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Report

MACROSEA: A Knowledge Platform for Industrial Macroalgae Cultivation in Norway

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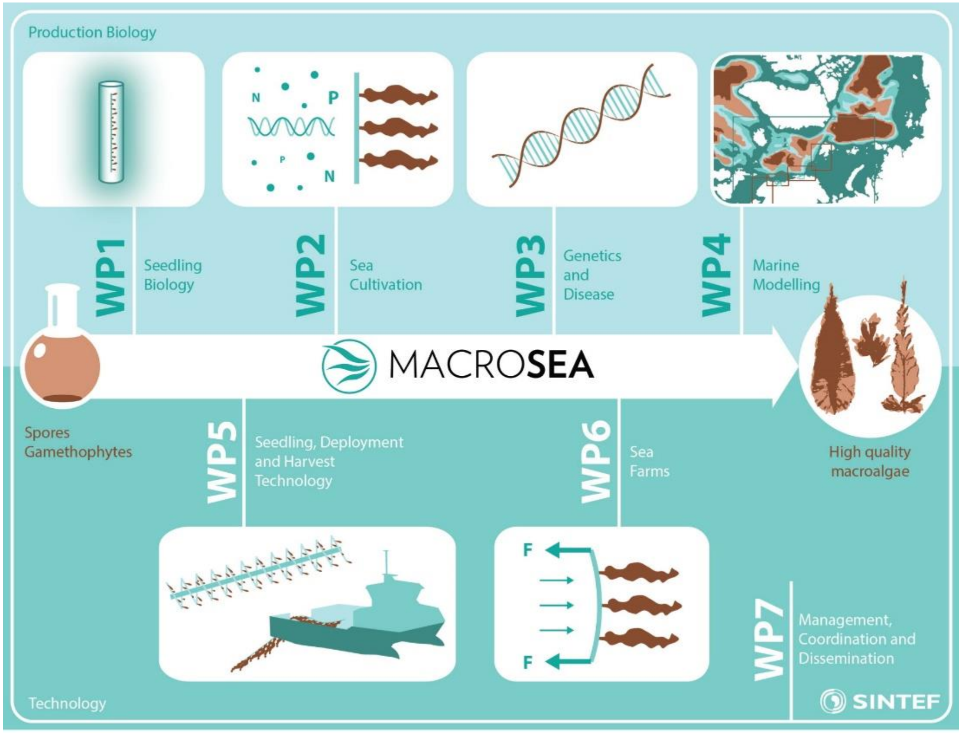

SINTEF Ocean AS
Aquatic Bioresources
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Report

MACROSEA: A Knowledge Platform for Industrial Macroalgae Cultivation in Norway

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ABSTRACT
The MACROSEA project has contributed to new knowledge on production biology and technology for selected brown and red macroalgae species in Norway. Seedling quality and growth, fouling and biochemical composition during sea cultivation have been studied. Population genetics along the coast and in two fjords have been characterized. Growth models have been developed and coupled with 3D hydrodynamics-ecosystem models to estimate site-dependent biomass production, and design studies of methods and prototyping of new technology for efficient seeding, deployment and harvest have been performed. Drag forces and deformation of farm systems at different sea states have been determined in tank experiments, and numerical tools for simulation and visualization of farm designs in dynamic marine systems have been developed.

The results will contribute to predictable production of biomass and development of enabling technologies for industrial macroalgae cultivation in Norway.

The project (2016-2019) was funded by the Research council of Norway.

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MACROSEA

MACROSEA - A KNOWLEDGE PLATFORM FOR INDUSTRIAL MACROALGAE CULTIVATION

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1 Key Figures

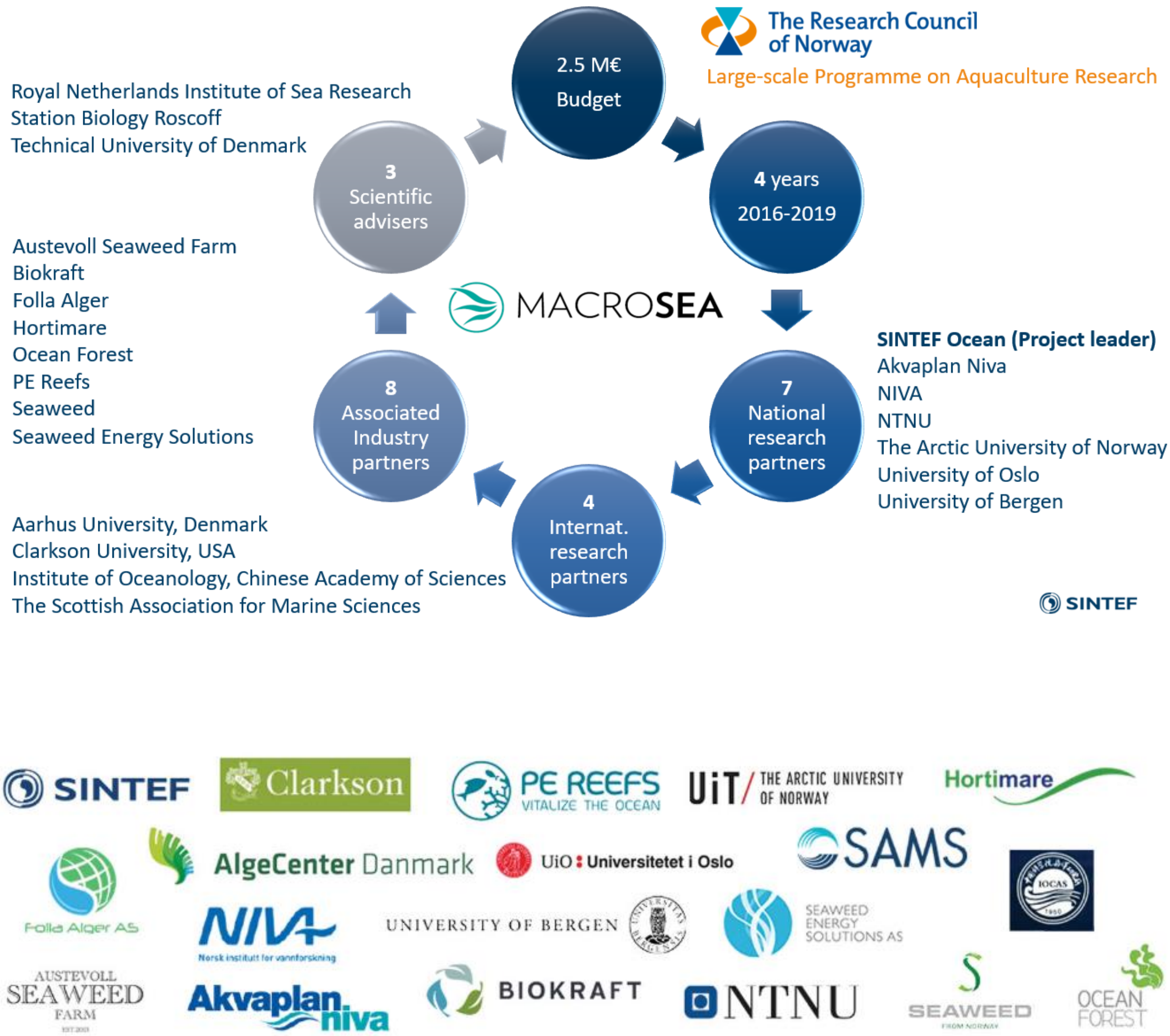


Figure 1. Key numbers, national and international research partners, associated industry partners and scientific advisory board in MACROSEA.

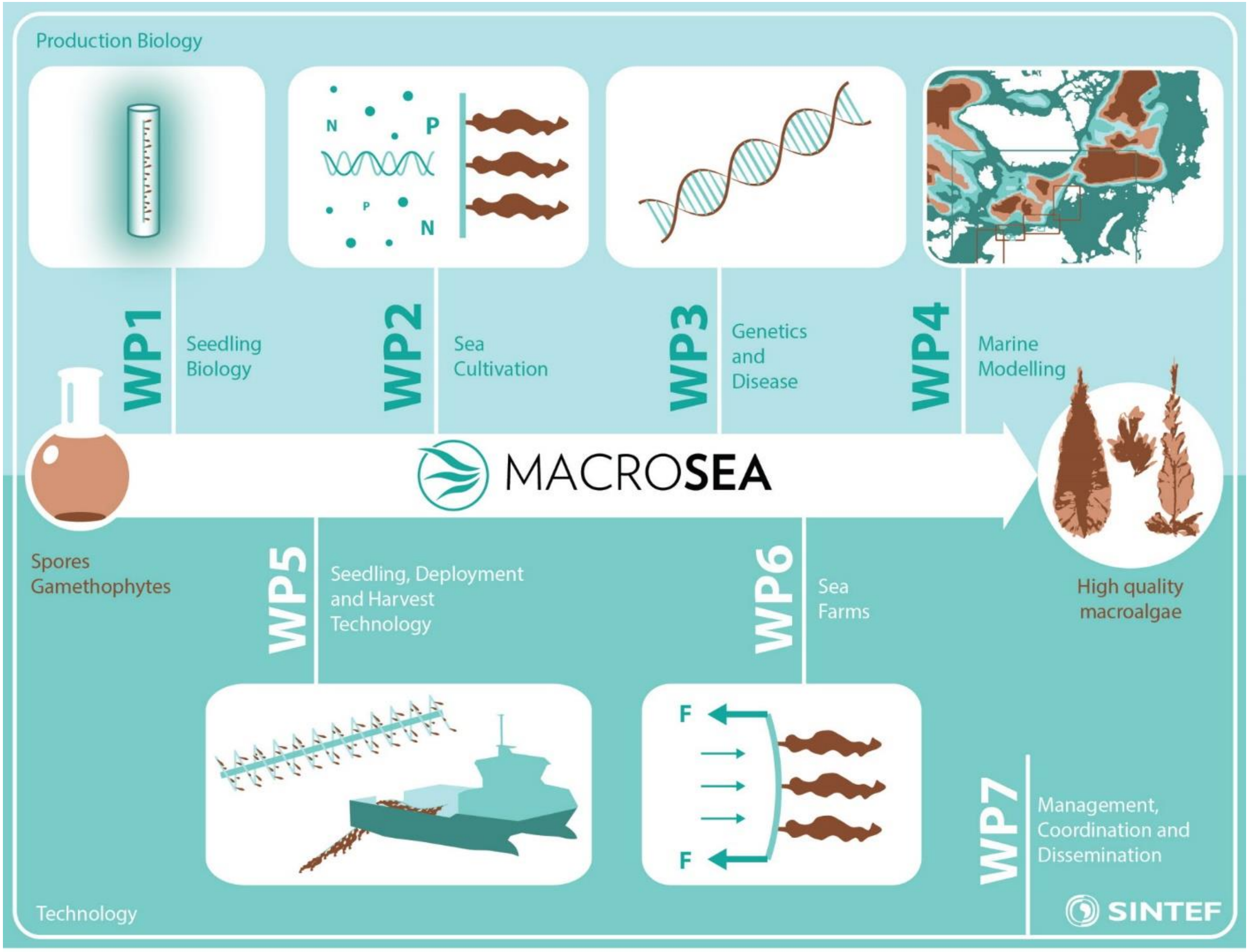


Figure 2. MACROSEA envisages an interdisciplinary knowledge platform on fundamental macroalgae production biology and technology, to make significant steps towards industrial cultivation in Norway.

2 Popular Science Summary (English)

Cultivation of the oceans is required to meet demands for food, feed, materials and energy for a growing global population. Norway, with one of the world's longest tempered and productive coastlines, can take a leading role. With MACROSEA (2016-2019), Norway has created an interdisciplinary knowledge platform on macroalgae production biology and technology, to make significant steps towards industrial cultivation.

MACROSEA has contributed to systematic research and delivered generic knowledge on seedling quality, sea cultivation, and genetics of selected brown and red macroalgae species. Models of brown and red species have been developed and coupled to 3D hydrodynamic-ecosystem models to estimate site-dependent biomass production. Methods for efficient seedling, deployment and harvest have been assessed and drag forces and deformation of different farm systems determined at different sea states by using numerical tools.

Three prototypes of seaweed cultivation equipment, a model for kelp cultivation potential and a numeric tool for structural design of kelp farms have been developed. The project has reached out through 80 talks, 10 popular- and 10 scientific articles, 10 reports, 1 book chapter, 55 media articles and 4 movies, and contributed to the education of 6 PhD and 10 MSc-students. The final "MACROSEA Open Day", which was arranged as a joint conference with SIG Seaweed and the new RCN-funded Knowledge platform *Norwegian Seaweed Biorefinery Platform* (2019-2023) in Trondheim in November 2019, gathered about 150 participants from national and international industry and research and national funding bodies and authorities.

Seedling biology: For cultivation of macroalgae in sea farms a sufficient number of seedlings of good quality on rope or other substrates is required. In MACROSEA the seedlings production protocols for sugar kelp *Saccharina latissima*, winged kelp *Alaria esculenta* and dulse *Palmaria palmata* have been improved to enable upscaled cultivation. For sugar kelp the industrialised cultivation protocol developed by SINTEF Ocean has been optimized suggesting 6 weeks cultivation on thin lines in large vertical flow-through incubators before transfer to thicker carrier ropes using a specialized "spinning-machine" before deployment at sea. Winged kelp is usually cultivated from gametophyte cultures due to a very short period with natural available spore containing sporophytes. MACROSEA have improved cultivation techniques including a method for fertility induction and for measurement of density and quality of gametophytes. Dulse is not cultivated commercially in Norway due to a complex life cycle and a very low yield of both seedlings for deployment and of harvestable biomass. MACROSEA has brought forward new knowledge on timing of natural fertility, induction of spore release, methods to increase the number of seedlings from the mother plants, methods for seeding of ropes and net, and testing of conditions for incubation of spores and seedlings. The work resulted in a successful sea cultivation trial with seedlings on nets in a sea farm in Mid-Norway.

Sea Cultivation: The variation in growth performance, biochemical composition and biofouling of cultivated *S. latissima* is mainly caused by seasonality and depth, varying systematically along a latitudinal gradient. The results from a monitoring program showed that maximum frond length and biomass yield occurred up to 2 months earlier at southern locations than at locations further north in Norway, suggesting a potential to supply the consumer market or processing industry for an extended period. Protein content showed a decreasing seasonal trend before onset of biofouling and the seasonal decrease was delayed at higher latitude. The same delay with latitude was observed for biofouling organisms, suggesting that a cultivation and harvesting strategy should follow these latitudinal patterns. Production, expressed in terms of frond length and biomass yield, was higher at shallow cultivation depths than deeper, whereas protein, ash and internal nitrogen compounds were generally higher at deeper depths. Salinity appeared to have an important impact by diminishing seaweed biomass yield and frond length, ash content and biofouling cover. Less accumulated light at deeper cultivation depths enhanced the protein content and altered the biofouling species composition.

Genetics: Sugar kelp is one of the most relevant species for cultivation in Norway, and a main object of MACROSEA was to examine genetic diversity and adaptation of sugar kelp along the coast. Growth and gene expression of sugar kelp cultures from Tromsø, Trondheim and Bergen cultivated together under laboratory conditions simulating the growth environment in North-, Mid- and South-Norway in May (sea temperature and day length) ("common garden experiment") showed a clear tendency of better growth of sugar kelp originating from the Tromsø culture. In order to examine the genetic structure and gene flow in populations of sugar kelp, samples from 21 stations, where 12 were placed in the coastal area from outer Oslofjord to Porsanger and nine in Hardangerfjord and Sognefjord, were analyzed. The results showed good gene flow and little genetic structure, except for a reduced gen flow between South- and North-Norway and between coastal stations and the innermost fjord stations. The results also suggested some degree of genetic adaptation of sugar kelp populations in the Skagerrak area and in the two fjords. Another study of sugar kelp was carried out on material collected from Scotland and the west coast of Sweden. The results of these studies show that gene flow between populations is strongly dependent on local currents, at the same time as clear genome-wide signatures of adaptation was evident.

Marine modelling: Maps of the potential for sugar kelp production along and outside the coat of Norway have been developed based on simulation results from the physical-biological ocean model system SINMOD coupled with a growth model for sugar kelp. The results will be made available for use in GIS-systems and googlemaps. Improvements to the biomass calculations have also been made based on the results from the field cultivation trials in MACROSEA. The model results supported maximum frond length and biomass yield up to 2 months earlier at southern locations than at locations further north in Norway. Estimates for biomass production (yields per unit ocean area) have also been made suggesting an average potential at 75 tons per hectare at harvest in June (range 20-220 tons per hectare depending on deployment time). The growth model for sugar kelp has been further developed to account for interactions with the light field (self shading). These developments entail an improved and more flexible system for calculations of kelp biomass production.

Seedling, deployment and harvest technology: A lab system has been designed for automatic seedling production, allowing the seedlings to be grown in monitored, optimal conditions. An industrial production line for seedlings has been assessed and outlined based on state of the art for such systems. Methods for image processing of substrate for quantification of sporophytes and spore density have been developed enabling processing of many images with little effort, compared to manual counting. A prototype for preparation of substrate-spools has been made, spinning 300m of substrate line on two plastic cylinders in 90 sec. A machine referred to as a "seaweed spinner" has been developed to automate the wrapping of substrate line around a carrier rope before deployment at sea. Spools of seeded twine are loaded into the machine and rotated around the rope at a processing speed of 110m carrying rope in 14min. A design study targeting automation and standardization of kelp cultivation (SPOKe) has been performed. The concept encompasses circular modules to allow for an easy platform for automation. A gantry robot on rails is used to interact with the modules, equipped with tools depending on its task. A VR demo and a prototype, demonstrating the principle for control of the gantry robot are available for display at SINTEF Ocean.

Sea Farms: Design and dimensioning of seaweed farms at sea are dependent on knowledge of forces exerted on the farm due to the e.g. sugar kelp being exposed to current and waves, and numerical methods that can simulate the sea farms when subjected to design conditions. Results from measurements of drag forces and tensile forces on cultivated sugar kelp from towing experiments in a closed test facility containing sea water showed a clear relation between the size of the sugar kelp and the drag forces. Numerical methods have been developed and are available as part of SINTEF Ocean's software FhSim for simulation of seaweed farms.

3 Popular Science Summary (Norwegian)

Dyrking av våre produktive hav er nødvendig for å dekke behovene for mat, fôr, materialer og energi til en stadig økende global befolkning. Norge, som har en av verdens lengste kystlinjer, kan ta en ledende rolle i en slik utvikling. Gjennom MACROSEA (2016-2019) har Norge etablert en tverrfaglig kunnskapsplattform for produksjonsbiologi- og teknologi, som kan bidra til videre utvikling av industriell makroalgedyrking.

MACROSEA har bidratt til systematisk forskning og levert ny generisk kunnskap om kimplantekvalitet, dyrking i sjø av røde og brune arter og genetikk hos en brun. Det er utviklet vekstmodeller av brune og røde arter som er koblet til 3D hydrodynamiske-økosystemmodeller for å estimere lokalitetsavhengig produksjon. Metoder for effektiv påsåing, utsett og høsting er utredet, og dragkrefter har blitt målt som input til numeriske modellverktøy som kan visualisere deformasjon av sjøanlegg under ulike miljøforhold.

