene (PE)). The tanks were placed in a shelf system with light, air and water circulation. Each tank contained approx. 24 rolls of seaweed line, and therefore approx. 1.13 km of line per tank, or 10.15 km of line in total in the cooling container.

Two different light sources were used: half of the tanks were supplied with 4 ×"58 W Philips MASTER TL-D 90 De LUXE 58W/950" fluorescent tubes in double splashproof fluorescent luminaires, whereas the other half of the tanks were supplied with "Cosmorrow LED 40W Blomst Grolys Secret Jardin". Each tank was equipped with a pump delivering approx. 2000 L per hour per tank. The pumps were of different types, but mainly "Tunze Silence 200 - 2,400 liter aquarium pump". Each tank had its own pump to reduce the risk of contamination between the tanks. In addition, each tank was equipped with two × 4 cm air stones to ensure supply of CO_2 and de-oxygenation of the water.

In both hatcheries, 0.2 µ filtered and UV treated seawater cooled down to 10-12 °C was used.

2.1.6 Seaweed starting material

On June 10th, 2022, approximately 100 individuals of sugar kelp were collected by snorkeling at Middelfart Old Harbour. The individual fronds were cut approx. 20 cm above the basis stipe, and transported under cooled conditions to DTU Aqua, Mors, after which they were stored in 10 °C seawater in darkness with aeration and weekly nutrient addition for approx. 7 weeks to induce sori formation following procedures by Boderskov et al. (2021b).

2.1.7 Seeding of lines

On the day of seeding, f/2 medium (Guillard and Ryther 1962) was added to all tanks to achieve a nutrient concentration of approximately 150 µM nitrate-N.

Sugar kelp with distinct sori formation was selected for spore extraction. Spores were extracted according to Boderskov et al. (2021b), and the concentration of spores was determined. The spores were then distributed over the

rolls in filtered seawater, achieving a spore concentration of 3,500 spores per ml in the seeding tanks.

The majority of the lines in Hatchery 2 were seeded on August 18th, 2022. On August 25th the remaining lines in Hatchery 2, as well as the lines in Hatchery 1 were seeded following the same procedure.

New seawater was frequently added as well as low doses of nutrient stock solution (f/2). To prevent the growth of diatoms, GeO2 was added in half the dose (0.5 mg/L) to the seeding tanks after 3 weeks (Shea and Chopin 2007). Unexpectedly, a whitish biofilm formed on rope surface in several of the tanks. At the point where the tiny seedlings (the juvenile sugar kelp sporophytes) had reached a visible size, the nutrient additions were increased to 440 µM nitrate-N, added once a week.

The hatchery period was between 47 and 69 days for all lines. In both hatchery setups, unexpected patchy dispersal of seedling growth was observed. On some areas of the lines, normal/expected growth was observed, while others had reduced or no growth. The distribution of the affected areas indicated that the variation could be due to chemicals released from the lines or bacterial growth/biofouling potentially induced in connection with the soaking and rinsing of the lines. For optimising field growth after deployment and in order to allow the poorest looking seeded lines to have more time in the nursery, the best-looking lines were initially deployed in the farm sections with the single horizontal line design (SSE and SNW).

2.1.8 Deployment of seeded lines

A total of approx. 8 km of seeded line was deployed in the cultivation area: 1.95 km of line as single horizontal lines in two sections (SSE and SNW), 3.7 km of line as loops in one section (SNE), and 2.3 km of line on double horizontal lines in one section (SSW).

Autumn is generally regarded as the optimal deployment time for sugar kelp in Denmark (Boderskov et al. 2021a). Therefore, all lines were deployed between October 4th and 26th 2022, making use of a total of 7 working days at sea, where weather conditions were suitable.

2.1.9 Monitoring of growth and loss of *S. latissima*

To monitor the growth of the sugar kelp, biomass samples from the various line deployment designs were collected on December 13th, 2022, February 28th, 2023, April 24th, 2023, and May 24th, 2023, (in May only of the horizontal lines in SSE and SNW). Biomass samples of 3 x 44 cm seeded line were taken from 3 seaweed lines within each area on December 13th, 2022, and 3 x 22 cm of line for the remaining sampling days. The sample length of 44 and 22 cm line corresponded to 2 and 1 rounds of a roll, respectively, to eliminate potential effects of seeding variation between top and bottom of lines on the rolls.

In the area with loops (SNE), triplicate samples were taken from the top (1 m) and from the bottom (3.5 m) of the loops, and in the area with double horizontal lines (SSW), triplicate samples were taken from both lines (0.75 m and 1.25 m depth).

All biomass samples were stored in 5 °C seawater until the following day, where samples were drained and the fresh weight (FW) determined. One sample from each line was also used for frond area determination, where the seaweed fronds were laid out in one single layer on a white plate and a photo was taken. Following, the total frond area of all seaweeds of the sample was determined based on a pixel analysis (ImageJ). After fresh weighing and area determination, samples were transferred to individual tinfoil trays and dried at 80 °C until stable weight to determine the dry weight (DW).

Additionally, 50 intact kelps on two different longlines were labeled and subjected to the 'punch hole method', where a small hole was made in the lower part of the blade in February to estimate blade elongation and loss of distal tissue during growth (Nielsen et al. 2014; Parke 1948). The positions of the holes in the fronds were measured on April 14th and May 24th, 2023.

2.1.10 Harvest of *S. latissima*

The majority of the harvest was carried out by 2 boats between April 24th and May 3rd, 2023. Boat 1 was a commercial longline mussel aquaculture boat (owner: Alex Mikkelsen). Boat 2 was owned by DTU AQUA, Nykøbing Mors.

On April 12th, a test harvest was carried out with boat 1, investigating if an extra hauler should be attached to the front of the boat to make the harvest process more efficient. The harvest on Boat 1 was carried out by running the seaweed line through the hauler and pulling it through two rubber wheels, from where the sugar kelp fronds were cut off manually onto a conveyer belt and from there conveyed into a big bag. During test harvest, several challenges were encountered: 1) **Biofouling by hydroids on the seaweed lines**: As epi-fouling (mostly hydroids) was growing on the seaweed lines near the sugar kelp holdfast, it was decided to cut the biomass approx. 10 cm above the holdfast to minimise the amount of fouling of the harvested biomass. 2) **Biofouling by ascidians on the longlines**: At the test harvest, it was also experienced that a substantial abundance of ascidians had developed on the longlines, and by using the hydraulic hauler to work on the longline, the majority of these ascidians were unintendedly harvested onto the conveyor belt. This biofouling would severely compromise the quality of the biomass harvest, and for this reason, all longlines were cleaned by boat 2 before the harvest was made by boat 1. 3) **Twisted seaweed lines**: Twisted seaweed lines in some areas of the most exposed parts of the farming sections constituted an additional challenge during the harvest phase which substantially slowed down the pace of harvest, both because of the extra handling time to detangle the lines, and also because biomass was lost due to the tangling and tear.

During the harvest phase on Boat 1, the leftover biomass on the lines (mainly haptera) was separated from the line and collected. This discarded biomass accounted for approximately 9% of the total harvest. On Boat 2, the lines were hauled on board manually, and the seaweed cut off the lines as on Boat 1, but left-over biomass was left on the lines. On Boat 2, the harvested sugar kelp was manually harvested directly into big bags.

On land, the big bags with sugar kelp biomass were loaded into a container and within the same day transported to a pilot scale processing plant at Aarhus University, Department of Biochemical Engineering, Foulum, for processing. Here, the biomass was screw-pressed and afterwards the pulp and juice mixed, finely chopped and finally fermented in sealed IBC containers

using a lactic acid bacteria inoculum supplied by the feed company, Fermentation Experts. The fermented biomass was stored for a minimum of six months to ensure biomass stability and following used in experimental feed for pigs as part of the project SMARTTANG (GUDP).

2.1.11 Analyses of sugar kelp tissue and seawater

From each of the three sampled seaweed lines pr. area and on each sampling day, a sugar kelp DW sample was analysed for tissue carbon (C), nitrogen (N), phosphorus (P) and ash. This resulted in tissue analyses from triplicate samples from each area and depth. At sampling on April 24th 2023, triplicate samples were also analysed to determine tissue trace metal and iodine concentrations.

Dry matter (DM)

The DM content of the biomass samples was determined by oven drying of the samples at 80°C until achieving a stable DW. The DM content was hereafter calculated as % of fresh biomass: DW/FW × 100. Hereafter the dry samples were finely milled and homogenised before further analysis.

Ash

For determination of ash content, a known amount of DM was combusted at 550°C for 2 h, and the ash fraction was calculated as % of DM.

Carbon and Nitrogen

Tissue concentrations of carbon (C) and nitrogen (N) were determined by Pregl-Dumas ignition in pure oxygen atmosphere followed by chromatographic separation of C and N with detection of the individual elements by thermal conductivity (Marcó et al. 2002).

Phosphorus

Total phosphorus (P) content was determined spectrophotometrically following standard methods (Grasshoff et al. 1999). Prior to analysis, the dried and homogenised samples were heated at 550°C for 2 h, autoclaved with 2 M hydrogen chloride (HCl) (20 mg DM for 7 mL acid), and finally filtered through GFF filters (Whatman).

Trace metals

Metal concentrations were determined using inductively coupled plasma mass spectrometry (iCAPq ICP-MS, Thermo Fischer, Bremen, Germany). Briefly, a 0.2 g dry sub- samples were digested in closed quartz vessels in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) using 5 ml of concentrated nitric acid (SPS science, Courtabeuf, France). The digests were subsequently diluted with Milli-Q water followed by quantification using ICP-MS using external calibration with internal standardisation (97Rh).

Iodine

For determination of total iodine tissue content (I), the principles of the standardised method EN17050:2017 (EN_17050 2017) was followed. Briefly, 0.15–0.20 g of dry samples was weighed into tubes (Sarstedt, Nümbrecht, Germany). Subsequently, 5 mL Milli-Q® water and 1 mL 25% tetra-methyl-ammonium-hydroxide (TMAH, Merck, Darmstadt, Germany) was added. The tubes were then sealed and placed in a preheated oven at 90 ± 3.0 °C for 3 h followed by cooling and diluting to a final volume of 20 mL with Milli-Q water. To remove coarse particles, the samples were centrifuged at 10,000 ×g for 20 min. Prior to analysis, the supernatant was filtered through a 0.45 µm filter

and samples were diluted with Milli-Q water prior to analysis. The iodine quantification was performed by ICP-MS (iCAPq) using external calibration with internal standardisation (125Te). Certified stock solutions were used for preparation of the calibration standard and internal standard (SPS Science).

2.2 Small-scale test cultivation of sugar kelp for modelling

2.2.1 Citizen science concept

To test how different environmental conditions across inner Danish waters, in particular salinity, affect the biomass production and nutrient removal potentials of sugar kelp, an existing collaboration with the Danish Blue Community Gardens (BCG) was continued. In Denmark, at the time of project start, 23 BCGs were established spread over the entire country and run by volunteers to grow and harvest seaweed and mussels for their own consumption. The association 'HAVHØST' is the overarching organisation connecting the BCGs. In collaboration with HAVHØST, a citizen-science program established during the project Tang.nu was re-established and expanded. All BCGs and a number of sugar kelp farming companies were invited to participate, and hereof 14 gardens and 4 companies engaged in the collaboration (table 2). These were distributed over most of the inner Danish waters except for the North Sea, representing areas with an ecological status ranging from moderate to bad, and providing very different growth conditions for sugar kelp due to different salinity regimes and environmental conditions in general (fig. 4. Table 2).

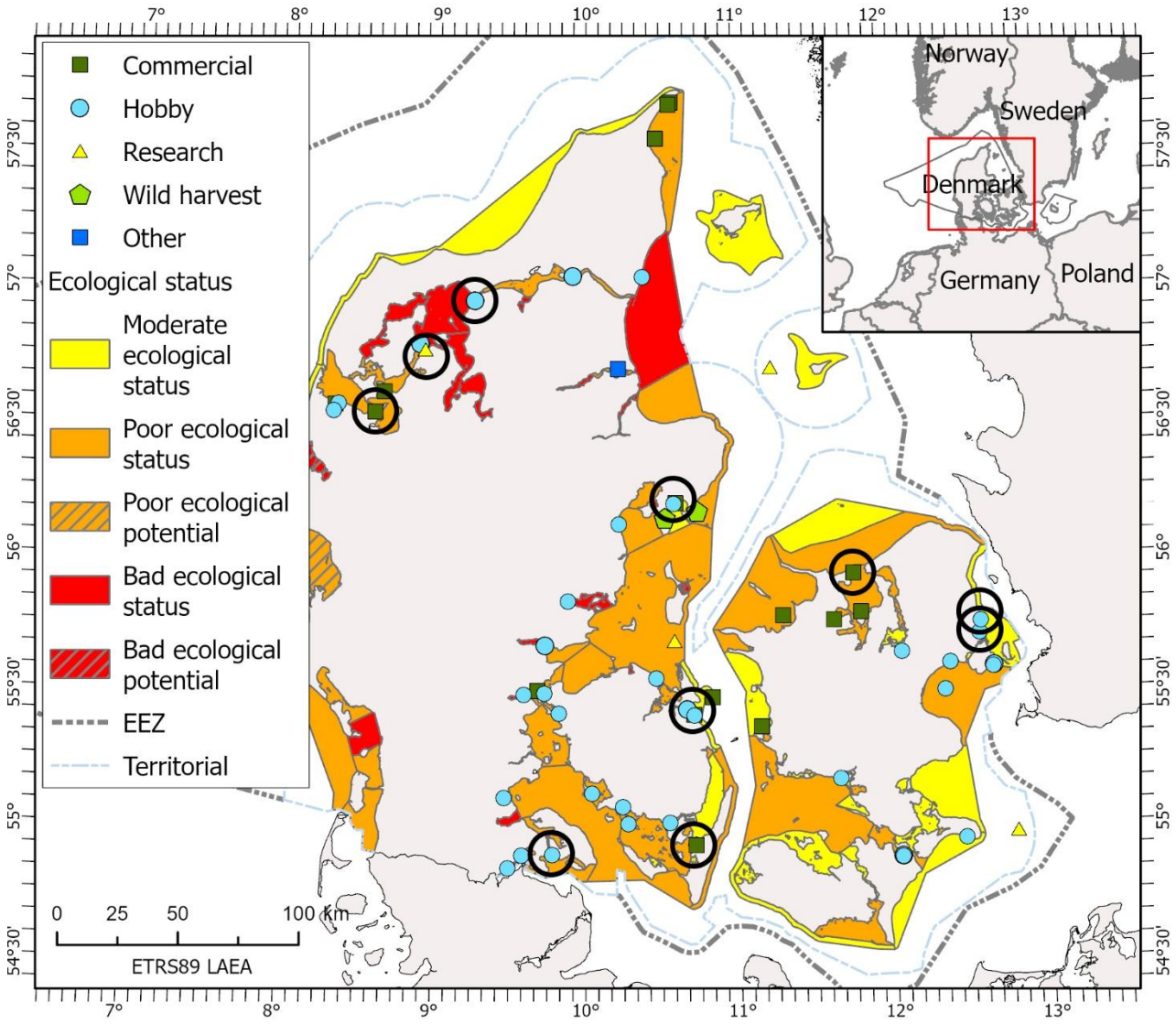

Figure 4. Overview of the Ecological Status of all Danish water areas and seaweed production sites in Denmark combining data from the Danish Coastal Authorities and Havhøst (havhøst.dk), updated with expert knowledge. Hobby sites refer to Blue Community Gardens (BCG) shown in the map on havhøst.dk, which may include marine gardens solely producing mussels. All other categories are classified as seaweed production in the Danish Coastal Authority's data on structures in Danish waters. Black open circles indicate the locations for the single large-scale (research) and nine small scale (three commercial and six BCG) deployments of sugar kelp in this project. The extent of the territorial Waters and the EEZ are indicated with hatched lines.

The 14 involved BCGs and the 4 companies received a minimum of 20-30 m of seeded line with juvenile sugar kelp, all deriving from the same batch of seeded lines used for the large-scale deployment in Sallingsund. The seeded lines were delivered in a cooling box. In addition, all participants received a full 'toolbox' with detailed protocols for data collection, a refractometer, a Secchi dish, a thermometer and a white tarp to serve as background for the photos of the growing seaweed (fig. 5).

Figure 5. Toolboxes packed and ready to be delivered at the 15 Blue Community Gardens participating in the test cultivation of sugar kelp in different Danish waters (Table 2). Teis Boderskov (right) and Sofie Laage Christiansen (left), both Aarhus University (AU), organized the cooperation between AU and the Blue Community Gardens.

The BCGs and company volunteers had the responsibility to deploy the seaweed lines, monitor growth and collect data from October 2022 to April/May 2023 and deliver representative biomass samples upon harvest in April/May 2023 to Aarhus University for further analysis. All surplus biomass was kept by the volunteers for consumption.

Coordinators from AU (Teis Boderskov and Sofie Laage Christiansen) organised the effort in collaboration with HAVHØST coordinators (Hardy Jensen and Line Bøttern).

Table 2. The small-scale cultivation of sugar kelp involved 14 Blue Community Gardens (BCG) and 4 companies (C). A total of 7 BCG and 3 C (in **bold**) managed to deliver data of good quality for sugar kelp growth and biomass composition, and for environmental parameters. The data from these BCG and C was included in the modelling of the efficiency of sugar kelp to act as a marine measure t for nutrient uptake and marine mitigation (Larsen and Erichsen 2024).

Blue Community Garden (BCG) /Company (C)	Water body	Ecological status
Havhaven Ebeltoft Vig (BCG)	Ebeltoft Vig	Moderate
Kerteminde Maritime Haver (BCG)	Storebælt	Moderate
Aalborg Fjordhave (BCG)	Limfjorden	Poor
Løgstør Fjordhave (BCG)	Limfjorden	Bad
Nykøbing Fjordhave (BCG)	Limfjorden	Bad
Fjordhaven Horsens (BCG)	Horsens Fjord	Bad
Vejle Fjordhave (BCG)	Vejle Fjord	Poor
Grønsund Havhave (BCG)	Grønsund	Moderate
Den Blå Kolonihave, Sønderborg (BCG)	Als Sund	Poor
Sønderborg Naturskole (BCG)	Als Sund	Poor
Svendborgsund Havhave (BCG)	Svendborg Sund	Poor
Skovshoved Havhave (BCG)	Øresund	Moderate
Havhøst (KBH) (BCG)	Øresund	Moderate
Mogens Suveren (Havnsø) (BCG)	Sejerø Bugt	Poor
Havsmag (C)	Jegindø	Poor
Dansk Tang (C)	Isefjord	Poor
Betina Flies (Orø) (C)	Isefjord	Poor
Blå Biomasse (C)	Oddesund	Poor

2.2.2 Deployment of seeded lines, monitoring of growth and harvest at Blue Community Gardens and Companies

The seeded lines were deployed by the volunteers of each BCG or company in October 2022 and harvested in May/June 2023. The aim was to deploy the lines horizontally as in Sallingsund, however many lines were deployed vertically due to already established local cultivation setups.

During the growth period, the BCGs and company volunteers measured a set of environmental parameters approximately monthly: Seaweed growth (taking photos), salinity, water samples for nutrient determination, temperature and water turbidity (secchi depth). In March and at harvest in May, 6 biomass samples were taken from each seaweed line, frozen (-20 °C) and brought to Aarhus University for handling in the same manner as previously described for the seaweed samples from Sallingsund (area, FW, DW, DM, CNP content determined, in few cases also trace metals and iodine).

Data on growth, tissue content of C, N and P, as well as environmental data were used by DHI to develop model estimates for the efficiency of sugar kelp cultivation to affect key indicators of the EU Water Framework Directive (summer Chlorophyll a concentrations and the water clarity, expressed as the light extinction coefficient) (Larsen and Erichsen 2024).

2.3 Modelling of sugar kelp growth

A fundamental model for growth of sugar kelp was developed by AU (Holst et al. 2025) for 1) developing a specific Dynamic Energy Budget model (DEB) for the growth of sugar kelp, which applies detailed knowledge about the internal carbon balance in the individual macroalgae to estimate the growth and 2) integrate

the DEB macroalgae growth model into the biogeochemical models of DHI, in order to predict and quantify the effect of sugar kelp cultivation on two specific environmental indicators to evaluate the condition of a given water body: Summer chlorophyl a concentrations and the light extinction coefficient (Kd).

The DEB model was implemented into the DHI biogeochemical model for Limfjorden, but not for any other Danish water bodies (Larsen and Erichsen 2024).

Within the project, the growth model of sugar kelp, was further developed towards the simplest possible, biologically based model to simulate the biomass growth from autumn to summer of *S. latissima* cultivated on lines in Scandinavia. The model was developed based on a well-established framework for modelling terrestrial crops, using data from the one growing season of large-scale cultivation in Sallingsund where *S. latissima* was cultivated on lines in Limfjorden, Denmark (Section 2.1). The model was subsequently compared to two independent data sets obtained from previously published studies carried out in Denmark and Norway.

2.4 Economy of large-scale sugar kelp production

The economy of the cultivation of sugar kelp in Sallingsund at the 12-hectare site using Best Available Technology (BAT) was estimated using the actual costs of materials and services (water, electricity, labour etc). The lifetime of equipment was set to 10 years for the hatchery and the facility, while the lifetime of buoys and blocks were estimated to 5 years in the Limfjorden environment, guided by Zhang et al. (2022). A minimum salary of DKK 250 per hour was assumed.

2.5 Environmental monitoring at the large-scale cultivation of sugar kelp

To monitor the fundamental environmental parameters during the sugar kelp growth season, a suit of data loggers was installed at each end of the seaweed farm, fastened to an empty long line between the SNE and SNW areas, and between the SSE and SSW areas (fig. 2). The loggers continuously recorded salinity, temperature (Star Oddi, DST CT) and light (Odessey PAR). A reference mooring was established 300 m west of the farm (reference station / station R) (fig. 6), continuously monitoring temperature, salinity, pH, dissolved oxygen, and chlorophyll-a fluorescence with sondes (InSitu Aquatroll 600) positioned 50 cm above the seafloor and 1 m below the water surface. During the study, the Star Oddi loggers got fouled with barnacles, compromising the salinity data quality, so salinity data from the reference mooring was used. The loggers were manually cleaned every 2-3 weeks, and additionally, the light loggers were equipped with a mounted automatic wiper. During each visit, triplicate water samples were collected for analysis of dissolved inorganic nutrients.

In addition to continuous measurements, three field campaigns were conducted to assess the potential environmental interactions between the seaweed farm and both the water column and the benthic environment (table 3).

Table 3. Overview of the environmental monitoring campaigns and the connection to the laboratory experiments conducted using sugar kelp and in-situ water from the cultivation site in Sallingsund. X indicate that monitoring and/or experiment was carried out. E1-E3 refers to the 3 experiments carried out to determine the release of dissolved organic carbon (DOC) from the sugar kelp at various times during the season. * indicates that sugar kelp biomass was not from the cultivation site, as in November, the juvenile kelps in Sallingsund were too small to be used in experiments.

Date	Sugar kelp biomass	Environmental monitoring				Experiments		
		Hydrology	CTD	Light	Sedimentation	DOC	POC	Flux
11-18.11.2022	Negligible, baseline (0 g DM/m line)	X	X	X	X	X (E1)*	(X)*	-
17-24.02.2023	Low to medium (50 g DM/m line)	X	X	X	X	X, E2	X	-
14-21.04.2023		X	X	X	X	X, E3	X	X

The first campaign, conducted from November 11-18th, 2022, immediately followed the farm's establishment and characterised conditions with negligible seaweed biomass. The second campaign, from February 17-24th, 2023, assessed mid-production-cycle conditions. The final campaign, from April 14-21st, 2023, was conducted just before harvest to characterise conditions under maximum seaweed biomass.

A comprehensive monitoring strategy was employed, sampling at multiple fixed positions within and around the farm and profiling the water column at higher spatial resolution (fig. 6). Sampling positions were determined based on the predominant NNE-SSW wind-driven and tidal current regime. Water depths in the study area ranged from approximately 5 to 8 meters.

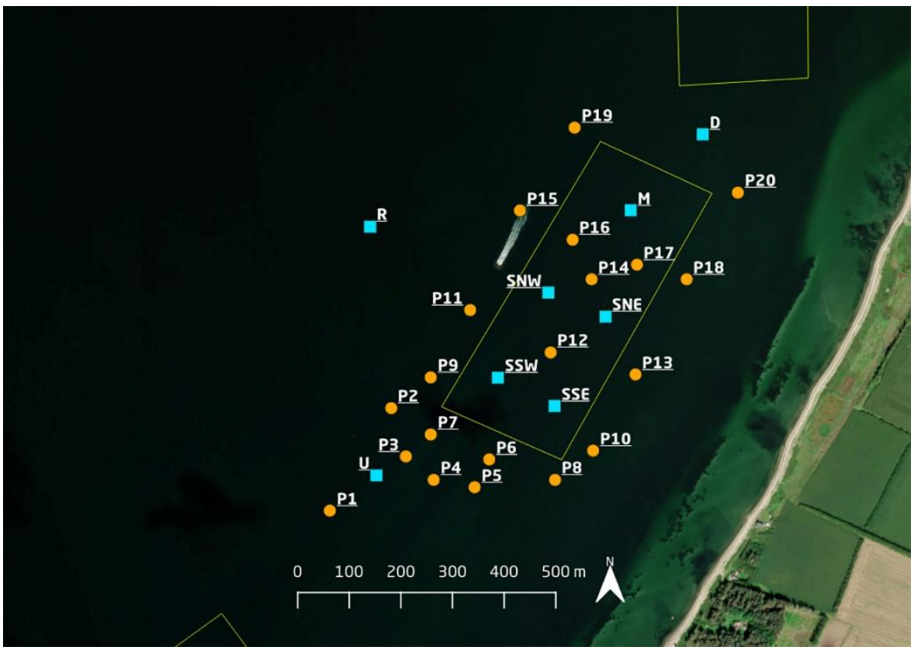

Figure 6. Sampling configuration for the environmental campaigns. Blue stations included profiling and sediment traps in the seaweed areas (SNW, SNE, SSW, SSE), the mussel area (M), upstream (U) and downstream (D) as well as on a reference site (R). Orange stations (P1-20) included only daily profiles. The farm perimeter is outlined in yellow, as are the two neighboring farms to the north and south. The area south (upstreams) of the experimental site was not in service during the monitoring period.

2.5.1 Physical parameters (CTD)

Vertical profiling for biophysical parameters was conducted at all stations, daily during the campaigns. The profiling package included a Licor Li-193 spherical underwater quantum sensor (PAR) coupled to a logger, a Sequoia Scientific LISST-200X, and a CTD package. The PAR logger recorded at 1Hz, and the LISST recorded at 1Hz using 25 samples per recording.

In the first campaign, an In Situ AquaTroll 600 was used as the CTD package, equipped with sensors to measure pressure, salinity, temperature, dissolved oxygen, chlorophyll-a fluorescence (chl-a), and pH, recording at 1 Hz. In the second and third campaigns, an RBR Maestro was used, recording the same parameters at 16 Hz. All instrument sensor interfaces were positioned at the same depth on the package frame. The frame was lowered at approximately 1-1.5 m m^{-1} while the vessel maintained position.

The diffuse downwelling light attenuation coefficient (KdPAR) was calculated from the exponential slope of PAR between the surface and measurements at depth (Mobley and IOCCG 2022). Beam attenuation (c) and particle volume concentrations were calculated using the LISST software with the irregular shape model.

As part of the environmental monitoring just before harvest in April from April 14th to 20th, 2023, light (PAR) loggers (Odyssey) were placed in the area to quantify the effect of the seaweed cultivation on the light attenuation in the area below and between the seaweed lines. Two loggers were placed in the reference area and two loggers were placed between and underneath the seaweed lines in area SSE. After the campaign, the logger placed between the lines was missing. Therefore, only light data immediately underneath a seaweed line provided data for evaluating the light environment below a seaweed farm inside the area SSE.

2.5.2 Hydrodynamics

Two Acoustic Doppler Current Profilers (ADCPs) were used to monitor hydrodynamics. A Teledyne RD 600 kHz Workhorse Sentinel was placed on a frame, upward looking, on the seafloor at station R during each campaign and between campaigns as part of the continuous monitoring program. This ADCP recorded in 50cm bins for 1-minute ensembles during the campaigns, and 10-minute ensembles between campaigns, with a blanking distance of 1.6m.

Additionally, a Nortek Signature 1000 5 beam 1mHz ADCP was deployed on a frame, upward looking, at stations R, D, M, SSW, and U during the campaigns (fig.6). Two concurrent recording plans were used: (1) average velocities in 50 cm bins in 1-minute ensembles, and (2) burst sampling in at 4Hz for 5-minute intervals in 2 cm bins, with a blanking distance of 20 cm. The high-resolution vertical fifth beam data allowed for the estimation of turbulent kinetic energy (TKE) dissipation (ε) rates (m^2s^{-3}) using a structure function method (Wiles et al. 2006; Guerra and Thomson 2017).

Stratification of the water column was assessed using the potential energy anomaly parameter φ (J m^{-3}) (Simpson and Bowers 1981). In-situ density (ρ) and the Brunt–Väisälä frequency squared (N2) were calculated from absolute salinity and conservative temperature by TEOS-10 (Roquet et al. 2015). Combining profiles and ADCP data, gradient Richardson numbers (Rigr) were derived from the quotient of N2 and velocity shear, where Rigr < 0.25 indicates shear velocity conditions overcoming buoyancy stability (Stevens and Petersen 2011).