Det er utviklet 3 prototyper av utstyr for taredyrking, en modell for produksjonspotensial og et beregningsverktøy for dimensjonering av sjøanlegg. Det er formidlet 80 foredrag, 10 populærvitenskapelige- og 10 vitenskapelige artikler, 10 rapporter, 1 bokkapittel, 55 mediesaker og 4 filmer. Prosjektet har bidratt til utdanning av 6 PhD-er og 10 MSc-studenter. Avslutningskonferansen, som ble arranger sammen med SIG Seaweed og den nye NFR-finansierte kunnskapsplattformen *Norwegian Seaweed Biorefinery Platform* (2019-2023) samlet rundt 150 deltagere fra nasjonal og internasjonal industri, forskning og myndigheter.

Kimplantekvalitet: Ved dyrking av makroalger i sjøanlegg er det behov for kimplanter av god kvalitet. MACROSEA har bidratt til forbedrede dyrkingsprotokoller for sukkertare *Saccharina latissima*, butare *Alaria esculenta* og søl *Palmaria palmata* til å bli mer robuste og tilrettelagt for bruk i en industriell skala. Resultatene viser at sukkertare vokser godt etter 6 ukers dyrking på tynn line i vertikale gjennomstrømmings-inkubatorer etterfulgt av spinning på tykt bæretau med bruk av en såkalt tarespinnemaskin før utsett i sjø.
Butare dyrkes vanligvis opp fra gametofyttkulturer på grunn av sesong-avgrenset tilgang på planter med sporer ute i naturen. MACROSEA har bidratt til ny kunnskap om fertilitetsinduksjon og metodikk for måling av tetthet og kvalitet av gametofyttkulturer. Søl dyrkes ikke kommersielt i Norge, blant annet på grunn av komplisert livssyklus og lavt utbytte både av antall kimplanter og høstbar biomasse. MACROSEA har bidratt til ny kunnskap om kjønnsmodning i naturen, induksjon av sporeslipp, metoder for å øke antall kimplanter fra morplantene, metoder for på-såing av tau og nett, og testing av betingelser for inkubering av sporer og kimplanter. Forskningen resulterte i et vellykket dyrkingsforsøk av søl på nett i sjøanlegg i Midt-Norge.

Dyrking i sjø: Variasjon i vekst, biokjemisk sammensetning og begroing av dyrket S. latissima varierer hovedsakelig med sesong og dyp og systematisk med breddegrad. Resultatene fra et måleprogram viste at sukkertare som ble dyrket i sjø fra februar oppnådde maksimal lengde og biomasse 2 måneder tidligere på lokaliteter i Sør-Norge enn på lokaliteter i Nord-Norge. Proteininnholdet ble redusert utover våren i perioden før plantene ble begrodde, og på same måte som vekst, biomasse og begroing ble også nedgangen i proteininnhold funnet å være forsinket med økende breddegrad. Denne sesongmessige forskyvningen av vekst og begroing og proteininnhold fra sør mot nord kan bidra til å forlenge perioden det kan dyrkes og høstes sukkertare langs norskekysten. Resultatene kan bidra til nye produksjonsstrategier. Produksjon målt som lengde og biomasse, var høyere på grunnere- (2 m) enn på større (8 m) dyp, mens protein, aske og internt nitrat generelt var høyere på større dyp. På lokaliteter med høy ferskvannstilførsel førte lavere salinitet til dårligere vekst, og mindre askeinnhold, begroing og akkumulert lys på dypere dyp, som igjen ga økt proteininnhold og en annen sammensetning av begroingsorganismer enn på lokaliteter uten ferskvannstilførsel.

Genetikk: Sukkertare er en av de mest aktuelle artene for dyrking i Norge. I MACROSEA har vi undersøkt genetisk mangfold og tilpassning av sukkertare langs kysten. Vekst og genuttrykk av sukkertarekulturer fra Tromsø, Trondheim og Bergen ble undersøkt i laboratoriet, der de tre kulturene ble dyrket sammen i laboratorie-miljø som simulerte vekstforholdene i Nord-, Midt- og Sør-Norge i mai (sjøtemperatur og dag lengde). Resultatene viste en tydelig tendens til at veksten av Tromsø-kulturen var best, spesielt under vekstforhold som simulerte miljøet i Nord-Norge. For å undersøke genetisk struktur og utveksling av gener hos sukkertarepopulasjoner ble materiale fra 21 stasjoner, der 12 var plassert i kystområdene fra ytre Oslofjord til Porsanger og ni i Hardangerfjorden og Sognefjorden, undersøkt. Resultatene viste god genflyt og liten forskjell i genetisk struktur, bortsett fra redusert genflyt mellom Sør-Norge og Nord-Norge, og også mellom kyststasjonene og de innerste fjordstasjonene. Resultatene tydet også på en viss grad av genetisk tilpassing av sukkertare-populasjoner i Skagerrak og i de to fjordene. Et annet studie ble gjort på sukkertare innsamlet i Skottland og på vestkysten av Sverige. Resultatene fra disse undersøkelsene viste at genflyten mellom populasjoner var sterkt påvirket av lokalt strømmønster, samtidig som populasjoner viste klar genetisk tilpassing til miljøet.

Marin modellering: Kart over produksjonspotensialet for sukkertare langs og utenfor norskekysten er utviklet. Kartene er basert på modellresultater fra det fysisk-biologiske havmodellsystemet SINMOD koblet med en vekstmodell for sukkertare. Det er gjort forbedringer i hvordan biomassen i tarekulturene beregnes, basert på resultater fra feltforsøkene i måleprogrammet. Vekstmodellen viste samme trend som feltmålingene, med 2 mnd senere maksimal plantestørrelse og biomasse på lokaliteter i Nord-Norge sammenlignet med i Sør-Norge. Modellbaserte estimater for biomasseproduksjon (utbytte per enhet sjøareal) antyder at det i gjennomsnitt kan dyrkes 75 tonn per hektar langs norskekysten ved høsting i juni (20-220 tonn per hektar avhengig blant annet av utsettingstidspunkt). Resultatene vil bli gjort tilgjengelige til bruk i GIS-systemer og googlemaps. Vekstmodellen for sukkertare er videreutviklet slik at den nå kan ta hensyn til hvordan tarekulturene vekselvirker med lyset (selvskygging). Resultatet av forbedringene er at modellene for biomasseproduksjon nå er mer fleksible og tar hensyn til flere viktige faktorer enn tidligere.

Teknologi for påsåing, utsett og høsting: Et laboratoriesystem for automatisk kimplanteproduksjon med overvåking har blitt designet. En industriell produksjonslinje for kimplanter er utredet basert på state of the art for slike systemer. Det er utviklet metoder for bildebehandling av substrat for kvantifisering av sporofytter og sporetetthet som muliggjør behandling av et stort antall bilder med lite tidsforbruk sammenlignet med manuell telling. En prototype for klargjøring av spoler for kimplanteproduksjon spinner 300 m substratline på to plastsylindere på 90 sek. En "tarespinner" er utviklet for å automatisere spinning av kimplanteline rundt bæretau før utsett i sjø. Spoler av kimplanteline plasseres i maskinen og roteres rundt tauet med en prosesshastighet på 110 m bæretau på 14 minutter. Et nytt dyrkingssystem kalt SPOKe: Standardized production of kelp, er designet med tanke på automatisering og standardisering. Konseptet omfatter sirkulære moduler for å gi en dedikert plattform for automatisering. En gantry-robot på skinner brukes til å interagere med modulene, utstyrt med verktøy avhengig av oppgave. En VR-demo og en prototype som demonstrerer prinsippet for gantry-roboten er tilgjengelige for visning på SINTEF Ocean.

Sjøanlegg: For å designe og dimensjonere tareanlegg som skal stå i sjø er det behov for datagrunnlag mht. krefter taren påfører anlegget når den blir utsatt for bølger og strøm, samt numeriske metoder og verktøy for simulering av anleggene under ulike miljøforhold. MACROSEA har bidratt til ny kunnskap om mekaniske egenskaper, morfologi og dragkrefter til sukkertare. Målinger av slepehastighet, drag-krefter og strekkrefter i slepeforsøk med dyrket sukkertare (middels store og store planter) på tau med forskjellige konstante hastigheter i en dokk med sjøvann viste klare sammenhenger mellom tarens vekst og påførte drag-krefter. Det er utviklet numeriske metoder i SINTEF Oceans simuleringsverktøy FhSim for simulering av anlegg i sjø.

4 Outcomes and Impacts

4.1 Seedling Biology

- An industrial seedlings production protocol for *Saccharina latissima.*
- Pre-incubation of *S. latissima* seedlings gives superior growth at sea.
- Improved hatchery techniques for *Alaria esculenta*.
- An improved hatchery protocol for *Palmaria palmata.*
- Successful sea cultivation of *P. palmata.*

4.2 Sea Cultivation

- A Monitoring program showed a great potential for cultivation of *S. latissima* from 58 to 69°N, except in areas with high local environmental variations, as high freshwater run-off
- A latitudinal pattern in a south to north gradient was found for growth (length and biomass), protein content and biofouling.
- Different deployment dates yields different amount of biofouling organisms, growth and shedding.
- Protein content can be manipulated by lowering cultivation lines during the growth period
- Linear uptake of nitrate was found for substrate concentration up to 18 µM

4.3 Genetics

- Growth of *S. latissima* was found to be higher in sporophytes from the Tromsø area than in sporophytes from the Bergen area, in conditions simulating the sea environment in Tromsø during mid-May
- A population genetic analysis using microsatellites suggested good gene flow between stations with *S. latissima* along the coast, except between some fjord populations and coastal populations, and between coast stations in South-Norway (Skagerrak-Frøya) and North-Norway (Troms-Finnmark).
- Microsatellites potentially under selection or associated with a part of the genome under selection were found.
- Removing a microsatellite potentially under positive selection caused changes in the genetic structuring in the Skagerrak region and in some fjord populations, suggesting adaptation of populations
- The results suggest that during cultivation of *S. latissima* special care should be taken to prevent exchange of genetic material between South- and North-Norway, and between the coast and enclosed fjords.
- A consistent method for dd-RADseq sequencing of *S. latissima* was developed allowing the development of high-resolution genome-wide SNP marker sets for genotyping and population analysis.
- Genome-wide selection scans and genomic-environmental association scans provided strong evidence for local adaptation and selection in *S. latissima* in Scotland and Sweden.
- Adaptive loci were found to be in association with numerous environmental variables including mean temperature, summer temperature, chlorophyll and minimum salinity.

4.4 Marine Modelling

- Model based maps and estimates for the cultivation potential of *S. latissima* along and outside the Norwegian coast.
- Some of the results (maps) available for use in site selection with google maps (DST).
- Dynamical growth model for bryozoan fouling on *S. latissima* fronds.
- Mechanistic light shading model for kelp cultures, taking into account the interactions between the kelp fronds and the light field.
- Updated *S. latissima* growth model
- Simplified model based assessment for the cultivation potential for *P. palmata*.

4.5 Seedling, Deployment and Harvest Technology

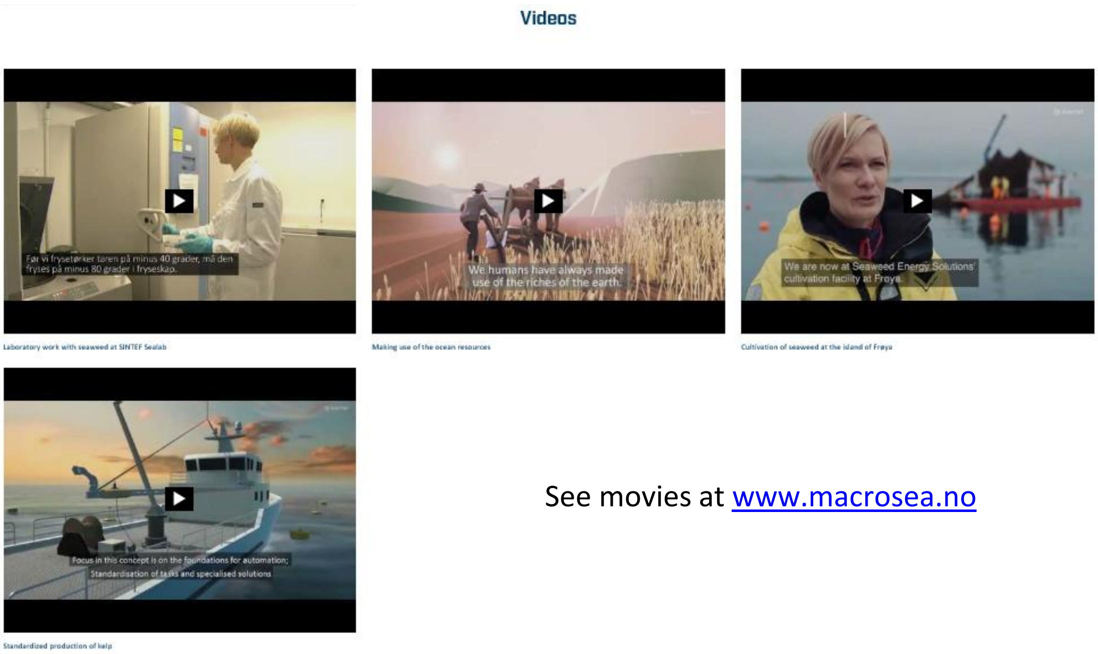
- A lab system pilot for seedling production.
- Machine vision for rapid assessment of seedling quality (size and density) before deployment.
- An onspinning machine for seedling twine on cylinders.
- A spinning machine for deployment of seeded ropes (from cylinders to ropes).
- A design concept for Standardized Production of Kelp (SPOKe).

4.6 Sea Farms

- Increased knowledge of mechanical properties of *S. latissima*.
- Forces on ropes grown with *S. latissima* from steady currents for a variety of growth densities and specimen growth.
- Parametrized force model for ropes grown with *S. latissima* – enables development of numerical methods and more precise modelling and design of seaweed farms

5 Outreach

- 80 Talks
- 55 Media
- 4 Movies
- 10 Popular articles
- 1 Book chapter
- 10 Scientific papers
- 19 Sci. papers in prep
- 10 Reports
- 10 MSc-thesis

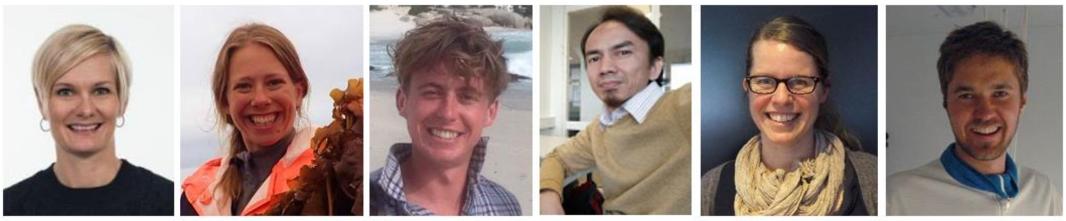


See movies at www.macrosea.no

6 Education

6 PhD | 10 MSc | 3 BSc | 5 Summer students

6.1 PhD-students



Silje Forbord **NTNU** | Sanna Matsson **APN** | Alexander Thomson **SAMS** | Saifullah Saifullah **NTNU** | Siv Anina Etter **NTNU/DTU** | Peter Schmedes **DTU/NTNU**

- **Silje Forbord** (NTNU, 2016-2020). Tentative title: "Cultivation of *Saccharina latissima* (Phaeophyta) in temperate marine waters; Quality, growth and nitrogen metabolism". Supervisor: Yngvar Olsen (NTNU), co-supervisor: Jorunn Skjermo (SINTEF Ocean).
- **Sanna Matsson** (APN, 2016-2020). Tentative title: Fouling of macro epibionts on cultivated *Saccharina latissima*: local, latitudinal and time dependent variation. Supervisor: Bodil Bluhm (UiT), co-supervisors: Hartvig Christie (NIVA), Anna Metaxas (Dalhousie University).
- **Alexander Thomson** (SAMS, 2016-2020). Tentative title: "Seascape genomics in the sugar kelp *Saccharina latissima*". Supervisor: Michele Stanley (SAMS), co-supervisor: Kjersti Sjøtun (UiB).
- **Saifullah** (NTNU, 2017-2020). Tentative title: Variation in growth and biochemical composition of sugar kelp (*Saccharina latissima*) as an effect of different abiotic conditions and resource availability. Supervisor: Yngvar Olsen (NTNU), co-supervisor: Aleksander Handå (SINTEF).
- **Siv Anina Etter** (NTNU, 2017-2021). Tentative title: Cultivation potential of brown macroalgae species integrated with open Salmon fish Aquaculture. Supervisor: Kjell Inge Reitan (NTNU), co-supervisor: Yngvar Olsen (NTNU).
- **Peter Søndergaard Schmedes** (DTU Aqua, 2016-2020). Tentative title: Investigating hatchery and cultivation methods for improved cultivation of *Palmaria palmata* and *Saccharina latissima*. Supervisors: Mette Møller Nielsen, Jens Kjerulf Petersen (DTU Aqua). Co-supervisor: Kjell Inge Reitan (NTNU).

6.2 MSc-students

- **Renate Rimstad Bøe** (2019) at NTNU: WP1 Seedling biology
  MSc-thesis: Investigation of important steps in *Palmaria palmata* cultivation
  Supervisor: Kjell Inge Reitan (NTNU), co-supervisor: Silje Forbord (NTNU/SINTEF Ocean) and Jorunn Skjermo (SINTEF Ocean).
- **Andreas Eggesvik** (2019) at NTNU: WP5 Seedling, Deployment and Harvest Technology
  MSc-thesis: Robot for automated seaweed deployment and harvesting
  Supervisor: Morten Alver (NTNU), co-supervisor: Torfinn Solvang (SINTEF Ocean).
- **Oliver Evans** (2018) at The University of Akron: WP4 Marine modelling
  MSc-thesis: Modeling the light field in macroalgae aquaculture.
  Supervisor: Shane Rogers (Clarkson University), co-supervisor: Ole Jacob Broch (SINTEF Ocean).
- **Guri Elilla Brodahl** (2018) at NTNU: WP2 Sea Cultivation
  MSc-thesis: The effects of variable environmental conditions on growth, nutritional state and protein content in cultivated *S. latissima* in Norway
  Supervisor: Yngvar Olsen (NTNU), co-supervisor: Silje Forbord (NTNU/SINTEF OCEAN).
- **Solveig Foldal** (2018) at NTNU: WP2 Sea Cultivation
  MSc-thesis: Morfologiske relasjonar til dyrka *S. latissima* ved tre stasjonar langs Norskekysten
  Supervisor: Yngvar Olsen (NTNU), co-supervisors: Ole Jacob Broch (SINTEF Ocean) and Silje Forbord (NTNU/SINTEF Ocean).
- **Vegard Rønning Dahlen** (2018) at NTNU: WP2 Sea Cultivation
  MSc-thesis: Characterization of the initial ammonium uptake in *Saccharina latissima*: Possible implications for an IMTA system with intensive salmon farming.
  Supervisor: Kjell Inge Reitan (NTNU), co-supervisor: Siv Anina Etter (NTNU).
- **Tonje Næss** (2018) at UiB; WP3: Genetics and disease
  MSc-thesis: Analyses of population genetics of *Saccharina latissima* (sugar kelp) in Norway
  Supervisor: Kjersti Sjøtun (UiB), co-supervisors: Geir Dahle (UiB) and Lars Asplin (UiB).
- **Carina Norvik** (2017) at NTNU: WP6 Sea Farms
  MSc-thesis: Scaling and simplified representation of aquatic vegetation in a steady flow
  Supervisor: Dag Myrhaug (NTNU), co-supervisors: Pierre-Yves Henry (NTNU) and Andreas Myskja Lien (SINTEF Ocean)
- **M Balasubramaniam** (2017) at UiO: WP1 Seedling Biology
  MSc-thesis: Veksteksperiment av makroalger med kommersiell interesse; med hovedfokus på *Palmaria palmata*.
  Supervisor: Stein Fredriksen (UiO)
- **Aires Duarte** (2017) at University of Porto: WP1 Seedling Biology
  MSc-thesis: Optimization of seedling production using vegetative gametophytes of *Alaria esculenta*
  Supervisor: Isabel Sousa Pinto (CIIMAR/University of Porto), co-supervisor: Jorunn Skjermo (SINTEF Ocean).