2.5.3 Sedimentation rates and CN content of the sedimented material

Sedimentation rates were estimated from particular material captured at stations R, D, M, SSW, SSE, SNE, SNW and U during the campaigns (fig. 6). Environmental campaigns typically began by deploying sediment traps on the Friday before the campaign week.

At each station, eight sediment traps (71 mm diameter) were placed 1 m above the sea floor and exchanged every 1-3 days by divers. The contents of the sediment traps were filtered using pre-combusted GF/F filters (Whatman), washed with 0.5 M ammonium formate, dried for 24 hours at 80°C, weighed for Total Particulate Matter (TPM), then combusted at 550°C for four hours, and reweighed for Particulate Organic Matter (POM).

The content of C and N of the sedimented POM was analysed as described for seaweed tissue in section 2.1.7. Statistical differences were analysed in R using the vegan package (Oksanen et al. 2022) with permutational two-way analysis of variance, due to non-normal distribution of residuals.

For the final campaign in April, the stable isotopes of C and N in the sedimented POM were analysed on a C- and N-analyser and isotope ratio mass spectrometer (Thermo Analytical Flash EA 2000 Elemental Analyzer coupled via a ConFlo IV interface to a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer).

2.5.4 Sediment samples for determining fluxes of carbon, oxygen and inorganic dissolved nutrients between sediment and water

Sediment cores were collected on April 19th, 2023 at station R in Limfjorden (56°47'01.4"N, 8°54'20.5"E) by divers. The sediment was collected in 30 cm long acrylic kayak tubes with an internal diameter 50-52 mm (KC Denmark, Silkeborg, Denmark). The tubes were inserted ~16 cm into the sediment, filled with seawater from the collection site (salinity 31 ppt) and sealed with rubber stoppers. The sediment cores were stored in thermal boxes and transported to the laboratory at Aarhus University, where they were placed in a thermostatic room (15 °C).

2.5.5 Nutrient concentrations in seawater

The seawater concentrations of dissolved inorganic nitrate, nitrite, ammonium and phosphate were measured using a five-channel SKALAR San plus segmented flow autoanalyser (Breda, The Netherlands). All methods were adopted from Grasshoff et al. (1999).

2.6 Laboratory experiments

Three laboratory studies were carried out in connection to the environmental campaigns to analyse in more detail the carbon cycle of the cultivated sugar kelp. The three studies focused on: 1) Excretion, composition and degradation of Dissolved Organic Carbon (DOC) from the cultivated *S. latissima*, 2) Loss and Degradation of *S. latissima* Particulate Organic Carbon (POC) in the water column, and 3) Degradation of the *S. latissima* Particulate Organic Carbon in the sediment and effect on fluxes of carbon, oxygen and dissolved inorganic nutrients between sediment and water.

2.6.1 Excretion, composition and degradation of *S. latissima* Dissolved Organic Carbon

Three laboratory experiments were conducted to quantify the excretion of DOC from *S. latissima* exposed to different light intensities. The first experiment was carried out in October 2022 (E1), the second in March 2023 (E2) and the third in April 2023 (E3), just prior to harvest. In E1, the effect of nutrient availability on DOC excretion and degradation was also investigated.

In general, the experimental design was similar between the three experiments. The differences are highlighted in the following.

2.6.1.1 Collection and preparation of seaweed material for the DOC experiments

For the first experiment (E1), samples of intact *S. latissima* individuals were collected from 1-2 m depth by snorkelling from a natural kelp forest in Lillebælt at Middelfart Old Harbour on October 26th, 2022. The seaweed had to be collected from natural kelp forests, as the cultivated kelp in Sallingsund had not yet grown to a sufficient size. For the second (E2) and third (E3) experiments, samples of intact *S. latissima* individuals were sampled by boat from the cultivation lines at the 12-ha seaweed cultivation site in Sallingsund. Thirty specimens were gathered during the environmental campaign on February 28th, 2023 and an additional thirty specimens during the final campaign on April 24th, 2023. In all cases, after sampling, the macroalgae was transported to the laboratory at Aarhus University in tanks with seawater collected in situ.

At Aarhus University, the macroalgae were kept aerated in natural seawater at in situ salinities and temperatures (table 4) at a light intensity of 60 µmol photons m^{-2} s^{-1}, following a 12:12 light:dark cycle for one day before the experiment.

Table 4. Information on collection site, date and acclimation conditions for the sugar kelp sampled for the three DOC experiments (E1-E3).

Experiment	E1	E2	E3
Collection date	26.10.2022	28.02.2023	24.04.2023
Collection site	Middelfart	Sallingsund	Sallingsund
Position	55°30'25.8" N	56°47'4.9" N	56°47'4.9" N
	9°43'43.1" E	8°55'5.7" N	8°55'5.7" N
Preparation/ Acclimation	Nutrient enriched versus ambient	None	None
Wild (W) or cultivated (C)	W	C	C
Origin of cultivar	Middelfart	Middelfart	Middelfart
Temperature (°C)	10	5	10
Salinity (ppt)	26	30	30

For E1, a pre-experimental treatment was conducted in order to acclimate the macroalgae to two different environmental nutrient regimes: ambient and eutrophic conditions, aiming for different nutrient tissue contents reflecting either nutrient limited (NL) or nutrient replete (NR) conditions. The target tissue nitrogen concentration for the nutrient replete conditions was 3% N of algae dry matter (DM) (Weigel and Pfister 2021). The acclimation was performed as follows: Four plastic containers were prepared with each 20 litres of artificial seawater (Aquarium Systems Instant Ocean, France) with a salinity of 25 ppt. In two of four containers no nutrients were added (NL), whereas in the remaining two containers (NR), the nutrient concentration was increased to 883 µM nitrate-N and 42 µM ortho-phosphate by adding 20 mL of f/2 nutrient stock solution (Guillard and Ryther 1962) on days 1 (October 26th,

2022) and 3 (October 28th, 2022). Twelve randomly selected *S. latissima* individuals were acclimated to NL conditions and 12 were acclimated to the NR conditions. The acclimation period lasted 7 days starting on October 26th, 2022 and ending on November 02, 2022. During the acclimation, the light intensity was 60 µmol photons m^{-2} s^{-1} in a 12:12 light:dark cycle, with a constant temperature of 10 °C. Each container was aerated with atmospheric air to ensure water circulation and inorganic carbon availability.

2.6.1.2 Laboratory experiments for determining DOC excretion

Experimental design

Macroalgae incubations for the DOC release experiments were made by incubating series of single intact individual of *S. latissima* for approximately four hours in a two-litre blue cap bottle sealed with a rubber stopper. An 18 cm glass tube with a 6 mm diameter was inserted through the stopper, allowing the oxygen concentration to be continuously logged during the incubation using an O_2 mini optode (Unisense, Denmark). The incubation bottles were placed on magnet tables to ensure stirring for a homogeneous oxygen concentration throughout the bottle. To secure the macroalgae from the spinning magnet, 30 cm of bird-gutter net was inserted between the magnet and the macroalgae (fig. 7a). The in-situ seawater from Aarhus Harbour (E1), or Limfjorden (E2 and E3) was filtered through 0.2 µm and 0.5 µm filter for E1 and E2, and E3, respectively. Immediately prior to the experiment, the filtered seawater was bubbled with nitrogen gas with atmospheric concentrations of carbon dioxide (99.96% N_2 / 0.04% CO_2). This was done to reduce the oxygen saturation to approximately 60-70% to avoid oxygen supersaturation during the experiments, as this could affect the photosynthesis process as well as the accuracy of the measuring O_2 optode (Unisense A/S, 2020).

In all experiments, the *S. latissima* individuals were incubated at one of three different light intensities: E1 (0, 60, 180 µmol photons m^{-2} s^{-1}), E2/E3 (0, 60, 500 µmol photons m^{-2} s^{-1}) on three consecutive days. Five replicate bottles with macroalgae and 3 replicate control bottles were used on each day. The control bottles contained the same as the experimental bottles, but no macroalgae (fig. 7.b). Every day, new experimental macroalgae were used for the incubations, and following the incubation period, the macroalgae were harvested and used for subsequent analyses. Thus, no macroalgae individuals were reused and exposed to several incubations.

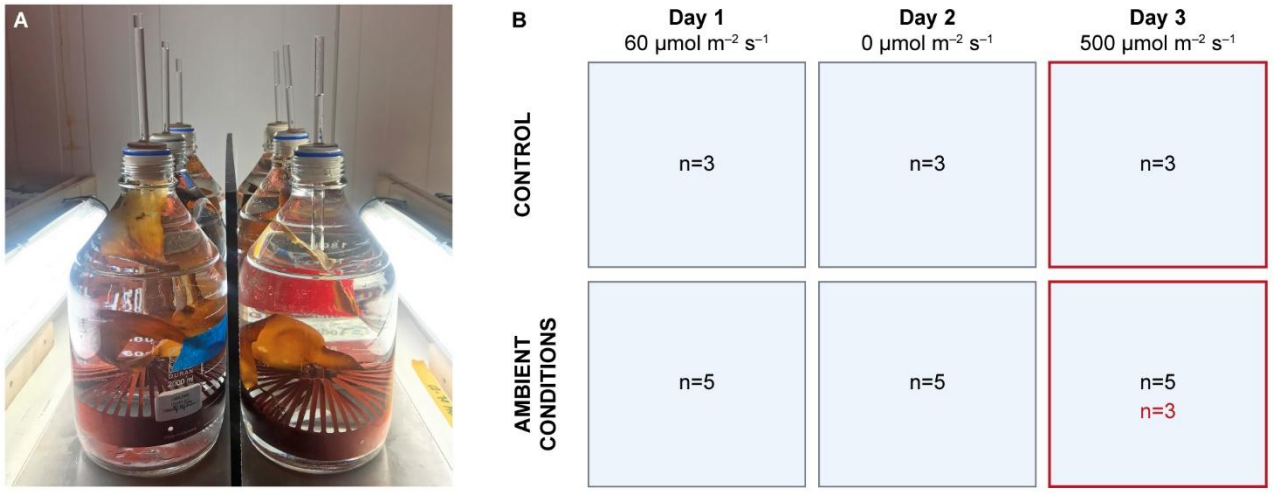

Figure 7. The experimental set-up for determining the excretion of Dissolved Organic Carbon (DOC) from sugar kelp (*Saccharina latissima*). a) Photo of the experimental set-up with the intact sugar kelp inside the 2 l blue cap bottles with seawater, rubber stoppers with penetrating glass tubes for inserting the oxygen optodes, and bird gutter separating the kelp from the stirring magnet, b) Incubation set-up to measure the oxygen exchange rates and DOC excretion. Control bottles contained no macroalgae. Ambient conditions; *S. latissima* individuals from ambient conditions in Limfjorden, Denmark, exposed to three light intensities (0,60 and 180/500 µmol m^{-2} s^{-1}). Oxygen production and DOC excretion were measured on three consecutive days. The red line indicates bottles from which water for the degradation experiment was extracted. N=number of replicate bottles.

The applied light intensities of 60, 180 and 500 µmol photons m^{-2} s^{-1} corresponded to the measured light intensity at 2.8, 1.8 and 0.7 meters depth at the seaweed farm in Sallingsund, Limfjorden; the latter two approximately resembled the light environment at the cultivation depths of *S. latissima* at the cultivation site.

Measurements and analyses

In all three experiments, the oxygen exchange rates and DOC excretions were measured over a four-hour period. Prior to use, glassware and filters were all muffled at 450°C for 4 hours, while non-muffled equipment was acid-washed in a 1 N HCL solution and rinsed with demineralised water to eliminate organic carbon contamination.

Photosynthesis

Oxygen exchange rates in each bottle were measured using an O_2 mini-optode (Unisense, Denmark).

Oxygen measurements began shortly after adding macroalgae to the bottle, recording oxygen concentrations every second. Oxygen concentrations were logged for 15 minutes at the start, 30-45 minutes in the middle, and 15 minutes at the end of the 4-hour incubation. Respiration was measured as oxygen consumption in darkness, while net photosynthesis was measured as oxygen production in light. Following the incubation experiment, supplementary measurements were conducted on both the macroalgae biomass and the incubation water.

Analyses of algae biomass

After finalising the four-hour incubation, the macroalgae individuals were carefully retrieved from the bottles, gently blotted with paper tissue, and weighed (FW). The algae were oven-dried at 60°C until a stabilised weight was attained. Dry weights were 1.49±0.42 g $individual^{-1}$, 0.62±0.023 g $individual^{-1}$ and 0.64 ± 0.03 g $individual^{-1}$ for E1, E2 and E3, respectively. The dried algae were finely milled for subsequent tissue analyses of carbon (C), nitrogen

(N) and ash content using a Retsch mill with 1700 rotations for two minutes as per the manufacturer's recommendation. Tissue content of C, N and ash was determined as described in section 2.1.7. Unfortunately, the ash samples from E2 were lost due to a technical malfunction of the oven.

Analyses of water from incubation

Dissolved organic Carbon (DOC): As soon as the macroalgae individuals were removed from the experimental bottles, three 40 mL incubation water samples were extracted for DOC analysis from each bottle. All samples were extracted using a Swinnex filter holder and a GF/F filter (Whatman, Ø= 25 mm). The filter was pre-muffled at 450 °C for 4 hours and a new filter was used for each bottle. A separate water sample for pH measurement was also extracted. Immediately following water extraction from the incubation bottles, 200 µL of phosphoric acid solution was added to each DOC sample to lower the pH to < 2, halting microbial degradation of DOC. Samples were stored in darkness at 5 °C until further processing.

Analysis of DOC concentrations were conducted using a Shimadzu TOC-L analyzer, which measures total organic carbon (TOC) based on non-purgeable organic carbon (NPOC). Inorganic carbon (IC) was eliminated, and the ratio between NPOC and TOC was determined. A standard curve was established using a stock solution of Acetanilide 99%. The DOC excretion (in units of µmol C (g DW)-1 h-1) was then calculated according to Reed et al. (2015):

$$\text{DOC excretion} = (\text{DOC} - \text{DOC control}) \cdot V / T\ \text{MDW}$$

Where MDW is the algal dry weight (g), T is the incubation time (h) and V is the incubation volume (L). The DOC control was the mean DOC concentration measured in the control bottles.

Dissolved Inorganic nutrients: The concentrations of dissolved inorganic nutrients of the incubation water were analysed in one sample from each replicate bottle as described in section 2.1.7.

2.6.1.3 Analysis of the components of DOC

At the end of the E3 incubations, the 0 and 500 µmol photons m^{-2} s^{-1} light intensity treatments were subsampled for analysis of Dissolved Organic Matter (DOM) composition. Two controls and two macroalgae bottles were subsampled in each case. The samples (500 mL) were solid-phase-extracted using PPL cartridges (Agilent Bond Elut), and DOM composition was analysed using UHPLC-qTOF-MS (See details in Appendix 1).

2.6.1.4 Degradation of DOC

To assess the degradation rate of the DOC excreted by *S. latissima*, 30 mL water samples from three out of the five replicate bottles with macroalgae, and from the triplicate control bottles exposed to 500 µmol photons m^{-2} s^{-1} in E1, were used (fig. 7b). These samples were collected from GF/F filtered residual water and transferred to 40 mL vials, assuming that the headspace in each vial could sustain aerobic microbial degradation of DOC. From each replicate bottle, 21 water samples were extracted, totaling 126 samples. The samples were stored at 15 °C in darkness to prevent photochemical degradation of DOC.

The DOC degradation experiment ran for 150 days, defining the remaining DOC concentration after 150 days as refractory DOC (RDOC) (Watanabe et al. 2020). On days 0, 3, 7, 11, 31, 90, and 150 after the incubation experiment, 150

µL of phosphoric acid was added to three of each biological replicate to reduce the pH to < 2 and halt microbial degradation of DOC. The DOC concentrations were quantified as described in section 2.6.1.2.

The degradation rate (k) and the RDOC fraction were computed using an exponential decay model defined as: An offset (represented by RDOC) accounts for the refractory component of DOC (Pedersen et al. 2021). The models utilise the DOC-corrected value, obtained by subtracting the DOC concentration measured in the macroalgae bottles from that in the control bottles. The turnover time is calculated as 1 divided by the degradation rate (k).

2.6.1.5 Emission of climate gases

Nitrous oxide and methane

Water samples for determining of dissolved concentrations of CO_2, CH_4 and N_2O, were collected straight after the end of the four hour incubation of Experiment 3 on April 24th, 2023, using the headspace equilibration (McAuliffe 1971), where 50 mL of water were collected from the incubation water using a plastic syringe. The 50 ml of water was reduced to 40 ml while eliminating bubbles. Subsequently, 10 ml of ambient air was added to the syringe, and the water/air was shaken continuously for 1 minute to release the gas from the water phase to the air phase. The air was then transferred into a 5.9 ml exetainer. Samples of ambient air were also collected and served as a baseline. The gas concentration in the headspace was measured with an Agilent 7890A Gas Chromatograph (Agilent, Nærum, Denmark) (Petersen et al. 2012) using 3 detectors: TCD for CO_2, ECD for N_2O, and FID for CH_4. The dissolved concentrations of the gases were calculated from the headspace gas concentration (ppm) using Henry's law and constants correcting for temperature and salinity (Weiss 1974; Weiss and Price 1980; Wiesenburg and Guinasso 1979).

Volatile organic carbons (VOC)

For analysis of emitted halocarbons, intact *S. latissima* individuals (same batch as used for quantification of nitrous oxide and methane emissions) were incubated 10 °C in 2 L gas-wash bottles filled with 1 L of artificial seawater. Three incubations were performed under light (160 µmol photons m^{-2} s^{-1}) and three under dark conditions. The inlets and outlets remained sealed for 1 h to allow the VOC accumulation in the headspace. To collect the VOC, the headspace was flushed onto the adsorbent tube containing 68 mg Tenax TA (60/80 mesh, Gerstel) with high-purity synthetic air at a flow rate of 150 ml min^{-1} (sampling time=20 min). To test what compounds could be further mobilised from the water by mild disturbance, the plants were removed, and the incubation media was purged onto the adsorbent tube as described above. Six incubations were conducted in total and an artificial seawater blank was taken to check for contamination. The VOC adsorbent tubes were analysed by thermal desorption (TDU, Gerstel, Germany) and gas chromatography-mass spectrometry (GC-MS) with an Agilent 7890B GC and 5977A MSD. Separation was achieved with DB-624 GC column (30 m × 0.25 mm × 1.40 µm) with helium as carrier gas. Chromatograms were analysed using Agilent MassHunter 10 software, and the VOC was identified according to their mass spectra in the NIST data library, at a match factor ≥85.

2.6.1.6 Statistical analysis

Statistical tests of the experimental data were performed in R studio; R version 4.2.2 (2022-10-31) (R Core Team, 2022). One-way ANOVA was used to examine whether wet weight, dry weight or area were significantly different within the light- and nutrient treatments of both experiment 1 and experiment 2 treatments.

Assumptions of normal distribution and Homogeneity of Variance were checked through the Shapiro-Wilk normality test and Levene's test/ Bartlett test for homogeneity of the variance respectively, before performing the t-test and ANOVA test. The parameters "N%" and "C/N ratio" were log-transformed to meet the criteria of variance homogeneity.

In scatterplots, data were fitted with the lm function and here the R-squared value indicates how much of the data are explained with the model and the p-value indicates the significant relationship in the model. All tests were conducted with a significance level of $p < 0.05$.

2.6.2 Degradation of *S. latissima* Particulate Organic Carbon in the water column

2.6.2.1 Seaweed tissue degradation experiment

The tissue degradation of *S. latissima* was studied using young (less than one year) and 2-3-year old individuals acclimated to high and low availability of dissolved inorganic nutrients (Vinbæk 2023).

Seaweed collection and acclimation

Saccharina latissima of different age, was collected from two different locations in October 2022 (fig. 2.1). Old (minimum 2-3 years of age) sporophytes were collected from near shore waters in Aarhus Bay, Jutland (56°10' 02.9"N, 10°13' 49.5"E). Young (less than one year of age) *S. latissima* was collected in the coastal waters near Middelfart, Fyn (55°30' 33.7"N, 9°43' 12.4"E).

Artificial seawater (ASW) (salinity of 27) was made using (Red Sea salt, Instant Ocean, France) and demineralised water. The growth media used was f/2 (Guillard and Ryther 1962).

All collected seaweeds were placed in different aerated containers with saltwater, old *S. latissima* in

ASW and young *S. latissima* in in-situ seawater with a light intensity of 60 µmol m^{-2} s^{-1} using a 12:12 light:dark cycle in a 10 °C climate room. Half of the young (Y) and old (O) sugar kelp individuals were acclimated to ambient (low) concentrations of dissolved inorganic nutrients (-), while the other half were acclimated to high nutrient availability (+). The treatments were named accordingly: O- and O+ , Y- and Y+.

Seaweed tissue degradation process

Two sporophytes from each treatment of old *S. latissima* and three sporophytes from each treatment of young *S. latissima* were randomly chosen. 15-20 circular discs with a diameter of 1 cm were cut from each sporophyte using a hollow metal cylinder. The discs were cut from older, distal part of the sporophyte, to imitate the tissue of the sporophytes most likely to be lost in situ. Fertile tissue was avoided. Approximately 45 discs from each treatment were placed in a 20 x 20 cm mesh polyester bag with a metal zipper opening (one mesh bag for each treatment).

A container was filled with 40 l of natural seawater from Aarhus Bay (salinity 27) and aerated with atmospheric air and the temperature was kept at 10 °C. The seawater was filtered through a 0.5 mm sieve to avoid bigger organisms disturbing the experiment. The four polyester bags were added to the same container, hanging from a steel wire, to make sure that the metal zippers did not

touch the water. The discs were kept in darkness to avoid photosynthesis in the algae discs. After approximately one week of degradation, the algae tissue, in especially the nutrient acclimatised discs, had disintegrated. To make sure that the algae tissue would not dissolve, leak through the mesh bag and disperse into the surrounding seawater, the different treatments were added to each their own bucket (degradation containers), all with the same water as before.

Measurements of seaweed tissue oxygen consumption - measurements and controls. General experiment setup

On day 1, 2, 3, 7, 21, 30, 69, and 153, oxygen consumption of three discs from each treatment was measured, using a Unisense MicroRespiration System (Unisense, Denmark, https://unisense.com

/products/microrespiration-system/). The Unisense MicroRespiration contained an O2 microsensor, Uniamp Multi Channel, eight approximately 4 ml closed glass chambers and a rack specialised to the glass chambers. Each chamber was equipped with a glass-coated magnetic stirrer. For the experimental setup, a container was filled with a water level high enough for the chambers to be immersed in water, sealing the chambers and keeping the temperature constant at 20 °C. The temperature of water was controlled by a thermoregulator (LAUDA ECO RE415), and mixing was created by a pump. Further, seawater from the container surrounding the discs was taken out of the climate room, the day before the experiment. This thermo-acclimation was to make sure that the temperature was constant to eliminate changes in water oxygen level caused by temperature change. The container was covered with black plastic bags, to make sure that light could not affect the oxygen measurements.

Algae disc measurements

On the experiment day, three replicate discs of every treatment were carefully taken out of the nets in the degradation container with a tweezer and transferred to the chambers in darkness, to avoid photosynthesis. In each chamber, one disc was placed on a small metal mesh, separating the mixing magnet from the sample. Sterile filtered 20 °C seawater, was carefully filled into the chambers. The O_2 consumption of the enclosed water and seaweed disc was monitored in darkness by an O_2 microsensor (Unisense) penetrating the lid. The oxygen microsensor was calibrated according to the manufacturer's instructions. Measurements were done over approximately 10-15 min to test if the algae discs still were able to photosynthesize, a second round of measurements was made with a light intensity of approximately 60 µmol photons m^{-2} s^{-1}. All measurements were performed against three control chambers containing only sterile filtered seawater. Three control measurements were made for each treatment (all four degradation containers (bucket)).

Calculation of the rate constant k

All oxygen measurements were detected with the software SensorTrace Rate (Unisense). From each measurement an oxygen consumption or production rate was given. The rate in the software was calculated based on the slope and the volume of the chamber. This rate was used to calculate the initial concentration of carbon (Gt) in the samples from the end carbon concentration ($G(t+\Delta t)$), given by the results from the CN-analysis. From this, and the time interval of the measuring period, the rate constant k was calculated.

Seaweed tissue analyses

After measurements, surplus water was removed from each algae disc using paper tissue, and the discs were weighted (FW) and placed in a 60°C oven for

at least 48 h to dry. When dry, the algae were weighted again (DW) and crushed using a small mortar.

C/N analysis: 1-2 mg crushed tissue was filled into a tin capsule. When degradation had caused loss of algae structure, the algae tissue was filtered on to a pre-weighted, muffled GF/F filter. The filters were then cut in half, and carefully packed in a tin capsule. The CN analysis was performed as described in section 2.1.7.

2.6.2.2 Degradation of particulate organic matter (POM) experiment

The degradation of POM was studied in material from sediment traps, deployed in five stations in

and around the seaweed and mussel farm during one growth season in Limfjorden, see section 2.1.1 (figs. 1 and 6).

Sampling of POM

Particulate Organic Matter was collected using sediment traps deployed in the seaweed cultivation site (see section 2.5.3). One set of sediment traps from each station (four bottles) was used to measure sedimentation rate, content of dry matter (DM) and ash content of the POM as described in section 2.5.3. The content of the second set of sediment traps was transferred to a bucket (one bucket for each station) and transported to the climate room with a temperature of 10 °C, where the buckets were kept throughout the experimental period. Each bucket was aerated with atmospheric air and placed in darkness.

POM oxygen consumption – measurements and controls.

On day approximately 1, 2, 3, 7, 14, 30, 60, 90 and 150 after collection of the POM, oxygen consumption measurements were conducted using the three replicate samples of POM from each station yielding a total of 15 samples per measuring day. Measurements were performed using the Unisense MicroRespiration system as previously described in section 2.6.2.2 with the following modifications. A sub sample of POM and the seawater supernatant was transferred to bluecap bottles and taken out of the climate room the day before measurements, to ensure a constant temperature and thereby eliminating changes in water oxygen concentrations caused by temperature change.

Oxygen and POM measurements

On the experimental days, oxygen consumption of three replicates of POM from each station were measured as described in section 2.6.2.2 with the following modifications. Approximately 1-1.5 ml

POM was transferred from the thermos acclimated bluecap bottle to the chamber with a Pasteur pipette. Hereafter, the supernatant seawater was carefully filled in the chamber using a Pasteur pipette until the chamber was fully filled, ensuring no presence of air bubbles. All measurements were performed in darkness and against control chambers containing seawater from the bluecap bottles from each station, filtered through muffled GF/F filters (Whatman). This filter size was chosen for controls because the same filter size was used for filtration of POM later in the experiment. To ensure that the filtering process did not cause air-water mixing (leading to oversaturated filtered water and air bubbles), the supernatant water from the bluecap bottles was filtered as the first thing in the morning, on each experimental day. The filtered seawater was filtered into another set of bluecap bottles which had been sterilised with boiling demineralised water. After each measurement, all content

from each of the chambers was filtered onto each their GF/F filter (Whatman). Every chamber was rinsed at least three times with demineralised water to make sure all material in the chamber was filtered. The filters were folded and dried in the oven at 60°C for at least 48 h.

C/N analysis and Inorganic carbon removal

When filters were dry, inorganic carbon was removed from the sediment of all sample filters by incubating the filters in an exicator containing 1 M HCl for approximately 24 hours. Following, all filters were carefully folded and packed in tin capsules. With a scalpel, all filters were cut in two, to fit the capsule. The C/N analysis was carried out as described in section 2.1.7. The removal of inorganic carbon was only done on samples from February and April, not for the November samples.

2.6.2.3 Statistical analysis

To compare k-values and CN-ratios between stations, treatments and days of measurements statistical analysis was conducted in R studio version 2022.12.0. All data sets were tested for the assumptions of normality of the residuals and homoscedasticity. Normality was tested using a Shapiro-Wilk test, and Bartlett's test was performed to test homoscedasticity. If the assumptions were confirmed, a one-way ANOVA test was performed. If the assumptions were not met, a non-parametric Kruskal Wallis test was performed. If the p-value showed significance ($p < 0.05$), pairwise multiple comparisons were tested with a non-parametric Dunn test. Linear regression analysis of the first-order constant (k) and CN-ratio was performed with a linear model (lm function I R Studio).

2.6.3 Degradation of *S. latissima* Particulate Organic Carbon in sediment

2.6.3.1 Sediment collection

Sediment was collected during the final environmental campaign on April 4, 2023, at station R in Limfjorden (56°47'01.4"N, 8°54'20.5"E) by divers, as described in section 2.5.4 (fig. 6).

To de-faunate the sediment cores, they were exposed to anoxia for 4 days by bubbling the overlying water with a N_2/CO_2 gas-mixture (0.04% CO_2 and 99.96% N_2). All fauna on the surface of the sediment was removed. The cores were then each equipped with a stirrer and submerged in an incubation tank with aerated water for 143 days until the addition of macroalgae.

2.6.3.2 Macroalgae collection and preparation

Macroalgal material of *S. latissima* was collected September 13th, 2023 in Aarhus Bay (56°10'03.5"N, 10°13'45.1"E) by snorkelling at 1-3 meters water depth and transported to the laboratory at Aarhus University in saltwater from the collection site (salinity 20) (Ehrenreich et al. 2025).

In the laboratory, the algal material was inspected and ~10 g FW was used for the experiment. We selected the tissue close to the meristematic region of *S. latissima*, as we wanted to avoid reproductive areas.

Macroalgae nutrient incubation

The collected *S. latissima* were each incubated for 7 days in buckets with 1 l artificial seawater, salinity 24 (Red Sea salt, Instant Ocean, France) with and without nutrient enrichment to achieve nutrient limited and nutrient replete

tissue, respectively. All macroalgae incubations included vitamins and metals of the f/2 media (Guillard and Ryther 1962), whereas nutrients were only added to the nutrient enriched treatment. Nutrients were added on day 1, 3 and 6 to avoid nutrient limitation. The buckets were placed in a thermostatic room at 10 °C and were continuously aerated.

2.6.3.3 Experimental set-up

To investigate the degradation of macroalgae in the de-faunated sediment cores, ~0.5 g FW of algae was added to each core (Boldreel et al. 2023) with four replicates (n=4) per treatment and three replicates of the control treatment (n=3). The algae material was cut into ~5x5 mm tissue fragments and placed on the sediment surface. To keep the algae in place, a circular piece of green plastic net, was placed on top of the algae/sediment. A magnetic stirrer was placed ~4 cm above the sediment surface in each core. The cores were submerged and aerated in an incubation tank with water from the collection site at a constant temperature of 15°C and kept in darkness during the entire experiment.

To monitor the degradation of the macroalgae over time, flux incubations were conducted on day 1, 5, 12, 20, 40 and 83 after the addition of macroalgae. Initial concentrations of O_2, dissolved inorganic carbon (DIC) and nutrients were taken by draining and thoroughly mixing the incubation water from the incubation and the overlying water in each sediment core. A polystyrene disc was placed in each core to prevent water-sediment mixing when refilling the water. The sediment-water flux incubations were initiated by sealing the cores with a gas-tight rubber stopper and incubating them for ~2 hours, while a magnetic stirrer in each sealed core ensured mixing. We aimed for a 10-20% decrease in oxygen concentration to ensure a linear decline over time (Glud 2008). The water volume in each core was determined by measuring the height of the water column. The incubation was terminated by removing the rubber stopper, measuring the O_2 concentration immediately, and then taking water samples for DIC and nutrient concentrations.

2.6.3.4 Flux measurements

Dissolved oxygen concentrations were measured using a handheld LDO101-probe with a precision of ±0.1 mg/l at O_2 concentration 0-8 mg/l and ±0.2 mg/l at >8mg O_2/l (HACH HQ40D, Loveland, Colorado, USA). Water samples for DIC analysis were taken from the middle of the water column using a glass-syringe and transferred to 12 ml exetainers and preserved with 100 µl 5% $HgCl_2$. The water samples were taken as triplicates per core. The samples were stored in darkness at room temperature until analysis. The analysis was conducted using a Dissolved Inorganic Carbon Analyzer model AS-C3 (Apollo SciTech Inc., Georgia, USA). Each exetainer was measured tree times, or until a mean value could be calculated with a %SE ≤ 0.1. Data were corrected for instrument background and drift due to temperature and density differences by use of a standard solution (concentration: 2052.75 µmol kg^{-1} ±0.62 µmol kg^{-1}). Water samples for nutrient analysis (NH_4, NO_3, NO_2, PO_4 and Si) were collected with a 60 ml plastic syringe and filtered through 0.7 µm pore size GF/F filter (Whatman, Maidstone, UK). The water samples were frozen immediately and stored at -20 °C until analysis. Dissolved nutrient concentrations were measured with a five-channel SKALAR SanPlus segmented flow autoanalyzer (Breda, The Netherlands). All methods were adopted from (Grasshoff et al. 1999).

Data analysis

The DIC, O_2 and nutrient fluxes were calculated per sediment core, using the formula described in Politi et al. (2019) with following modifications: The time for DIC fluxes was defined as the time from when water was poured into the core

until the experiment was completed. Whereas time for O_2 and gas fluxes were defined as time from plugging the core until the experiment was completed.

To calculate the cumulative fluxes, an interpolation of data between the measure dates was made. The interpolation assumed a linear relationship in concentrations between two days of measuring. After interpolation, the daily fluxes were cumulatively summed.

To quantify the decay rate constant (k d^{-1}) an exponential function was fitted to the cumulative O2 consumption for each core:

$$flux(t)=b \cdot e^{(-k \cdot t)} \quad \text{Eq. 1}$$

Where the flux(t) is the total O_2 flux at time (t), b is the initial O_2 flux to t=0 and k is the decay constant. The fitting was preformed using the nls function in Rstudio with the algorithm "port", which is chosen for solving nonlinear least squares problems.

The amount of algae added to each core, was not the same in all treatments. To correct for this, the fluxes were normalised to the added amount of algae using the formula:

$$\text{Algae normalised flux} = (flux_i(t))/(\text{added algae}_i) \quad \text{Eq. 2}$$

Where flux_i(t) is the flux (mmol O2 m^{-2} day^{-1}) at time t for core i. The added algae is the amount of carbon added to each core in mmol. The algae corrected flux is in mmol O2 m^{-2} mmol $(C)^{-1}$.

In most other studies, carbon mineralisation is assessed from the O_2 consumption in darkness and the respiratory quotient (RQ). Here, the carbon mineralisation was calculated based on both the O_2 consumption and the release of DIC. The carbon turnover is the percentage added macroalgae carbon released over the entire experimental period, and this was calculated using either the O_2 or DIC method (Eq 3 and 4).

O_2 method:

$$\text{Carbon turn over (\%)} = ((flux(total)i \cdot RQi) - (flux(total)\_control \cdot RQ\_control))/(\text{added algae}_i) \cdot 100\% \quad \text{Eq. 3}$$

Where flux(total)i is the total flux (mmol O_2 m^{-2}) over the entire experimental period in core i, flux(total)control is the mean total flux (mmol O_2 m^{-2}) over the entire experimental period for the control, added algaei is the amount of algae added to core i (mmol C m^{-2}) and RQi and RQcontrol is the mean DIC:O2 ratio over the entire experimental period for core i and control, respectively.

DIC method:

$$\text{Carbon turn over(\%)} = (flux(total)\_i - flux(total)\_control)/(\text{added algae}_i) \cdot 100\% \quad \text{Eq. 4}$$

Where flux(total)i is the total flux (mmol DIC m^{-2}) over the entire experimental period in core i, flux(total)control is the mean total flux (mmol DIC m^{-2}) over the entire experimental period for the control and added algae_i is the amount of algae added to core i (mmol C m^{-2}).

Sediment and algae measurements

The sediment was analysed for density, dry matter content, C/N and loss of ignition (LOI), at the start of the experiment (initial sediment), and after the experiment. Each sediment core was cut into sections at depth intervals: 0-0.5, 0.5-1, 1-2, 2-3, 3-4, 4-7 and 7-10 cm. Each section was placed onto pre-weighed alu foil trays, and the wet weight (WW) was determined. The sediment sections were dried in the oven at 105 °C for two days. The dry sediment was analysed as described in Boldreel et al. (2023) with exception of the procedure for LOI. Here 0.5 and 2.0 g of the dried sediment was added to pre-weighted crucible and dried at 550 °C for 2 hours.

The algae tissue was analysed for DM content, tissue N and C as described in section 2.1.7.

2.6.3.5 Statistical analysis

All statistical analysis were performed in Rstudio, R version 4.3.3, 2024-02-29 ucrt (R Core Team, 2024). Most data were fitted into a generalised linear model (GLM), which is an extended version of a linear regression model, where varying types of error distributions can be handled. In some cases, the data was not linear, and a generalised additive model (GAM) was then used to fit the data. After creating a model, different analysis was performed to investigate if the model assumptions were met, including QQ-plot (distribution of residuals), residual plot (linearity and homoscedasticity) and Levene's test (homogeneity of variance). When all assumptions were met, a one- or two-way ANOVA analysis was performed. If the criteria of homogeneity of the variance in data were not met, evident for nutrient data, a Kruskal-Wallis test was applied. When the Kruskal-Wallis test was significant, a Dunn's test with Bonferroni adjustment was used as a post hoc test to test the differences between treatments. In one case the assumption of homogeneity of variance was slightly violated, but this appears clearly in the result section. All data was analysed from a significance level of $p < 0.05$.

3 Results

3.1 Sugar kelp production in large scale - Biomass yields, tissue composition and nutrient removal potential

3.1.1 Environmental conditions

The photosynthetically Active Radiation (PAR) generally ranged between 25 and 250 µmol photons m^2 s^{-1} in 3 meters depth (fig. 8.a). The light intensity was lowest during the winter months and increased from March onwards. The average Secchi depth in the area was 3 meters.

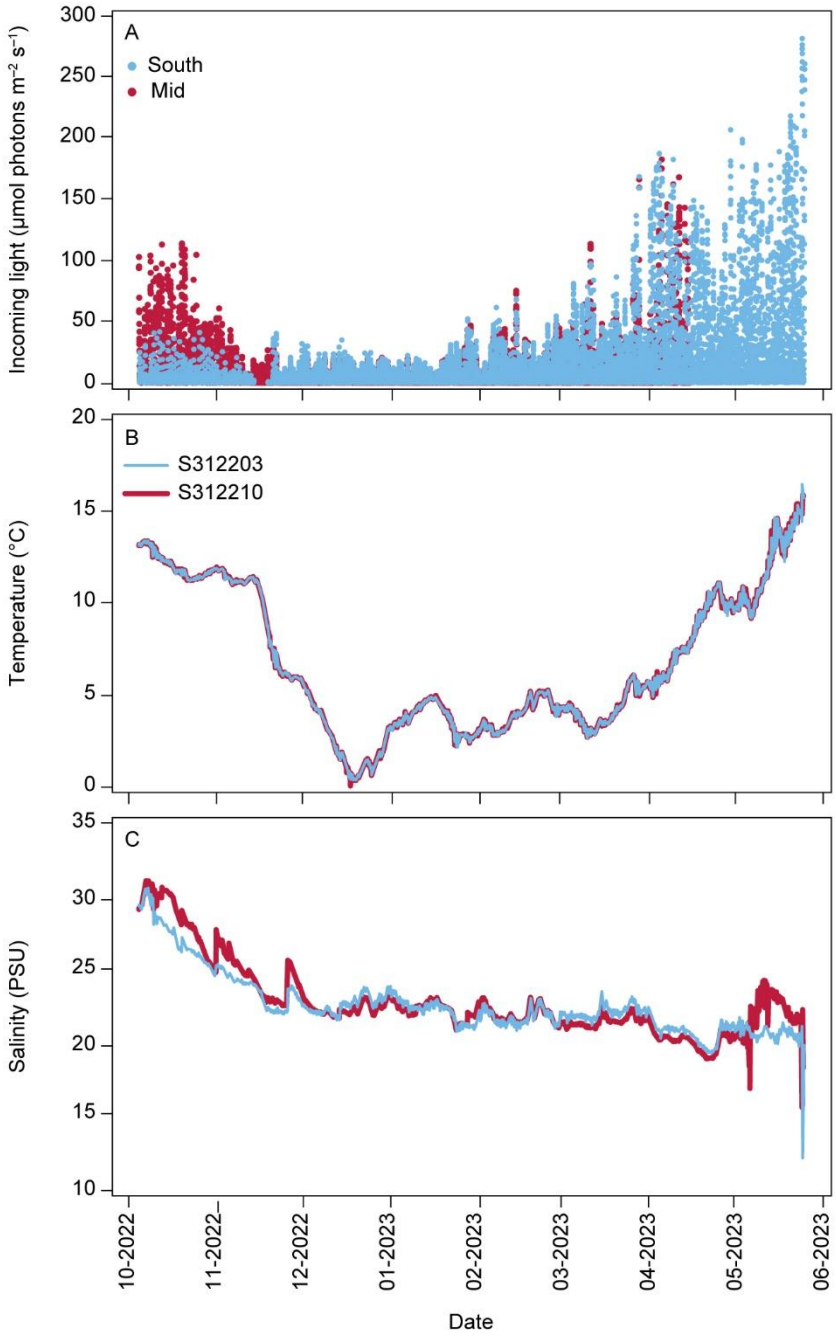

Figure 8. Ambient environmental conditions within the large-scale seaweed cultivation site in Sallingsund, Limfjorden, during the sugar kelp growth season from deployment in October 2022 over harvest in April to final sampling and monitoring in May 2023. A) Incoming light (Photosynthetic Active Radiation (PAR)) (µmol photons m^{-2} s^{-1}), b) Temperature (°C) and c) Salinity (PSU). All were measured by continuous loggers deployed with the seaweed cultivation systems. The blue and red colors refer to data from each of the two monitoring stations (blue =south and red=mid) within the farm as described in section 2.5 (fig.1).

During the main growth season, temperatures ranged from 0°C to 13°C at the main harvest in April (fig. 8.b). Post-harvest, temperatures increased to approximately 15°C at the time of the final sampling and monitoring in May.

Salinity ranged between 26 and 31 PSU, with a tendency for a lower salinity at the end of the cultivation period (fig. 8.c). The highest salinities were observed from October to December, while a stable salinity of 21-24 PSU was observed from December to May.

Ambient concentrations of dissolved inorganic nutrients exhibited a seasonal pattern, with concentrations increasing during the autumn and winter months and subsequently decreasing towards spring and the harvest period (fig. 9). Ortho-phosphate (PO_4) concentrations declined more rapidly than concentrations of dissolved inorganic nitrogen (DIN), resulting in an N/P-ratio >60 in February and March 2023, indicating that phosphate may be the limiting nutrient during the growth season.

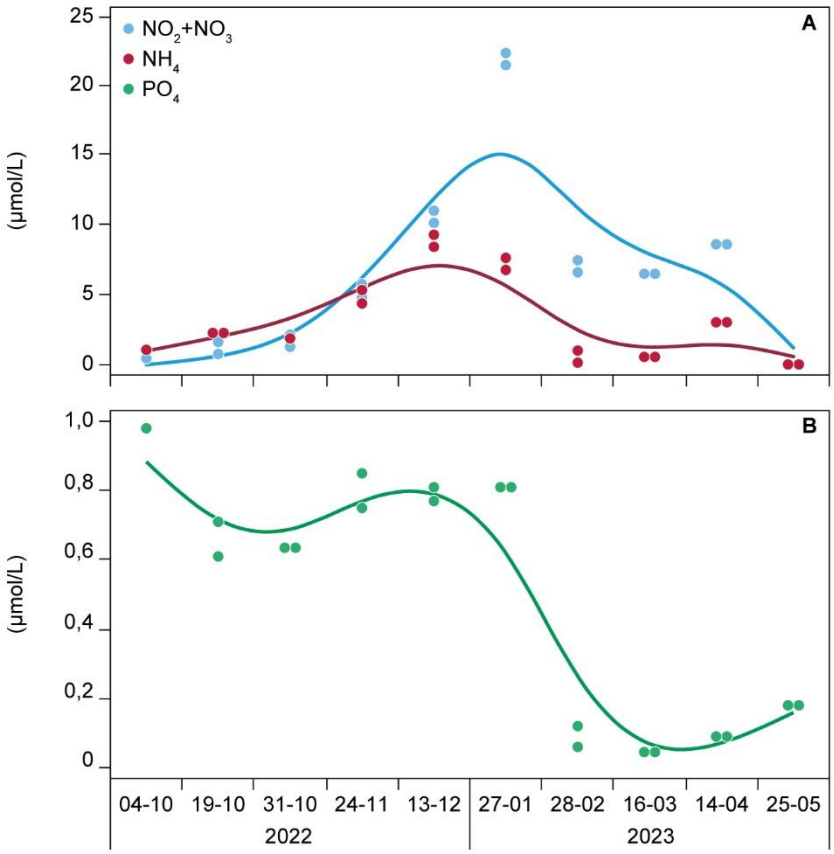

Figure 9. Ambient concentrations of dissolved inorganic nutrients within the large-scale seaweed cultivation site in Sallingsund, Limfjorden, during the sugar kelp growth season from deployment in October 2022 over harvest in April to final sampling and monitoring in May 2023. Concentrations of a) dissolved inorganic nitrogen (nitrite + nitrate, ammonium) and b) dissolved inorganic phosphorus (ortho-phosphate).

3.1.2 Sugar kelp growth and biomass yields

The sugar kelp grew across all cultivation areas up until the main harvest in late April 2023 (fig. 10). A few lines were kept in the water beyond this period to monitor growth and quality post-harvest, and on these lines the sugar kelp growth either stagnated or declined. This observation suggested that late April was the optimal time for harvesting to mitigate the risk of biomass loss.

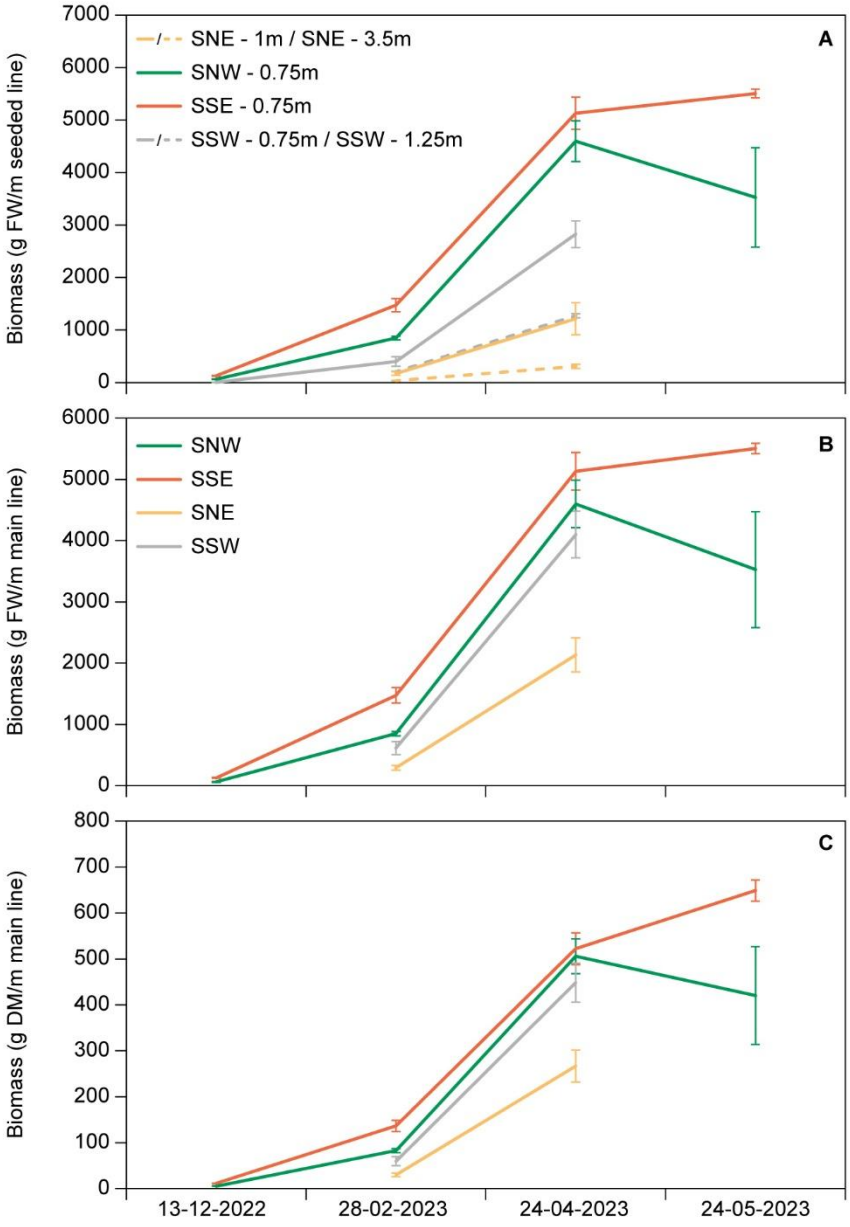

Figure 10. Temporal development in biomass yield in the four cultivation areas: a) wet biomass yield pr. meter seeded line given in two depths representing top and bottom of the two cultivation designs, b) wet biomass yield pr. meter main line and c) dry biomass yield pr. meter main. The cultivation designs used in the four areas were: SSE and SNW - single horizontal line, SSW - doble horizontal lines, and SNE – loops (see fig.3). Data is presented as mean±SE (n=9).

At the main harvest in late April, a clear distinction was observed between different cultivation areas and systems. The single horizontal lines produced the highest yield in terms of wet weight (WW) per meter of seeded line, whereas the loop system and the deep line of the double horizontal line system exhibited the lowest yields ($p<0.001$). When considering the yield per meter of the main line, no significant differences were observed between the SNW, SSE, and SSW areas, all of which used horizontal line systems. However, the loop system had a significantly lower yield per meter of mainline ($p=0.002$). This pattern was consistent for both WW and dry weight (DW) per meter, as only minor differences were found in the dry matter content (fig. 10).

There was no additional biomass gain in the double horizontal system as compared to the single horizontal line system. This suggests reduced growth even at relatively shallow depths (1.25 meters) in the area, as compared to growth nearer the surface (0.75 meters). Additionally, a potential system or design effect may have caused the upper line in the double horizontal system to perform worse than the single horizontal line system, possibly due to tangling and extra handling, wear and tear, or lower quality seed lines.

Individual growth and loss

The length and width of the sugar kelp fronds increased until mid-April, after which the length decreased while the width stagnated. Between April and May, the pattern of length loss was opposite to that of biomass, with sugar kelp in the SSE area showing greater length loss than kelp in the SNW area (data collected 10 days prior to harvest, as shown in fig. 11). Meristematic growth was highest from February to April, with fronds growing on average 65 cm over 45 days, compared to 27 cm over 40 days from April to May. During the February to April period, the overall frond growth was 67 cm, which was comparable to the meristematic growth, indicating no overall loss of frond length during this time. No significant differences were observed between the two areas.

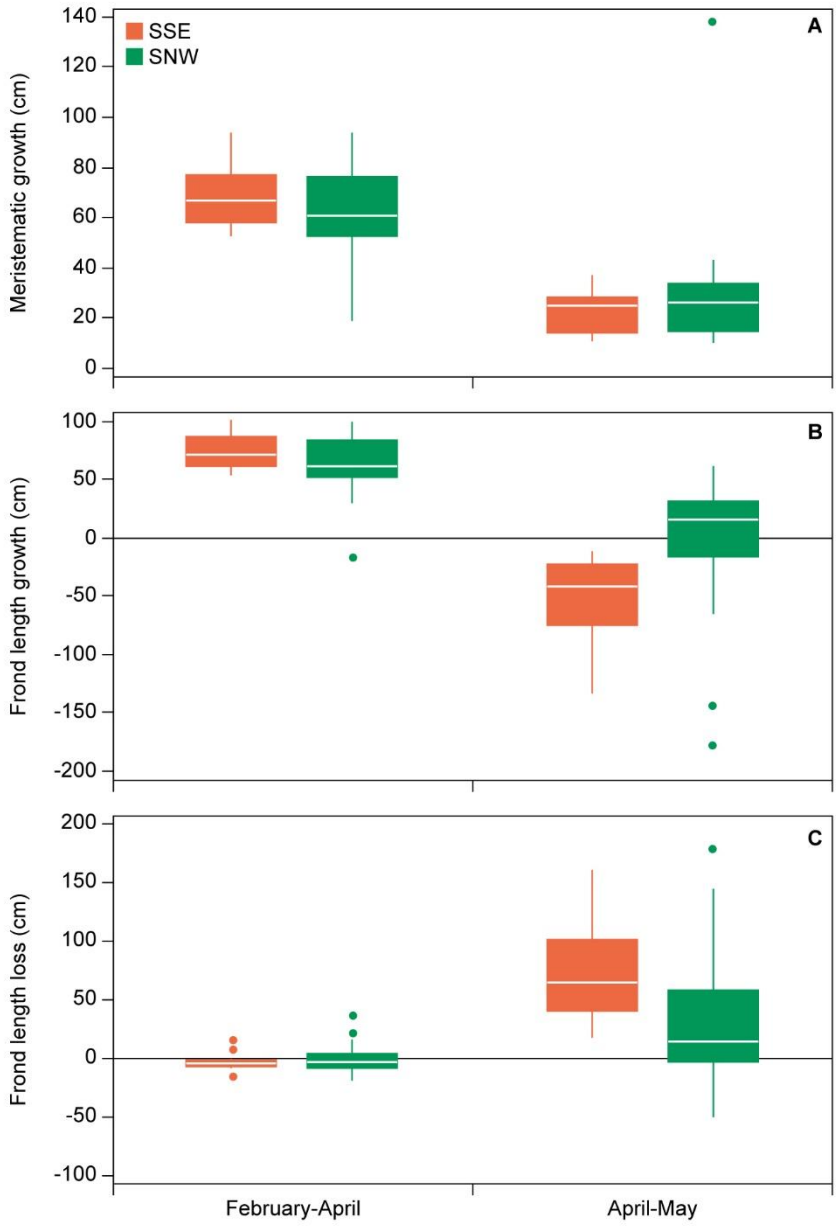

Figure 11. Growth and loss of sugar kelp individuals measured using the 'punch hole' method (Parke et al, 1948. Nielsen et al, 2014). A) Meristematic growth (cm) (top), b) Overall frond growth (cm (middle), and c) Frond length loss (cm) (bottom) in the two areas with single horizontal line setup (SSE (orange) and SNW (green)) during two periods: 1. The period of maximal growth up to harvest (Feb-April (45 days) and 2. The post-harvest period (April-May (40 days)). Data is presented as box plots with outliers (n=17-20).

In contrast, from April to May, the meristematic growth was reduced to 27 cm, and overall frond growth was no longer comparable to meristematic growth due to the onset of distal loss. This distal loss was more pronounced in SSE than in SNW, with losses of 71 cm and 34 cm, respectively over the 40-day monitoring period (p=0.0137).

3.1.3 Sugar kelp biomass composition

Only minimal differences were observed in the composition of seaweed from the four areas (fig. 12). The only significant difference was a higher DM content in the sugar kelp from the SNE area as compared to sugar kelp from the SSE area. The fact that the N content (N% of DM) did not differ between the sugar kelp cultivated in the four areas indicated that the mussel farming in the area north of the seaweed areas (downstream) did not impact the results.

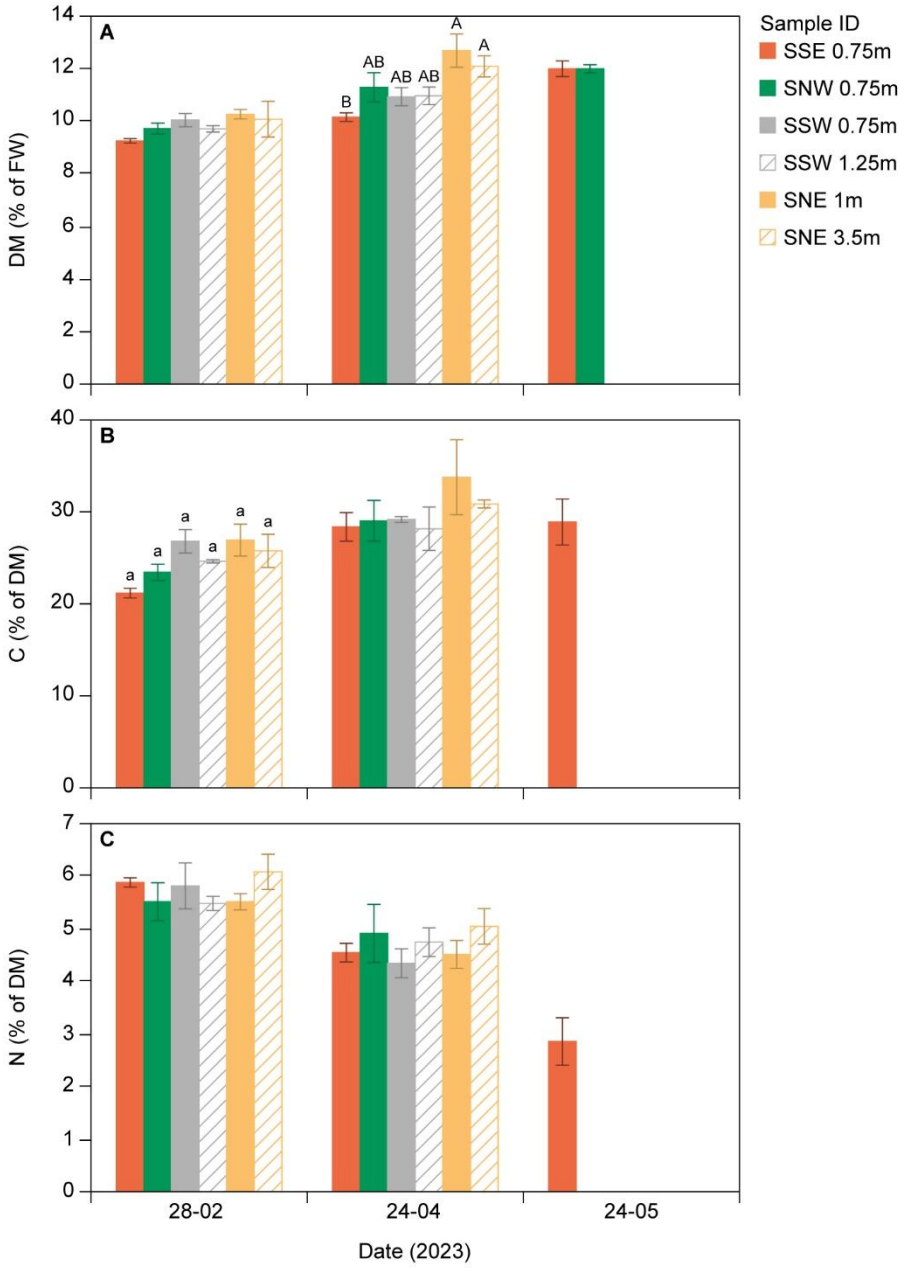

Figure 12. Dry matter content (DW (% of FW)), carbon (C) content (% of DM), and nitrogen (N) content (% of DM) from February, April and May for the four different areas: SSE - single horizontal line, SNW - single horizontal line, SSW - doble horizontal lines, and SNE - loops. For the areas SSW and SNE, samples were taken from two different depths. Data is presented as mean±SE (n=3, except SSE 24-05-2023, where n=2).