7 Approaches, Ambitions and Hypothesis

The macroalgae cultivation phase consists of four main stages: seedlings production, deployment, on-growing and harvesting. Basic biological and technological knowledge is strongly needed in all these phases to enable industrial cultivation. The ambitions for how MACROSEA could contribute to increase this knowledge and develop a knowledge platform for industrial cultivation of the selected macroalgae species *Saccharina latissima*, *Alaria esculenta* and *Palmaria palmata* in Norway are shown in Figure 3.

The hypothesis in MACROSEA was that macroalgae cultivation can be industrialized. To test the hypothesis and fulfil the Research Council of Norway's Large-scale program for aquaculture's call for a fundamental knowledge platform for industrial macroalgae cultivation, the project addressed the following main challenges to reach scientific understanding and support industrial development:

- The potential for macroalgae cultivation along the wide range of climatic, ecological and physical regimes in Norway are not exploited.
- Threshold levels for environmental variables that regulate and modify growth and quality of macroalgae are not known.
- The importance of ecological and physiological factors on biofouling are not understood.
- Technological solutions for industrial cost-effective cultivation does not exist.
- Numerical tools for estimating biomass production potentials and dimensioning of sea farms does not exist.

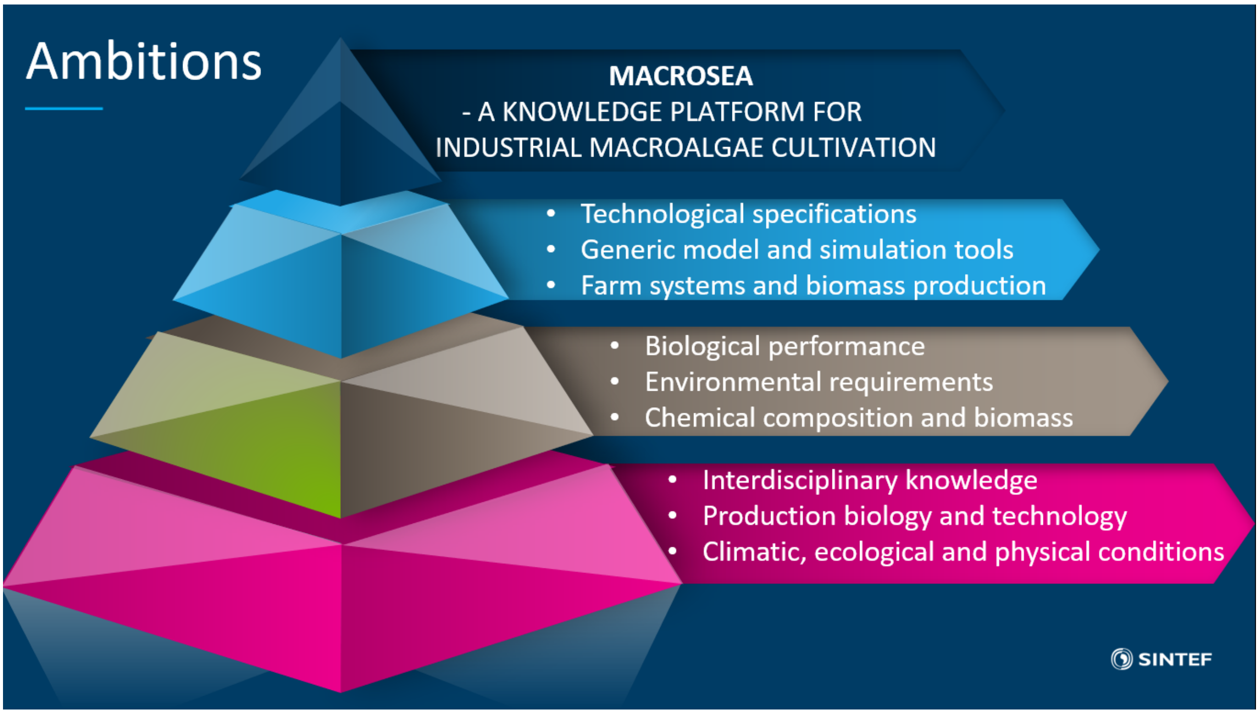


Figure 3. The ambitions in MACROSEA for how to develop a knowledge platform for industrial macroalgae cultivation.

8 Background for MACROSEA

Cultivation of the oceans is required to meet demands for food, energy and biomaterials for a growing global population expected to reach 9.5 billion before 2050[1]. Total freshwater and marine aquaculture production was ~96 million tons in 2013; macroalgae accounted for 26 million tons, of which Europe contributed to only ~0.1%[3]. However, new market demands and opportunities for multiple uses of macroalgae, such as food and feed ingredients, fertilizers and biofuels[2,4,5,6] (Figure 4), have strengthened the motivation for industrial cultivation within Europe[7,8]. Norway, with one of the world's longest tempered and productive coastlines and comprehensive knowledge and industry related to aquaculture and marine technology, can take a leading role in this developing industry.

The large kelp species of the brown algal order Laminariales, are among the fastest growing plants in the world. Annual production capacities of *S. latissima* have been estimated to be 114 - 170 tons wet weight ha-1 9, with 14-15% dry matter content[10]. While *S. latissima* has been found to contain up to 63% carbohydrates and 7% protein, peak values in *A. esculenta* of 72 and11 %, respectively, have been reported[5]. The production capacities of *P. palmata* at sea are unknown and remain to be assessed and validated. Due to high productivity and/or high protein content the above mentioned species are regarded as attractive for biomass production. The production capacity of **the red algae** *P. palmata*, belonging to the order Palmariales, has been estimated to be 33 - 113 tons dry weight ha^{-1}, containing up to 12% protein in land-based cultivation[11].

Growth rate and biochemical composition of macroalgae depend on environmental, physiological and developmental factors, whereof the availability of the key resources nutrients and light energy are the most important during cultivation. Temperature and physical regime (i.e., wave/current exposure) are important modifying factors, and also epiphytic fouling affects production yields. Nitrate (NO_3) is the main nitrogen source, available from autumn until the planktonic spring bloom. NO_3 supplied from deep water can

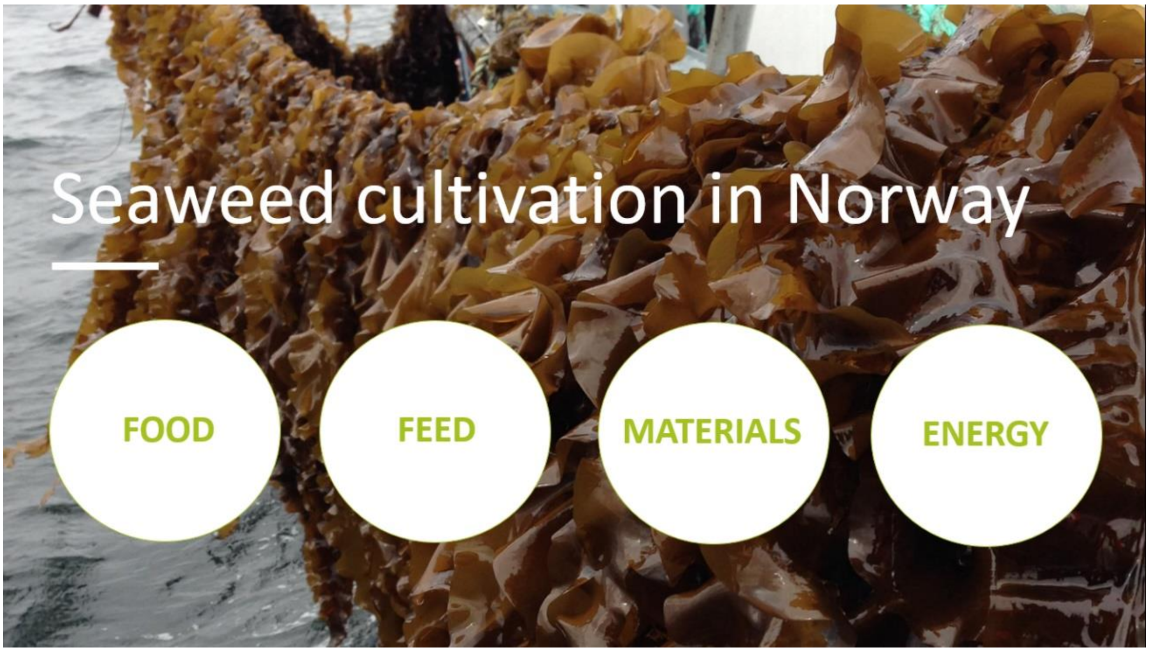


Figure 4. New market demands and opportunities for multiple uses of macroalgae, such as food and feed ingredients, fertilizers and biofuels, have strengthened the motivation for industrial cultivation within Europe. Norway, with one of the world's longest tempered and productive coastlines and comprehensive knowledge and industry related to aquaculture and marine technology, can take a leading role in this developing industry.

limit growth from after the spring bloom until the autumn turnover (April-September), the main season of biomass increase. A second source is inorganic ammonia (NH_4) originating from fish metabolism, which can be an important N source for macroalgae in integrated multi-trophic aquaculture (IMTA) with e.g. salmon[9,12,13,14].

NO_3 availability is closely linked with cell divisions and growth in macroalgae whereas photosynthesis is primarily determined by light and only indirectly controlled by nutrient availability. Algae that experience temporary nutrient limitation may continue photosynthesis at a high rate, then produce endogenous carbohydrates for storage and later use for anabolic processes and growth. This situation will normally result in low contents of N metabolites and protein in the algae. Preliminary results have shown that the internal pool of nitrate (NO_3-I) in *S. latissima* is closely related to growth rate and protein contents for light sufficient growth[15].

Biofouling is a major factor influencing the quality and growth of macroalgae; it can destroy a complete crop within few weeks during mid-summer[16,17]. Biofouling is shown to vary significantly between geographical regions and between different coastal waters within regions[18]. Physical and ecological factors that influence biofouling in macroalgae cultivation are insufficiently described in scientific literature and are poorly understood. The lack of models for prediction of biofouling hinders precise evaluation of suitable sites for macroalgae cultivation. Hence, laboratory experiments and field studies of these factors, and attempts to model physical and ecological dynamics that influence biofouling, are prerequisite for successful cultivation of macroalgae.

Protocols have been developed for hatchery of the kelps *S. latissima*[19] and *A. esculenta*[20] and the red *Palmaria palmata*[21]. Kelp species have a diplo-haplontic, heteromorphic lifecycle with large sporophytes that produce spores in meiosporangia and microscopic dioecious heteromorphic gametophytes. *P. palmata* has a diplo-haplontic, heteromorphic life cycle, with a small female gametophyte, a macroscopic male gametophyte and a foliose tetrasporophyte. These two life cycles are very different, and require different approaches along the entire cultivation line. Methods for hatchery and sea cultivation of *S. latissima* and *A. esculenta* are established at the Norwegian Seaweed Technology Center[9,12,13,22] hosted by SINTEF Ocean and the Norwegian University of Science and Technology (NTNU); however, variations in productivity, quality and chemical composition during grow-out at sea needs to be better understood with regard to industrial processes. Quality control must be improved and domestication of selected species must be assessed[23]. Existing methods for *P. palmata* need to be improved and adapted to Norwegian conditions.

Industrial cost-effective cultivation requires novel technology with a high degree of mechanization and automation along the entire production chain comprising hatchery, deployment, cultivation, monitoring and harvesting. Cultivation methods and prototypes for mechanized twisting of seeded ropes onto carrier ropes for deployment at sea have been developed at NSTC. New cultivation substrates have been tested in EU-projects, and methods for direct seeding of materials are being patented; however, equipment for industrial handling and simulation tools for drag forces and deformation of farm systems does not exist. In order to facilitate transitions from "low-tech" (labor intensive) to "high-tech" (cost-effective) cultivation there is accordingly a need to; (i) facilitate technology-transfer from successful marine and maritime industries to adapt efficient methods for seedling, deployment and harvest, and (ii) develop model tools for simulation and visualization of dynamic systems to perform reliable structure analysis that supports rational design of efficient sea farm concepts for different species and environmental conditions. Better understanding of forces acting upon growing systems will allow rational design of systems that are often oversized to avoid potential failure and loss of algae biomass. Better models of fluid dynamics within macroalgae cultivation systems may also improve our understanding of, and ability to predict, nutrient exchange, macroalgae growth rates and production for different sea farm concepts and different species.

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9 Work Packages

MACROSEA has contributed to systematic research and delivered generic knowledge on seedling quality (WP1, Figure 5), sea cultivation (WP2), and genetics (WP3) of selected brown and red macroalgae species. Models of brown and red species have been developed and coupled to 3D hydrodynamic-ecosystem models to estimate site-dependent biomass production (WP4). Methods for efficient seedling, deployment and harvest have been assessed (WP5) and drag forces and deformation of different farm systems determined at different sea states in tank experiments (WP6).

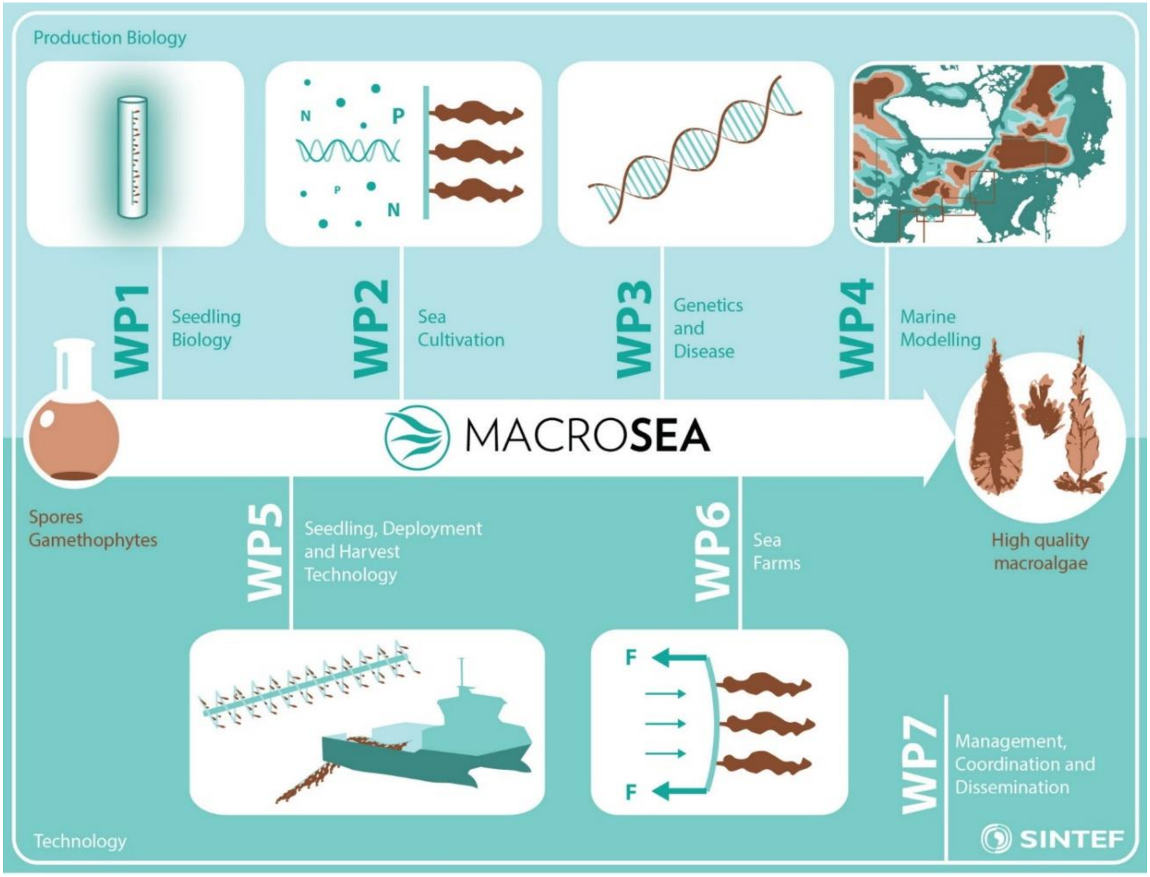

Figure 5. MACROSEA envisages an interdisciplinary knowledge platform on fundamental macroalgae production biology and technology, to make significant steps towards industrial cultivation in Norway.

10 Main Results

10.1 Seedling Biology

Lead: Senior scientist Dr. Jorunn Skjermo, SINTEF Ocean
R&D: SINTEF Ocean, NTNU, DTU Aqua, UiO, IOCAS, SAMS, AaU, CU, SES, Hortimare

Objectives

i) Establish quality parameters for seedling production of S. latissima, A. esculenta and P. palmata.
ii) Characterize biological requirements and threshold levels for environmental variables on seedling quality.
iii) Develop a protocol for industrial production of P. palmata.

10.1.1 Quality parameters and biological requirements for *S. latissima* and *A. esculenta* seedlings

Winged kelp *Alaria esculenta*

To increase the biomass yield and lower the overall production costs industrial scale is needed, including optimizing of the cultivation protocols as well as mechanization and automation of the processes. In this study we focused on the hatchery phase and how optimizing of different steps in the protocol used for seedlings production of winged kelp *A. esculenta* can improve the efficiency.

As the commercial seedlings production takes place during autumn and winter, unlike the natural cycle with spore production at spring, gametophyte cultures are maintained under red light throughout the year to have access to *A. esculenta* seedlings. Fertility needs to be induced in white light before the seedlings production can start and an experiment, testing photoperiods of 16, 23 and 24 hours light per day, demonstrated that 23 hours light gives the fastest transition from vegetative tissue to reproductive growth, with cultures ready for seeding after 6 days. Dense cultures may however need a longer induction period for optimum development due to higher light absorption.

Little is known about the phlorotannin content and mode of action in *A. esculenta* but as phlorotannin production in macroalgae often is related to stress the phlorotannin content was analyzed to examine whether this can be used as a quality parameter for gametophytes and seedlings in optimization of hatchery conditions. This experiment also included addition of a plant growth stimulant to the gametophyte cultures. The phlorotannin concentration in non-fertile gametophytes was on average 0.4±0.1 mg/g DW with no differences between cultures with or without addition of the growth stimulant. After fertility induction the concentration increased significantly, to 1.4±0.2 mg/g DW tissue in the control condition and 2.6±0.4 mg/g DW tissue in cultures added growth stimulant. Phloroglucinol and dihydrophloroglucinol were identified. More work is needed to explain the phlorotannin dynamics in *A. esculenta* seedlings.

The study also demonstrated estimation of the gametophyte culture density by use of optical density measurement (OD_{750}) and estimation of seedlings growth by image analysis, both techniques that can be developed further and implemented in automatized macroalgae seedlings production to replace time-consuming manual monitoring methods.