Also, in relation to cultivation depth, no differences in composition were observed. On a temporal scale, the DM content (% of FW) (Feb: 9.8±0.13, Apr: 11.4±0.20, May: 12.0±0.13) and carbon content (C % of DM) (Feb: 24.8±0.65, Apr: 29.9±0.90, May: 28.9±2.50) appeared to increase from February to April/May. Conversely, the nitrogen content (N % of DM) was generally high, with the highest N content observed in February (5.7% +/-± 0.11), and decreasing to 4.7% ±+/- 0.13 at harvest in April, and further decreasing after the main harvest in April to 2.9+/±0.45% of DM (the observation in May being based only on two samples).

3.1.4 Nutrient and carbon removal potential – large scale production

The nutrient removal potential, harvest of C and N through harvest of the seaweed biomass, closely followed the biomass development (fig. 13), as no major significant differences were observed in the biochemical composition (fig. 12). The highest N removal potential was recorded at the main harvest in April. The N yield per meter of seeded line was highest for the single horizontal lines (SSE and SNW), while no differences were found for the N yield per meter of main line. After April, the N yield decreased due to a reduction in the tissue N content (fig. 13).

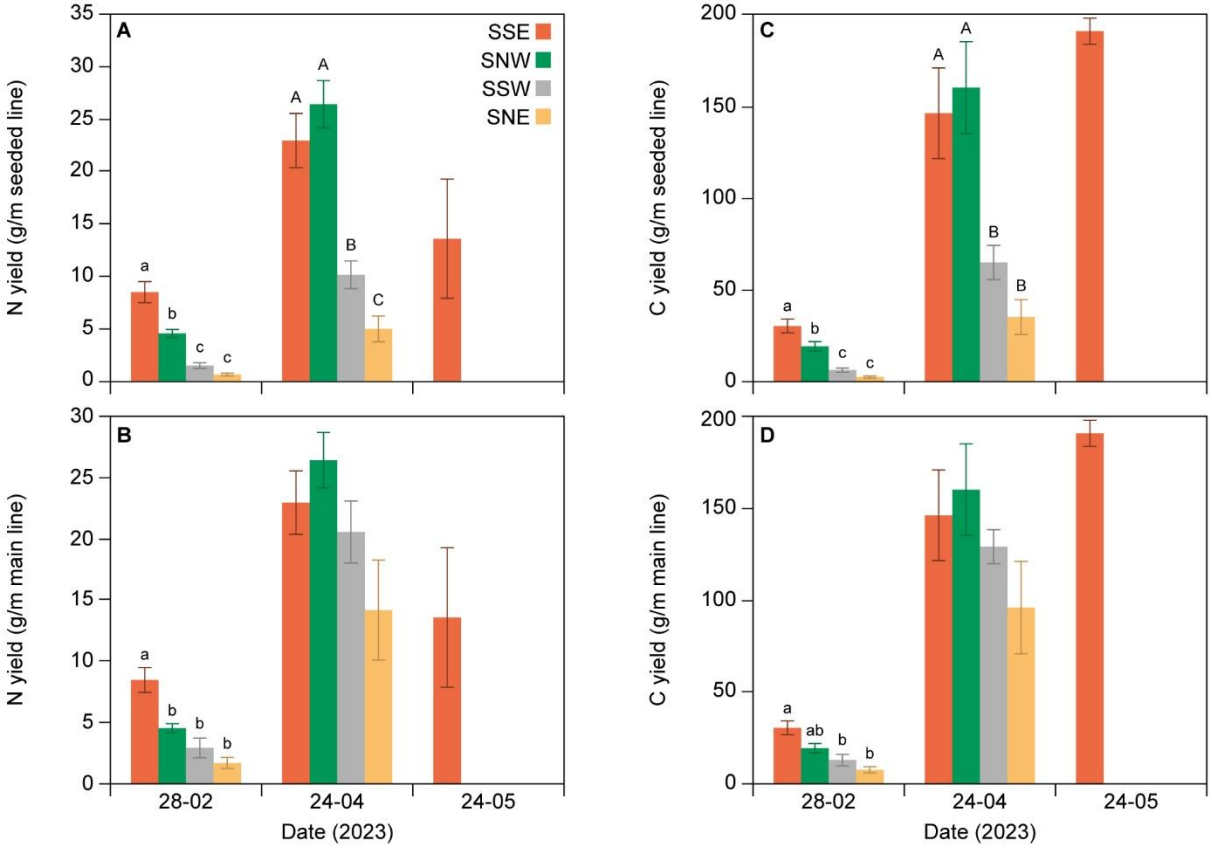

Figure 13. Removal potentials of nitrogen (N) (left) and carbon (C) (right). Nitrogen or carbon removal potential (yield) pr. meter of seeded line (top) and main line (bottom) from February-May for sugar kelp grown in four areas with different design of line systems: SSE and SNW - single horizontal line, SSW - double horizontal lines, and SNE - loops. Data is presented as mean±SE (n=3, except N-data from SSE 24-05-2023, where n=2).

The highest C removal potential was observed in the SSE area in May, following the main harvest. This increase was attributed to an increase in both tissue DM content and C content (C % of DM) in the SSE area from April to May (fig. 13). At the main harvest, the pattern of C removal potential mirrored that of the N removal potential.

Table 5. Results on biomass yields, composition and nutrient removal potential for the small-scale cultivation trials carried out at the Blue Community Gardens (BCG) and commercial sugar kelp producers in Denmark. Data from the large-scale site in Sallingsund, area SSE, are in **bold**. Data is presented as average ± SE, n = 3-9.

Area	Harvest date (all 2023)	Biomass yield (g DM/m line)	DM (% FW)	C (% DM)	N (% DM)	P (% DM)	N removal potential (g N/m)	P removal potential (g P/m)
Ebeltoft	03.05	240 ± 14	13.6 ± 0.1	36.9 ± 3.9	1.3 ± 0.6	0.15 ± 0.01	3.75 ± 1.82	0.41 ± 0.03
Havnsø	27.05	132 ± 34	20.8 ± 0.8	38.7 ± 6.3	0.6 ± 0	ND	0.89 ± 0.28	ND
Jegindø	27.04	558 ± 59	11 ± 0.5	30.7 ± 4	3.1 ± 0.4	0.11 ± 0	18.33 ± 3.49	0.67 ± 0.02
Kerteminde	12.05	208 ± 16	12.9 ± 0.3	36.7 ± 2.5	1 ± 0	0.3 ± 0.02	1.77 ± 0.13	0.54 ± 0.06
Københavns havn	12.05	4 ± 1	10.7 ± 1.5	33 ± 2.3	1 ± 0	ND	0.03 ± 0	ND
Løgstør	12.05	91 ± 26	16.6 ± 3.5	25.1 ± 1.2	3.5 ± 0.1	ND	2.3 ± 0.27	ND
Oddesund	19.04	293 ± 59	12.7 ± 0.4	32.7 ± 1	4.1 ± 0.2	0.2 ± 0.04	12.26 ± 2.98	0.62 ± 0.22
Odsherred	12.05	120 ± 16	12.5 ± 0.4	45.9 ± 3.6	1.2 ± 0.1	0.09 ± 0	1.5 ± 0.27	0.11 ± 0.01
Sallingsund	**24.04**	**522 ± 35**	**10.2 ± 0.2**	**28.4 ± 0.9**	**4.5 ± 0.1**	**0.16 ± 0.01**	**22.96 ± 1.48**	**0.81 ± 0.06**
Skovshoved	07.05	1 ± 0,4	6.8 ± 0.7	24.6 ± 0.9	1.8 ± 0.2	ND	0.03 ± 0.01	ND
Sønderborg	09.05	231 ± 21	15.6 ± 0.8	36.9 ± 0.5	1.4 ± 0.1	0.12 ± 0.01	3.35 ± 0.54	0.29 ± 0.05

3.2 Sugar kelp production in smaller scale for modelling of yield and nutrient removal potential

3.2.1 Biomass yields and environmental conditions

The biomass was harvested by the BCG and commercial producers within a period from April 19th to May 27th, 2023 (table 5). Biomass yields ranged from 1±0.4 g DM m^{-1} of seeded line in Skovshoved just north of Copenhagen to 558 g DM m^{-1} of seeded line in Limfjorden, Jegindø, not far from the large-scale site in Sallingsund, where the biomass yield was comparable: 522 g DM m^{-1} of seeded line.

The environmental conditions observed by the BCG and commercial sugar kelp producers demonstrated large variations between sites (table 6). The measured salinities spanned from ranges of 10-20 PSU measured in Havnsø to 19-31 PSU measured in Sallingsund at the large-scale site. The temperature varied from 3-12 °C measured in Ebeltoft to 3.2-17.6 °C measured in the Harbour of Copenhagen. Also, the clarity of the water varied between sites with the lowest Secchi depths measured in Løgstør (1.5-2.5 m) to 5.0-8.5 m in Ebeltoft.

Table 6. Environmental parameters (temperature, salinity and water clarity (Secchi depth) for the small-scale cultivation trials carried out at the Blue community Gardens (BCG) and commercial sugar kelp producers in Denmark. Data is presented as average ± SE, n=3-9. Data from the large-scale cultivation site in Sallingsund is included (in **bold**) for comparison.

Area	**Temperature (min-max °C)**	**Salinity (min-max PSU)**	**Secchi depth (min-max m)**
Ebeltoft	3-12	19-27	5-8.5
Havnsø	4-14	10-20	5-6
Jegindø	ND	ND	5.2
Kerteminde	5-15	13-28	4-(>6)
Harbour of Copenhagen	3.2-17.6	10-16	3.3-6.5
Løgstør	3-13	16-25	1.5-(>2.5)
Oddesund	ND	ND	1.6
Odsherred	0-14	10-21	2.4
Sallingsund	**0-13.4**	**19-31**	**2.9**
Skovshoved	ND	10-25	ND
Sønderborg	4-13	13-22	3.5-(>3.75)

3.2.2 Biomass composition and nutrient removal potential

The biomass composition of the sugar kelp cultivated at different locations in inner Danish waters also differed with DM content ranging from 6.8±0.7% of FW in Skovshoved to 20.8±0.8% of FW in Havnsø (table 7). The N content varied from 0.6% of DM in Havnsø to 4.5% of DM in Sallingsund. The four sites with the highest sugar kelp N contents were all found in the Limfjorden. The P content varied from 0.09% of DM in Odsherred to 0.16% of DM in Sallingsund. The C content did not fluctuate as much as the N and P, with tissue C concentrations ranging from 24.6% of DM to 45.9% of DM.

The variation in biomass yields and nutrient contents across the different water bodies affected the nutrient removal potentials (table 7): The largest N removal potential was observed in Sallingsund, at the large-scale cultivation site, where 22.93 g N and 0.81 g P m^{-1} seeded line could be removed. In contrast, in the Harbour of Copenhagen and in Skovshoved only 0.03 g N m^{-1} of seeded line could be removed. A strong correlation between biomass yield and N removal potential was observed (R^2 = 0.86), as well as a correlation between biomass yield and minimum salinity observed (R^2=0.54).

Table 7. Results on biomass composition and nutrient removal potential (g m^{-1}) for the small-scale cultivation trials carried out at the Blue Community Gardens (BCG) and commercial sugar kelp producers in Denmark. Data is presented as average±SE, n=3-9. Data from the large-scale cultivation site in Sallingsund is included (in **bold**) for comparison.

Location	Biomass composition				Nutrient removal potential	
	DM	C	N	P	N	P
	(% FW)	(% DM)	(% DM)	(% DM)	(g m^{-1})	(g m^{-1})
Ebeltoft	13.6 ± 0.1	36.9 ± 3.9	1.3 ± 0.6	0.15 ± 0.01	3.75 ± 1.82	0.41 ± 0.03
Havnsø	20.8 ± 0.8	38.7 ± 6.3	0.6 ± 0.0	ND	0.89 ± 0.28	ND
Jegindø	11 ± 0.5	30.7 ± 4	3.1 ± 0.4	0.11 ± 0	18.33 ± 3.49	0.67 ± 0.02
Kerteminde	12.9 ± 0.3	36.7 ± 2.5	1 ± 0.0	0.3 ± 0.02	1.77 ± 0.13	0.54 ± 0.06
Harbour of Copenhagen	10.7 ± 1.5	33 ± 2.3	1 ± 0.0	ND	0.03 ± 0	ND
Løgstør	16.6 ± 3.5	25.1 ± 1.2	3.5 ± 0.1	ND	2.3 ± 0.27	ND
Oddesund	12.7 ± 0.4	32.7 ± 1	4.1 ± 0.2	0.2 ± 0.04	12.26 ± 2.98	0.62 ± 0.22
Odsherred	12.5 ± 0.4	45.9 ± 3.6	1.2 ± 0.1	0.09 ± 0	1.5 ± 0.27	0.11 ± 0.01
Sallingsund	**10.2 ± 0.2**	**28.4 ± 0.9**	**4.5 ± 0.1**	**0.16 ± 0.01**	**22.96 ± 1.48**	**0.81 ± 0.06**
Skovshoved	6.8 ± 0.7	24.6 ± 0.9	1.8 ± 0.2	ND	0.03 ± 0.01	ND
Sønderborg	15.6 ± 0.8	36.9 ± 0.5	1.4 ± 0.1	0.12 ± 0.01	3.35 ± 0.54	0.29 ± 0.05

3.2.3 Content of critical minerals in sugar kelp

The content of critical minerals in the sugar kelp: Iodine (I), Cadmium (Cd), Lead (Pb), Mercury (Hg) and Arsenic (total As) varied between the different sites (table 8). The DM content of Cd, Pb and Hg did not in any case exceed the EU limit values for dietary supplements. In contrast, the tissue content of total As exceeded in 5 of the 7 cases the EU limit values for use in animal feed, including the sugar kelp produced in Sallingsund. The iodine content of the sugar kelp ranged between the lowest content of 2,903 ± 205 mg kg^{-1} found in Odsherred (Dansk Tang) to 10,380 ± 540 mg kg^{-1} found in the BCG in Ebeltoft. No limit values for the content of iodine or total As in food exist in the EU, but limit values for inclusion of As in feed exist (EU 2002).

Table 8. The content of critical minerals in the sugar kelp harvested at the large-scale cultivation site in Limfjorden (Sallingsund), and at a selected number of Blue Community Gardens and commercial sugar kelp producers. Numbers are given as mg kg^{-1} (ppm) of DM, and as average ± SE (n =3). Limit values are given according to the EU limits for dietary supplements (EU 2008) or feed (*) (EU 2002). No limit values for content of iodine exist in EU. Data from the large-scale cultivation site in Sallingsund is included (in **bold**) for comparison.

Location	Iodine (I)	Cadmium (Cd)	Lead (Pb)	Mercury (Hg)	Arsenic (total As)
Sallingsund	**3379 ± 253**	**0.81 ± 0.11**	**0.17 ± 0.02**	**0.04 ± 0.00**	**48.20 ± 7.32**
Jegindø	5145 ± 257	0.63 ± 0.16	0.17 ± 0.03	0.07 ± 0.01	47.54 ± 4.52
Odsherred	2903 ± 205	0.41 ± 0.06	0.35 ± 0.08	0.04 ± 0.01	36.29 ± 3.62
Ebeltoft	10380 ± 540	0.32 ± 0.04	0.15 ± 0.00	0.08 ± 0.01	38.53 ± 2.59
Kerteminde	6682 ± 229	0.35 ± 0.03	1.76 ± 0.79	0.06 ± 0.00	33.69 ± 1.40
Oddesund	7978 ± 225	0.48 ± 0.03	0.29 ± 0.09	0.07 ± 0.01	39.72 ± 1.27
Sønderborg	4435 ± 371	0.40 ± 0.03	0.25 ± 0.02	0.06 ± 0.00	45.77 ± 1.91
Limit values	-	< 3	< 3	< 0.1	< 40*

3.3 Economy of large-scale sugar kelp production and nitrogen removal

3.3.1 Identification of Best Available Technology

Prior to calculating the costs related to producing sugar kelp in large-scale at the cultivation site in Sallingsund, the Best Available Technology (BAT) was defined from the different designs tested as 1) Hatchery in cooling container, 2) single horizontal line design, 3) area density of seeded lines and 4) harvest by modified mussel harvest technology:

The hatchery design – permanent hatchery or mobile, temporal hatchery in cooling container. To accommodate a solution to be flexible on a temporal as well as spatial scale for the hatchery, the cooling container solution was selected as BAT, assuming full areal use of the container space (in contrast to the 50% use in this study).

The line design – single horizontal lines, double horizontal lines or loops

To select the best performing design, the yield and costs were compared: Since the biomass yield per main line was highest – and similar - for the two vertical line designs – single horizontal lines (areas SSE and SNW) and double horizontal lines (area SSW), and since both material and handling costs were lower for one single line as compared to two, the single horizontal line design was identified as the BAT.

Area density of seeded lines – in order to navigate the boats for deployment, maintenance and harvest between the lines, the optimal density of lines was set to 1000 m of seeded line per ha, equivalent to a distance of 10 m between mainlines.

Harvest technology – harvest by hand (Boat 1) or mechanised harvest (Boat 2)

The harvest of sugar kelp per long line varied from 140 to 515 kg FW per hour. Before harvest, it proved necessary to handle each long line twice, to first untangle the seaweed line from the longline and remove biofouling (ascidians primarily) from the longline to avoid contamination of the harvested sugar kelp, and second to carry out mechanised harvest of the lines. We estimated that a full day of work was needed to clean all 28 longlines and disentangle seaweed lines before harvest. As boat 1 had a higher level of automation for

handling the lines, the work environment was better suited for fast harvest using the mechanised harvester modified from mussel harvesting as compared to harvest by hand. However, the harvest performance per boat type was greatly influenced by the level of entanglement of each seaweed line.

The experiences and results from area SSE was following used as input for the calculations of economy of BAT (table 9, Appendix 2), and the key cost figures for large-scale production of seaweed in Limfjorden, Denmark was accordingly calculated (table 10).

The production costs of the sugar kelp biomass were calculated to 12.6 DKK kg FW^{-1} or 123.4 DKK kg DM^{-1}. The grow-out of the sugar kelp at the cultivation site in the Limfjorden, amounted to 82% of the costs (16% for establishing of the site (CAPEX) and 66% for the operating costs (OPEX)), whereas the costs of the hatchery phase amounted to 18% of the total costs (12% for the establishing of the hatchery and 6% for operating the hatchery) (fig. 14).

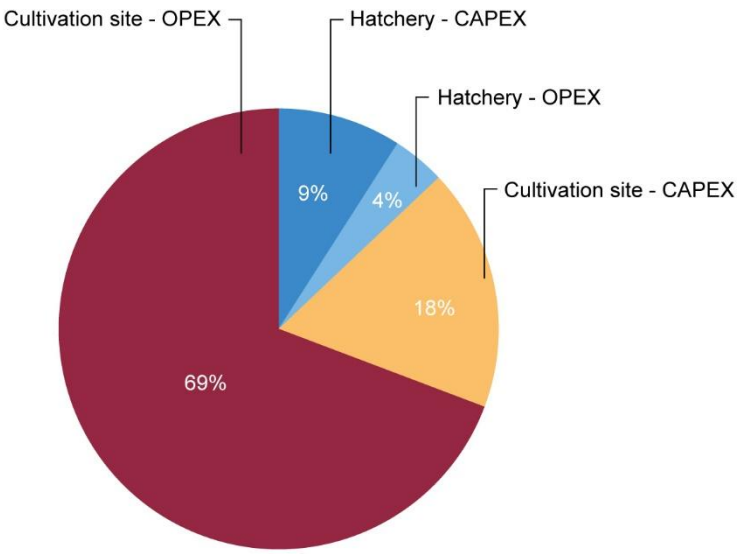

Figure 14. a) Cost of 12-hectare cultivation of sugar kelp in Sallingsund 2022-2023; broken down in capital costs (CAPEX) and operation costs (OPEX) for the hatchery and grow out phase at the cultivation site (for details see Appendix 2).

The production cost of seeded line was 8.3 DKK m^{-1}. Hereof, 67% of the costs were the establishing of the hatchery (CAPEX), while 33% was the operating costs of the hatchery (OPEX).

Table 9. Inputs for cost calculations based on results from the large-scale site in Sallingsund. See details in Appendix 2.

Parameter	Number
Number of mainlines in area SSE (number)	7
Total meters of seaweed line in area SSE (m)	987
Total efficient area used in large-scale test production (ha)	9,3
Total efficient area used in area SSE (ha)	2,3
Optimal density of seeded lines (meters of seeded line ha^{-1})	1000
Biomass yield in area SSE (kg FW m^{-1} of seeded line)	5.1
Biomass yield in area SSE (g DM m^{-1} of seeded line)	522
Nitrogen removal potential (g N m^{-1} of seeded line)	22.96
Minimum salary (DKK h^{-1})	250
Cost of boat rental (no crew) (DKK h^{-1})	1600
Cost of deployment of drill anchors (DKK $anchor^{-1}$)	4000

Table 10. Key cost figures for large-scale production of sugar kelp in the Limfjorden, Denmark. See details in Appendix 2.

Service	Cost (unit)
Seeded line (m)	8.34 DKK m^{-1}
Total cost per m of mainline	64.40 DKK m^{-1}
Total cost of cultivation site (18.8 ha)	1,159,232 DKK
Total cost per hectare	64,402 DKK ha^{-1}
Total cost of sugar kelp produced	12.63 DKK kg FW^{-1}
Total cost of sugar kelp produced	123.39 DKK kg DM^{-1}

Assuming the same production costs in all areas of Danish waters and based on the results on N removal potential for sugar kelp production in different Danish water bodies (table 4), the cost of N removal through production of sugar kelp was calculated for all sites that provided data for the small-scale cultivation (table 11). The cost of nitrogen removal varied by almost a factor of 1000, ranging from 2805 DKK kg N^{-1} to more than 2 million DKK kg N^{-1}, with the lowest costs estimated for three of the four sites in Limfjorden (Sallingsund, Jegindø, Oddesund), where the biomass yield and tissue nutrient contents were high, and the highest costs for the cultivation sites in the Copenhagen area (Copenhagen Harbour, Skovshoved), where biomass yields were negligible.

Based on the large-scale processing of the harvested sugar kelp for animal feed, a production price for the sugar kelp biomass was calculated to 12.63 DKK kg FW^{-1} or 123.39 DKK kg DM^{-1}. The processing costs for transporting, washing, milling and fermenting the fresh sugar kelp were estimated at 6.57 DKK kg FW^{-1} (table 11). Hence the potential profit of producing sugar kelp for animal feed in Sallingsund, Limfjorden, without including potential subsidies for N removal, would amount to 0.80 DKK kg FW^{-1} or 73.3 kDKK per standard cultivation site (18 ha) per year. The break-even price of the sugar kelp would accordingly be 11.83 DKK kg FW^{-1}.

Table 11. Calculations of break-even prices and potential profit for cultivation of sugar kelp for animal feed.

Unit	Value
Standard cultivation site (ha)	18
Line density (m ha^{-1})	1000
Biomass yield (kg FW m^{-1})	5.1
Cost of production (DKK kg FW^{-1})	12.63
Cost of processing (DKK kg FW^{-1})	6.57*
Estimated income, feed (DKK kg FW^{-1})	20**
Profit (DKK kg FW^{-1})	0.80
Break-even price (DKK kg FW^{-1})	11.83
Production (ton $site^{-1}$)	91.8
Profit (DKK $site^{-1}$)	73309
Standard cultivation site (ha)	18
Line density (m ha^{-1})	1000

*Processing including transportation of biomass from cultivation site to processing plant.
**Price estimate based on personal communication with the commercial feed industry.

3.4 Sugar kelp growth model

The simple model provided a good fit to the Sallingsund observations (fig. 15). The percentage deviation between observations and model predictions indicated no bias with a median deviation of 0% and an interquartile range of

-29% to +7%. The largest deviations occurred for the three single lines on February 28th with model estimates at -60% to -72% below the observed densities. At 0.75 m depth, the single lines were placed closest to the surface. Apart from these systematic deviations, we consider the calibration satisfactory. We demonstrated that the model—driven by irradiation only—was flexible enough to accommodate the observed dynamics of *S. latissima* growth. Note that the growth of both small and large sporophytes over a 100-1000 fold range were well accounted for by the model (Holst et al. 2025).

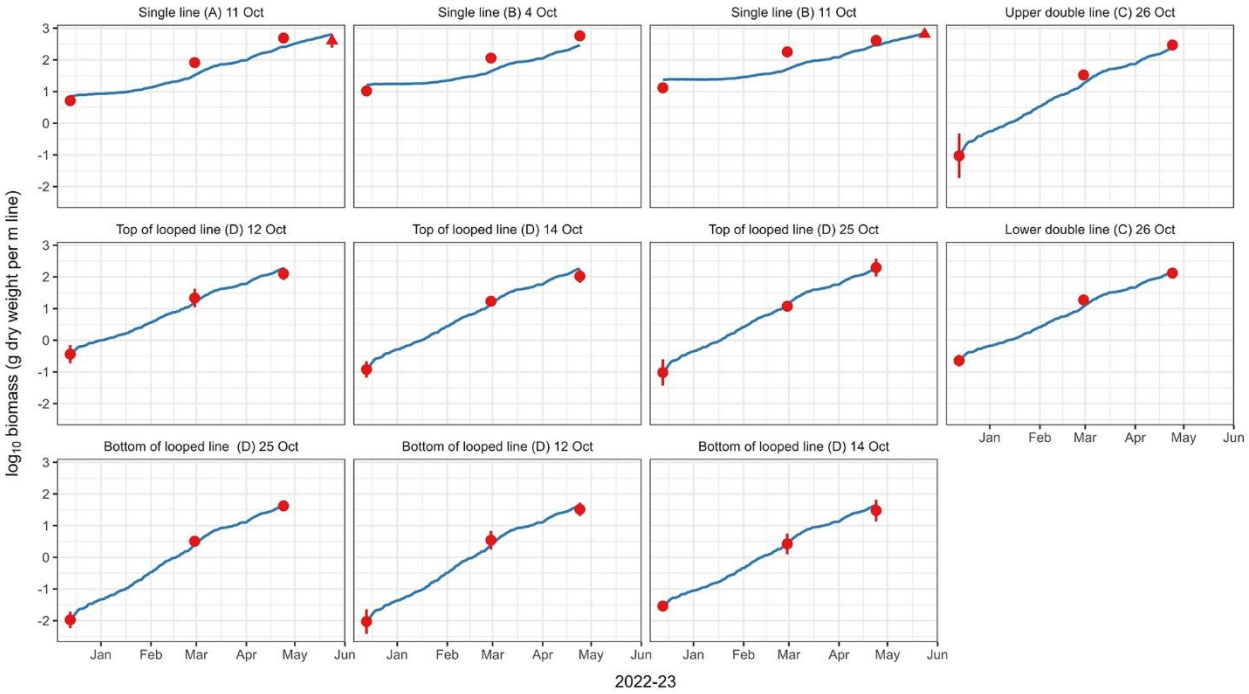

Figure 15. Observed sugar biomass per meter seeded line (red points ± 2 SE) cultivated in Sallingsund, together with model predictions (curves). The two late observations (red triangles) were not included in model calibration; hence, curves from April 24th to May 24th are model extrapolations. Sub-plot titles refer to the cultivation design (A: Single lines deployed at 0.75 m depth in SNW, and B: in SSE. C: Double lines deployed at 0.75 m and 1.25 m depth in SSW. D: Looping line between 0.8-3.5 m depth, deployed in SNE (see fig. 3) and the deployment date (Holst et al, 2024).

Sea temperature was not needed as a variable to obtain an accurate model; global irradiation was the only environmental variable necessary to drive the model. Model validation was successful as the predicted time trends of *S. latissima* biomass mimicked both the pattern and the magnitude of the biomass trends observed in the two validation data sets. Due to a lack of data, the model did not include the effects of sub-optimal levels of nutrients or salinity; however, thanks to the model's simplicity and its reliance on basic biological principles, we expect that it can easily be re-calibrated to simulate growth also under sub-optimal environmental conditions. Its plain formulation makes it straightforward to incorporate into complex models of marine cropping systems and provides a readily available prediction of the expected benefits of sugar kelp cultivation, in terms of the biomass produced and the amount of fixated carbon and nitrogen.

3.5 Ecosystem and climate effects of large-scale cultivation of sugar kelp

3.5.1 Physical and chemical properties - Light, pH and oxygen

In all campaigns, no clear patterns of dissolved oxygen concentrations were observed, and no statistical spatial relationships were found. Variability between days was more pronounced than spatial variability. Periodic patterns of higher pH were observed in the farm, though daily variability and 'patchiness' in the whole study domain provided inconsistency in patterns.

In the period immediately before harvest with the highest biomass standing stock at the cultivation site, higher pH, higher O_2 concentrations and areas of increased light attenuation to the bottom (Kd) were periodically observed (fig. 16). The changes were not unambiguous, due to a high degree of mixing and water exchange, which also contributed to significant daily variation.

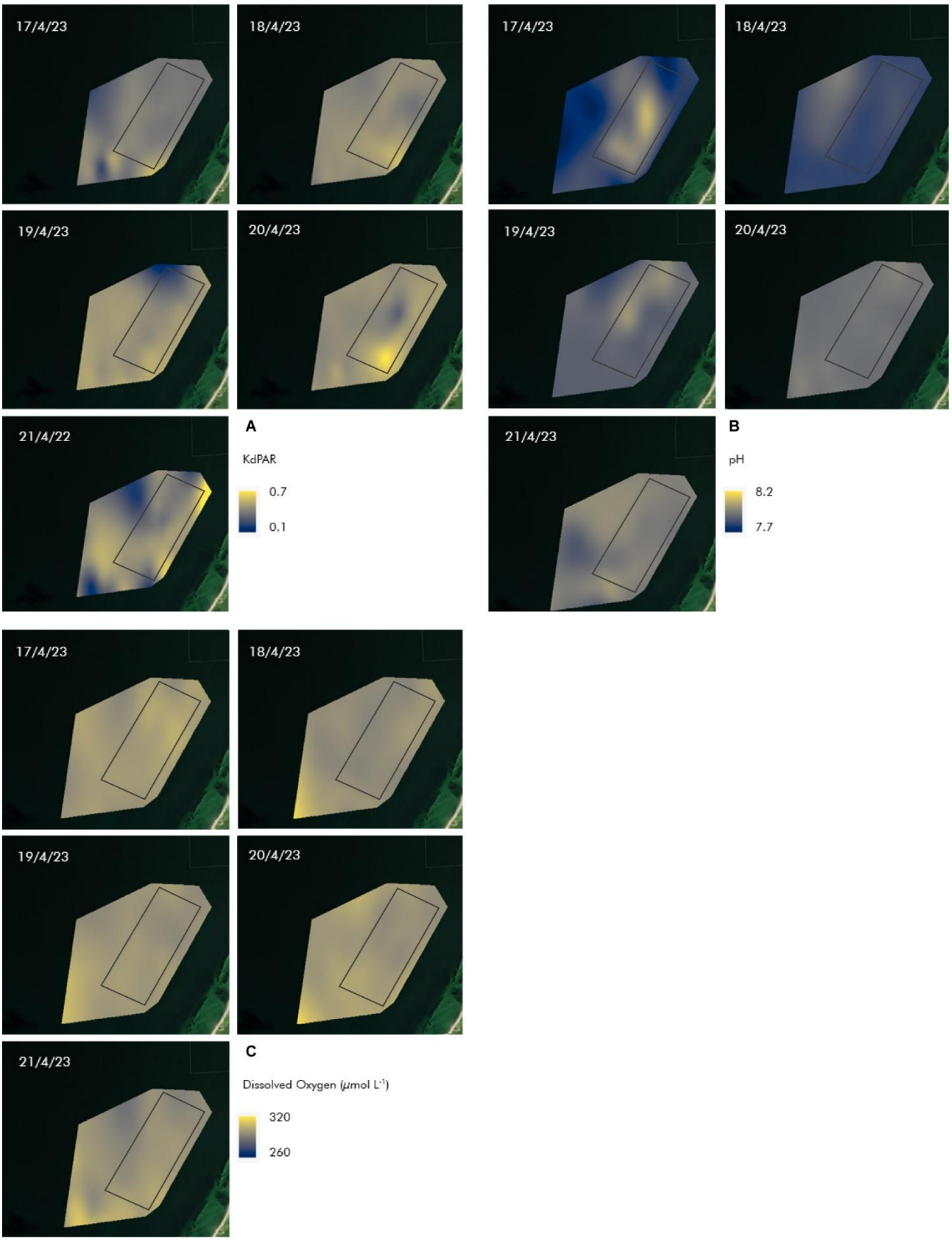

Figure 16. Heatmaps of the cultivation site during the final environmental campaign (3) in late April 2023 at the period of highest sugar kelp biomass standing stock, showing a) light attenuation to the bottom (Kd PAR), b) pH and c) O_2 concentrations (µmol O_2 L^{-1}). The outline of the cultivation area is indicted with a thin grey line. The date of data collection is indicated in each heatmap.

Direct in-situ studies from the site in Sallingsund documented no significant "shadow effect" to the seabed from the sugar kelp biomass hanging in the upper water column. Light loggers, deployed directly below the cultivation lines at a depth of 4.5 meters, showed an average of 50% reduction of light directly under a cultivation line, indicating that the shadow effect was limited to a narrow band with an extension of a few meters on each side of the cultivation lines. The shadow from the cultivation line moved and varied throughout the day with the path of the sun. Light loggers deployed between the cultivation lines registered a reduction of incoming light of 3% on average to the sediment, as compared to the reference site. Vertical profiling within the cultivation site also did not show clear patterns in the impact of light penetration to the sea floor.

Farm effects on optical properties due to potential redistribution of suspended particles or direct shadowing in the water column were indistinguishable from ambient variability.

3.5.2 Hydrology – current velocity and stratification

Possible impacts on local current patterns were observed at the large-scale cultivation site at Sallingsund, where a higher degree of mixing and turbulence at depths consistent with farm structures and associated biomass, as well as weakened stratification, were observed in the cultivation site (fig. 17). The effect was most pronounced in April at the maximal biomass density immediately before harvest but observed also earlier in the production cycle (February) when the seaweed biomass was not yet significant and where a possible effect could be attributed to the physical structures of the cultivation site. The bathymetry in the area could also have contributed to reduced current velocities and reduced stratification in the cultivation site compared to the reference area.

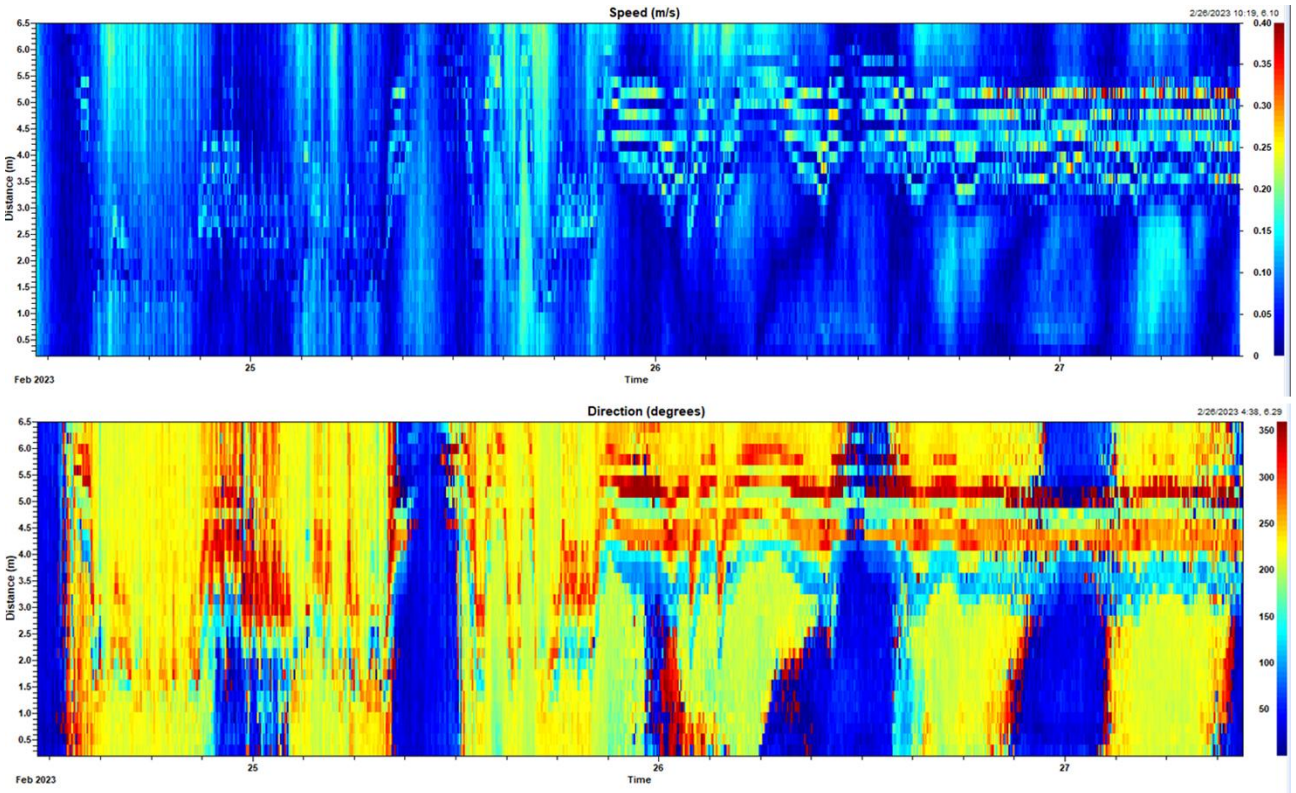

Figure 17. High current speeds (top) and changing current directions around the seaweed canopy (bottom) indicate turbulent eddies during campaign 2 in February 2023. Note that the position and intensity of the observed turbulent flows are responding to the changing position of the seaweed lines relative to the fixed position of the Acoustic Doppler Current Profiler (ADCP). Tidal phases are indicated by the full-column directional change.

3.5.3 Sedimentation

Even though the current velocity was often reduced within the seaweed system, this did not give rise to increased sedimentation under the seaweed lines. The highest sedimentation rates were observed in Autumn, decreasing through February to April prior to harvest (fig. 18.a).

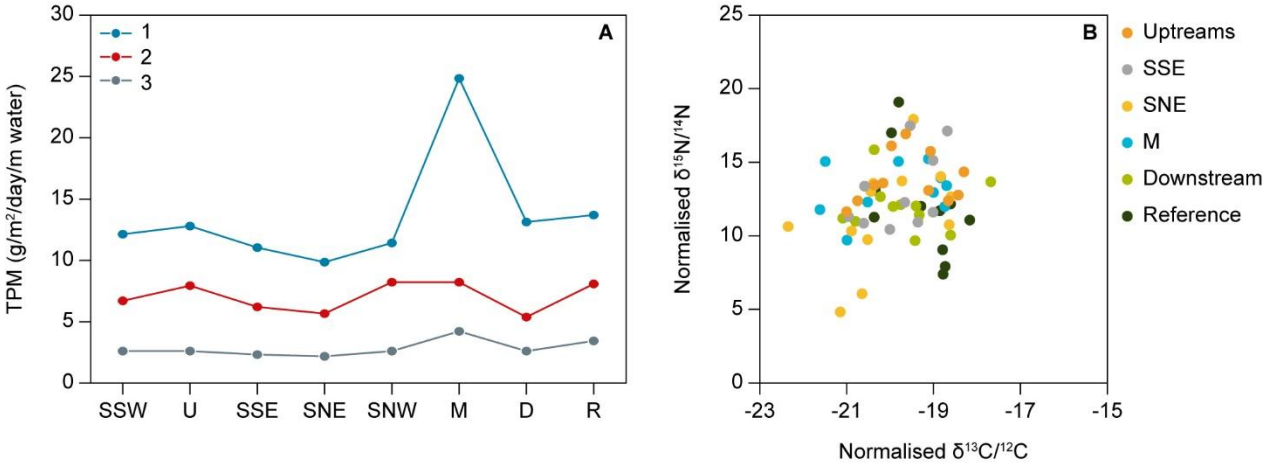

Figure 18. Sedimentation rates and isotopic characterisation of organic matter sedimented below and around the large-scale cultivation site. U: Upstream, D: Downstream, M: Mussels, R: Reference, SSW: Seaweed Southwest, SSE: Seaweed Southeast, SNE: Seaweed Northeast and SNW: Seaweed Northwest (see figure 6). a) Total particulate material (TPM) sedimentation rates normalised to the water depth per station observed during the three campaigns 1 (October 2022), 2 (February 2023) and 3 (April 2023). The x-axis represents the different stations in the sugar kelp cultivation area. b) The isotopic signatures of the POM sedimented and sampled at the different stations in and around the seaweed cultivation site.

Higher sedimentation rates were observed below the mussel cultivation lines at station M in campaign 1 (autumn productivity), and the lowest sedimentation rates were observed at the seaweed stations during campaign 3, where the sugar kelp canopy was largest.

It is likely that the seaweed lines and the seaweed biomass generated turbulent channelled flow in the seaweed cultivation site, as previously observed in mussel farms (Delaux et al. 2011), and potentially promoting transport of particles out of the area which could reduce sedimentation rates within the farm. At the same time, the mussel cultivation immediately north of the seaweed lines may have contributed to the filtration of particles in the water transported into the seaweed system in the direction of the south-westerly current (Taylor et al. 2024).

The isotopic signature of the C and N in the particulate organic material sedimented below the seaweed, mussels, at the reference station, and upstream and downstream to the cultivation site in April, did not differ between stations (fig. 18.b), with $^{13}C/$ratios ranging between -17.7 to -22.3, and $^{15}N/^{14}N$ ratios ranging between 4.8 and 19.8.

3.5.4 Excretion and degradation of Dissolved Organic Carbon

Overall, a limited proportion (2-7%) of the photosynthetically fixed carbon was excreted by the sugar kelp as dissolved organic carbon (DOC)(Pedersen et al. In prep; Christiansen 2023).

The photosynthesis rate of the sugar kelp from the cultivation site increased at high light intensities (500 µmol photons m^{-2} s^{-1}) as compared to low light (60 µmol photons m^{-2} s^{-1}) and was higher at both low and high light in

February as compared to April (fig. 19.a). The sugar kelp excreted dissolved organic carbon (DOC) with rates up to 15 µmol of C g^{-1} DM h^{-1} when exposed to low and high light intensities, and up to 7-8 µmol of C g^{-1} DM h^{-1} in darkness (fig. 19.b). The absolute rate of DOC excretion was significantly higher in February than in April, but when normalised to the amount of C fixed by photosynthesis, no significant difference was found between the DOC excretion in February and April. In darkness, 3% of the fixed C was released as DOC, as compared to 6-7% at low light and 2-4% in high light (fig. 19.c). Tendencies were observed towards a higher DOC excretion in February as compared to April and towards a higher DOC excretion at low light as compared to high light, however, these differences were not significant.

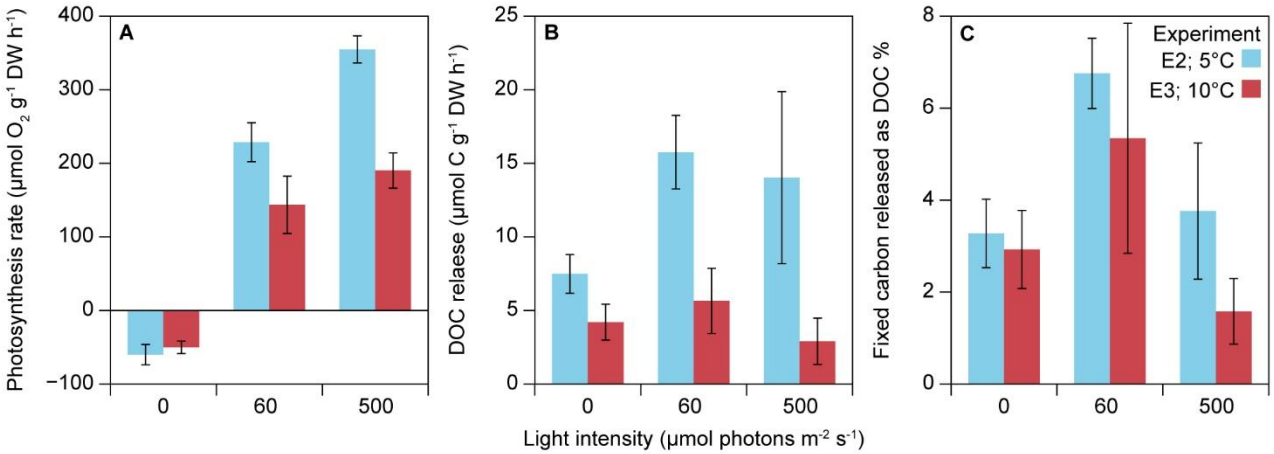

Figure 19. Photosynthesis and DOC excretion of sugar kelp from Sallingsund measured during the environmental campaigns in February/March (E2) and April 2023 (E3) using three different light intensities 0, 60, and 500 µmol photons m^{-2} s^{-1}. a) Photosynthesis rate of sugar kelp (µmol O_2 g^{-1} DW h^{-1}). b) Sugar kelp DOC excretion (µmol C g^{-1} DW h^{-1}) from sugar kelp exposed to light intensities of 0, 60, and 500 µmol m^{-2} s^{-1}. c) percentage of fixed carbon released as DOC from sugar kelp at light intensities of 0, 60, and 500 µmol photons m^{-2} s^{-1}. Fixed carbon released as DOC at light intensities of 0 µmol photons m^{-2} s^{-1} was calculated based on carbon fixed at light intensities of 60 µmol photons m^{-2} s^{-1}.

The sugar kelp DOC excretion introduced new compounds into the water, and the composition of the excreted DOC changed between night and day (darkness and light), with a more diverse composition in darkness (327 compounds) than in light (247 compounds) and with partially overlapping signatures of the molecules (114 compounds) (fig. 20.a)(Pedersen et al. In prep). For the compounds specifically excreted in either day or night, the broad molecular properties i.e., the proportions of aliphatic (H/C≥1.5), highly unsaturated and phenolic (H/C<1.5 & AI<0.5), and unambiguously aromatic formulas (AI≥0.5), did not differ substantially between day and night (fig. 20.b.1,3). In both cases, the 'highly unsaturated and phenolic' and unambiguously aromatic groups comprised ca. 40% and 12% of the total number of molecules, respectively (fig. 20.b).

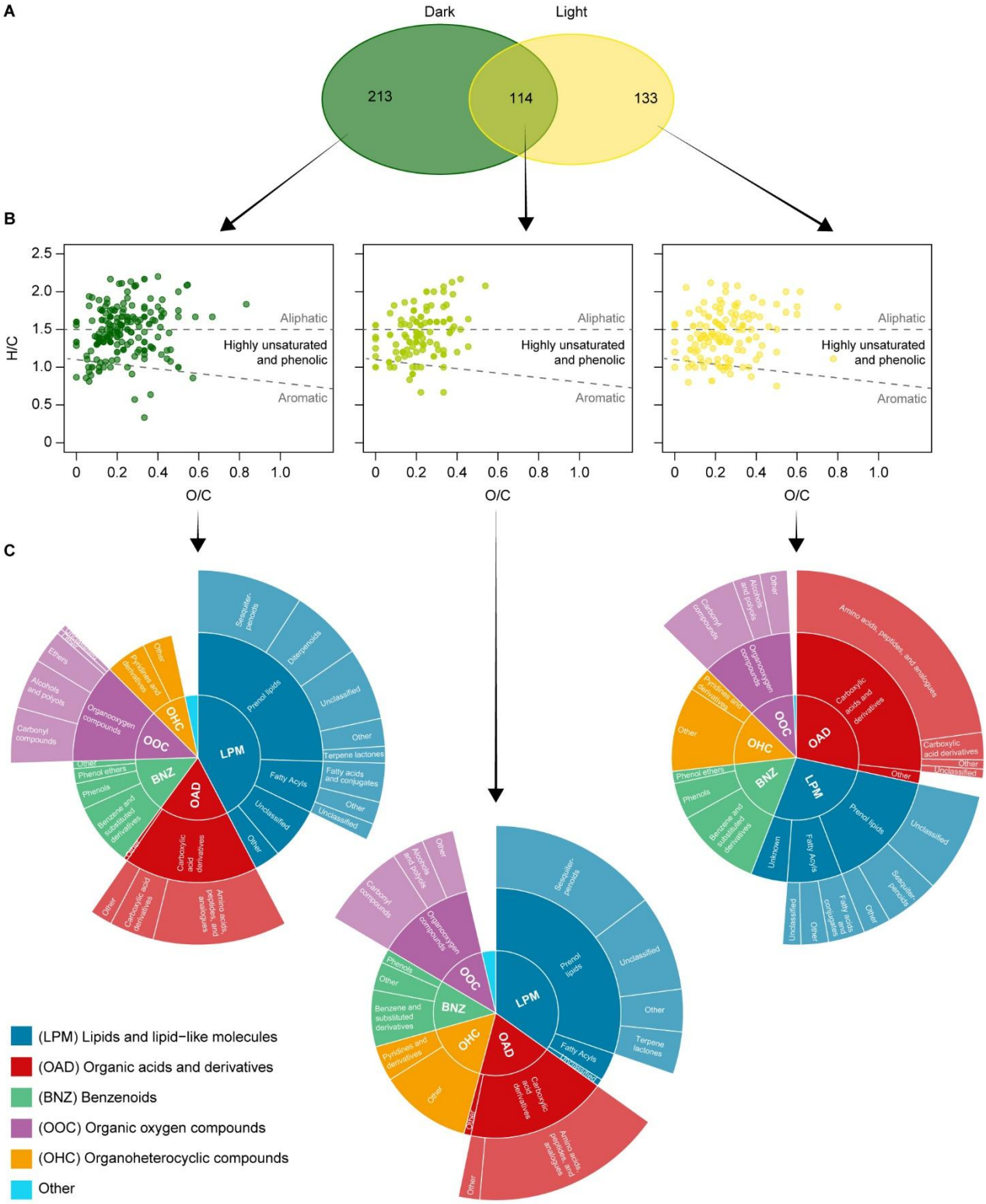

Figure 20. Molecular composition of the DOC excreted by the sugar kelp in April immediately prior to harvest. (a) Number of molecules detected in light and darkness, with a partial overlap. (b) The van Krevelen diagrams categorising excreted molecules into the groups of aliphatic (H/C≥1.5), highly unsaturated and phenolic (H/C<1.5 & AI<0.5), and aromatic formulas (AI≥0.5). (c) More detailed characterisation of the DOC compound classes at three levels: superclass, class, and subclass (where available). Main detected superclasses: Lipids and lipid-like molecules (LPM), Organic acids and derivatives (OAD), Benzenoids (BNZ), Organic oxygen compounds (OOC), and Organo-heterocyclic compounds (OHC).

Lipids (LPM) and organic acids (OAD) accounted for more than 50% of the excreted DOC molecules (fig. 20.c). During day, the proportion of organic acids and derivatives (OAD) was larger than during night, and in contrast, the excretion of lipids and lipid-like molecules (LPM) was higher in darkness. The

proportion of benzenoids (BNZ) in the excreted DOC, comprised 13-17% and did not differ markedly between day and night.

Modelling predicted that 23.46% of DOC excreted by sugar kelp in February was refractory (not degraded after 150 days), with a decay rate of 0.042 d^{-1} or 0.0261 d^{-1}, depending on whether the model included an offset (assuming a refractory DOC pool) or not (fig. 21) (Christiansen 2023).

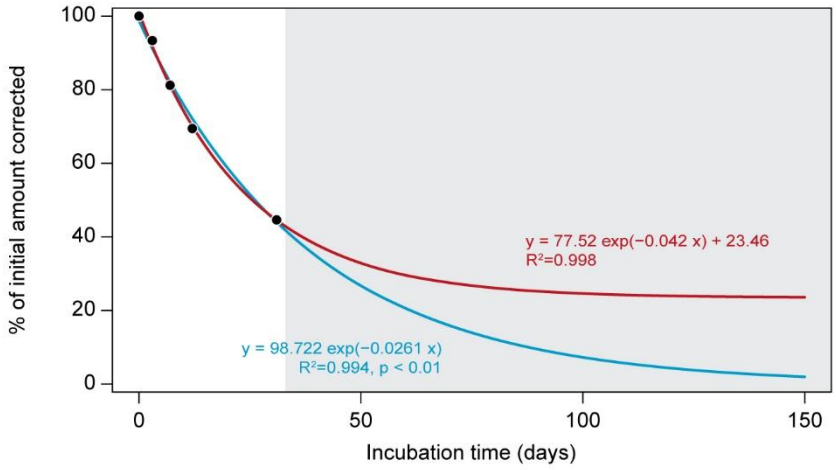

Figure 21. Degradation of Dissolved Organic Carbon (DOC) over time during a 31 day degradation experiment. The percentage of initial DOC concentration from one macroalgae (based on control corrected values). The grey box indicates estimated degradation until day 150. The blue regression line indicates an exponentially fitted curve, and the red line indicates an exponentially fitted curve including offset. The DOC degradation was investigated based on sugar kelp harvested in February and exposed to ambient high light (500 µmol photons m^{-2} s^{-1}).

3.5.5 Turn-over of particulate organic carbon in water and on sediment

The lability of both sugar kelp tissue and POM from beneath the sugar kelp farm decreases with time (fig. 22). During the first week of degradation the decay rate constant increased to up to 0.12 d^{-1} for the kelp tissue and 0.08 d^{-1} for the POM, then gradually decreased to 0.03-0.04 d^{-1} over the experimental period of 153 or 80 days, respectively. No significant effect of neither tissue age or nitrogen content was found for the sugar kelp tissue, and no significant difference in degradation rate was found in the lability of POM from the five stations in and around the sugar kelp and mussel farm (fig. 22)(Vinbæk 2023).

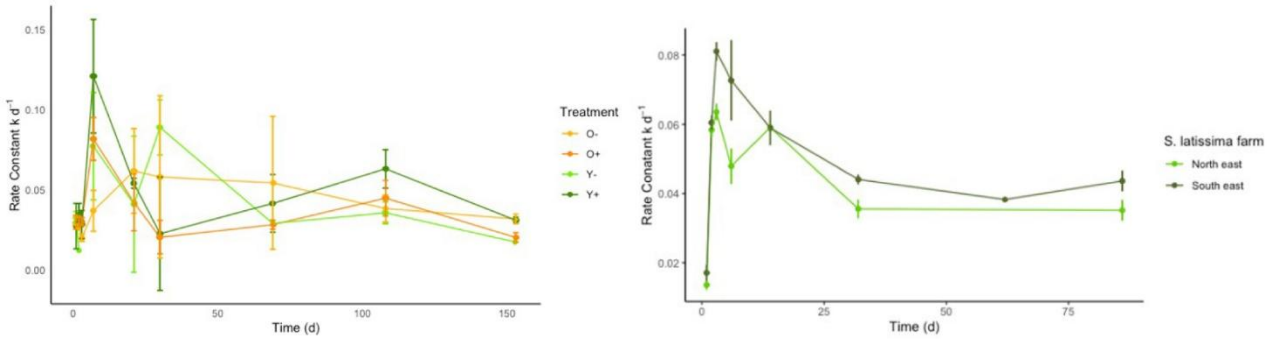

Figure 22. Degradation rates over time of a) old (O) and young (Y) sugar kelp tissue with high (+) and low (-) nitrogen content, and b) particulate organic matter sedimented below the seaweed cultivation in the Northeast (SNE) and Southeast (SSE) section of the cultivation site (figure 6). Data are presented as mean±SE, n=3.

Assuming a constant decay rate constant from day 153, calculations indicated that all sugar kelp tissue would be fully degraded after 100 years (Hurd et al. 2022), and hence no carbon would be sequestered.

3.5.6 Benthic flux of carbon and nutrients

Adding sugar kelp to the sediment cores increased the content of organic matter in the upper 2-3 cm of the sediment (fig. 23.a), where also the C/N ratio was reduced, albeit insignificantly (fig.23.b). The turnover of carbon in the sediment was stimulated by the addition of fresh sugar kelp tissue, indicated by an increase in C turnover compared to sediments without algae. This increase was equivalent to more than 100% of the added C, measured by both the uptake of oxygen and the release of dissolved inorganic carbon from the sediment (figs. 24.a and b). This indicated a 'priming effect' where the degradation of recalcitrant carbon stored in the sediment was stimulated by the addition of new labile organic material.

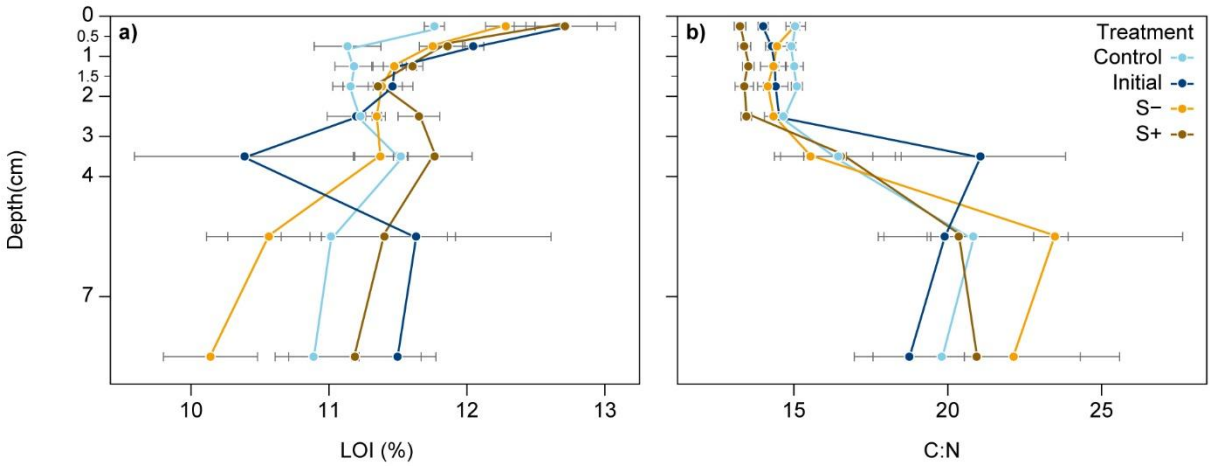

Figure 23. Depth profiles for sediment cores from Limfjorden at the beginning and end of the experiment showing: a) Content of organic matter (Loss of ignition (LOI)) and b) C:N-ratio. The sediment cores were added sugar kelp tissue with and without nutrient enrichment (S+ and S-). No algal biomass was added to the control sediment cores. The 'initial cores' (cores with no addition of algal biomass) were analysed before the initiation of the experiment. The data are means±SE for the treatment cores (n=4), initial cores (n=3) and control cores (n=3).