Sugar kelp *Saccharina latissima*

Saccharina latissima is interesting for cultivation at the Atlantic coasts in Northern Europe and many farmers start up production of this species. There is a need to optimize the production technology to reduce cultivation costs, and one important step is to increase the quality and predictability in the seedlings production. The hatchery protocol developed at SINTEF is now available in the book "Protocols for macroalgae research" as an own chapter by Forbord et al. (2018).

For vector seeding, spores and gametophytes were seeded and pre-cultivated on twine for periods of up to 6 weeks in hatchery before twisting on to carrier ropes at deployment in sea, in total 7 different treatments. Image analysis, developed in WP5, was used as a method for effective estimation of seedling coverage on seeded material before deployment and showed later a good correlation with frond lengths in sea. For direct seeding fertile gametophytes and juvenile sporophytes pre-cultivated in tumbling cultures were seeded directly on cultivation ropes using a binder at the day of deployment, in total 4 different treatments.

We evaluated the biomass production potential from these seedlings at SINTEFs licensed seaweed site at Skarvøya, which lays between Hitra and Frøya at the coast off Central-Norway. All 11 treatments were deployed at the same day in February and the growth registered after 3 (May) and 4 (June) months. The biomass yield peaked at 7,22±0,14 kg wet weight pr meter in June for vector seeding of ropes with spores 6 weeks prior to deployment, whereas direct seeding gave 1/3 of the maximum biomass (Figure 6).

The sporophyte density on the ropes in June (Figure 7) was similar for these two conditions but the individual weight of the direct seeded sporophytes was only 32% of the vector seeded sporophytes, suggesting that the direct seeded gametophytes or juvenile sporophytes used much of the spring to develop holdfast and start growing instead of immediate fast growth and biomass production. The results further show that twine seeding with spores or gametophytes and a hatchery period of 2-4 weeks gave a biomass yield of only 45-58% of the maximum yield. More details can be found in Forbord et al. (2019).

Figure 6. *S. latissima* after 4 months cultivation in sea (Forbord et al. 2019). Left: Glue seeding at deployment. Right: Twine seeding and 6 weeks incubation before deployment.

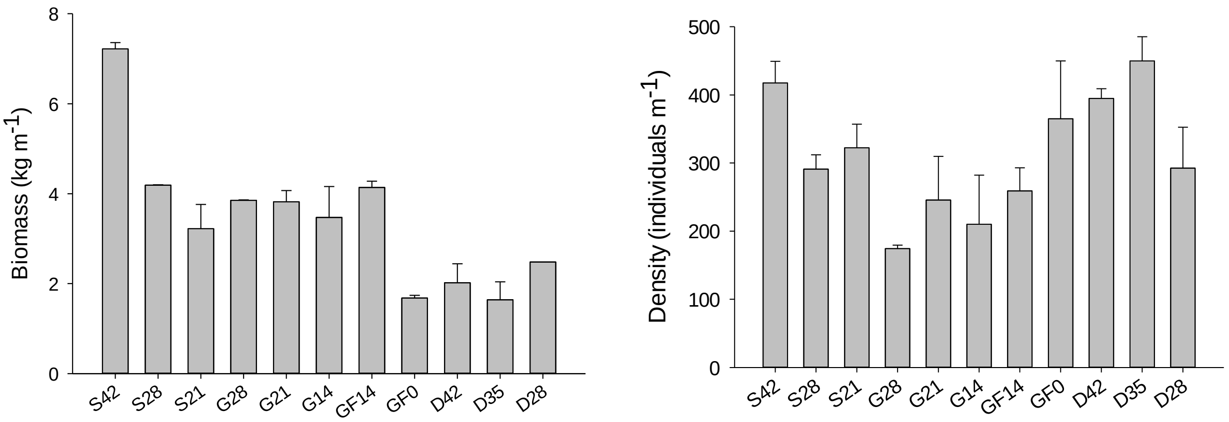

Figure 7. *Saccharina latissima* biomass (left) and density (right) on ropes after a 4 months cultivation period in the sea (Forbord et al. 2019). S were seeded as spores, G as gametophytes and D as microscopic sporophytes using a binder. Numbers denotes days in the hatchery, either on rope (S and G) or in white light tumbling culture, and GF0 was seeded using binder. GF are gametophytes with a one-week fertility induction period prior to seeding.

10.1.2 Optimizing of the seedlings protocol for *Palmaria palmata* cultivation

Due to its good taste, nutritious composition and nice appearance and colour dulse *Palmaria palmata* attracts a lot of interest among both consumers, companies and researchers in the Northern Europe. The increased demand raises the interest of cultivating this specie in Norway and more research within important steps in the life stages and cultivation of *P. palmata* is decisive in order to enable a successful large-scale cultivation. The focus in MACROSEA was thus to solve some of the bottleneck in *P. palmata* seedling cultivation, including studying the seasonal dependency for fertility, surface disinfection of sorus, different methods for spore release from fertile tissue and methods to increase the seedlings yield from the obtained spores. Finally, a sea cultivation experiment with Palmaria was also carried out as the first in Norway starting with spore release and attachment on nets. The work on Palmaria has resulted in the MSc-thesis "Veksteksperiment av makroalger med kommersiell interesse; med hovedfokus på *Palmaria palmata* by M. Balasubramaniam (2017) and «Investigation of important steps in *Palmaria palmata* cultivation» by R. R. Bøe (2019), and several scientific papers by P.S. Schmedes (Schmedes et al. 2019ab). Some main findings are summarized below.

A survey on the year-round health and reproductive status of *P. palmata* in Trondheim revealed that collecting sporophytes during winter may secure high amount of fertile and healthy sporophytes suitable for spore release. In Trondheim the access to fertile Palmaria is best in January. The survey also investigated the distribution of fertile tetraporophytes and fertile male gametophytes, suggesting that high availability (20-40 %) of fertile male gametophytes during the reproduction season can be utilize.

Two spore release trials over three days, one in January and one in November, examined when spore release had its highest density. Spore release rate seemed to be seasonal dependent, and spore release with sori from sporophytes collected within the reproductive season demonstrated highest spore density after 28 hours of spore release. The effect of four chemical disinfectants at different concentrations was investigated in order to reduce diatom contamination. The most promising disinfection treatment was to immerge sori from sporophytes collected within the reproductive season in 0.5 mL L^{-1} germanium dioxide solution (GeO_2) for 2 minutes in an exposure temperature of 10 °C followed by two rinsing baths of sterile seawater for 30 seconds. This treatment significantly reduced diatom contamination without affecting seedling development and survival and gave the longest seedlings after 21 days of incubation. Disinfection of sori from sporophytes collected outside the reproductive season in GeO_2 showed a delaying effect on spore to seedling development. 0.1 mL L^{-1} GeO_2 added in growth medium in *P. palmata* cultures was also investigated as a way of controlling diatom contamination. The addition of GeO_2 showed no significant effect on seedling survival. Sori disinfection with 0.02 % and 0.2 % Lugol, 1 % and 7 % acidic acid and 300 ppm and 600 ppm of sodium hypochlorite reduced tetraspore survival and should not be recommended for *P. palmata*. For sea cultivation nets and twine were seeded and incubated in tanks for 3 months before deployment in IMTA in February 2018. While the seedlings on twines did not grow in the sea, the seedlings on nets showed good growth (Figure 8). The plants grew slowly in spring, but in June a high density and blades up to 15 cm were measured.

Figure 8. *Palmaria palmata* (Dulse) for spore release (left) and cultivated on nets in SINTEF/ACE's salmon and seaweed farm at Rataren outside Frøya, Norway in 2018. The seeded nets were deployed 16.02.2018.

10.1.3 Publications

- Balasubramaniam M (2017). Veksteksperiment av makroalger med kommersiell interesse; med hovedfokus på *Palmaria palmata*. MSc-thesis, University of Oslo (UiO).
- Bøe RR (2019). Investigation of important steps in *Palmaria palmata* cultivation. MSc-thesis, Norwegian University of Science and Technology (NTNU).
- Duarte A (2017). Optimization of seedling production using vegetative gametophytes of *Alaria esculenta*. MSc-thesis, University of Porto.
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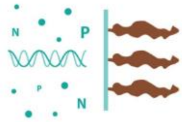
- Schmedes PS and Nielsen MM (2019b). New hatchery methods for efficient spore use and seedling production of *Palmaria palmata* (dulse). *Journal of Applied Phycology*. doi: 10.1007/s10811-019-01998-0

- Su L, Pang S, Shan T, Li X (2017). Large-scale hatchery of the kelp *Saccharina japonica*: a case study experience at Lvshun in Northern China, *Journal of Applied Phycology*. https://link.springer.com/article/10.1007/s10811-017-1154-y

Scientific publications in prep.

- Schmedes PS, Nielsen MM, Andersen KL, Petersen JK. Cultivation of *Palmaria palmata* in open water - the effect of seedling size at deployment and farm location on harvest yield.
- Schmedes PS, Nielsen MM, Petersen JK. Productivity and yields of *Palmaria palmata* affected by irradiance, salinity and nutrient availability – interventional cultivation as a production method?

10.2 Sea Cultivation



Lead: Professor Yngvar Olsen, NTNU
R&D: NTNU, SINTEF, APN, NIVA, UiO, UiT, AaU, ALL industry partners

Objectives

i) Characterize for Saccharina latissima;
- *nitrogen metabolism and storage capabilities of protein,*
- *biochemical composition as a function of physical (light, temperature) and nutritional state for regional cultivation regimes and*
- *importance of physical, ecological and physiological factors for biofouling.*

ii) Cultivate Palmaria palmata to evaluate a seedling protocol established in Part 10.1 Seedling Biology.

10.2.1 N metabolism in *Saccharina latissima* and the potential for IMTA

Several laboratory experiments have been conducted with nitrogen-saturated and nitrogen-limited *S. latissima* to study the capacity to take up nitrogen under various substrate concentrations. The initial uptake rates of nitrate (NO_3^-) and ammonia (NH_4^+) were investigated in relation to available nitrogen source and nutritional history of juvenile, cultivated *Saccharina latissima* by using the whole sporophytes (8-12 cm in length). Substrate concentrations of 2-18 µM NO_3^- and NH_4^+ were chosen to be representative of Norwegian coastal surface waters in winter and spring when the cultivated kelp biomass has its highest growth. It was shown that *S. latissima* with deprived tissue nitrogen pools of 19.5 mg N g^{-1} DW experienced a significantly higher uptake of NO_3^- than sporophytes with higher tissue nitrogen pools of 29.5 mg N g^{-1} DW over a short time period of 90 minutes. Uptake rates were shown to be directly related to the substrate concentration as there was observed a linear increase in uptake with an increase in available substrate concentration (Figure 9), indicating that the substrate concentrations should be higher than the assayed concentrations to reach saturation. The internal nitrate pools were significantly lower for the N-limited sporophytes compared to the N-saturated ones before initiating the experiments, and even after 5 hours of incubation in highest external NO_3^-, the internal pools did not reach more than 0.6 mg NO_3^- g^{-1} DW.

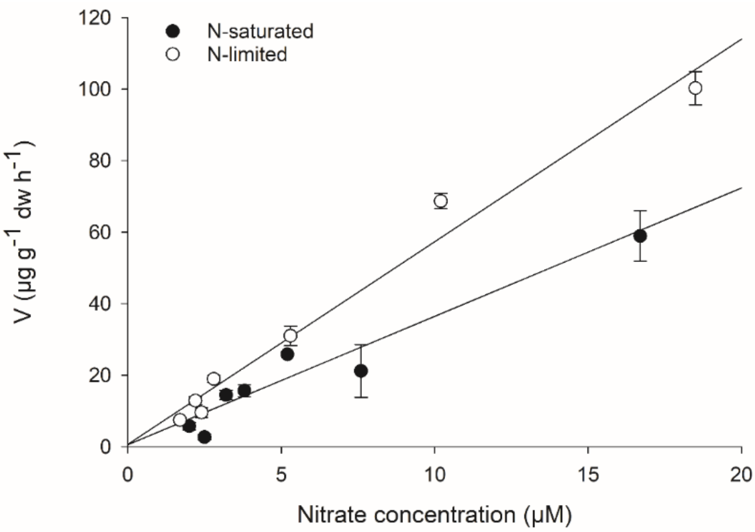


Figure 9. Mean uptake rates of NO_3^- (V, µg g^{-1} DW h^{-1}) for N-saturated and N-limited sporophytes as a function of initial ambient NO_3^-concentration. The value for N-saturated sporophytes perturbed with 7.6M deviated somewhat from the other values and were not included in the line fitting. Mean ± SE, n=5-6.

S. latissima was observed to rapidly adapt to different concentrations of ammonium (NH_4^+), with a swift increase in uptake rates and maximal uptake rates within 50 minutes (Dahlen 2018). Furthermore, uptake of NH_4^+ increased linearly with augmenting concentration, regardless of nutritional history. The nutritional history of *S. latissima* appeared to affect the uptake, as N-limited sporophytes had a significant faster uptake of NH_4^+ than N-saturated sporophytes. The results from this experiment indicate that juvenile *S. latissima* have the ability to utilize pulses of NH_4^+ released from the fish farms for growth, independent of the nutritional state.

10.2.2 A one-year monitoring program for *Saccharina latissima*

The Norwegian coastline covers more than ten degrees in latitude and provides a range in abiotic and biotic conditions for seaweed farming. In this task, we compared the effects of cultivation depth and season on the increase in biomass (frond length and biomass yield), chemical composition (protein, tissue nitrogen, intracellular nitrate and ash content), and biofouling (total cover and species composition) of cultivated *S. latissima* at nine locations along a latitudinal gradient from 58°N to 69°N (Figure 10).

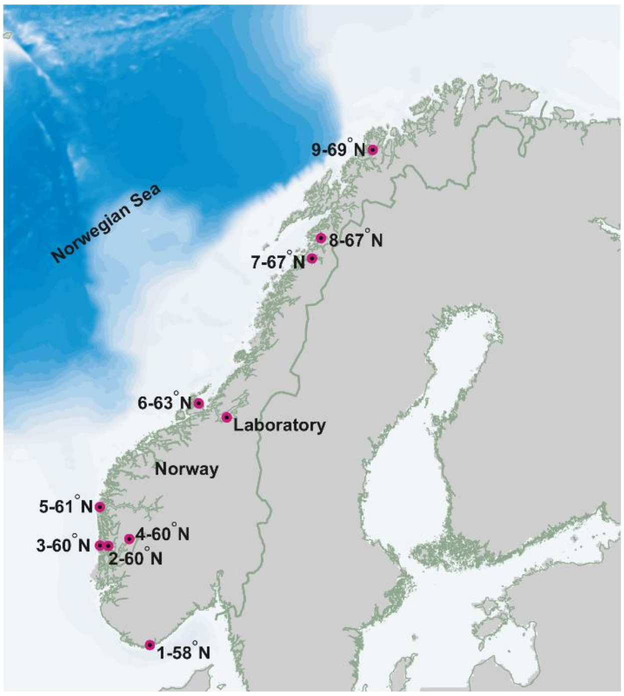


Figure 10. The locations of the experimental sites along the Norwegian coastline and the seaweed laboratory where seed lines were produced and distributed from. The name for each site is composed of a consecutive number and the latitude.

Sporophytes of *S. latissima* with mature sori were collected near each study site in December 2016 and shipped to the seaweed laboratory (63°N, Figure 9) for production of seed lines. This procedure is according to the recommendations of the Norwegian Environment Agency requiring that cultivated algae should be of local genetic origin, applying the precautionary principle. When the seedlings reached an average length of ~0.5 cm, the seed ropes from each location were express-shipped to the location where the fertile sporophytes were collected. Deployments took place as soon as possible after the delivery of seedlings to the site. The effects of light and temperature on frond length and biofouling were evaluated along with their relevance for selecting optimal cultivation sites.

Growth performance was greater at 1-2 m than at 8-9 m depth and showed large differences among locations (Figure 11). Maximum frond lengths varied between 15 and 100 cm, and maximum biomass yields between 0.2 and 14 kg m^{-2}, mainly in relation to local salinity levels (Brodahl 2018; Foldal 2018; Forbord et al. 2020. Timing of maximum frond length and biomass yield varied with latitude, peaking 5 and 8 weeks later in the northern location (69°N) than in the central (63°N) and southern (58°N) locations, respectively. The nitrogen-to-protein conversion factor (averaged across all locations and depths) was 3.8 while protein content varied from 22 to 109 mg g^{-1} DW, with seasonality and latitude having the largest effect (Forbord et al. 2020).

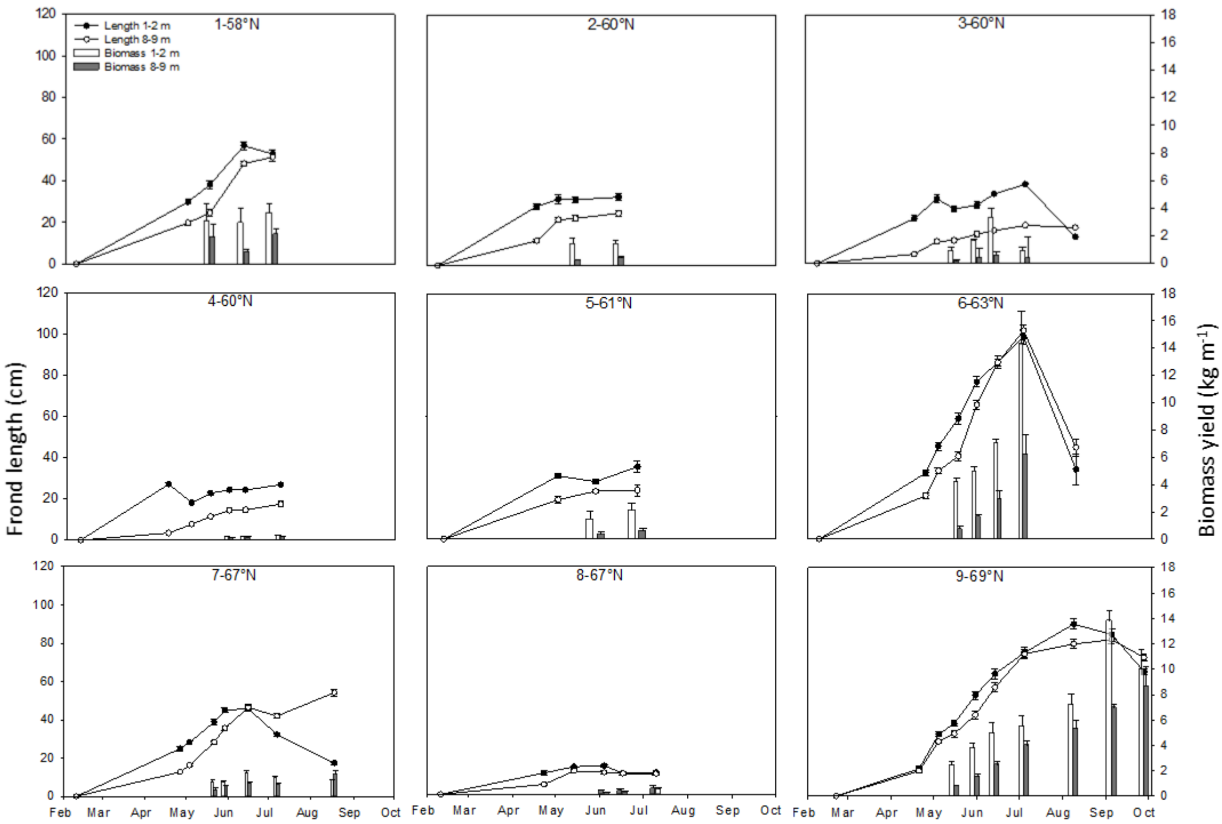


Figure 11. Length (solid line, left y-axis) and biomass (bars, right y-axis) for both cultivation depths for all nine locations during the experimental period (February-September). Mean ± SE, n = 50 for length and n = 4 for biomass.

The onset of biofouling also followed a latitudinal pattern, with a delayed onset in northern locations and at freshwater-influenced sites. The dominant epibiont was the bryozoan *Membranipora membranacea* (Figure 12). Our results demonstrate the feasibility of *S. latissima* cultivation along a wide latitudinal gradient in North Atlantic waters and underscore the importance of careful site selection for seaweed aquaculture (Forbord et al. 2020).

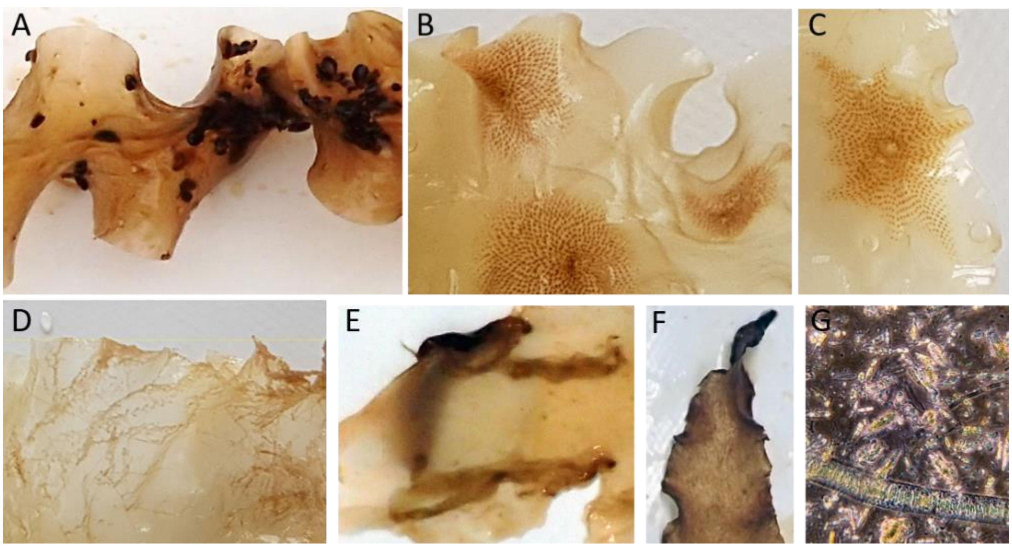


Figure 12. Images of the epibionts found and registered in this study, A: Bivalvia, B: *Membranipora membranacea*, C: *Electra pilosa*, D: Hydroids, E: Filamentous algae, F: Diatoms, G: Diatoms at 40x magnification.

10.2.3 Cultivation strategies to optimize protein

During the Monitoring program, an additional experiment was carried out at location 6-63°N (Figure 10). After 14 weeks of cultivation (mid-May), five of the ropes were turned upside down so that the sporophytes cultivated at 1-2 m depth were moved down to 8-9 m depth and vice versa. Five control ropes kept their original cultivation depth during the whole growth period. Growth was measured until September, and samples were analysed for amino acids (protein). Results showed an increase in protein content in the biomass moved from 1-2 m to 8-9 m depth during the cultivation experiment, and this could be a future method to increase valuable components to optimize the value of the cultivated biomass. The experiment will together with earlier experiment be used to analyse the importance of light intensity and nitrogen availability for growth rate and biochemical composition of *S. latissima*.

10.2.4 Importance of physical, ecological and physiological factors for biofouling

An article has been published in Journal of Aquaculture on local/regional variation of total biofouling, fouling species and growth of *S. latissima* (Matsson et al. 2019). Here the effect of blade age (as in area of blade), depth, and the environmental parameters temperature and salinity were evaluated. Only temperature showed a significant positive effect on total biofouling cover. A field trial has been conducted on the effect of out-planting time (Feb, Apr, May) of *S. latissima* related to growth rate, shedding rate, internal nitrate-N, C/N and amount of fouling organisms in Tromsø. Preliminary results show a difference between the deployment dates in amount of biofouling organisms, growth and shedding. Total cover of biofouling and biofouling species was also a part of the Monitoring program where the effect of temperature, light, internal nitrate-N, frond length and biomass were tested, but only temperature had a significant positive effect and light a significant negative effect on biofouling cover. Salinity also had a major impact on biofouling cover and type of fouling species.

10.2.5 Sea cultivation of *Palmaria palmata*

For sea cultivation nets and twine were seeded and incubated in tanks for 3 months before deployment in IMTA in February 2018. Seedlings on twines did not grow in the sea but on the nets a good growth was eventually observed. The plants grew slowly during the spring, but in June a high density and blades up to 15 cm were registered (Figure 7). This task was done in connection with WP1 and a master student (Bøe 2019).

10.2.6 Publications

- Brodahl GE (2018). The effects of variable environmental conditions on growth, nutritional state and protein content in cultivated *S. latissima* in Norway. MSc-thesis, Norwegian University of Science and Technology (NTNU).
- Bøe RR (2019). Investigation of important steps in *Palmaria palmata* cultivation. MSc-thesis, Norwegian University of Science and Technology (NTNU).
- Dahlen V (2018). Characterization of the initial ammonium uptake in *Saccharina latissima*: Possible implications for an IMTA system with intensive salmon farming. MSc-thesis, Norwegian University of Science and Technology (NTNU).
- Foldal S (2018). Morfologiske relasjonar til dyrka *S. latissima* ved tre stasjonar langs Norskekysten. MSc-thesis, Norwegian University of Science and Technology (NTNU).
- Forbord S, Matsson S, Brodahl G, Bluhm B, Broch OJ, Handå A, Metaxas A, Skjermo J, Steinhovden KB, Olsen Y. Latitudinal and seasonal variation of growth, chemical content and biofouling of cultivated

Saccharina latissima (Phaeophyceae) along the Norwegian coast. *Journal of Applied Phycology.* doi:10.1007/s10811-020-02038-y

- Matsson S, Christie H, Fieler R (2019). Variation in biomass and biofouling of kelp, *Saccharina latissima*, cultivated in the Arctic, Norway. *Aquaculture* 506, p 445-452.
- Skjermo J (2019). Høsting av havets planter – tang og tare. In: Nye Muligheter for verdiskaping i Norge, Almås K (ed). https://www.nho.no/contentassets/6ffd5dbbe6e54616b9565b737c7e94ca/sintef_verdiskaping2019.pdf

Scientific publications in prep.

- Bruhn Annette, Teis Boderskov, Helle Buur, Morten Foldager Pedersen, Silje Forbord, Jørgen LS Hansen, Signe Høgslund, Dorte Krause-Jensen, Stiig Markager, Sanna Matsson, Morten Holtegaard Nielsen, Michael Bo Rasmussen, Rumakanta Sapkota, Peter Søndergaard Schmedes, Peter Anton Stæhr, Sanjina Upadhyay, Anne Winding. Ecosystem effects of large-scale macroalgae cultivation
- Etter et al. Possibility of high quality macroalge production in IMTA by recycling nutrients from aquaculture.
- Etter et al. Bioremediation potential by nitrate and ammonia assimilation in *Saccharina latissima*
- Etter et al. Importance of phosphate assimilation in *Saccharina latissima* for bioremediation potential.
- Etter et al. Optimal configuration of environmental and economical macroalgae IMTA cultivation systems.
- Forbord et al. Protein content and growth of *Saccharina latissima* grown at various light and nitrate supply in an open sea environment.
- Forbord et al. Initial short-term nitrate uptake responses in young, cultivated *Saccharina latissima* (Phaeophyceae) under nitrogen-saturated and nitrogen-limited conditions.
- Jevne L., Forbord S., Olsen Y. The effect of nutrient availability and light conditions on growth and intracellular nitrogen components of tank cultivated *Saccharina latissima* (Phaeophyceae).
- Matsson et al. Variations in biofouling as an effect of deployment. Can a prolonged growing season delay the onset of biofoulers?
- Saifullah et al. Seasonal variation in the carbohydrate composition of sugar kelp (*Saccharina latissima*) from different farming stations along the Norwegian Coastline.
- Saifullah et al. Improving carbohydrate composition of sugar kelp (*Saccharina latissima*) through algal movement

10.3 Genetics

Lead: Professor Kjersti Sjøtun, UiB
R&D: UiB, SAMS, IOCAS, NTNU

Objective

Identify genetic markers associated with specific functional performance in local strains of S. latissima.

10.3.1 Quality indicators (genetic markers) for cultivated macroalgae

The functional traits of a species are dependent on its genetic composition, and intraspecific genetic variation in a species may consequently result in variation in its functional traits. Functional traits are ultimately determined by how genes are expressed or remain unexpressed, which has a genetic basis. Vital functional traits of macroalgae can for example be differential growth in relation to environmental factors, uptake rate of nutrients, or tolerance to stress factors or pathogens. Intraspecific variations in such functional traits are of interest and importance for macroalgal cultivators.

Saccharina latissima is at present one of the most relevant macroalgae for cultivation in Norway. Sugar kelp is a circumpolar species which is distributed from the Arctic to warm temperate areas, and in Norway it is common along the entire coast. It is consequently adapted to a wide range of variation of environmental factors such as temperature, light and salinity. The period of highest seasonal growth of *S. latissima* is normally between March and June. A common garden experiment was carried out in order to examine the potential for growth of strains of *S. latissima* from three different sites in Norway, where environmental conditions at each site in mid-May was simulated. *S. latissima* was sampled from stations close to Tromsø, Trondheim and Bergen. Pieces of lamina covered with sporangia were cut from 10-12 sporophytes on each site and transported in cooling boxes to Bergen, where spores from the three localities were seeded on granite stones (10 cm x 10 cm, Figure 13).

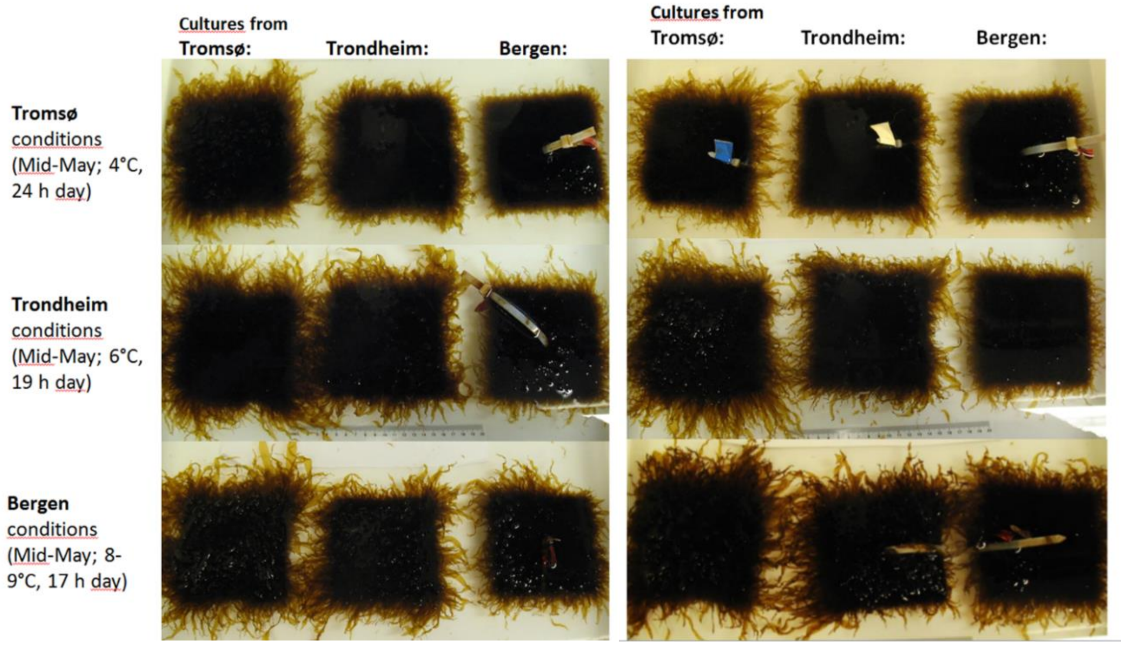


Figure 13. Panels of granite stones with sporophytes from Tromsø, Trondheim and Bergen, cultivated together in three months in conditions simulating those of each of the three sites in mid-May (two replicates).

Granite stones with sporophytes from Tromsø, Trondheim and Bergen were cultivated together in tanks with running sea water. Three sets of tank conditions were applied with two replicates of each; sea temperatures and day-lengths in mid-May in North-Norway (Tromsø), Mid-Norway (Trondheim) and South-West Norway (Bergen). The common garden experiment was run for three months. Midway and at the end of the experiments a number of sporophytes of each of the three cultures and in each category were sampled, and frozen in RNAlater solution for transcriptomic analyses and production of RNA libraries. The largest sporophytes on each stone were sampled for the transcriptomic analyses. All the granite plates were photographed when terminating the experiment.

A very dense growth of sporophytes developed on all the granite stones, in spite of several attempts to reduce the density of the sporophytes on the stones. As a result of this large differences in size developed, presumably due to light or nutrient competition. There was however a clear gradient in size development of the longest sporophytes present during the common garden experiment, with sporophytes reaching greatest lengths in the culture from Tromsø (Figure 13). The difference in length development between the cultures was most pronounced in the tanks simulating the conditions of North-Norway in mid-May (4 ˚C and 24 h day-length), where the sporophytes originating from Bergen were considerably smaller than those originating from Tromsø. The sporophytes of the Trondheim culture showed intermediate development. The difference in length development was least conspicuous in the cultures which had grown in the tanks simulating the conditions of South-West Norway (8-9 ˚C and 17 h day-length).

The transcriptomics of the samples are not yet ready, since some methodical adjustment of the analyses has to be done in order to extract RNA. The RNA library will show potential differences in gene expression between the three different cultures of *S. latissima*, when grown in the different conditions.

10.3.2 Genetic diversity of target species at different spatial scales.

Even though sea cultivation of seaweeds has relatively low impact on the environment, it is not without environmental risks. In particular, there is a risk of genetic contamination of local populations from the cultivated species. Even with precautions taken by the farmers there is a considerable risk that "crop-to-wild" gene flow will occur, which may as a worst case scenario cause genetic alterations of wild populations. Seaweed aquaculture is at present in an upstart phase in Norway with *S. latisssima* as one of the most commonly cultivated species. A large-scale baseline study along the coast of Norway has been carried out in order to evaluate the impact of a possible genetic contamination from cultivated strains on wild populations of *S. latissima*. Localities both on the coast and in fjords have been included, which makes it possible to compare gene flow in different spatial scales. In addition, results from studies of population genetics of *S. latissima* in Scotland and on the west coast of Sweden are included. This study is part of a PhD study of Alexander Thomson at SAMS, Scotland.

In Norway samples of *S. latissima* from a total of 21 stations were included in the study. The stations range from the outer part of Oslofjord in South-East Norway to Porsangerfjord in North-Norway (Figure 14). In two large fjords in South-West Norway (Hardangerfjord and Sognefjord) several stations were sampled inside each fjord, in order to test if local isolation of *S. latissima* populations inside the more enclosed fjords was evident. Initially the plan was to analyze the samples using RADseq markers, but it proved difficult to achieve clean enough DNA. As an alternative method microsatellites was applied, and the material was analyzed using 9 microsatellite loci (two were removed due to presence of null alleles) (Næss 2018).

The allele distribution of the microsatellites was analyzed by STRUCTURE. This analysis finds the spatial distribution of the most likely number of genetic groups in the material, based on the allele frequencies at the stations. Further, when checking the microsatellites, one of them was found to be potentially under positive selection, and one under balancing selection. This means that these microsatellites are not neutral, and especially the microsatellite under potential positive selection (or associated with an area of the genome under positive selection) may have an impact on the pattern of genetic structure. Analyzing the material with or without the microsatellite potentially under selection showed different results of the STRUCTURE analyzes, but all analyses showed that the most likely number of genetic groups were four (K=4) (Figure 15). When analysing the material using all the microsatellites, there was a tendency of domination of one of the genetic groups in Skagerrak (stations L, C), in some of the fjord populations (Ha2, Ha3, So1) and in the stations in Troms and Finnmark (stations G, F, E). After removing the microsatellite potentially under positive selection the dominating genetic group disappeared in Skagerrak, and the pattern with dominating genetic groups in the fjord stations Ha3 and So1 becomes less clear. A dominating genetic group in the northernmost stations remained. When excluding the fjord stations in a new STRUCTURE analysis the four genetic groups remained, and also the dominating genetic group in Troms and Finnmark remained (Figure 14c). There were no differences in the genetic structure in the coastal stations in South- and Mid-Norway.

The results suggest that there is good gene flow and little genetic structure along the coast, except for reduced gene flow and some isolation between Mid- and North-Norway, and between the coastal stations and some fjord populations. Removing a locus potentially under positive selection caused changes in genetic structure in Skagerrak and some fjord populations, suggesting possible effects of selection here.

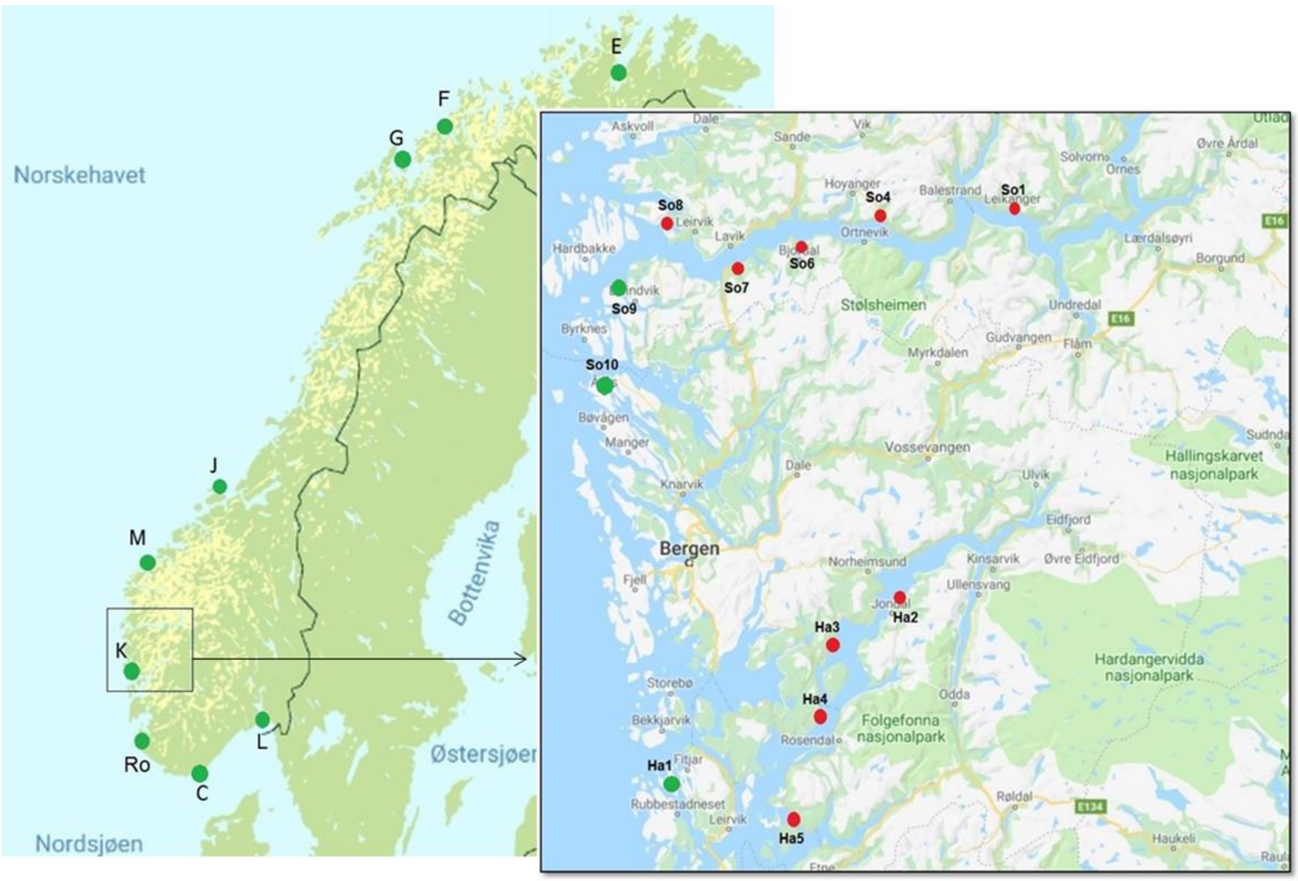


Figure 14. Stations with samples of *Saccharina latissima* (n=8-32) in Norway. A detailed sampling was done in Hardangerfjord (Ha) and Sognefjord (So). The coastal stations are shown with green symbols and the fjord stations with red symbols.