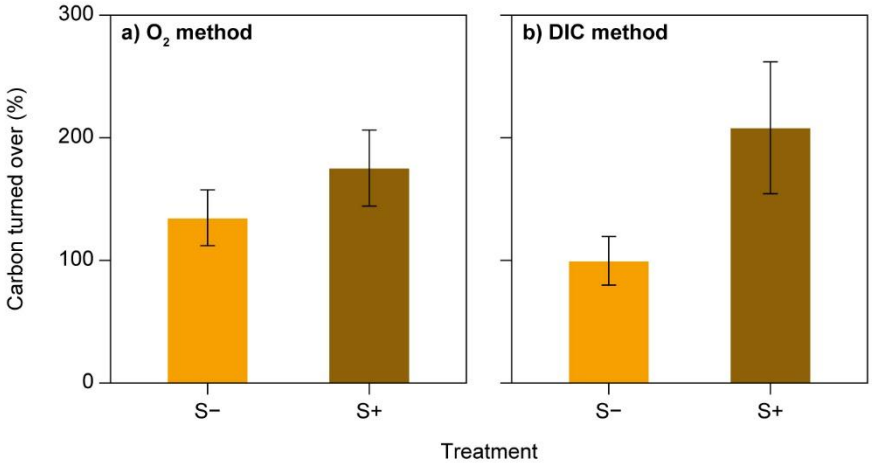

Figure 24. Total carbon turnover, expressed as the percentage of carbon release from sediments with macroalgae minus control sediments over the 83-day experimental period relative to the amount added macroalgae carbon. Carbon turnover was estimated from O_2 consumption and a) the DIC:O_2 ratio or b) DIC release. The study compared sediment cores with addition of sugar kelp with and without nutrient enrichment (S+ and S-) and a control treatment without algal material. The data is presented as mean±SE (n=4).

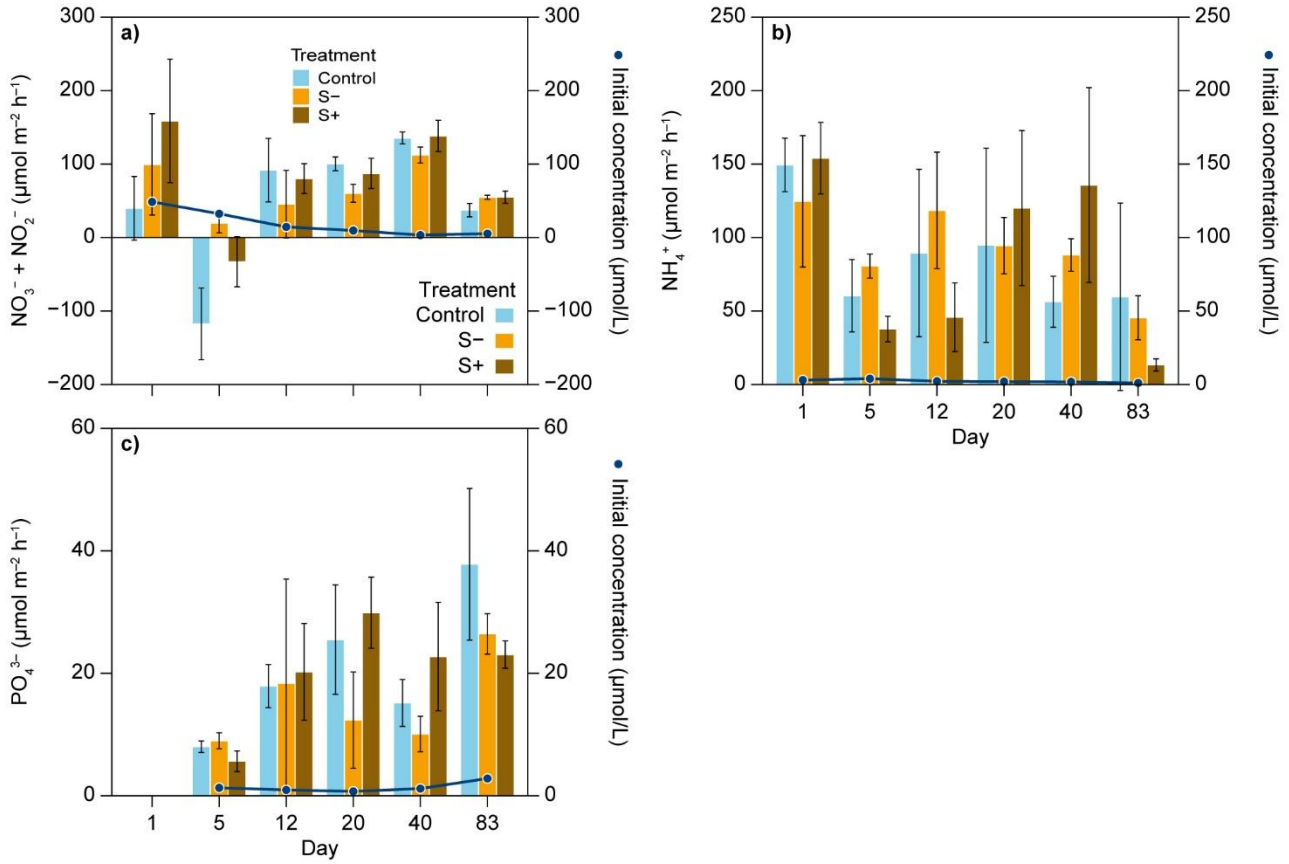

Figure 25. The flux of inorganic dissolved nutrients from sediment to water in sediment cores over an 83-day incubation period with or without addition of sugar kelp tissue: a) NO^{3-} + NO^{2-}, b) NH_4^+ and c) PO_4^{3-}. The sugar kelp added was with and without nutrient enrichment (S+ and S-), and the control sediment cores were without algal material. The initial concentration of inorganic dissolved nutrients the incubation water prior to the flux incubation is presented on a second y-axis in each plot (connected black dots). The data is presented as mean±SE (n=4 for treatments and n=3 for the control).

In contrast, the flux of dissolved inorganic nutrients from the sediment to the water was not increased by the addition of sugar kelp to the sediment (figs. 25, a, b and c).

3.5.7 Emissions of climate active gases from sugar kelp : methane, nitrous oxide and VOC

No emissions of methane (CH_4) or nitrous oxide (N_2O) were documented from the incubations of actively growing sugar kelp (including microbiome) in the laboratory incubations, as sea water concentrations of methane and nitrous oxide were not significantly different between incubations with or without (controls) sugar kelp in neither light nor darkness (fig. 26).

The VOC analysis identified several climate-relevant halocarbons (table 12). No notable differences were observed between light and dark incubations, but more compounds were identified by purging the incubation water than by purging the incubation headspace.

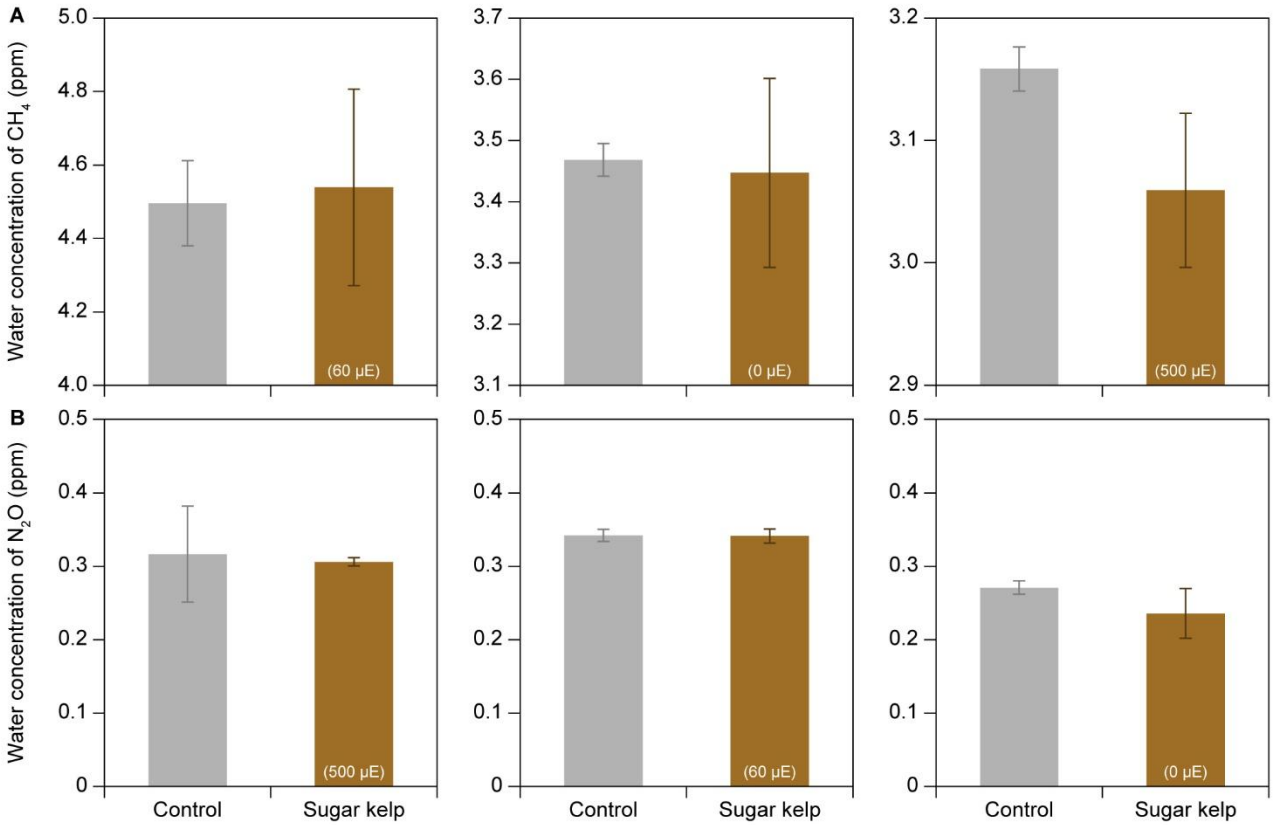

Figure 26. Seawater concentrations of a) methane and b) nitrous oxide after 4-hour incubations with intact, live sugar kelp at the time of harvest (late April) in in-situ water with either low light, high light or darkness (60, 150 or 0 µmol photons m^{-2} s^{-1} (µE), respectively). Data are shown as mean±SE, n=3.

Table 12. Climate-relevant halocarbons identified in incubations of sugar kelp.

Compound	Environmental relevance	VOC sampling method	Other reports of emission from *S. latissima*
Dibromomethane (CH_2Br_2; Methylene bromide)	Precursors for reactive bromide species, which deplete ozone, alter HOx and NOx radical budgets and drive mercury deposition into the ecosystems (Parrella et al. 2012; Saiz-Lopez and von Glasow 2012; Schmidt et al. 2016)	Headspace purging, Water purging	(Schall et al. 1994; Nightingale et al. 1995; Carpenter et al. 2000; Laturnus et al. 2010)
Tribromomethane ($CHBr_3$; Bromoform)		Water purging	
Trichloromethane ($CHCl_3$; Chloroform)	Contributes to reactive chlorine burden and ozone destruction (Claxton et al. 2019)	Headspace purging, Water purging	(Nightingale et al. 1995; Laturnus et al. 2010; Ferraces-Casais et al. 2013)
2-iodopropane (C_3H_7I; Isopropyl iodide)	Rapidly photodissociate into reactive iodine atoms, with further reactions leading to ozone depletion, changes to NO_x and HO_x ratios, and new particle formation (Saiz-Lopez et al. 2014; Saiz-Lopez et al. 2012)	Water purging	(Ferraces-Casais et al. 2013; Schall et al. 1994)
Diiodomethane (CH_2I_2; Methylene iodide)			(Schall et al. 1994; Carpenter et al. 2000; Laturnus et al. 2010; Kundel et al. 2012)
Iodoethane (C_2H_5I; Ethyl iodide)			(Carpenter et al. 2000; Kundel et al. 2012)

4 Discussion

4.1 Best Available Technology for Cultivation of sugar kelp in Denmark

The Best Available Technology (BAT) for cultivation of sugar kelp in large-scale in Limfjorden was defined as a long line system with 10 m spacings between 200 m long lines equipped with seeded lines in a single horizontal line design. This equals a total line density of 1000 m seeded line ha^{-1}.

The single horizontal line system provided the same biomass yield as systems with double horizontal lines, while using only 50% of the seeded lines needed for the double line system and less handling time during both hatchery, deployment, maintenance and harvest. The single horizontal line system also provided 2.3-fold higher biomass yields (FW) per meter seeded line as compared to seeded lines arranged in loops, again with less material costs for the seeded lines, and less resources spent in all handling processes. In conclusion, multiple horizontal lines or loops was not the optimal way for increasing biomass yields through increased density of seeded lines in the cultivation area.

Another option for increasing the line density was tested by increasing the number of longlines per area on site by reducing space between lines. This was not successful, as it proved to be practically impossible to maneuverer with boats between the lines during handling processes. Hence, the distance of 10 m between lines is still recommended as BAT.

The line density of 1000 m seeded line ha^{-1} is a five-fold reduction to a line density of 5000 m ha^{-1} which has been assumed earlier (Petersen et al. 2021; Bruhn et al. 2020a; Zhang et al. 2022) based on a design where up to 5 horizontally parallel lines were tested in similar scale set-ups in Horsens Fjord (Boderskov et al. 2023). In contrast to the trial in Horsens Fjord, where the seeded lines were deployed with weights below the longlines (Boderskov et al. 2023), the seeded lines in the Limfjorden trial were deployed above the long line, which could have made the seeded lines more prone to tangling. The tangling caused loss of biomass due to tear, and also made harvest considerably more time consuming as an additional handling process (detangling of lines) was needed prior to harvesting of the biomass. Substantial cultivation experiences from Norway indicate that the number of horizontal seeded lines can successfully be increased by fixing the horizontals lines in a grid design between neighbouring longlines (Seaweed Solutions, personal communication). Substantial physical differences between Norwegian and Danish cultivation conditions may preclude transferability of designs. In respect to the line entanglement and shear, it could be relevant to design systems according to their hydrodynamic regime, where the Limfjorden is characterised by low amplitude high frequency waves and often opposing surface-column velocities that may benefit from different tensions, spacing, etc. of lines, than in Norwegian systems, where otherwise biomass loss exceeds optimal space use.

The BAT of cultivation of sugar kelp is continuously developing (Sæther et al. 2024), with increasing success in mechanisation of direct seeding, as well as coppicing allowing for multiannual harvest of the same seeded lines with success in both Faroe Islands and Southwestern Norway. The direct seeding has been tested in smaller scale in Denmark without achieving similar or

improved yields after one cultivation season, but coppicing has been successfully tested in Danish waters, however with increased fouling pressure compared to first year lines (Boderskov et al. 2021a; Boderskov et al. 2023). Recent trials at commercial producers in Great Belt in Denmark (Kerteminde Seafarm) has also achieved promising results applying both technologies and a horizontal deployment design, confirming the site-dependency of success of different cultivation technologies. The high fouling pressure and the shallow water depths in areas like the Limfjorden would challenge the use of multiannual cultivation and direct seeding of a species like *S. latissima*. The results from Kerteminde Seafarm are not scientifically documented and for that reason not included in the determination of BAT.

Cultivation designs with another configuration than traditional longlines are being developed and tested in Denmark and abroad. Development towards cultivation systems with even higher line density (up to 10,000 m seeded line ha^{-1}) as in Asia (Zhang et al. 2017) is to be expected also in Europe. Systems designed for cultivation of mussels on large vertical nets (SMART farm systems) has been tested in Denmark (Boderskov et al., 2023), with indications of achieving larger biomass yields on nets than lines using the same area of sea and are presently being tested for cultivation of sugar kelp in Limfjorden (SMART tang, GUDP). Positive results emerge on scaling the different procedures, while challenges persist in the seeding and harvesting of the large net structures.

The Faroese system from Ocean Rainforest (https://www.oceanrainforest.com), the Macroalgae Cultivation system (MACR) (Bak et al. 2018), that relies on vertical growth lines are being produced and sold, either as it is or tailormade for specific areas. Also, a smaller scale system (2 ha) with longlines in a closer configuration allowing for 3 km of seeded line ha^{-1} to be operated from smaller boats is provided by the Swedish company Koastal (https://www.koastal.se) and Artic Seaweed in Bergen, Norway (https://aseaweed.com). Two of these systems will be tested from 2025 in Denmark as part of the CoolBlue project (HAVHØST (https://xn--havhst-eya.dk)).

4.2 Cultivation of sugar kelp for nutrient removal

The maximal N removal potential per m of seeded line was improved by more than a factor of 2 from 10.88 to 22.96 g N m^{-1} seeded line as compared to previously reported N removal potentials of sugar kelp in Denmark (Bruhn et al. 2020a; Petersen et al. 2021). This was caused by the high sugar kelp biomass yield pr m cultivation line and the high N content achieved at harvest in the large-scale demonstration in Limfjorden. In contrast, adapting the 1000 m seeded line ha^{-1} as BAT instead of the previous assumption of 5000 m line ha^{-1} will reduce the assumptions of the density of seeded lines by a factor of five compared to former predictions of the nutrient removal efficiency of cultivation of sugar kelp as a marine measure for mitigating marine environmental quality towards Good Ecological Status (GES) (Petersen et al. 2021; Bruhn et al. 2020a), (Petersen et al. 2021; Bruhn et al. 2020a).

Consequently, the maximal nutrient removal potentials are reduced to 23 kg N ha^{-1} from 47.3 kg N ha^{-1} and to 0.8 kg P ha^{-1} from 5.2 kg P ha^{-1} (Petersen et al. 2021; Bruhn et al. 2020a).

The nitrogen removal potential per m of seeded line documented within Danish waters varied by 765-fold from 0.03 to 23 g N m^{-1} of line, depending primarily on the biomass yield achieved, but also on the tissue DM content and

concentration of N in the cultivated sugar kelp. An important distinction is that biomass yields outside Limfjorden were based on data from Blue Community Gardens provided by volunteers, and not larger scale scientific experiments as in Limfjorden, and for this reason, the results are not fully comparable. Limfjorden appeared to be an optimal location for sugar kelp production, regarding both biomass and nitrogen yields, presenting the highest achieved biomass yields, but also with the highest documented tissue N concentrations up to 4.5% of DM. The biomass yields achieved in this large-scale demonstration were higher than previously documented in smaller scale in Limfjorden (Boderskov et al. 2021a; Bruhn et al. 2016; Nielsen 2015) emphasising the importance of farm design, operation, site selection and site knowledge for optimising biomass yields through timing of deployment and harvest. The tissue N content was also high in the harvested sugar kelp, but still within range of previous results from sugar kelp in Limfjorden (Bruhn et al. 2020a).

Optimal biomass and N yields depend on the timing of the deployment and harvest of the sugar kelp, and it was confirmed that biomass harvest in late April is key for 1) high biomass yield, avoiding both distal loss of biomass and biofouling resulting in loss of biomass and reduced biomass quality (Boderskov et al. 2021a; Boderskov et al. 2023), and 2) high tissue N, as the tissue N concentrations decreased in the sugar kelp from April to May.

Overall, the small-scale trials documented large geographic variability in biomass yield and tissue nutrient content in Danish waters, emphasising that cultivation of sugar kelp for N removal may be feasible in certain areas, but not in others. It cannot be ruled out that the differences in cultivation setups or physical disturbance during the cultivation phase have contributed to the observed differences in yields. Therefore, these results should only be used as indicative. Still, careful selection of a cultivation site is needed, taking into consideration the local ambient environmental conditions, in particular the salinity, but also the availability of nutrients. Smaller-scale cultivation trials experiencing production deficiencies or problems due to cultivation design differences or physical disturbance may contribute to the apparent geographic variability, requiring repeated controlled trials over several growth seasons to capture interannual variation and better characterise site-specific potential yield variability.

Surprisingly, it was observed that in locations with salinity range minimums down to 10-13 PSU such as Sønderborg, Odsherred and Kerteminde, relatively high biomass production yields of up to 120-231 g DM m^{-1} of seeded line were achieved, but the N removal potentials were low, ranging from 1.5 to 3.35 g N m^{-1}, due to low tissue N contents. These good yields from low salinity areas again emphasise the heterogeneity of Danish waters where local conditions, i.e. current patterns and mixing may sustain better or worse growth of sugar kelp than predicted from salinity only, calling for detailed 3D ecological site-selection models, such as developed for mussel production and nutrient removal (Maar et al. 2023a).

Since the Limfjorden is categorised an area in need for nutrient removal actions, and since the large-scale trial provided data for Limfjorden, the effects of sugar kelp cultivation in Limfjorden towards achieving GES was modelled in a number of scenarios as part of the overall project (Larsen and Erichsen 2024). The two environmental indicators "summer chlorophyll-a" and "light attenuation" are defined as key performance indicators for GES, and the effect on these key indicators of sugar kelp nutrient removal was modelled in two scenarios differing in intensity:

Scenario 1: The first scenario assumed sugar kelp cultivation at the 35 existing mussel cultivation sites in Limfjorden over 5 years, assuming the productivity achieved in this project. With 35 sugar kelp cultivation sites in Limfjorden, the model predicts that an average of 1.8 tonnes of N would be removed, thus summing up to a limited proportion of the amount of total N added to (11 kton N) - or transported through the Limfjorden (4.2 kton N) during the year (Larsen and Erichsen 2024). Thus, given the assumptions of scenario 1, the effects of sugar kelp cultivation on the GES indicators would be small to negligible. There could be several explanations for this:

- The results of modelling scenario 1 indicated no effect on the two key indicators, potentially because the cultivation season of the sugar kelp (September to April) is complementary to the time of measuring the two environmental indicators; summer chlorophyll-a (May to September) and the light attenuation coefficient (Kd) during the growing period (March to September).

- In future modelling studies the assumptions will need revision: According to scenario 1, only about 50 kg of N would be removed per cultivation area of 10 hectares, which is considerably less than the nutrient removal potential documented in the large-scale trial, where more than 400 kg of N would be removed from a 18 ha cultivation site. This 8-fold underestimation of modelled N removal potential, as compared to the on-site quantitative data may in part be explained by 1) each standard cultivation site is 18 ha, but in the model, this was set to 10 ha to reflect the size of the large-scale test cultivation site in Sallingsund (Larsen and Erichsen 2024); 2) heterogeneity of environmental conditions among the 35 sites included in the model, which is also supported by the N removal potential between the four sites in Limfjorden differing by a factor of 2, if only including the three sites with cultivation experience and excluding the BCG in Løgstør, that provided a considerably lower potential.

Scenario 2: To give an indication of the degree of area-specific productivity improvement or increased number of farm sites that will be necessary for seaweed cultivation to function as a marine measure for nutrient removal, a second modelling scenario was set up. In this scenario, the nutrient removal efficiency of the sugar kelp cultivation was increased, assuming a 15 times increase in production yields, which in theory could be achieved through more seaweed farms, higher line density in existing farms and/or increased productivity. According to this scenario 2, about 27 tonnes of N would be removed over the growth period of the seaweed. This still constituted less than 1% of the nitrogen that is added or transported through Limfjorden on an annual basis. However, cultivation on a scale as modelled in this scenario resulted in a detectable reduction of up to more than 30% of the summer and year-round concentrations of dissolved inorganic N in the areas around the seaweed cultivation sites and with detectable reductions in DIN concentrations also downstream of the cultivation sites. Still in model scenario 2, the seaweed cultivation had no effect on the two key performance indicators for GES "summer chlorophyll-a" and "light attenuation" (Larsen and Erichsen 2024).

Comparing the assumptions of this second modelled scenario to the results achieved in the large-scale cultivation site in 2022/2023, the '15-fold increase in production yield' is not unrealistic: 1) the farm areas assumed were 10 ha not 18 ha, and 2) the measured N removal potential was a factor 4-8 higher

than assumed in the model. Taking this into consideration, the second model scenario better reflects the actual potential and production yield, meaning that for achieving the modelled effects on reductions in summer DIN in the Limfjorden, the production yields only need to increase by a factor of up to two, which is realistically within reach with future technology improvements in both farm design and seaweed productivity. Alternatively, more sugar kelp farms could be licenced in Limfjorden.

In contrast to the effect of restoration of eelgrass meadows suggested as a tool for improving the environmental status of the Danish waters, but in parallel with cultivation of line mussels, the N absorbed in the sugar kelp is permanently removed from the system, when the sugar kelp is harvested (Maar et al. 2023b; Timmermann et al. 2024). After harvest, the sugar kelp biomass can be used as a resource for food, feed or materials. Cultivated seaweed has low environmental impact regarding footprints of both nutrients and carbon (Gephart et al. 2021).

Importantly, this also implies that the economy of using sugar kelp as a marine measure for environmental mitigation does not need to rely (solely) on N credits or subsidies, as the biomass presents a tangible market value (World_Bank 2023).

4.3 Feasibility of large-scale production of sugar kelp for marine mitigation

Based on the costs of cultivation with the Best Available Technology at the large-scale cultivation site in Sallingsund, the cost price for sugar kelp was calculated to DKK 12.18 per kg FW and DKK 64 per m of seeded line produced.

The price for nitrogen removal will thus correspond to DKK 2795 per kg of nitrogen harvested at Sallingsund in Limfjorden, provided that the sugar kelp is only cultivated for nutrient removal and not representing a value for any other purpose. The price for harvested nitrogen will be significantly higher in other water areas, since both the yield and nitrogen content of sugar kelp cultivated in other Danish waters were lower than in Limfjorden.

A standard cultivation site of 18 hectares will, based on the cultivation site in Sallingsund, be able to produce 96 tonnes of seaweed (FW) per year with current BAT. The processing costs of the sugar kelp for feed (transport, chopping, fermentation) were estimated based on the large-scale test processing at DKK 6.57 per kg FW. When selling sugar kelp to produce feed (approx. DKK 20 per kg FW), the estimated profit per kg of fresh seaweed, after subtracting production and processing costs, can be approx. DKK 1.25 per kg FW. Under these assumptions, a standard cultivation site can thus, with a production of 96 ton FW, gain an annual profit of approx. DKK 120,000. It needs to be strongly emphasised that all economic estimates are subject to large uncertainty, as data only derive from one single year of large-scale cultivation.

While a major part of European sugar kelp is presently traded for production of feed or biostimulants, the World Bank estimates that on medium and long term, expansion of more lucrative market for use of seaweed for nutra- and pharmaceuticals will increase the market value (World_Bank 2023).

4.4 Ecosystem effects of large-scale sugar kelp production not relating to GES

When discussing the ecosystem effects documented in this project, it needs to be taken into consideration, that while the production scale of 12 ha (28 × 150 m longlines) is considered as being 'large-scale' sugar kelp cultivation in Denmark, it only aligns with the definition of 'small-medium' scale (0-50 × 200 m lines) and not 'large-scale' (> 50 × 200 m lines) as defined by the Scottish government (Marine_Scotland 2017) and used by Campbell et al. (2019). At the 12-ha cultivation site, the line density did not fully align with the suggested BAT of 1000 m line ha^{-1} due to test of different designs, and also the density of sugar kelp on the system varied as a consequence of variation in seeded line quality and deployment time. Also, the study carried out at the 12-ha site spanned only one single cultivation cycle and therefore cannot account for interannual variations in yield and environmental conditions in general.

Consequently, a true 'large-scale' cultivation can be expected to have a different degree of ecosystem impact.

4.4.1 Effects on the physical environment

Light: With the actual line density applied in the trial in Sallingsund, which was lower than assumed using BAT, a negligible (3%) reduction of light to the seabed was documented in the cultivation site between the lines at maximal biomass density just before harvest of the biomass. A larger shadow effect was only observed immediately below the lines in a narrow band of few meters just below the seaweed canopy, where up to 50% of incoming light reached the seabed. This is in line with observations from another large-scale sugar kelp production site at similar water depths in Horsens Fjord, where a light reduction of 1.4% to the seabed was observed between lines also at maximal biomass density (Bruhn et al. 2020b). This reduction in light below the lines is less or in range with what is observed from below natural kelp forests, where approximately 50% of incoming light reached the benthos (Reed and Foster 1984).

Oxygen and pH: In periods of high biomass and high irradiance, minor increases in oxygen concentration and pH in the cultivation area were observed as a consequence of the photosynthesis of the sugar kelp, however in most cases, potential effects were masked by temporal variability in environmental conditions. Increased pH of up to 0.1 has been reported from large-scale seaweed cultivation areas of *S. japonica* (Xiao et al. 2021) and also in natural kelp forests (Krause-Jensen et al. 2016) suggesting, that cultivated seaweed biomass can provide a local refuge from ocean acidification of calcifying organisms (Young et al. 2022).

Hydrology: The hydrology in and around the sugar kelp cultivation site was affected by the cultivation structures and biomass at the time of maximal biomass standing stock prior to harvest: Channelized flow was observed between the seaweed lines, resulting in overall reduced current speed and potentially reduced sedimentation in the cultivation area overall, with locally increased current velocities between and below the lines and turbulent flows around the biomass and farm structures. Such effects have been predicted in modelling of flow in large-scale kelp cultivation in China (Shi et al. 2011) and also observed in mussel cultivation systems (Delaux et al. 2011). Proximity to a mussel farm may have influenced observed sedimentation rates within the sugar kelp area.

The increased current and reduced sedimentation rates below seaweed cultivation systems is in agreement with the reduced sedimentation rates observed. This could reduce the potential impact on benthic turnover, as observed below fish and mussel farms, where the POM deriving from the cultivation system is transported out of the system to sediment elsewhere (Valdemarsen et al. 2015; Maar et al. 2023b). The potential increase in current speed and reduction in sedimentation rates below the seaweed cultivation system as well as potentially increased sedimentation rates downstream of the cultivation system could also affect the benthic sediment structure, benthic communities in and around a seaweed cultivation system, the balance between benthic suspension feeders and deposit feeders (Rhoads and Young 1970), and benthic vegetation.

While effects on hydrodynamics and sedimentation were observed, in physically dynamic systems such as the Limfjorden, temporal variability (daily to seasonally) due to physical forcing can be dominant. To fully understand the effect of changes in hydrology on the complex interactions in the benthic community structure was beyond the scope of this study and will require a baseline study of the targeted area prior to the deployment of the system, followed by dedicated investigations of the area below the system and adjacent areas over a longer time period, to take into account interannual variations caused by other interacting factors, such as weather induced resuspension and fluctuations in phytoplankton primary production.

4.4.2 Carbon uptake, loss and degradation

The environmental effects in the following relate to the C cycle mechanisms documented in this project, extrapolated to a production of sugar kelp of an average of 522 g DM m^{-1} on 12 ha with a line density of 1000 m ha^{-1} as defined by BAT.

At the time of harvest, assuming BAT, the standing stock of carbon stored in the sugar kelp in Sallingsund would be approximately 148 kg C ha^{-1}. This is defined as the amount of C that is removed from the system via harvest of the sugar kelp and following can be accounted for and used in a carbon budget for the system (Zhang et al. 2022; van Duinen et al. 2023).

The carbon uptake driven by photosynthesis of the growing seaweed was high, and assuming a respiratory quotient of 1, the net C uptake ranged from 150-200 µmol C g DM^{-1} h^{-1} in late April at the time of maximal biomass. The loss of C as particulate or dissolved organic carbon (POC or DOC) in time until harvest was measured in the laboratory experiments to be relatively low:

No loss of POC through distal erosion of the sugar kelp fronds was observed until harvest in April, and loss of DOC was <7% of fixed carbon in the spring period until harvest in late April. The loss of DOC was not measured at times after harvest, whereas the loss of POC was followed through sections of seaweed left at two individual lines, confirming that distal erosion increased in late spring, as previously reported from Danish natural forests of sugar kelp (Nielsen et al. 2014), and from cultivated sugar kelp in Norway (Fieler et al. 2021). In a cultivation site in Norway, the loss of sugar kelp constituted 8% of the accumulated DM up until harvest in June, whereas if the kelp was not harvested, the C loss increased over July to nearly 50% of the annual production in Augst (Fieler et al. 2021). In China, the loss of POC from cultivation of *S. japonica* was estimated at 61% of the gross production with the major loss happening through distal erosion (91.3%), with a rate of loss increasing from

January to April (Zhang et al. 2012). At the cultivation site in Sallingsund, no average detectable distal erosion took place before harvest in late April whereas post-harvest, the sporophytes lost an average of 38 to 71 cm of frond tissue from the distal end. In agreement, the isotopic 13C/15N signature of the POM sedimented below the seaweed cultivation system, did not indicate specific contribution of kelp POC to the sedimented material below the seaweed cultivation system.

The change in rate of loss of distal material from the fronds emphasises the importance of the harvest and management praxis of sugar kelp cultivation, as an early harvest will reduce the loss of POC to the environment. At harvest, the risk of losing whole sporophytes to the environment increases in the harvest process (Fieler et al. 2021). While loss of sporophytes was observed at harvest, it was not possible to quantify the loss.

During growth, macroalgae release dissolved organic carbon (Weigel and Pfister 2021; Paine et al. 2021). Brown algae release 0–89.92 µmol C g DW^{-1} h^{-1} (Paine et al. 2021). The DOC loss from the sugar kelp in Sallingsund was in the lower range of this, as up to 15 µmol C g DW^{-1} h^{-1} was observed as a maximum in February decreasing to 5 µmol C g DW^{-1} h^{-1} in April at the time of maximal biomass. This resulted in a loss of up to 7% of C fixed through photosynthesis, with DOC loss rates decreasing from February to April. The loss of DOC was not driven by light intensity nor tissue N content, the latter indicating that eutrophication does not impact the rate of sugar kelp DOC excretion (Pedersen et al. In prep; Christiansen 2023).

Particulate and dissolved organic carbon lost from the sugar kelp will be degraded in the water and on/in the sediment. The composition of the POC and DOC determines the rate of degradation, and hence the fraction of C that is buried in the sediment and here potentially sequestered – defined as not turned over in a time frame of 100 years (Hurd et al. 2022; GESAMP 2019). After 150 days, 67% of the DOC released from the sugar kelp was degraded, while 33% persisted and was categorised as 'refractory DOC' (RDOC) (Christiansen 2023). The recalcitrant fraction of the DOC of up to 33% corresponded well with the finding that around one third of the DOC compounds excreted are either benzenoids, such as phenolic compounds, or organo-hetero-cyclic compounds, that are highly recalcitrant (Seo et al. 2009; Wada et al. 2008). Degradation rates of both POC and DOC decrease over time, as labile compounds are degraded and increasingly larger fractions of RDOC consequently persist (Vinbæk 2023). For this reason, predicting the sequestration potential of the sugar kelp POC and DOC remains challenging. Using the decay constants achieved at the latest experimental stages (approximately after 150 days) indicate that all C will be degraded after 100 years, and hence none of the lost sugar kelp DOC or POC will be sequestered. In contrast, the addition of sugar kelp tissue to the sediment can even increase the turn-over of buried RDOC in the sediment through 'priming', where addition of more labile organic matter accelerates the degradation of existing refractory organic matter (Kuzyakov et al. 2000; Jenkinson et al. 1985). Adding sugar kelp to sediment can increase the C turnover more than 200% of the C added (Ehrenreich et al. 2025; Boldreel et al. 2023), in particular in sugar kelp with high tissue N content (Ehrenreich et al. 2025).

Overall, the knowledge obtained on the release and degradation of sugar kelp DOC and POC in shallow, eutrophic systems like Limfjorden overall supports the assumption that the sequestration potential of sugar kelp C in Danish waters is discountable when harvesting the sugar kelp in late spring prior to distal loss of tissue and biofouling.

Climate aspects of cultivation of sugar kelp in Danish waters is reviewed by Gyldenkærne and Callisen (2024).

4.4.3 Climate gasses

No emissions of methane or nitrous oxide were detected from the sugar kelp. This is in accordance with existing knowledge, where only minor emissions of nitrous oxide have been detected from macroalgae, and only from a green macroalgae, *Ulva* sp. (Albert et al. 2013).

In contrast, but also in accordance with existing knowledge, emissions of various climate-active VOCs were documented, but not quantified, from the cultivated sugar kelp. Sugar kelp has been documented to be responsible for the emission of climate-relevant trace gases, such as elemental iodine (I2) and volatile halocarbons. The climate impact of these compounds are diverse and even counteracting. Some of these compounds have the potential to form particles, increase cloud formation and thus reduce the radiative forcing (McFiggans et al. 2004; Saiz-Lopez et al. 2012), while others can destroy ozone, perturb NOx and HOx budgets. The true contribution of macroalgal sources to these impacts is still unknown (Keng et al. 2020; Phang et al. 2015; Saiz-Lopez et al. 2012).

While our analysis shows that trace gases of environmental concern are detectable even in short-term incubations with *S. latissima*, we had no means of quantifying the emissions, and a comprehensive, in situ quantification of these emissions is recommended in the future. In the incubations, more compounds were mobilised into the headspace after a mild purge of incubation water. This illustrates that VOC emissions are influenced by physical conditions (e.g., waves and column mixing), and highlights that laboratory tests should be supplemented with in situ measurements and modelling of sea-to-air exchange.

4.5 Perspectives and knowledge gaps

Sugar kelp is the most produced seaweed in Europe and the production is expanding in Europe and North America (Sæther et al. 2024; Araújo et al. 2021). Labor and resource consuming cultivation procedures are being mechanised and made more efficient (Kerrison et al. 2018; Umanzor et al. 2020; Boderskov et al. 2021a; Kite-Powell et al. 2022), and cultivation technologies are being developed to fit various locations differing in scale and degree of exposure (Bak et al. 2018; Fernand et al. 2017). Knowledge on gametophyte biology and population genetics is emerging, preparing for selective breeding and up-scaling of production (Ebbing et al. 2020; Ebbing et al. 2021a; Ebbing et al. 2021b; Boderskov et al. 2022b; Ribeiro et al. 2022; Nielsen et al. 2016). Still, major knowledge gaps persist as barriers for the scaling up of sugar kelp production.

One major knowledge gap remains the development and adaptation of cultivation technology, along with long-term monitoring of cultivation efficiency in different sites to account for interannual variation in environmental conditions and to allow for an iterative adaptation and testing of structure design. As establishing and operating cultivation sites in large- scale is costly, this will most efficiently be done in close science-industrial cooperations, or as large strategic scientific infrastructure investments. Also, developing efficient site-selection models to identify the most suitable areas for biomass production as well as nutrient removal is pertinent to avoid loss of investments.

Another major knowledge gap is the effect of kelp cultivation on climate, where quantification and understanding of the underlying mechanisms behind emissions of climate active volatile carbons and loss of dissolved and particulate matter to the marine environment is needed (Pessarrodona et al. 2024). While this project has provided substantial knowledge about the loss of seaweed biomass as DOC and POC during a production season; the turnover of DOC and POC in the water column and sediment; the transport of DOC and POC in the water column vertically and horizontally; and the emission of VOCs from the production of sugar kelp, there is still a need to understand the mechanisms behind the dynamics, and the cumulative effects. Expanded production in restricted space, such as in estuaries and fjords, will generate cumulative effects on biological and physical conditions that while are largely negligible in at the single-farm, may culminate in substantial ecological modifications at larger scales; such as normal flow and particle exchange (Grant and Bacher 2001) and ephemeral habitat provision in impaired waters (Corrigan et al. 2024; Li et al. 2024). Some of the existing knowledge gaps on carbon cycle and climate effects can be closed by continued cultivation on a smaller scale in different water bodies supplemented by laboratory experiments. as the variation in both production yields and environmental effects between individual years is significant (Corrigan et al. 2022; Pessarrodona et al. 2024).

The effect of large-scale production on biodiversity remains another major knowledge gap. Due to the short nature of this project, the effects on biodiversity were not investigated. Having only one experimental growth season, required the use of an existing cultivation structure, not leaving options for taking a Before, After, Control, Impact (BACI) design approach, and not allowing to account for interannual variations. Hence, results on effect on biodiversity would not be consistent. Recent reviews indicate cultivated kelps do not provide the same positive effects on biodiversity as natural kelp forests in part due to the temporal nature of the habitat provided by the cultivated kelp (Forbes et al. 2022). To assess the effect on biodiversity of large-scale seaweed cultivation, there is a need to develop robust and standardised methods for mapping and quantifying biodiversity in seaweed cultivation areas – targeting both sessile and pelagic organisms, such as fish and mammals (Corrigan et al. 2022).

Closing knowledge gaps on production technology, the long-term fate of kelp carbon and biodiversity requires large-scale, long-term studies along with development of standardised and robust methods for both determining and developing the value of kelp farms, the fate of kelp C, the intermittently suspended biomass as a habitat and the cumulative effects on the marine environment of large-scale kelp cultivation (Corrigan et al. 2022; Pessarrodona et al. 2024).

5 Conclusions

By cultivation of sugar kelp, up to 23 g of N m^{-1} line $year^{-1}$ can be removed in Limfjorden from October to April where nutrients are most available. This is equivalent to an annual nutrient removal potential of 23 kg of N ha^{-1} and 0.8 kg of P ha^{-1}, and a carbon capture potential of 145 kg C ha^{-1} $year^{-1}$, using a standard cultivation system with 1000 m of cultivation line ha^{-1}. The documentation of nutrient removal potential in large-scale in this project documents an increase in production yield and nutrient removal efficiency, but a reduction of the area based nutrient removal potential as compared to earlier estimates (47.3 kg N ha^{-1} $year^{-1}$) (Bruhn et al. 2020a). Earlier potentials were based on extrapolations assuming a different cultivation design with a higher density of cultivation lines (5000 m ha^{-1}), which turned out not to be feasible in this case. This highlights the importance of testing assumptions at scale in different areas. The highest nutrient removal potentials were found in areas with high salinity and nutrient availability, such as Limfjorden, and the lowest potentials in areas with low salinity (<10 PSU) and limited cultivation experience. The cost of nutrient removal varies accordingly, with the lowest cost resuming 2805 DKK kg N^{-1}.

Modelling scenarios indicate that in areas optimal for cultivation and nutrient removal, a doubling of existing cultivation area or areal efficiency is required for achieving a significantly reduced concentration of dissolved inorganic nitrogen. Negligible effects was achieved for the key target environmental indicators for GES, "summer chloro-phyll-a" and "light attenuation", potentially due to a mismatch in time of cultivation and indicator monitoring.

At large scale seaweed cultivation (12 ha), the environmental effects include a) negligible reduction of light to the seabed, b) altered hydrology with reduced overall velocities, increased turbulence, and increased current velocities between and below lines, c) reduced sedimentation rates below cultivation system, and d) periodic increase in pH and oxygen concentrations in the cultivation area. These effects were limited in space and periodicity, where natural daily and seasonal dynamics more importantly contributed to variability.

Harvest no later than April will reduce the loss of both volatile, dissolved and particulate carbon (VOC, DOC and POC) from the seaweed to the environment, but while fractions of both DOC and POC are recalcitrant, it is unlikely that C is sequestered (>100 year) in inner Danish waters. In contrast, sugar kelp tissue deposited on/in the sediment can stimulate the degradation of buried recalcitrant carbon (priming effect).

The need remains for further large-scale, and long-term studies of the efficiency and environmental effects of cultivation of sugar kelp to address persisting fundamental knowledge gaps in particular in relation to 1) implementing more area intensive production systems, 2) lowering production costs, impact on and interaction with 3) the marine carbon cycle and climate effects in relation to C burial/sequestration, and emissions of climate active VOC, and 4) biodiversity. This requires cooperation with the industry or strategic infrastructure investments to ensure relevance and reduce costs.

6 Acknowledgement

In addition to the work by the authors, several others have contributed. We thank the Blue Community Gardens and companies, that on a volunteer basis, have contributed with data. In particular thanks to Hardy Jensen and Line Bøttern, HAVHØST. Thanks to the lab technicians at AU: Kitte Linding Gerlich, Lene Rechnagel, Dorte Søndergaard and Birgitte Kretzschmar Tagesen, and the technicians at DTU: Finn Bak, Kasper Lenda Maarbjerg, Kasper Meyer and research assistants Kathrine Habekost Hansen and Camilla Lynggaard Larsen. Thanks also to Tinna Christensen, AU, for assistance with figures.

7 References

Albert KR, Bruhn A, Ambus P (2013) Nitrous oxide emission from *Ulva lactuca* incubated in batch cultures is stimulated by nitrite, nitrate and light. Journal of Experimental Marine Biology and Ecology 448:37-45. doi:10.1016/j.jembe.2013.06.010

Araújo R, Vázquez Calderón F, Sánchez López J, Azevedo IC, Bruhn A, Fluch S, Garcia Tasende M, Ghaderiardakani F, Ilmjärv T, Laurans M, Mac Monagail M, Mangini S, Peteiro C, Rebours C, Stefansson T, Ullmann J (2021) Current Status of the Algae Production Industry in Europe: An Emerging Sector of the Blue Bioeconomy. Frontiers in Marine Science 7 (1247). doi:10.3389/fmars.2020.626389

Bak UG, Mols-Mortensen A, Gregersen O (2018) Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting. Algal Research 33:36-47. doi:https://doi.org/10.1016/j.algal.2018.05.001

Boderskov T, Nielsen MM, Rasmussen MB, Balsby TJS, Macleod A, Holdt SL, Sloth JJ, Bruhn A (2021a) Effects of seeding method, timing and site selection on the production and quality of sugar kelp, *Saccharina latissima*: A Danish case study. Algal Research 53:102160. doi:https://doi.org/10.1016/j.algal.2020.102160

Boderskov T, Rasmussen MB, Bruhn A (2021b) Obtaining spores for the production of *Saccharina latissima*: seasonal limitations in nature, and induction of sporogenesis in darkness. J Appl Phycol 33:1035-1046. doi:10.1007/s10811-020-02357-0

Boderskov T, Rasmussen MB, Bruhn A (2022a) Upscaling cultivation of *Saccharina latissima* on net or line systems in Danish waters; comparing biomass yields and nutrient extraction potentials In preparation for Frontiers in Marine Sciences

Boderskov T, Rasmussen MB, Bruhn A (2023) Upscaling cultivation of *Saccharina latissima* on net or line systems; comparing biomass yields and nutrient extraction potentials. Frontiers in Marine Science 10. doi:10.3389/fmars.2023.992179

Boderskov T, Rasmussen MB, Cassard CH, Svensgaard J, Enevoldsen LN, Bruhn A (2022b) Comparing effects of nutrient sources approved for organic seaweed production on hatchery stage development of sugar kelp, *Saccharina latissima*. Algal Research 61:102602. doi:https://doi.org/10.1016/j.algal.2021.102602

Boldreel EH, Attard KM, Hancke K, Glud RN (2023) Microbial degradation dynamics of farmed kelp deposits from *Saccharina latissima* and *Alaria esculenta*. Marine Ecology Progress Series 709:1-15

Bruhn A, Flindt MR, Hasler B, Krause-Jensen D, Larsen MM, Maar M, Petersen JK, Timmermann K (2020a) Marine virkemidler - Beskrivelse af virkemidlernes effekter og status for vidensgrundlag. Aarhus Universitet, DCE –

Nationalt Center for Miljø og Energi, 122 - Videnskabelig rapport nr 368 http://dce2audk/pub/SR368pdf

Bruhn A, Rasmussen MB, Pedersen HB, Boderskov T (2020b) Effekter af dyrkning af sukkertang på lys- og bundforhold samt biodiversitet ved Hjarnø Hage. Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi, 14 s Notat nr 2020|29 https://dceaudk/fileadmin/dceaudk/Udgivelser/Notatet_2020/N2020_29pdf

Bruhn A, Tørring DB, Thomsen M, Canal Vergés P, Nielsen MM, Rasmussen MB, Eybye KL, Larsen MM, Balsby TJS, Petersen JK (2016) Impact of environmental conditions on biomass yield, quality, and bio-mitigation of *Saccharina latissima* Aquaculture Evironmental Interactions 8:619-636

Campbell I, Macleod A, Sahlmann C, Neves L, Funderud J, Øverland M, Hughes AD, Stanley M (2019) The environmental risks associated with the development of seaweed farming in Europe - Prioritizing key knowledge gaps. Frontiers in Marine Science 6 (107). doi:10.3389/fmars.2019.00107

Carpenter LJ, Malin G, Liss PS, Küpper FC (2000) Novel biogenic iodine-containing trihalomethanes and other short-lived halocarbons in the coastal east Atlantic. Global Biogeochemical Cycles 14 (4):1191-1204. doi:https://doi.org/10.1029/2000GB001257

Christiansen FS (2023) Excretion and degradation of dissolved organic carbon from sugar kelp (*Saccharina latissima*). Masters Thesis, Aarhus University:1-62

Claxton T, Hossaini R, Wild O, Chipperfield MP, Wilson C (2019) On the Regional and Seasonal Ozone Depletion Potential of Chlorinated Very Short-Lived Substances. Geophysical Research Letters 46 (10):5489-5498. doi:https://doi.org/10.1029/2018GL081455

Corrigan S, Brown AR, Ashton IGC, Smale DA, Tyler CR (2022) Quantifying habitat provisioning at macroalgal cultivation sites. Reviews in Aquaculture 14 (3):1671-1694. doi:https://doi.org/10.1111/raq.12669

Corrigan S, Smale DA, Tyler CR, Brown AR (2024) Quantification of finfish assemblages associated with mussel and seaweed farms in southwest UK provides evidence of potential benefits to fisheries. Aquaculture Environment Interactions 16:145-162

Delaux S, Stevens CL, Popinet S (2011) High-resolution computational fluid dynamics modelling of suspended shellfish structures. Environmental Fluid Mechanics 11 (4):405-425. doi:10.1007/s10652-010-9183-y

Ebbing A, Pierik R, Bouma T, Kromkamp JC, Timmermans K (2020) How light and biomass density influence the reproduction of delayed *Saccharina latissima* gametophytes (Phaeophyceae). Journal of Phycology 56 (3):709-718. doi:https://doi.org/10.1111/jpy.12976

Ebbing APJ, Fivash GS, Martin NB, Pierik R, Bouma TJ, Kromkamp JC, Timmermans K (2021a) In-Culture Selection and the Potential Effects of Changing Sex Ratios on the Reproductive Success of Multiannual Delayed Gametophytes of *Saccharina latissima* and *Alaria esculenta*. Journal of Marine Science and Engineering 9 (11):1250

Ebbing APJ, Pierik R, Fivash GS, van de Loosdrecht NCJ, Bouma TJ, Kromkamp JC, Timmermans K (2021b) The role of seasonality in reproduction of multiannual delayed gametophytes of *Saccharina latissima*. Journal of Phycology 57 (5):1580-1589. doi:https://doi.org/10.1111/jpy.13191

Ehrenreich JL, Bruhn A, Taylor D, Nielsen MM, Schmedes PS, Hermannsen L, Olesen B, Boderskov T, Høgslund S (2025) Macroalgae detritus accelerates degradation of refractory organic matter in coastal marine sediments. Submitted to Science of the Total Environment

EN_17050 (2017) Animal feeding stuffs: Methods of sampling and analysis — determination of Iodine in animal feed by ICP-MS. European commitee for standardization (CEN), Brussels, Belgium

EU (2002) EU directive 2002/32/EC of the European Parliament and of the Council of 7th May 2002 on undesirable substances in animal feed.

EU (2008) Commission regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. The Commission of the European Communities

Fernand F, Israel A, Skjermo J, Wichard T, Timmermans KR, Golberg A (2017) Offshore macroalgae biomass for bioenergy production: Environmental aspects, technological achievements and challenges. Renewable and Sustainable Energy Reviews 75:35-45. doi:https://doi.org/10.1016/j.rser.2016.10.046

Ferraces-Casais P, Lage-Yusty MA, Rodríguez-Bernaldo de Quirós A, López-Hernández J (2013) Rapid identification of volatile compounds in fresh seaweed. Talanta 115:798-800. doi:10.1016/j.talanta.2013.06.049

Fieler R, Greenacre M, Matsson S, Neves L, Forbord S, Hancke K (2021) Erosion Dynamics of Cultivated Kelp, *Saccharina latissima*, and Implications for Environmental Management and Carbon Sequestration. Frontiers in Marine Science 8. doi:10.3389/fmars.2021.632725

Forbes H, Shelamoff V, Visch W, Layton C (2022) Farms and forests: evaluating the biodiversity benefits of kelp aquaculture. J Appl Phycol 34 (6):3059-3067. doi:10.1007/s10811-022-02822-y

Gephart JA, Henriksson PJG, Parker RWR, Shepon A, Gorospe KD, Bergman K, Eshel G, Golden CD, Halpern BS, Hornborg S, Jonell M, Metian M, Mifflin K, Newton R, Tyedmers P, Zhang W, Ziegler F, Troell M (2021) Environmental performance of blue foods. Nature 597 (7876):360-365. doi:10.1038/s41586-021-03889-2

GESAMP (2019) High level review of a wide range of proposed marine geoengineering techniques. In Boyd, P W & Vivian, C M G [Eds] IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UN Environment/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection Rep Stud GESAMP No 98, 144 pp

Glud RN (2008) Oxygen dynamics of marine sediments. Mar Biol Res 4 (4):243-289. doi:10.1080/17451000801888726

Grant J, Bacher C (2001) A numerical model of flow modification induced by suspended aquaculture in a Chinese bay. Canadian Journal of Fisheries and Aquatic Sciences 58 (5):1003-1011. doi:10.1139/f01-027

Grasshoff K, Ehrhardt M, Kremling K (1999) Methods of Seawater Analysis. ISBN: 3-527-29589-5. Wiley, Weinheim

Guerra M, Thomson J (2017) Turbulence Measurements from Five-Beam Acoustic Doppler Current Profilers. Journal of Atmospheric and Oceanic Technology 34 (6):1267-1284. doi:https://doi.org/10.1175/JTECH-D-16-0148.1

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana Hustedt* and *Detomula confervacae Cleve*. Canadian Journal of Microbiology 8:229-239

Gyldenkærne S, Callisen LW (2024) Muligheder og metoder for indregning af det marine område i den nationale drivhusgasopgørelse. Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi, 88 s - Videnskabelig rapport nr 625

Holst N, Boderskov T, Bruhn A (2025) A simple growth model of *Saccharina latissima* cultivated in Scandinavia: Theoretical and practical implications. Algal Research:103971. doi:https://doi.org/10.1016/j.algal.2025.103971

Hu Z-M, Shan T-F, Zhang J, Zhang Q-S, Critchley AT, Choi H-G, Yotsukura N, Liu F-L, Duan D-L (2021) Kelp aquaculture in China: a retrospective and future prospects. Reviews in Aquaculture 13 (3):1324-1351. doi:https://doi.org/10.1111/raq.12524

Hurd CL, Law CS, Bach LT, Britton D, Hovenden M, Paine ER, Raven JA, Tamsitt V, Boyd PW (2022) Forensic carbon accounting: Assessing the role of seaweeds for carbon sequestration. Journal of Phycology 58 (3):347-363. doi:https://doi.org/10.1111/jpy.13249

Jenkinson DS, Fox RH, Rayner JH (1985) Interactions between fertilizer nitrogen and soil nitrogen—the so-called 'priming' effect. Journal of Soil Science 36 (3):425-444. doi:https://doi.org/10.1111/j.1365-2389.1985.tb00348.x

Keng FS-L, Phang S-M, Abd Rahman N, Leedham Elvidge EC, Malin G, Sturges WT (2020) The emission of volatile halocarbons by seaweeds and their response towards environmental changes. J Appl Phycol 32 (2):1377-1394. doi:10.1007/s10811-019-02026-x

Kerrison PD, Stanley MS, Hughes AD (2018) Textile substrate seeding of *Saccharina latissima* sporophytes using a binder: An effective method for the aquaculture of kelp. Algal Research 33:352-357. doi:https://doi.org/10.1016/j.algal.2018.06.005

Kite-Powell HL, Ask E, Augyte S, Bailey D, Decker J, Goudey CA, Grebe G, Li Y, Lindell S, Manganelli D, Marty-Rivera M, Ng C, Roberson L, Stekoll M, Umanzor S, Yarish C (2022) Estimating production cost for large-scale seaweed farms. Applied Phycology 3 (1):435-445. doi:10.1080/26388081.2022.2111271

Krause-Jensen D, Marbà N, Sanz-Martin M, Hendriks IE, Thyrring J, Carstensen J, Sejr MK, Duarte CM (2016) Long photoperiods sustain high pH in Arctic kelp forests. Science Advances 2 (12):e1501938. doi:doi:10.1126/sciadv.1501938

Kundel M, Thorenz UR, Petersen JH, Huang RJ, Bings NH, Hoffmann T (2012) Application of mass spectrometric techniques for the trace analysis of short-lived iodine-containing volatiles emitted by seaweed. Anal Bioanal Chem 402 (10):3345-3357. doi:10.1007/s00216-011-5658-z

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32 (11):1485-1498. doi:https://doi.org/10.1016/S0038-0717(00)00084-5

Larsen TC, Erichsen A (2024) Kvantificering af virkemiddeleffekter i danske marine vandområder. DHI Rapport 11827025

Laturnus F, Svensson T, Wiencke C (2010) Release of reactive organic halogens by the brown macroalga *Saccharina latissima* after exposure to ultraviolet radiation. Polar Research 29 (3):379-384. doi:10.3402/polar.v29i3.6075

Li Z, Xu K, Meng M, Xu Y, Ji D, Wang W, Xie C (2024) Environmental heterogeneity caused by large-scale cultivation of *Pyropia haitanensis* shapes multi-group biodiversity distribution in coastal areas. Science of The Total Environment 931:172692. doi:https://doi.org/10.1016/j.scitotenv.2024.172692

Marcó A, Rubio R, Compañó R, Casals I (2002) Comparison of the Kjeldahl method and a combustion method for total nitrogen determination in animal feed. Talanta 57 (5):1019-1026. doi:https://doi.org/10.1016/S0039-9140(02)00136-4

Marine_Scotland (2017) Seaweed Cultivation Policy Statement. Available at: https://wwwgovscot/publications/seaweed-cultivation-policy-statement-2017

McAuliffe C (1971) Gas Chromatographic Determination of Solutes by Multiple Phase Equilibrium. . Chemical Technology 1:46-51

McFiggans G, Coe H, Burgess R, Allan J, Cubison M, Alfarra MR, Saunders R, Saiz-Lopez A, Plane JMC, Wevill D, Carpenter L, Rickard AR, Monks PS (2004) Direct evidence for coastal iodine particles from Laminaria macroalgae – linkage to emissions of molecular iodine. Atmos Chem Phys 4 (3):701-713. doi:10.5194/acp-4-701-2004

Mobley CDe, IOCCG (2022) The Oceanic Optics Book. Dartmouth, NS, Canada, International Ocean Colour Coordinating Group (IOCCG), 924pp DOI: http://dxdoiorg/1025607/OBP-1710

Maar M, Larsen J, Schourup-Kristensen V (2023a) FlexSem Biogeochemical model with mussel farming in Horsens Fjord V8 (Version v8). Zenodo https://doiorg/105281/zenodo7877743

Maar M, Larsen J, Schourup-Kristensen V, Taylor D (2023b) Nutrient extraction and ecosystem impact by suspended mussel mitigation cultures at two contrasting sites. Science of The Total Environment 888:164168. doi:https://doi.org/10.1016/j.scitotenv.2023.164168

Nielsen M, Krause-Jensen D, Olesen B, Thinggaard R, Christensen P, Bruhn A (2014) Growth dynamics of *Saccharina latissima* (Laminariales, Phaeophyceae) in Aarhus Bay, Denmark, and along the species' distribution range. Mar Biol:1-12. doi:10.1007/s00227-014-2482-y

Nielsen MM (2015) Cultivation of large brown algae for energy, fish feed and bioremediation. PhD thesis Aarhus University Department of Bioscience Denmark 170 pp

Nielsen MM, Paulino C, Neiva J, Krause-Jensen D, Bruhn A, Serrão EA (2016) Genetic diversity of *Saccharina latissima* (Phaeophyceae) along a salinity gradient in the North Sea–Baltic Sea transition zone. 52 (4):523-531. doi:doi:10.1111/jpy.12428

Nightingale PD, Malin G, Liss PS (1995) Production of chloroform and other low molecular-weight halocarbons by some species of macroalgae. Limnol Oceanogr 40 (4):680-689. doi:https://doi.org/10.4319/lo.1995.40.4.0680

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022) _vegan: Community Ecology Package_. R package version 2.6-4. https://CRANR-projectorg/package=vegan

Paine ER, Schmid M, Boyd PW, Diaz-Pulido G, Hurd CL (2021) Rate and fate of dissolved organic carbon release by seaweeds: A missing link in the coastal ocean carbon cycle. Journal of Phycology 57 (5):1375-1391. doi:https://doi.org/10.1111/jpy.13198

Parke M (1948) Studies on British Laminariaceae. I. Growth in *Laminaria Saccharina* (L.) Lamour. Journal of the Marine Biological Association of the United Kingdom 27 (3):651-709. doi:10.1017/S0025315400056071

Parrella JP, Jacob DJ, Liang Q, Zhang Y, Mickley LJ, Miller B, Evans MJ, Yang X, Pyle JA, Theys N, Van Roozendael M (2012) Tropospheric bromine chemistry: implications for present and pre-industrial ozone and mercury. Atmos Chem Phys 12 (15):6723-6740. doi:10.5194/acp-12-6723-2012

Pedersen MF, Filbee-Dexter K, Frisk NL, Sárossy Z, Wernberg T (2021) Carbon sequestration potential increased by incomplete anaerobic decomposition of kelp detritus. Marine Ecology Progress Series 660:53-67

Pedersen SG, Christiansen FS, Mostovaya A, Boderskov T, Nielsen MM, Schmedes PS, Holding J, Pedersen MF, Hermansen L, Taylor D, Bruhn A (In prep) Excretion and degradation of dissolved organic carbon from sugar kelp (*Saccharina latissima*) at a coastal cultivation site.