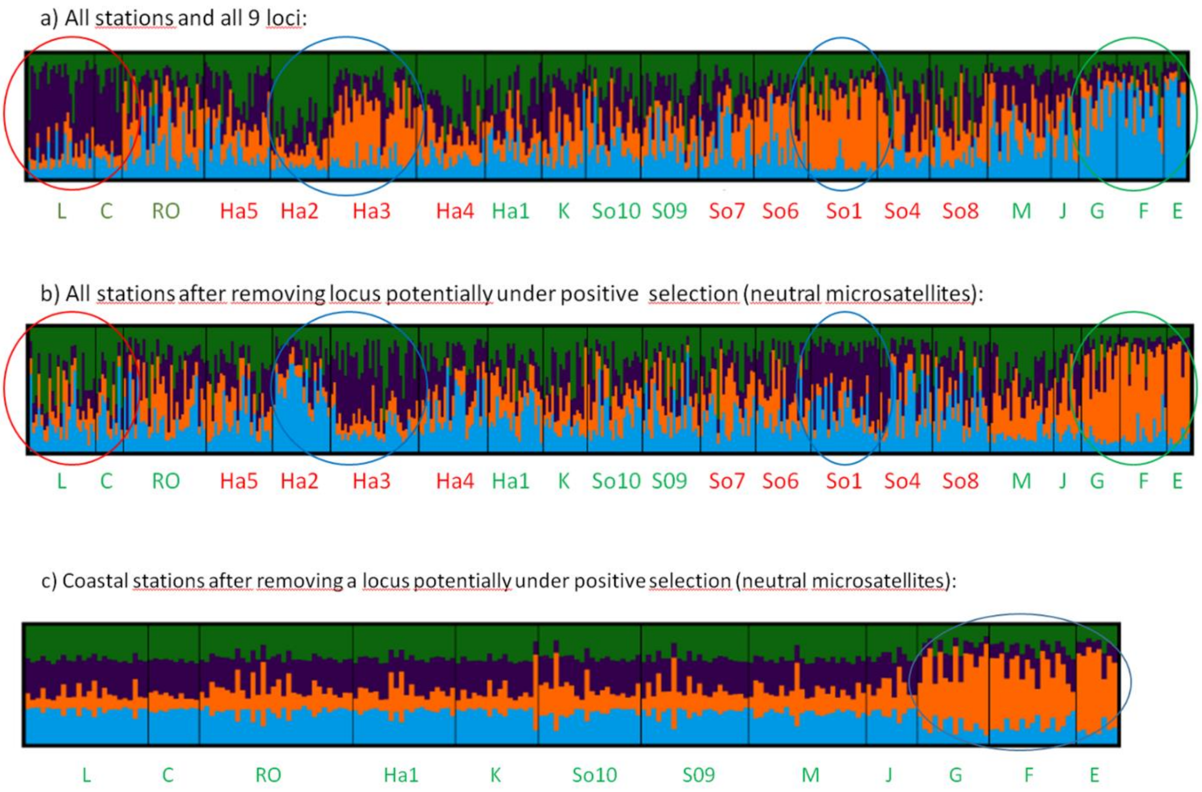


Figure 15. Different STRUCTURE analyses of *Saccharina latissima* from Norway; a) all stations and all microsatellites, b) all stations after removing microsatellite potentially under selection, c) coastal stations after removing microsatellite potentially under selection. The stations correspond to the labelling in Figure 2. Stations with a dominating genetic group are indicated by circles.

Additional population genetic studies were done in Scotland and Sweden. One of the aims of MACROSEA was to develop the use of Next Generation Sequencing and reduced representation sequencing in *S. latissima* for genotyping and the development of functional breeding markers. A method for a consistent and effective extraction of high quality gDNA for next generation sequencing of *S. latissima* was developed, resulting in two population libraries based on a ddRADseq protocol (Peterson et al. 2014), from a total of 400 individuals from 20 sampling stations across Scotland and Sweden (Figure 16A, C). A marker panel of 9,222 SNPs was developed for the west coast of Sweden, whilst a panel of 12,170 SNPs was developed for the west coast of Scotland. Analysis of the technical replicate samples showed a high degree of reproducibility between libraries demonstrating the consistency and reproducibility of the genotyping approach and allowing the direct comparison of the two regional data sets in further analyses (Figure 16).

Genomic marker sets for the Scottish and Swedish west coast populations were combined with empirical and modelled environmental datasets for the sampled stations, and hydrodynamic particle tracking models for each region. Results from Scotland highlighted strong population structuring along the west coast, with clear population breaks between northern and southern west coast populations, and between offshore Hebridean populations and northwest coastal populations (Figures 16B, 17). Genome-wide selection scans and environmental association scans identified a large number of loci putatively under selection or in association with environmental variables suggesting the importance of adaptation to local environment in sugar kelp across the region.

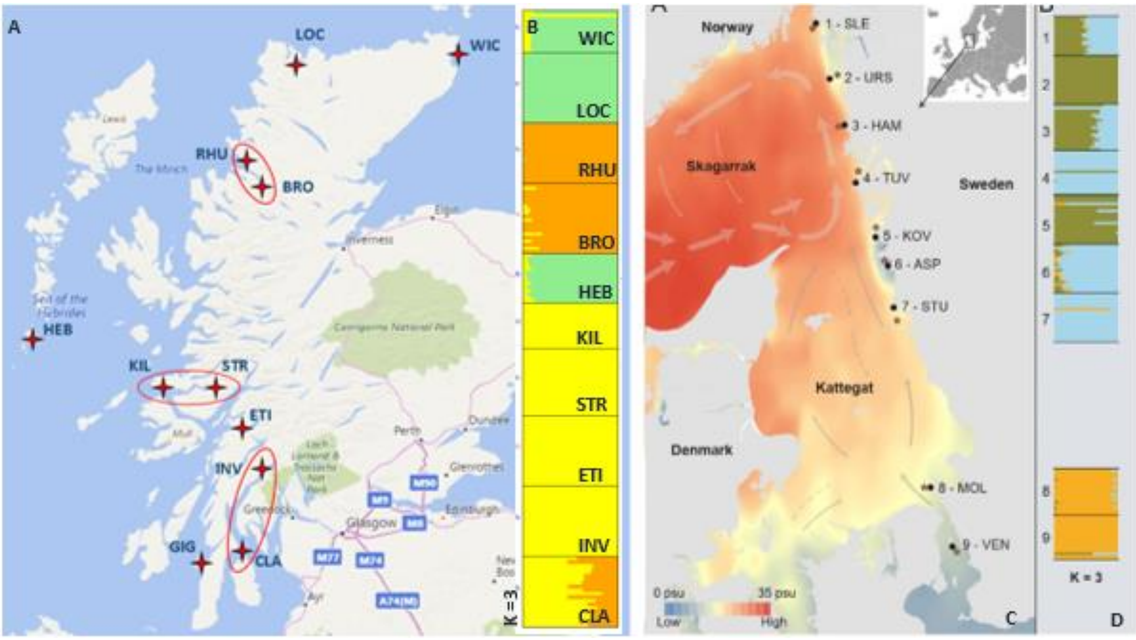


Figure 16. Population sampling maps for (A) Scotland and (C) Sweden. Red circles represent paired "Loch-to-Sea" stations. Arrows represent major and minor regional current systems in the Skagerrak and Kattegat (Svanson, 1972). The background heat map in (C) shows diffusion interpolation of minimum salinity values from the ICES data-base (1980-2018). Plots (B) and (D) show fast structure models of population structure for Scotland and Sweden respectively.

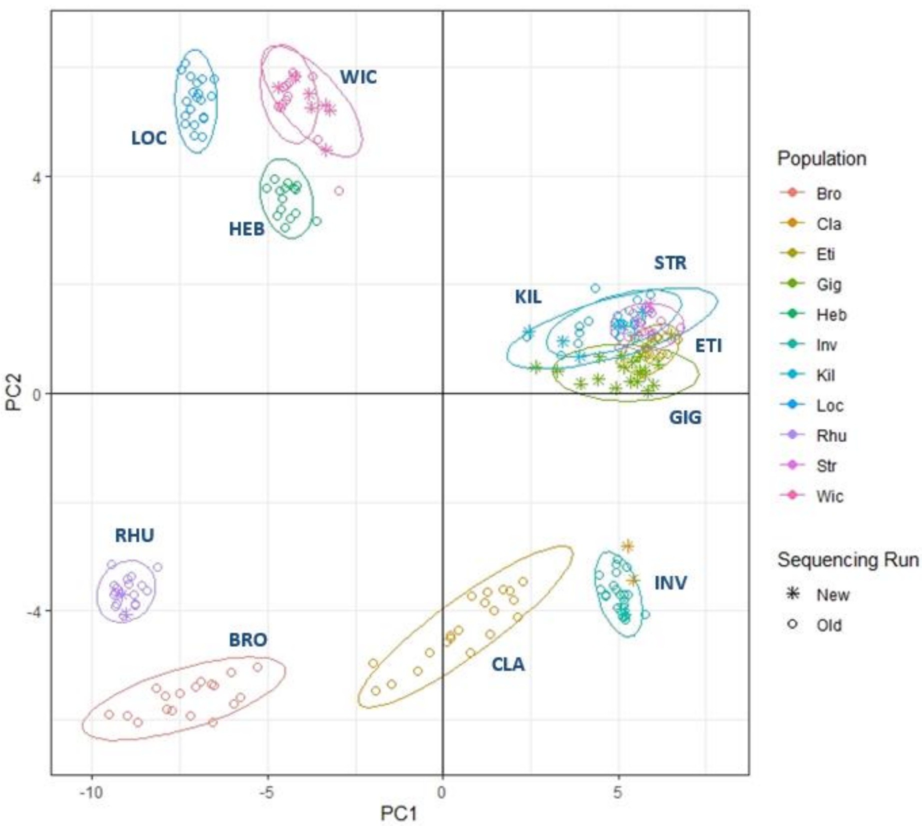


Figure 17. PCA of Scottish population genotypes from sequencing library 1 (old) and sequencing library 2 (new) showing population clustering along the west coast of Scotland. 95% confidence intervals are displayed by ellipses. Technical replicates from sequencing library 2 (new) are represented by stars. Sequencing library 1 samples are represented by circles.

Results from Sweden supported high levels of gene flow between populations, likely driven by strong directional currents in the region. Diversity and heterozygosity were much higher than in Scottish populations, reflecting the higher degree of connectivity and gene flow along the coastline (Thomson submitted). Nevertheless, genomic and hydrodynamic modelling results supported the presence of barriers to dispersal and gene flow between the southern and central Kattegat and between the Kattegat and Skagerrak (Figure 15D). Genome-wide selection scans and environmental association scans identified a large number of loci putatively under selection or in association with environmental variables, with the largest number being associated with mean sea temperature, mean summer sea temperature, mean chlorophyll and mean minimum salinity. Population differentiation and structure within adaptive loci showed much stronger differentiation, again supporting the roles of selection and local adaptation in driving population differentiation and diversification.

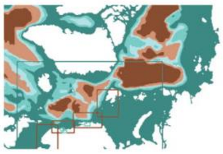
10.3.3 Publications

- Næss T (2018). Analyses of population genetics of *Saccharina latissima* (sugar kelp) in Norway. MSc-thesis, University of Bergen (UiB).
- Thomson A, Visch W, Jonsson P, Nylund GM, Pavia H, Stanley M. Drivers of local adaptation and connectivity in the sugar kelp*, Saccharina latissima* across the Baltic-North Sea environmental transition zone*. Submitted.*

Scientific publications in prep.

- Næss et al. Connections between cost and fjord populations of the kelp *Saccharina latissima* in Norway.
- Sjøtun et al. Functional gene mapping related to stress resilience and growth performance in cultivar strains.
- Thomson et al. Seascape genomics reveal strong adaptation and population structuring in sugar kelp across the heterogenous seascape of the Scottish west coast.
- Thomson et al. Comparative regional population genomics and adaptive ecological signatures of selection in the brown algae *Saccharina latissima*.

10.4 Marine Modelling



Lead: Senior scientist Dr Ole Jacob Broch, SINTEF Ocean
R&D: SINTEF Ocean, NTNU, APN, CU

Objectives

Develop model tools for site selection and prediction of biomass production and composition taking into account key biotic and abiotic environmental variables.

10.4.1 Kelp production potential in Norway

The kelp growth model for *S. latissima* was run coupled with the biology-physics ocean model system SINMOD (e.g. www.sintef.no/sinmod). The model was initialized with *S. latissima* in all model grid cells in September and February 2012-2015, and the model system was thus run for four entire cultivation cycles (September-July). The harvest was assumed to take place in the beginning of June. The total potential yields were integrated over all cultivation cycles and the water column down to 8 m depth. Model domains for the Norwegian coast of 800 m horizontal resolution were used. The results were normalized by dividing with the greatest potential yield over all the model domains, hence providing an "index" ranged from 0 (lowest potential yields) to 1 (highest potential yields). The index (Figure 18, *left panel*) thus tells us something about the basic potential for *S. latissima* cultivation, objectively, without considering cultivation technology and practices. It therefore makes sense to any compare different locations in the index map. Effects of integrating *S. latissima* with salmon farming to exploit the nutrient effluent (ammonium) has not been considered here. This an important subject that depends a lot on local conditions, the salmon production cycle etc, and requires high resolution modelling to avoid "artificial" (numeric) dilution of the released nutrients. A study on this has been made in (Fossberg et al. 2018).

Estimates for cultivation potential in terms of production yields for different regions of the Norwegian coast were also made. There was a clear North-South gradient in the *timing* of the maximum growth rates (up to 2 month differences in the timing of the maximum rates), results that have been partly confirmed by experiment (WP2, Forbord et al. 2020). The findings summarized here, along with further details and analyses, are published in (Broch et al. 2016, 2017, 2019). A simplified index for the production potential of *P. palmata* was also made (Figure 18, *right panel*). No actual growth model was used, but the relevant environmental variables (NO_3 concentrations, temperature and salinity) were scored based on their simulated values and available results on their importance for growth performance in *P. palmata*.

10.4.2 Updated growth model for *S. latissima*

Two important updates to the *S. latissima* growth model have been made, both with implications for calculations of biomass.

The first is the introduction of dynamically calculated weight:frond area-ratios. This is based on a form of allometric equation for the empirical weight:frond area relations in the MSc thesis at NTNU by Solveig Foldal (Foldal 2018). Previous versions of the model have used a fixed structural weight:unit area-ratio. (Note that only the *structural* weight:area ratio was previously assumed to be fixed. This provides a more detailed approach to calculating biomass in the model.

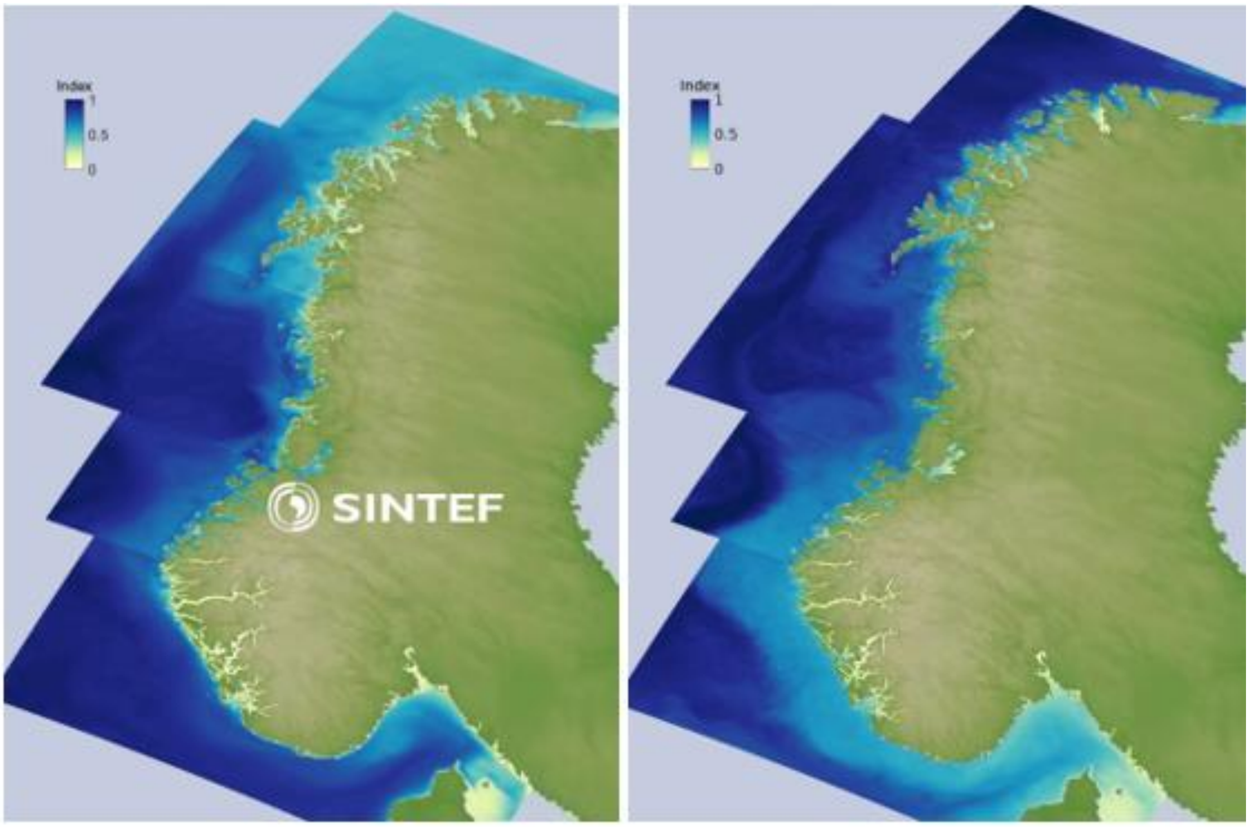


Figure 18. Index for the production potential for *S. latissima* (left) and *P. palmata* (right) along and outside the coast of Norway. The index for *S. latissima* is based on running the dynamical growth model coupled with the biology-physics ocean model system SINMOD for 4 years and 8 cultivation cycles and integrating the results. The index for *P. palmata* is based on only one season, and is an integrated scoring of environmental variables.

The second one is the introduction of a population model structure for cultivated kelp with density dependent mortality. The population structure involves the possibility of simulating not only single individuals, but a range of individuals with varying sizes and states within a single cultivation substrate (e.g. small rope segment). Mortality involves a base mortality (probability for dislodgement from the substrate) and further the potential for mechanical interactions with nearby individuals of smaller sizes. To wit, larger individuals bring smaller ones with them. The effect is a "natural" thinning out of the population: from a certain point on, increasing the seedling density will not increase the production.

Decision support tool

The results on the *S. latissima* production index are being made available online for use in googlemaps. Knowledge on suitable locations for kelp cultivation is much sought after by existing and prospective kelp farmers. Thus, the results may be used directly in siting decisions for kelp farmers and governance.

The population model structure is further important for another constraint to the kelp production potential which we describe next.

10.4.3 Light and nutrient shading

The denser the kelp culture (and natural strand), the greater the light shading. Oliver Evans, a MSc student in Akron, Ohio (through the collaboration with Shane Rogers/Clarkson University), developed and implemented an advanced model for light shading in a kelp culture (Evans 2018, Figure 19). This entailed developing a model for the density of kelp biomass in a culture. The light shading model itself was based on solving the radiative transfer function. The vector form is given by the following integro-differential equation:

$$\boldsymbol{\omega} \cdot \nabla L(\boldsymbol{x}, \boldsymbol{\omega}) + a(\boldsymbol{x}) L(\boldsymbol{x}, \boldsymbol{\omega}) = b \left(\int_{4\pi} \beta(\boldsymbol{\omega} \cdot \boldsymbol{\omega}') L(\boldsymbol{x}, \boldsymbol{\omega}') \, d\boldsymbol{\omega}' - L(\boldsymbol{x}, \boldsymbol{\omega}) \right) + \sigma(\boldsymbol{x}, \boldsymbol{\omega}).$$

Here, $L(x, \omega)$ denotes the radiance at the position x (a point in space) in the direction ω. We cannot go into the details of the solutions here. Through this equation it is possible to take into consideration different shapes, sizes, densities of kelp fronds, along with their individual light absorbance and calculate the effects on the light field and hence on the primary production of the culture.

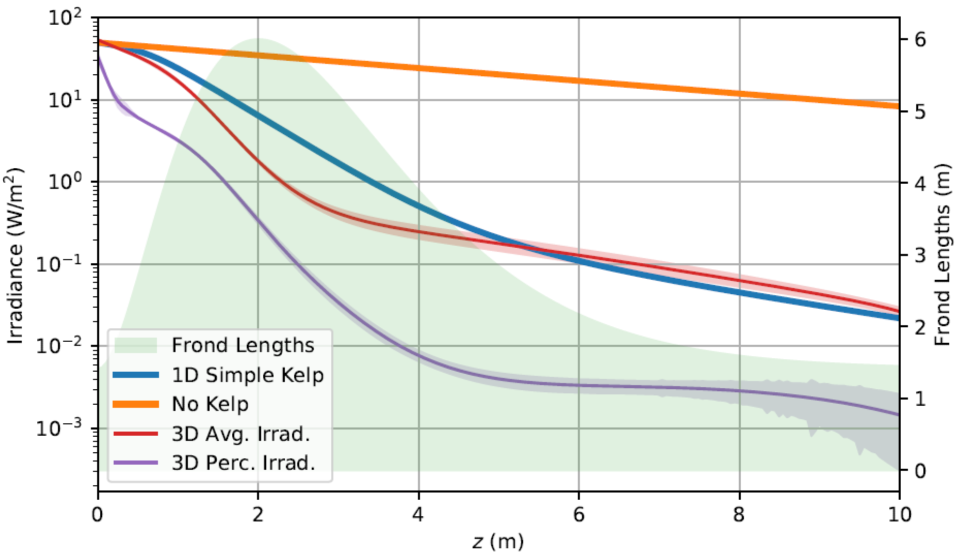


Figure 19. Illustration of the light shading by kelp fronds of different lengths. Average irradiance and perceived irradiance calculated with different implementations of shading models with realistic kelp growth. Frond lengths over depth are shown on the right-hand axis. Estimated discretization error is shown by error bands for perceived irradiance and average irradiance. Note the different scales in the two plots. The figure is taken from Oliver Evan's thesis. The caption is also from the thesis, and adapted by OJB.

10.4.4 Dynamical growth model for bryozoan fouling on kelp fronds

Biofouling is one of the most severe constraints for kelp aquaculture production in Norway (see S. Matssons's work in Chapter 6.2 Sea Cultivation). One of the most important taxa of fouling organisms is bryozoans. A simple dynamical growth model for bryozoans on kelp fronds was developed (Figure 20). The main state variable is the *fraction* of the frond covered. The model is based on the logistic equation: an exponential growth phase in the beginning, with reduced growth rates as the available substrate is limiting further growth (when the frond is nearly entirely encrusted, relative growth rates subside). The specific growth rate is calculated as a function of food (flagellates, provided by the SINMOD model system), temperature and salinity. The model was parametrized from available literature.

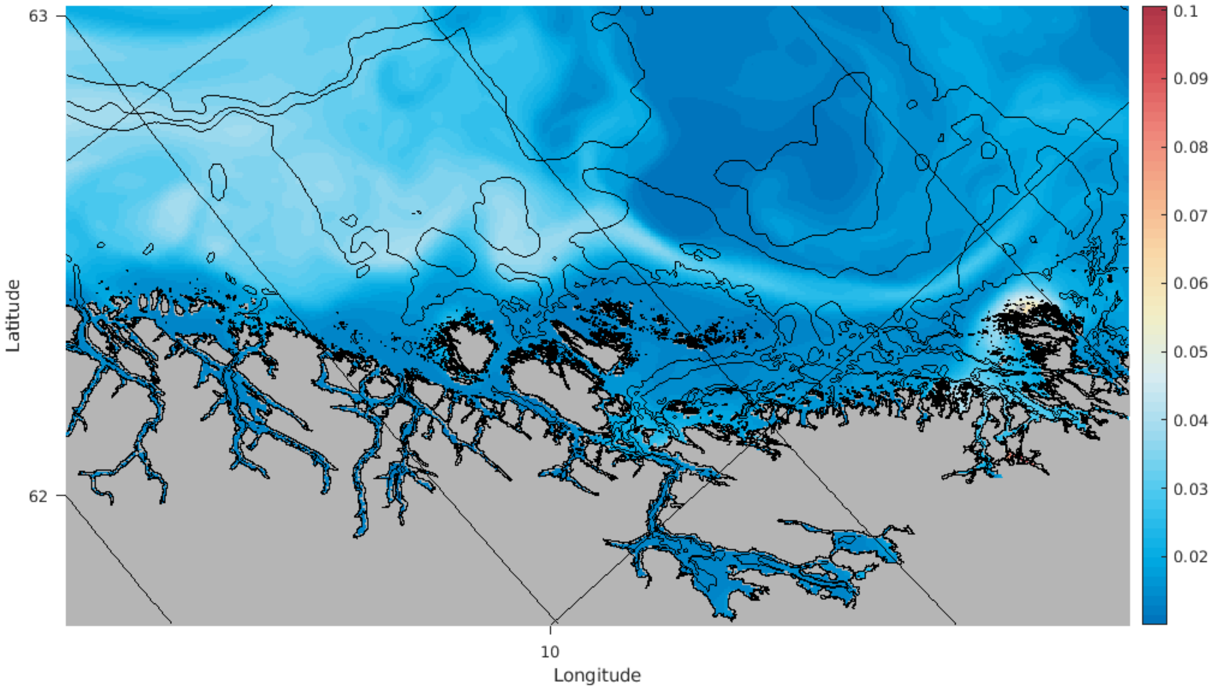


Figure 20. Simulated fraction of the kelp frond covered by bryozoans, early June. The colours indicate the fraction of the frond covered by bryozoans. The black curves are 200, 300 and 500 m isobaths. It may seem counter intuitive that fouling should be a problem at sea, where kelps do not grow naturally. It must be remembered that the model has been seeded with kelps everywhere in the model domain, and that the kelps have also been seeded with a start value at May 1. Hence the colours really represent the potential for bryozoan development rather than the risk of fouling. The model domain used here has 800 m horizontal resolution, and thus does not properly resolve the physics (or the biology) in narrow fjords.

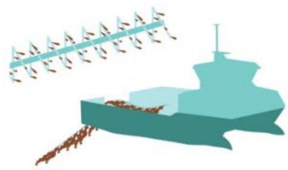
10.4.5 Publications

- Broch OJ, Alver MO, Bekkby T, Gundersen H, Forbord S, Handå A, Skjermo, J, Hancke K (2019). Kelp cultivation potential in coastal and offshore regions. *Frontiers in Marine Science.* doi.org/10.3389/fmars.2018.00529
- Broch OJ, Tiller R, Skjermo J, Handå A (2017). Potensialet for dyrking av makroalger i Trøndelag. SINTEF Ocean OC2017 – A200. ISBN 978-82-14-06099-7. https://sintef.brage.unit.no/sintef-xmlui/handle/11250/2457837
- Broch OJ, Skjermo J, Handå A (2016). The potential for large scale cultivation of macroalgae in Møre and Romsdal (in Norwegian: Potensialet for storskala dyrking av makroalger i Møre og Romsdal) SINTEF report. ISBN:978-82-14-06099-7. https://sintef.brage.unit.no/sintef-xmlui/handle/11250/2446958
- Evans, O. (2018) Modeling the light field in macroalgae aquaculture. MSc-thesis, The University of Akron.
- Foldal S (2018). Morfologiske relasjonar til dyrka *S. latissima* ved tre stasjonar langs Norskekysten. MSc-thesis, Norwegian University of Science and Technology (NTNU).
- Fossberg J, Forbord S, Broch OJ, Malzahn A, Jansen H, Handå A, Førde H, Bergvik M, Fleddum AL, Skjermo J, Olsen Y (2018). The Potential for Upscaling Kelp (*Saccharina latissima*) Cultivation in Salmon-Driven Integrated Multi-Trophic Aquaculture (IMTA). *Frontiers in Marine Science* 5:418. https://www.frontiersin.org/articles/10.3389/fmars.2018.00418/full

Scientific publications in prep.

- Broch OJ, Evans O, Foldal S, Forbord S, Rogers S, Handå A, Skjermo J, Olsen, Y, Matsson S et *al*. Constraints for large scale and industrial kelp cultivation.
- Skjermo J, Broch OJ et *al.* Biomass and composition of *S. latissima* in exposed, open IMTA systems.

10.5 Seedling, Deployment and Harvest Technology



Lead: Researcher Torfinn Solvang, SINTEF Ocean
R&D: SINTEF Ocean, NTNU

Objectives

Identify requirements and bottlenecks for industrial scale seedling production systems, and evaluate and develop concepts for deployment and harvest operations.

10.5.1 Industrial production line for seedlings

The report *Industrial production line for seedlings* (Alver 2019) summarizes an evaluation of the production process for macroalgae seedlings with regard to industrial scale production. For each part of the production process (Figure 21), the challenges and bottlenecks facing producers when increasing production scale has been identified, and a prioritized action plan suggested as a roadmap towards industrial production (Table 1), including process changes, technological developments and research needs. Most of the required actions goes beyond the scope of the MACROSEA project.

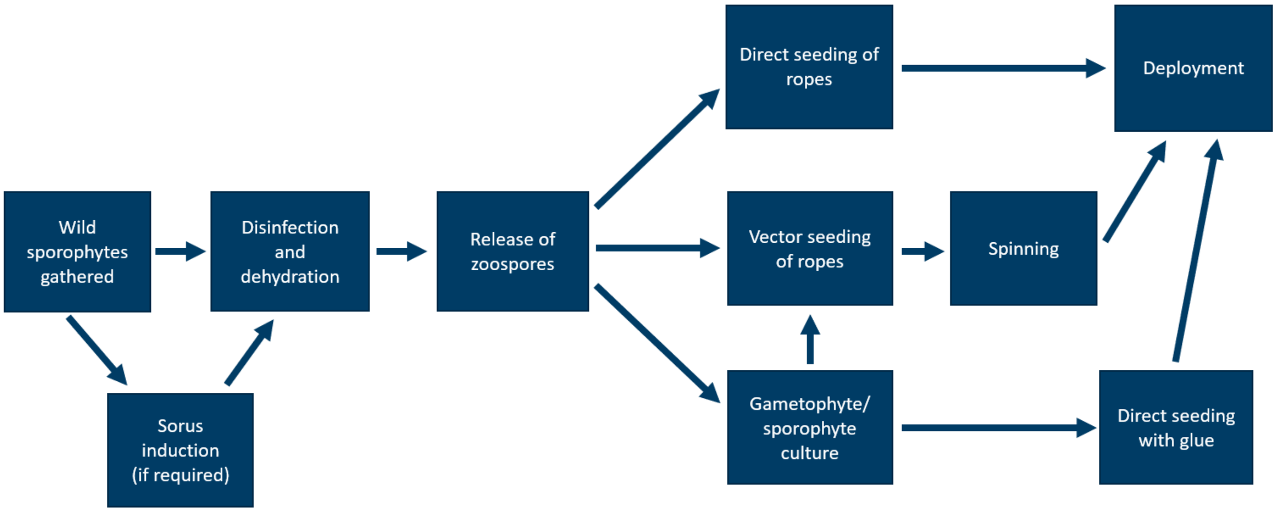


Figure 21. A process map of seedling production (Alver 2019).

A new concept for seaweed cultivation has been sketched up, the new design has a horizontal design is easy to scale in all dimensions and are ~50% more space efficient than the old vertical design. As well as space efficiency, the new design also eliminates the need for air bubbling for mixing the water around the spools due to strategically mounted nozzles on the water intake creating sufficient water mixture in the incubator. The new design also makes it easier for future robotization as the spools are placed in a slit in the incubator, bringing the autonomous seaweed cultivation system one step closer.

Table 1. Action plan for industrialization of seedling production (Alver 2019).

Priority / Time	MACROSEA	1-2 years	2-5 years	>5 years
High	Automated spinning.	Predictability of induction time. Biomass measurement of spores/gametophytes/sporophytes.	Optimization of sporophyte culture conditions and protocols. Gametophyte culture units. Design of seeding systems.	Eliminate wild gathered sporophytes. Detection of culture condition and contaminations.
Medium		Optimization of sorus induction. Sorus maturity assessment. Optimization of disinfection. Method to split large gametophytes. Control of rope seeding process. Detection of contaminants on substrate. Monitoring of seedling density on substrate. Direct seeding method without glue. Quality assessment of deployed seedlings.	Predictability of spore count from sorus induction. Predictability of size and density of seedlings on rope. Conditions to ensure that seedlings are well attached to substrate. Eliminate pre-seeding of substrates. Protocols and products for direct seeding with glue.	
Low		Protocols for repeated use of sorus for spore release. Removing gametophytes/sporophytes from container walls.		

10.5.2 Proof of concept on seeding systems

Standardization of production methods to improve quality control and predictability of produced quantity of seaweed biomass is needed for upscaling to industrial production volumes of macroalgae in high-cost countries, e.g. in Western Europe (Alver et al 2018b). Monitoring, automation and control techniques are necessary to replace manpower. Forbord et al. (2019) demonstrates a possible first step through the measurement of the substrate coverage as a form of early-stage control of the seedling quality and expected quantity of produced biomass.

A machine vision system was developed in order to quantify the number of seedlings covering twine substrate (Figure 22): Images were collected 2 days prior to deployment at sea for later processing. Cylinders with twine substrate were placed in a small water-filled glass tank for depiction using a DSLR camera and a macro lens. A LED ring light was used for even illumination in a brightfield setup with the lens positioned inside the ring light. Each substrate cylinder was depicted at three distinct locations, resulting in three images per substrate. Software was developed in LabVIEW which extracted the saturation color plane to identify seedlings on the white substrate. Using the saturation color plane is a robust method of segmenting the growth from the cultivation substrate. The output values were normalized to a percentage, where 0% were a clean, white substrate and 100% a substrate completely covered by sporophytes (no substrate-colored pixels visible).

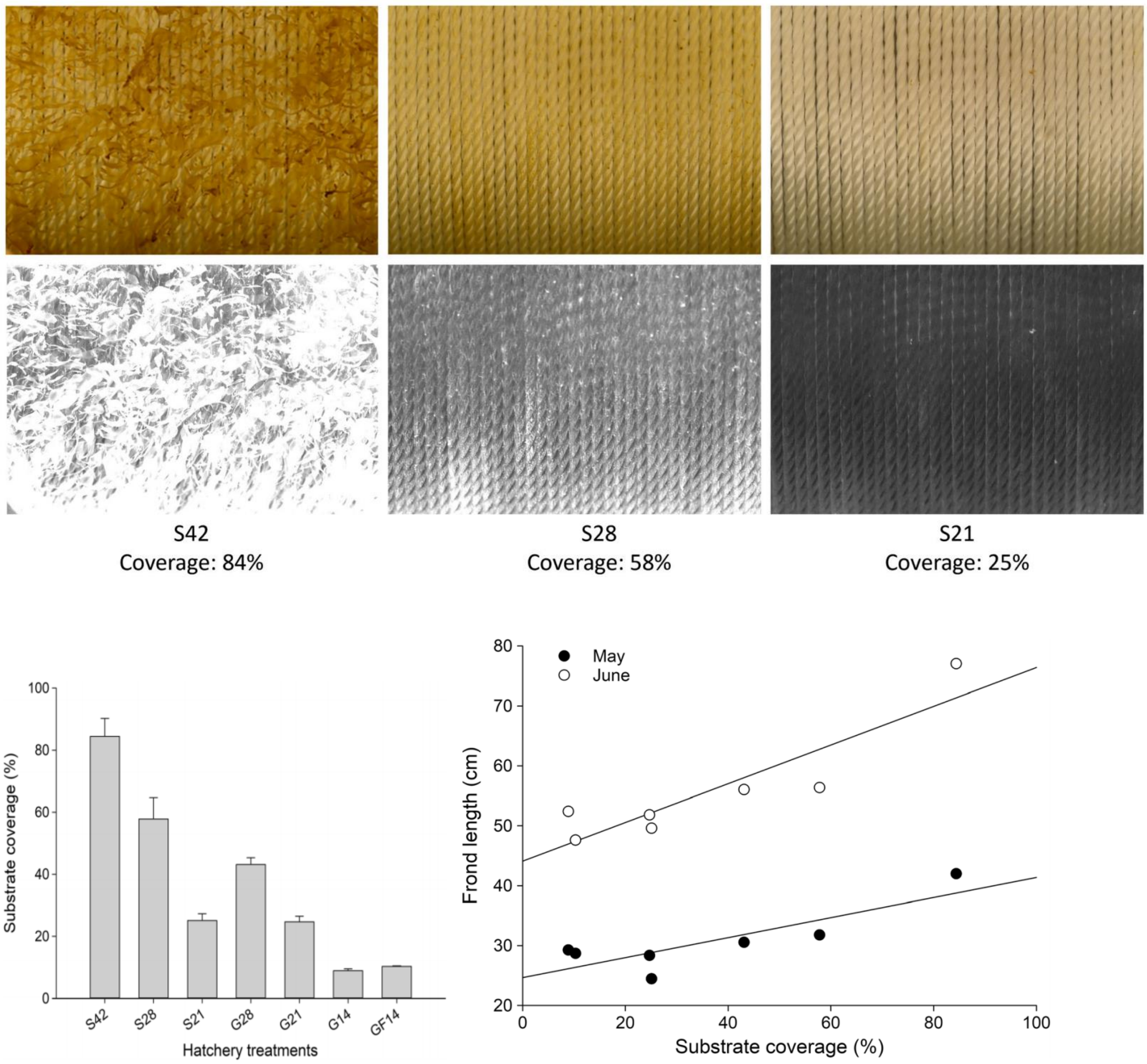


Figure 22. Examples of color images of substrate (TOP) and corresponding saturation image planes (MID). Left treatment had an average substrate coverage of 84%, mid treatment 58% and right treatment 25%. BOTTOM: The relationship between mean frond length and the substrate coverage before deployment revealed a strong positive correlation for both evaluation months (Forbord et al., 2019).