Pessarrodona A, Howard J, Pidgeon E, Wernberg T, Filbee-Dexter K (2024) Carbon removal and climate change mitigation by seaweed farming: A state of knowledge review. Science of The Total Environment 918:170525. doi:https://doi.org/10.1016/j.scitotenv.2024.170525

Petersen JK, Timmermann K, Bruhn A, Rasmussen MB, Boderskov T, Schou HJ, Erichsen A, Thomsen M, Holbach A, Tjørnløv RS, Canal-Vergés P, Flindt MR (2021) Marine Virkemidler - Potentialer og Barrierer. DTU Aqua-rapport nr 385-2021 Institut for Akvatiske Ressourcer, Danmarks Tekniske Universitet 49 pp

Petersen SO, Hoffmann CC, Schäfer CM, Blicher-Mathiesen G, Elsgaard L, Kristensen K, Larsen SE, Torp SB, Greve MH (2012) Annual emissions of CH_4 and N_2O, and ecosystem respiration, from eight organic soils in Western Denmark managed by agriculture. Biogeosciences 9 (1):403-422. doi:10.5194/bg-9-403-2012

Phang S-M, Keng FS-L, Mithoo Singh P-K, Lim Y-K, Abd Rahman N, Leedham EC, Robinson AD, Harris NRP, Pyle JA, Sturges WT (2015) Can seaweed farming in the tropics contribute to climate change throuhg eimssion of short-lived halocarbons? Malaysian Journal of Science 34 (1):8-19. doi:10.22452/mjs.vol34no1.2

Politi T, Zilius M, Castaldelli G, Bartoli M, Daunys D (2019) Estuarine Macrofauna Affects Benthic Biogeochemistry in a Hypertrophic Lagoon. Water 11 (6):1186

Reed DC, Carlson CA, Halewood ER, Nelson JC, Harrer SL, Rassweiler A, Miller RJ (2015) Patterns and controls of reef-scale production of dissolved organic carbon by giant kelp *Macrocystis pyrifera*. Limnol Oceanogr 60 (6):1996-2008. doi:https://doi.org/10.1002/lno.10154

Reed DC, Foster MS (1984) The Effects of Canopy Shadings on Algal Recruitment and Growth in a Giant Kelp Forest. Ecology 65 (3):937-948. doi:https://doi.org/10.2307/1938066

Rhoads DC, Young DK (1970) The influence of deposit-feeding organisms on sediment stability and community trophic structure Journal of Marine Research 78 (3)

Ribeiro PA, Næss T, Dahle G, Asplin L, Meland K, Fredriksen S, Sjøtun K (2022) Going With the Flow – Population Genetics of the Kelp *Saccharina latissima* (Phaeophyceae, Laminariales). Frontiers in Marine Science 9. doi:10.3389/fmars.2022.876420

Roquet F, Madec G, McDougall TJ, Barker PM (2015) Accurate polynomial expressions for the density and specific volume of seawater using the TEOS-10 standard. Ocean Modelling 90:29-43. doi:https://doi.org/10.1016/j.ocemod.2015.04.002

Saiz-Lopez A, Fernandez RP, Ordóñez C, Kinnison DE, Gómez Martín JC, Lamarque JF, Tilmes S (2014) Iodine chemistry in the troposphere and its effect on ozone. Atmos Chem Phys 14 (23):13119-13143. doi:10.5194/acp-14-13119-2014

Saiz-Lopez A, Plane JM, Baker AR, Carpenter LJ, von Glasow R, Martin JC, McFiggans G, Saunders RW (2012) Atmospheric chemistry of iodine. Chem Rev 112 (3):1773-1804. doi:10.1021/cr200029u

Saiz-Lopez A, von Glasow R (2012) Reactive halogen chemistry in the troposphere. Chemical Society Reviews 41 (19):6448-6472. doi:10.1039/C2CS35208G

Schall C, Laturnus F, Heumann KG (1994) Biogenic volatile organoiodine and organobromine compounds released from polar macroalgae. Chemosphere 28 (7):1315-1324. doi:https://doi.org/10.1016/0045-6535(94)90076-0

Schmidt JA, Jacob DJ, Horowitz HM, Hu L, Sherwen T, Evans MJ, Liang Q, Suleiman RM, Oram DE, Le Breton M, Percival CJ, Wang S, Dix B, Volkamer R (2016) Modeling the observed tropospheric BrO background: Importance of multiphase chemistry and implications for ozone, OH, and mercury. Journal of Geophysical Research: Atmospheres 121 (19):11,819-811,835. doi:https://doi.org/10.1002/2015JD024229

Seo JS, Keum YS, Li QX (2009) Bacterial degradation of aromatic compounds. Int J Environ Res Public Health 6 (1):278-309. doi:10.3390/ijerph6010278

Shea R, Chopin T (2007) Effects of germanium dioxide, an inhibitor of diatom growth, on the microscopic laboratory cultivation stage of the kelp, *Laminaria saccharina*. J Appl Phycol 19 (1):27-32. doi:10.1007/s10811-006-9107-x

Shi J, Wei H, Zhao L, Yuan Y, Fang J, Zhang J (2011) A physical–biological coupled aquaculture model for a suspended aquaculture area of China. Aquaculture 318 (3):412-424. doi:https://doi.org/10.1016/j.aquaculture.2011.05.048

Simpson JH, Bowers D (1981) Models of stratification and frontal movement in shelf seas. Deep Sea Research Part A Oceanographic Research Papers 28 (7):727-738. doi:https://doi.org/10.1016/0198-0149(81)90132-1

Stevens CL, Petersen JK (2011) Turbulent, stratified flow through a suspended shellfish canopy: implications for mussel farm design. Aquaculture Environment Interactions 2 (1):87-104

Sæther M, Diehl N, Monteiro C, Li H, Niedzwiedz S, Burgunter-Delamare B, Scheschonk L, Bischof K, Forbord S (2024) The sugar kelp *Saccharina latissima* II: Recent advances in farming and applications. J Appl Phycol. doi:10.1007/s10811-024-03213-1

Taylor D, Jakobsen H, Lyngsgaard MM, Darecki M, Werther M, Maar M, Saurel C (2024) Quantifying bivalve phytoplankton depletion in a eutrophic system: an integrated approach. Limnol Oceanogr 69 (10):2436-2452. doi:https://doi.org/10.1002/lno.12680

Timmermann K, Mostovaya A, Barnewitz A, Erichsen A, Petersen AH, Holbach A, Bruhn A, Nielsen B, Taylor D, Hansen FT, Hansen FH, Christiansen FS, Anker HT, Christensen J, Holding J, Baaner L, Hermannsen L, Callisen LW, Nielsen MM, Gommesen M, Lees MK, Flindt MR, Holst N, Canal-Vergés P, Schmedes PS, Kjær RA, Steinfurth RC, Stæhr SU, Pedersen SG, Vinbæk SJ, Gyldenkærne S, Andersen SS, Boderskov T, Jørgensen TS, Banke TL, Andersen TA, Larsen TC (2024) Ålegræstransplantering og tangdyrkning som marine virkemidler: Potentialer, effekter og juridiske aspekter. DTU Aqua-rapport nr. XXX-2024

Umanzor S, Li Y, Yarish C (2020) Effect of direct "seeding" binders and embryonic sporophyte sizes on the development of the sugar kelp, *Saccharina latissima*. J Appl Phycol 32 (6):4137-4143. doi:10.1007/s10811-020-02277-z

Valdemarsen T, Hansen PK, Ervik A, Bannister RJ (2015) Impact of deep-water fish farms on benthic macrofauna communities under different hydrodynamic conditions. Marine Pollution Bulletin 101 (2):776-783. doi:https://doi.org/10.1016/j.marpolbul.2015.09.036

van Duinen R, Rivière C, Strosser P, Dijkstra JW, Rios S, Luzzi S, Bruhn A, Nielsen MO, Göke C, Samarasinghe MB, Chassé E, Nielsen CH, Thomsen M (2023) Algae and Climate. Publication Office of the European Union, 2023, doi: 102926/208135

Vinbæk SJ (2023) Sedimentation and lability of *Saccharina latissima* derived POM in a large-scale seaweed and mussel farm. Masters Thesis, Aarhus University:1-63

Wada S, Aoki MN, Mikami A, Komatsu T, Tsuchiya Y, Sato T, Shinagawa H, Hama T (2008) Bioavailability of macroalgal dissolved organic matter in seawater. Marine Ecology Progress Series 370:33-44

Watanabe K, Yoshida G, Hori M, Umezawa Y, Moki H, Kuwae T (2020) Macroalgal metabolism and lateral carbon flows can create significant carbon sinks. Biogeosciences 17 (9):2425-2440. doi:10.5194/bg-17-2425-2020

Weigel BL, Pfister CA (2021) The dynamics and stoichiometry of dissolved organic carbon release by kelp. Ecology 102 (2):e03221. doi:https://doi.org/10.1002/ecy.3221

Weiss RF (1974) Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Marine Chemistry 2 (3):203-215

Weiss RF, Price BA (1980) Nitrous oxide solubility in water and seawater. Marine Chemistry 8 (4):347-359

Wiesenburg DA, Guinasso NL, Jr. (1979) Equilibrium solubilities of methane, carbon monoxide, and hydrogen in water and sea water. Journal of Chemical & Engineering Data 24 (4):356-360. doi:10.1021/je60083a006

Wiles PJ, Rippeth TP, Simpson JH, Hendricks PJ (2006) A novel technique for measuring the rate of turbulent dissipation in the marine environment. Geophysical Research Letters 33 (21). doi:https://doi.org/10.1029/2006GL027050

World_Bank (2023) Global Seaweed: New and Emerging Markets Report, 2023. © Washington, DC: World Bank http://hdlhandlenet/10986/40187 License: CC BY-NC 30 IGO"

Xiao X, Agustí S, Yu Y, Huang Y, Chen W, Hu J, Li C, Li K, Wei F, Lu Y, Xu C, Chen Z, Liu S, Zeng J, Wu J, Duarte CM (2021) Seaweed farms provide refugia from ocean acidification. Science of The Total Environment:145192. doi:https://doi.org/10.1016/j.scitotenv.2021.145192

Young CS, Sylvers LH, Tomasetti SJ, Lundstrom A, Schenone C, Doall MH, Gobler CJ (2022) Kelp (*Saccharina latissima*) Mitigates Coastal Ocean Acidification and Increases the Growth of North Atlantic Bivalves in Lab Experiments and on an Oyster Farm. Frontiers in Marine Science 9. doi:10.3389/fmars.2022.881254

Zhang J, Fang J, Wang W, Du M, Gao Y, Zhang M (2012) Growth and loss of mariculture kelp *Saccharina japonica* in Sungo Bay, China. J Appl Phycol 24 (5):1209-1216. doi:10.1007/s10811-011-9762-4

Zhang X, Boderskov T, Bruhn A, Thomsen M (2022) Blue growth and bioextraction potentials of Danish *Saccharina latissima* aquaculture — A model of eco-industrial production systems mitigating marine eutrophication and climate change. Algal Research 64:102686. doi:https://doi.org/10.1016/j.algal.2022.102686

Zhang Y, Chang Z, Zheng Z, Yang J (2017) Harvesting machine for kelp culture in floating raft. Aquacultural Engineering 78:173-179. doi:https://doi.org/10.1016/j.aquaeng.2017.07.005

8 Appendix

Appendix 1. Supporting information on DOM composition analysis

For analysis of DOM composition, a 500 mL water sample was filtered through a pre-combusted GF/F filter, acidified to pH 2 with 37% HCl, and solid-phase extracted using a PPL cartridge (Agilent Bond Elut, 1 g, 6 mL). After desalting the cartridge with 0.01M HCl, the DOM was eluted with methanol (Dittmar et al., 2008). The eluted sample was filtered through 0.22 µm PTFE hydrophobic syringe filter (Qmax), dried, and reconstituted in 200 µL of 10% acetonitrile with 1 µg mL−1 vanillin-(phenyl-13C6) as an internal standard. Reconstituted samples were stored at 4°C until analysis.

Samples (2 µL injections) were analysed on a Thermo UltiMate 3000 UHPLC coupled to Bruker compact qTOF-MS, with electrospray ionization (ESI) in positive mode (negative mode yielded a small number of features and therefore not discussed). The chromatographic separation was performed on an Acquity UPLC HSS T3 column (1.8 µm, 2.1×100 mm, Waters) with a gradient elution of mobile phase (0.1% acetic acid in water and 0.1% acetic acid in acetonitrile) at a flow rate of 0.2 mL min−1 and temperature of 45°C. The ESI settings included 4.5 kV capillary voltage, 1.5 bar nebulier pressure, 6 L min−1 drying gas flow, and 200°C capillary temperature. The MS was calibrated using 5mM sodium acetate, and the MS scans were performed over 20–1000 m/z. Data acquisition was carried out using Bruker Compass HyStar software (v. 6.2).

The acquired UHPLC-qTOF-MS raw data were centroided and converted to .mzXML format (Chambers et al., 2012) for processing in MZmine3, a software for feature detection in untargeted MS data (Schmid et al., 2023, Heuckeroth et al., 2024). Only features with signal-to-noise ratio >3 were considered. After alignment and filtering, the features were exported to SIRIUS 5 for tentative formula assignment and compound classification using the CANOPUS integrated tool (Dührkop et al., 2019, Dührkop et al., 2021). The Mzmine and SIRIUS output data merging and all subsequent analyses were performed in R v. 4.4.1 (R Core Team, 2024). The double bond equivalent was calculated as $DBE=1+C-\frac{1}{2}(H+Cl+Br+I)+\frac{1}{2}(N+P)$ and formulas with DBE<0, non-integer DBE, as well as H/C>2.2, O/C>1.2, and N/C>0.5 were disregarded (Koch & Dittmar, 2006). High-intensity outliers were removed from the dataset. Compounds found in the SPE method blanks, acetonitrile blanks, and in the control treatment were filtered out, leaving the compounds unique to the macroalgae treatment. For assessment based on formulas alone, aromaticity index was calculated as $AI=(1+C-O-S-\frac{1}{2}(N+P+H))/(C-O-N-S-P)$ (Koch & Dittmar, 2016), and compound groups were delineated as follows: aliphatic (H/C≥1.5), highly unsaturated and phenolic (H/C<1.5 and AI<0.5), and unambiguously aromatic (AI≥0.5). The .mzXML data files, MZmine batch file (.xml), Mzmine output file (.mfg), and SIRIUS output files are available upon request.

Table S1. Mzmine3 data processing workflow

Step	Settings
Mass detection	MS1, level=1 | Mass Detector: Centroid; Noise level: 1.0E3
Mass detection	MS2, level=2 | Mass Detector: Centroid; Noise level: 0
Mass calibration	Standard calibrant library (SCL): Standard calibrant library file: <calibration file>; m/z tolerance: 0.01 m/z or 5 ppm; Retention time tolerance: 0.1 min | Percentile range of errors: 10–90 | OLS regression: Polynomial degree: 2
ADAP Chromatogram Builder	Scan filters: Retention time: 0.4–33.0; MS1, level=1 | Min consecutive scans: 4 | Min intensity of consecutive scans: 3.0E3 | Min absolute height: 1.0E4 | m/z tolerance: 0.005 m/z or 15 ppm
Smoothing	Smoothing algorithm: Savitzky Golay; Retention time smoothing: 5
Local minimum feature resolver	Dimension: Retention time | Chromatographic threshold: 90% | **Minimum search range RT/Mobility (absolute): 0.05** | **Minimum relative height: 0** | **Minimum absolute height:** 1.0E4 | **Min ratio of peak top/edge: 1.70** | **Peak duration range (min/mobility): 0–1** | **Min scans (data points): 4**
13C-isotope filter	m/z **tolerance: 0.001 m/z or 3 ppm** | **Retention time tolerance: 0.04 min** | **Monotonic shape:** ✓ | **Maximum charge: 2** | **Representative isotope: Most intense**
Isotopic peaks finder	Chemical elements: H, C, N, O, S / m/z tolerance: 0.0005 m/z or 10 ppm | Maximum charge of isotope m/z: 1
Join aligner	**m/z tolerance: 0.001 m/z or 5 ppm** | **Weight for m/z: 3** | **Retention time tolerance: 0.1min** | **Weight for RT:1**
Peak finder	**Intensity tolerance**: 20% | **m/z tolerance**: 0.001 m/z or 5 ppm| **Retention time tolerance**: 0.1 min | **Minimum scans (data points)**: 2
Duplicate peak filter	**Filter mode**: new average | **m/z tolerance**: 0.001 m/z or 5 ppm | **RT tolerance:** 0.1 min
Feature filter	**Duration**: 0–1 | **FWHM**: 0–0.2 | **Keep only features with MS/MS scans** ✓
Feature list rows filter	**Minimum aligned features (samples)**: 2 | **m/z**: 80–750 | **Retention time:** 0.5–33 | Chromatographic FWHM: 0–2 **Keep or remove rows: keep** | Feature with MS2 scan ✓ | **Reset the feature number ID** ✓
Export for SIRIUS	Merge MS/MS ✓ | m/z tolerance: 0.003 m/z or 5 ppm

References:

Chambers MC, Maclean B, Burke R, et al. (2012) A cross-platform toolkit for mass spectrometry and proteomics. Nature Biotechnology 30: 918-920.

Dittmar T, Koch B, Hertkorn N & Kattner G (2008) A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnology and Oceanography: Methods 6: 230-235.

Dührkop K, Fleischauer M, Ludwig M, Aksenov AA, Melnik AV, Meusel M, Dorrestein PC, Rousu J & Böcker S (2019) SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. Nature Methods 16: 299-302.

Dührkop K, Nothias L-F, Fleischauer M, et al. (2021) Systematic classification of unknown metabolites using high-resolution fragmentation mass spectra. Nature Biotechnology 39: 462-471.

Heuckeroth S, Damiani T, Smirnov A, et al. (2024) Reproducible mass spectrometry data processing and compound annotation in MZmine 3. Nature Protocols.

Koch BP & Dittmar T (2006) From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. Rapid Communications in Mass Spectrometry 20: 926-932.

Koch BP & Dittmar T (2016) From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. Rapid Communications in Mass Spectrometry 30: 250-250.

Schmid R, Heuckeroth S, Korf A, et al. (2023) Integrative analysis of multimodal mass spectrometry data in MZmine 3. Nat Biotechnol 41: 447-449.

R Core Team (2024). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Appendix 2. Capital and operating costs (CAPEX and OPEX) of a large-scale hatchery and cultivation system for production of sugar kelp in Denmark

See next page.

Appendix 2. Capital and operating costs (CAPEX and OPEX) of a large-scale hatchery and cultivation system for production of sugar kelp in Denmark, based on experiences from the Sallingsund site.

Task	Materials/hours	Description	Number of units	Category	Cost of unit (DKK)	Lifetime (years)	Total price based on SSE (DKK)	Total price for full container (DKK)	Assumptions
Hatchery - CAPEX	Rent of container	Price of rent per month	5	Materials	6393	1	31965	31965	
	Delivery of container	Delivery, test, pick up	1	Materials	13648	1	13648	13648	
	IBC containers	IBC container for maturing of mother kelp	2	Materials	1585	10	317	317	2 ICB containers are sufficient for maturing kelp for 22 km of line
	Light tubes	TL-D 90 De LUXE 58W/950	20	Materials	153	10	306	612	
	Lighting fittings	Double doplet proof fittings	10	Materials	149	10	149	298	
	LED units	Cosmorrow LED	12	Materials	429	10	515	1030	
	Seaweed line	Nylon 4 mm	10800	Materials	1	1	11297	22594	
	Plastic pipes for seaweed line	63 mm PVC pipes	216	Materials	50	10	1080	2160	
	Water pump	Eheim pumps	10	Materials	542	10	542	1083	
	Tanks	Long, flat white plastic tanks	9	Materials	6130	10	5517	11033	
	Shelf system	Steel shelf system	3	Materials	21923	10	6577	13154	
	Plastic boards	Plastic	1	Materials	20000	10	2000	4000	
	Cutting of plastic pipes	Working hours	8,64	Man hours	250		2160	4320	
	Cutting of slot in plastic pipes	Working hours	1,08	Man hours	250		270	540	
	Cleaning of plastic pipes	Working hours	1,44	Man hours	250		360	720	
	Mounting of shelf systems etc.	Working hours	37,5	Man hours	250		9375	18750	Estimated number of hours, 2 persons for 2.5 day
	Total price - CAPEX						86076	**126223**	
Hatchery - CAPEX	Total price (CAPEX) per m of seeded line						7,97	5,84	
Hatchery - OPEX									
	Collection of mother seaweed	Working hours	8	Man hours	250		2000	2000	Estimated number of hours
	Nursing/maturing of mother seaweed	Working hours	8	Man hours	250		2000	2000	Estimated number of hours
	Preparation of spools	Working hours	5,4	Man hours	250		1350	2700	
	Rinsing/drying of spools	Working hours	6,67	Man hours	250		1667	3333,333333	
	1 person for 2 days	Working hours	15	Man hours	250		3750	3750	Estimated number of hours
	1 person, 1 day a week, 6 weeks	Working hours	45	Man hours	250		11250	11250	
	Inorganic nutrient stock	F/2 nutrient stock solution	1	Materials	100	1	100	200	
	Salt water	Filtered seawater	1	Materials		1			No cost of salt water
	Electricity for cooling	Electricity use, cooling container	2433	Materials	3,76	1	9148	9148,08	
	Electricity for light and pumps	Electricity use, light, punps etc	2607	Materials	3,76	1	9802	19604,64	
	Total price - OPEX						41067	**53986**	
Hatchery - OPEX	Total price (OPEX) per m of seeded line						3,80	2,50	
Total - OPEX+CAPEX							**11,77**	**8,34**	

Task	Materials/hours	Description	Number of units	Category	Cost of unit (DKK)	Lifetime (years)	Total price based on SSE (DKK)	Total price for full container (DKK)	Assumptions
Establishing cultivation site - CAPEX									
	Establishing of drill anchors	Price per anchor (Frank Tousgaard, 2022)	2	Materials/man hours	4000	10	800		Frank Tousgaard, 2022
	Work boat (hourly cost)	1 hour for streching of line, 1 hour for preparing line	2	Work boat	1600	10	320		Frank Tousgaard, 2022
	Salary hourly cost (2 persons)	2 persons	4	Man hours	250	10	100		Frank Tousgaard, 2022
	Main lines	Mainline - 150 meter of 16 mm DANLINE	1	Materials	1200	10	120		
	Buoys	15 x 18 L pr line	15	Materials	45	5	135		
	Blocks	30 x 10 kg concrete pr line	30	Materials	35	5	210		
	Area marker buoys as per legal request	4 yellow 'isolated danger' marker buoys	1	Total	42380	10	34		For a 18 ha site in Limfjorden
	Total price per mainline						1719		
Cultivation site - CAPEX	Total price per m of mainline						11,46		
Running the cultivation site - OPEX									
Deployment of sugar kelp	Work boat (hourly cost)	Use registered in this project	1,07	Work boat	1600		1714		
	Salary hourly cost (2 persons)	Use registered in this project	2,14	Man hours	250		536		Assming that 2 persons can harvest
Maintenance	Work boat (hourly cost)	Estimated	0,50	Work boat	1600		800		Estimated - half of time used for deployment
	Salary hourly cost (2 persons)	Estimated	1,00	Man hours	250		250		Estimated - half of time used for deployment
Harvest of sugar kelp	Work boat (hourly cost)	Use registered in this project	1,61	Work boat	1600		2583		
	Salary hourly cost (2 persons)	Use registered in this project	3,23	Man hours	250		807		Assming that 2 persons can harvest
	Total price per mainline						6690		
Cultivation site - OPEX	Total price per m of mainline						44,6		

TOTAL. Hatchery and grow-out		
Total cost per m of mainline (DKK m-1)	67,83	**64,40**
Total cost per cultivation site (DKK 18 ha-1)	1220963	**1159232**
Total cost per ha (DKK ha-1)		**64402**
Cost of sugar kelp (DKK kg FW-1))	13,30	**12,63**

CULTIVATION OF SUGAR KELP AS A MARINE MEASURE FOR MITIGATING EUTROPHICATION

Production in large-scale, nutrient removal efficiency, environmental impacts, and economy

The cultivation of sugar kelp has been suggested as a marine mitigation measure for the uptake and removal of nutrients from the marine environment. In 2022-2023, sugar kelp was cultivated in the Limfjorden in a 12-hectare experimental facility to document 1) biomass yields, 2) uptake of nitrogen, phosphorus and carbon and 3) effects on the environment by large-scale cultivation. The results show that large-scale cultivation of sugar kelp can remove up to 23 g N m^{-1} line $year^{-1}$, corresponding to an annual nutrient removal potential of 23 kg N ha^{-1} and 0.8 kg P ha^{-1} in a standard cultivation system with 1000 m cultivation line ha^{-1}. Yields and potentials for nitrogen removal in Danish waters vary and are highest in areas with high salinity and nutrient availability. The cost of nutrient removal varies accordingly, with the lowest cost of DKK 2805 kg N^{-1}. Modelling scenarios indicate that a significant upscaling of sugar kelp cultivation is required to achieve any effect on the key environmental indicators for GES: "summer chlorophyll-a" and "light attenuation". At high biomass densities, the environmental effects include 1) a negligible reduction of light to the seabed, 2) altered water flow, 3) reduced sedimentation rates, 4) limited periodic increases in pH and oxygen concentration under, in and near the seaweed cultivation site. Harvesting in April reduces the loss of carbon from the seaweed to the marine environment, and it is unlikely that seaweed cultivation can contribute to carbon sequestration in the inner Danish waters.

ISBN: 978-87-7156-970-4
ISSN: 2244-9981