The method makes processing of a large number of images possible with little effort, compared to manual counting/analysis of the substrate itself or images of it. A similar technique has been used to monitor the growth of gametophytes and sporophytes (Duarte 2017), and showed an exponential growth 14 days in the trail, and showed a good relation to the manual, traditional method of counting and length measurements.

The process of preparing seeded carrier rope for deployment consists of three process steps (Alver et al 2018b): Preparation of spools with twine, seeding of twine, and spinning of twine onto carrier rope. The technology for each of these steps as demonstrated at SINTEF Sealab is described in the following sections. The spools need to be prepared by spinning the twine to form a tight layer around the spool cylinder. To automate this task, a device has been developed in collaboration with the mechanical workshop M-Tech that has a capacity to prepare two spools in parallel (Figure 23). The twine is initially fastened at the bottom end of the empty spools, and the machine will automatically spin the twine tightly around the spools, to the top, and stop when finished. The spinning process takes approximately 90 seconds.

A new concept for seaweed cultivation has been sketched up, the new design has a horizontal design is easy to scale in all dimensions and is volume-efficient (Figure 24). As well as space efficiency, the new design also eliminates the need for air bubbling for mixing the water around the spools due to strategically mounted nozzles on the water intake creating sufficient water mixture in the incubator. The new design also makes it easier for future robotization as the spools are placed in a slit in the incubator, bringing the autonomous seaweed cultivation system one step closer.

When seedlings are ready for deployment, the seeded twine needs to be fastened to the carrier rope – either before or during the deployment process. A good way to fasten the twine is by wrapping it around the carrier rope, and fastening it at intervals of a few m, but the process of wrapping the twine is very time consuming if done manually. A machine referred to as a "seaweed spinner" has been developed (also in collaboration with the M-Tech workshop) to automate the wrapping of twine at a rapid rate (Figure 25). The seaweed spinner allows the carrier rope to be inserted radially (from the top of the machine), without requiring the end of the rope to be available (i.e. the rope can be inserted into the machine even while fastened at both ends). Spools of seeded twine are loaded into the machine, and easily replaced. During operation, the carrier rope is fed through the machine, and the spool is rotated around the rope at a speed coordinated to wrap the twine at a constant distance per round trip. The machine processes 110m carrying rope in 14min (8.2s / m), using 150m substrate. This yields 430m on-spun rope per hour, or 3.5km on an 8h working day.

Figure 23. On-spinning prototype of substrate line on plastic cylinders. The cylinders are later placed bioreactors in the lab for the growth-phase

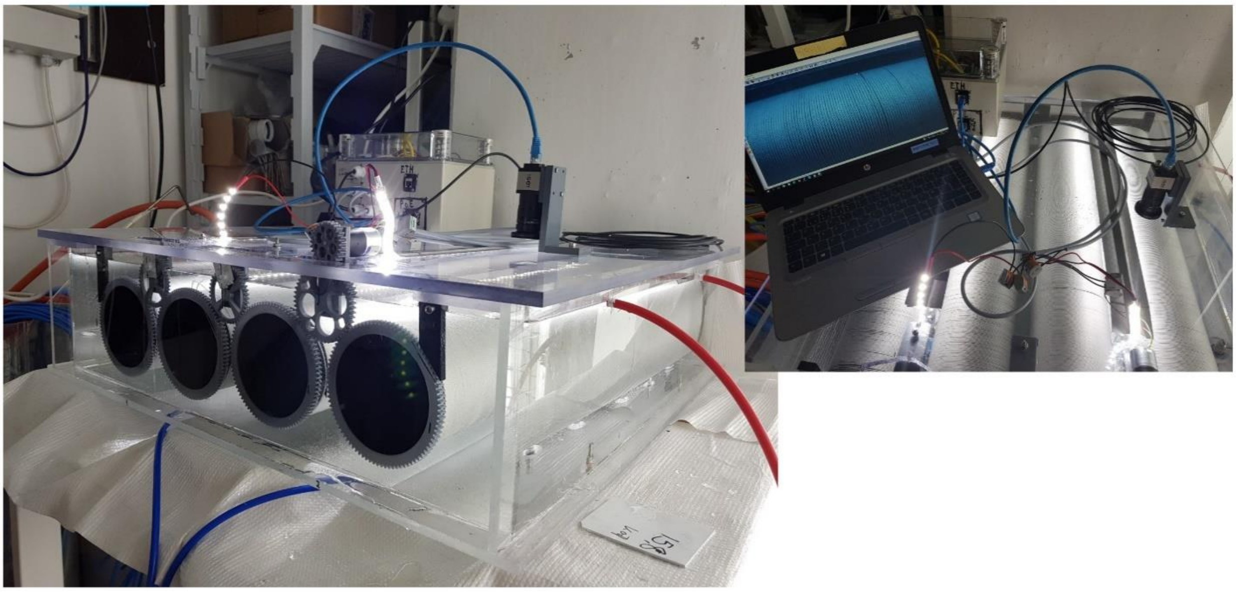


Figure 24. New concept for compact seedling cultivation, including robotized cylinder rotation and use of machine Vision.

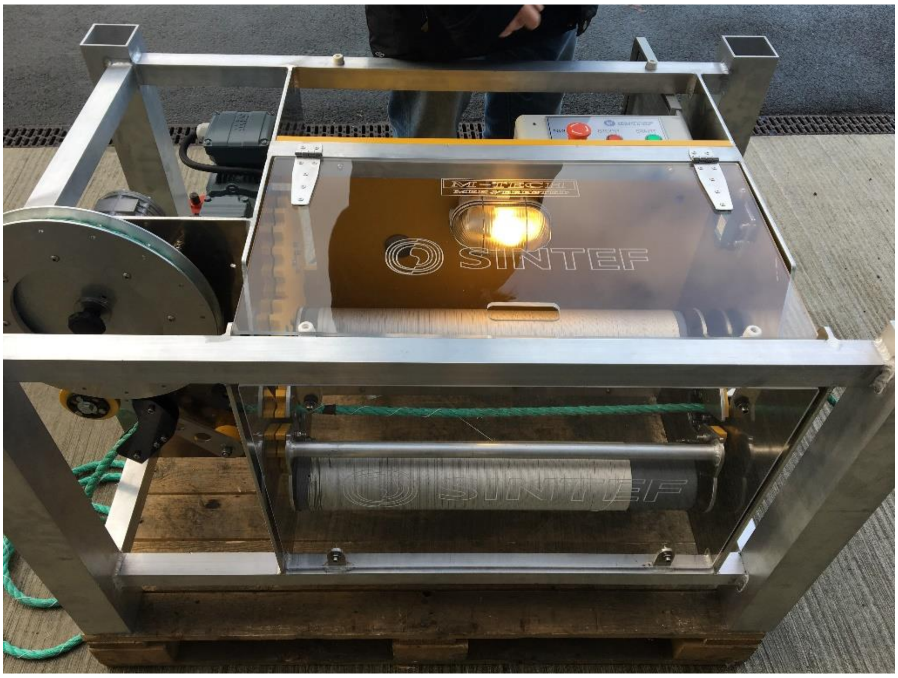


Figure 25. The seaweed spinner, developed in collaboration with M-Tech, allows spinning of seeded twine around a carrier rope.

10.5.3 State of the art on automatic deployment and harvesting technology

To achieve efficient large-scale cultivation, development of cultivation technology is an important component, and in MACROSEA the objective is to identify requirements and bottlenecks for industrial scale seedling production systems and evaluate and develop concepts for deployment and harvest operations. In order to understand the limitations and potential of today's cultivation methods, an overview of the methods used by today's producers has been sought. A literature survey was carried out, yielding remarkably few scientific publications covering cultivation technology. The opinions of producers on future challenges and bottlenecks was requested through a questionnaire and the results are published in the report *State of the art Seedling, Deployment and Harvest technology* (Alver et al. 2018a).

10.5.4 Design study and concepts for deployment and harvesting

Automation is a key component to develop large scale macroalgae cultivation, and it is important that a new cultivation system is designed with automation in mind. An ideal situation is if all the steps in the cultivation process are automated. To ensure that deployment and harvesting happens at the right conditions, monitoring of the biomass is a viable part of the concept. The concept should be easy to up-scale. Another important feature to allow up-scaling is standardization. Equipment used on one unit at one location should be applicable at another unit at another location. In order to automate, problems should be simplified, and standardization is the next natural step. Standard solutions give standard equipment with possibilities of the use of modules, scaling, and redundancy.

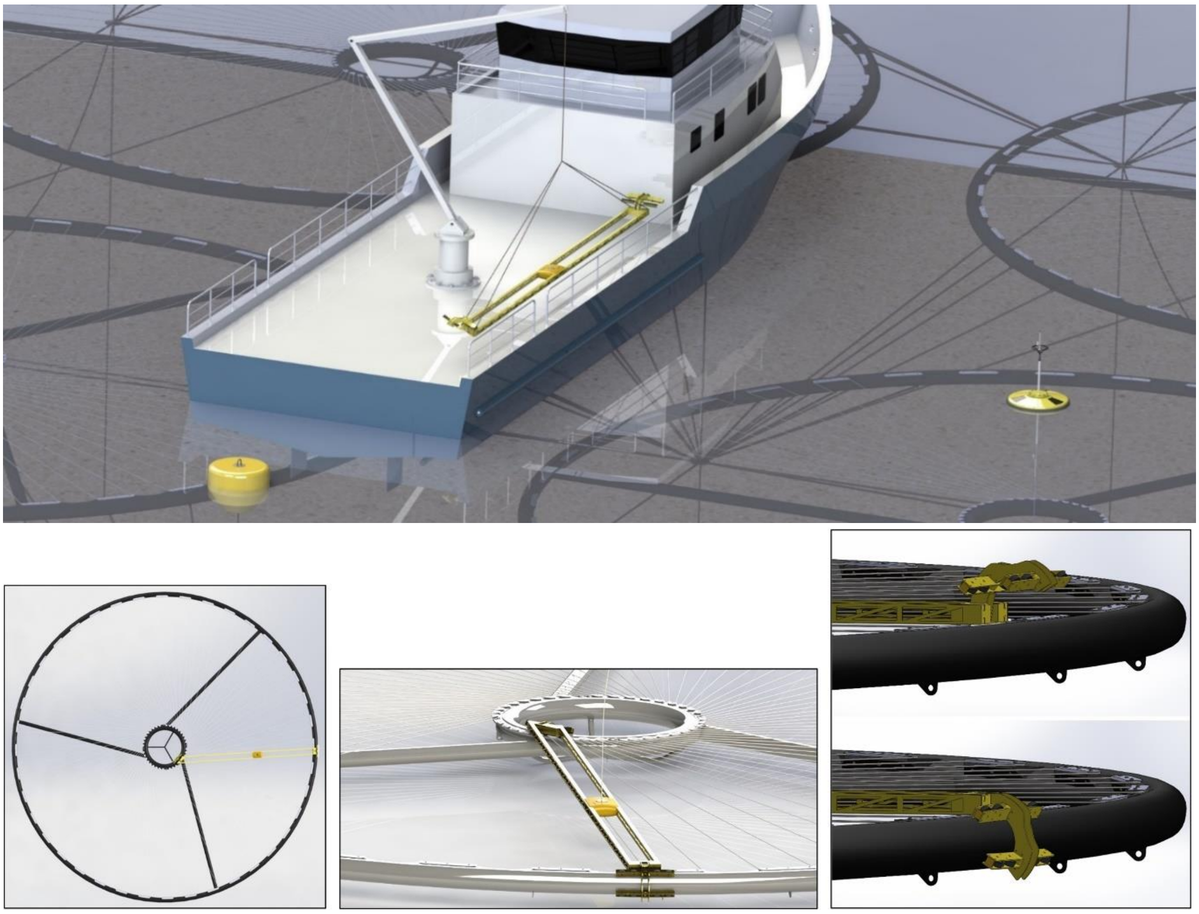


Figure 26. The concept SPOKe: Standardized production of kelp encompasses module-based farm design, robotic tools and instrumentation in buoys (Bale 2017).

After initial design studies (Dybvik 2016), The work in MACROSEA resulted in the suggested concept SPOKe: Standardized production of kelp (Figure 26, Bale 2017). The concept encompasses circular modules, to allow for both an easy platform for automation and being sturdy in rough conditions. The modules can make farm scaling easier. A gantry robot on rails is used to interact with the SPOKe modules. The robot might be equipped with different tools depending on the task; mounting or unmounting the substrate or harvesting (Wiik 2018). The idea of having few service vessels and specialized tools serving several modules / farms is inherited from salmon farming. The use of HDPE tubes in the construction is also proposed in SPOKe; this is an already proven choice which also allows for possible trading benefits from the salmon industry.

The concept is described in the report *Development of area efficient and standardized structures for large-scale macroalgae cultivation* (Bale 2017). The CAD drawings of the concept was also used to produce a VR demonstration and an animation, available at www.macrosea.no: https://youtu.be/oEbBfFy2H2w. The VR demo and a quadrant prototype, demonstrating the principle for control of the gantry robot (developed as a M.Sc. Thesis at dept. of engineering cybernetics in collaboration with SINTEF, Eggesvik 2019ab) are available for display at SINTEF Ocean.

10.5.5 Publications

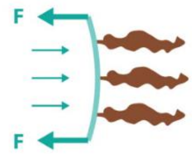
- Alver, MO (2019) Industrial production line for seedlings. SINTEF-report. ISBN: 978-82-14-06847-4. https://sintef.brage.unit.no/sintef-xmlui/handle/11250/2582466
- Alver MO, Solvang T, Dybvik H (2018a) State of the art Seedling, Deployment and Harvest technology. SINTEF-report. ISBN: 978-82-14-06931-0. https://sintef.brage.unit.no/sintef-xmlui/handle/11250/2478264
- Alver MO, Solvang T., Kvæstad, B (2018b) Proof of concept on seeding systems. SINTEF-report. ISBN: 978-82-14-06664-7. https://sintef.brage.unit.no/sintef-xmlui/handle/11250/2564187
- Bale ES (2017) Development of area efficient and standardized structures for large-scale macroalgae cultivation. SINTEF summer student report. https://www.sintef.no/globalassets/sintef-ocean/macrosea-prosjektweb/development-of-area-efficient-and-standardized-structures-for-large-scale-macroalgae-cultivation.pdf
- Duarte A (2017). Optimization of seedling production using vegetative gametophytes of *Alaria esculenta*. MSc-thesis, University of Porto.
- Dybvik H (2016) Concept development for macroalgae seeding, deployment and harvesting. SINTEF summer student report. https://www.sintef.no/globalassets/sintef-ocean/macrosea-prosjektweb/concept-development-for-macroalgae-seeding-deployment-and-harvesting.pdf
- Eggesvik AT (2019a) Robot for automated seaweed deployment and harvesting. MSc-thesis, Norwegian University of Science and Technology (NTNU).
- Eggesvik AT (2019b) SPOKe prototype. SINTEF summer student report.
- Forbord S, Steinhovden KB, Solvang T, Handå A, Skjermo J., (2019) Effect of seeding methods and hatchery period on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case-study. *Journal of Applied Phycology, DOI: 10.1007/s10811-019-01936-0.*
- Wiik I (2018) Concept development of details for macroalgae cultivation. SINTEF summer student report.

Scientific publications in prep.

- Solvang, T, Alver, MO, Bale, ES.: *Automation concepts for industrial scale production*

10.6 Sea Farms

Lead: Scientist Per Christian Endresen, SINTEF Ocean
R&D: NTNU, SINTEF, APN, NIVA, UiO, UiT, AaU, ALL industry partners



Objectives

To develop numerical tools for evaluation of new farm concepts or effective macroalgae biomass production.

10.6.1 Drag forces and deformation on different sea farm systems with different macroalgal densities and stadiums of growth.

The focus of this work has been forces on seaweed farms containing sugar kelp (*Saccharina latissima*). For design and dimensioning of seaweed farms at sea knowledge is needed regarding the forces exerted on the farm due to the kelp being exposed to current and waves. This knowledge need has resulted in a series of experiments and sea trials during the period 2016 to 2018 in order to obtain data on and knowledge of how currents affect the kelp and thus exert forces on seaweed farms. A study on mechanical properties of sugar kelp, a master thesis and a scientific publication have been produced. More knowledge on forces on sugar kelp from waves is needed.

Master thesis and study on mechanical properties
The study of mechanical properties and morphology of sugar kelp was done as part of a SINTEF summer student program in 2016 (Norvik 2016), and towing experiments with artificial sugar kelp were conducted at NTNU in 2017 as a part of a master thesis (Norvik 2017). Drag forces were measured and the movement of the models was recorded by an underwater camera.

Sea trials
Sea trials were performed in 2016 and 2017. In these tests ropes with cultivated sugar kelp was mounted to a test rig and either pushed by or towed behind a vessel. Forces in the towing direction and the velocity of the vessel were measured. Although the tests were performed in relatively sheltered areas it was evident that some of the results were affected by parameters (wind and waves) that could not be controlled or measured reliably.

Towing tests (2018)
The goal of the experiments conducted in 2018 was to obtain reliable data on forces due to water current on ropes grown with sugar kelp. When conducting experiments control of the environment in which the tests are performed is important in order to achieve results that are not affected significantly by parameters that you are not able to measure reliably. Tests of for instance nets and net cages for fish farming are usually performed in flume tanks, towing tanks or ocean basins as this will ensure a controlled environment in terms of water currents, waves and wind. Testing in specialized facilities usually also means larger availability and flexibility with regards to sensors and hence a wider range of what parameters can be measured, and it usually produces more accurate measurements than can be obtained in the field. However, most facilities use fresh water, sometimes chlorinated, and facilities often do not permit biological material in their tanks. Sugar kelp will deteriorate under these conditions and its mechanical properties will change which may affect test results. As a result of these considerations the tests in 2018 were conducted in the "Subsea Test Centre" (OceanTech, formerly run by Linjebygg/PREZIOSO Linjebygg) in Trondheim, Norway. The facility has a dock

(containing sea water from the fjord). It is closed off from the fjord during testing and has negligible water movement and circulation during tests, while it is partially sheltered from wind. For the experiments a specially built test rig and raft with suitable measuring equipment was designed and built. The tests themselves involved mounting ropes with cultivated sugar kelp to the rig and towing them through the water with a variety of constant velocities, thus simulating water current. The total force on the ropes as well as the tension in the ropes were measured. The ropes were mounted horizontally 1.1 m below the surface and perpendicular to the simulated current, i.e. the orientation for which the largest loads on the ropes were expected. Four ropes with a variety in growth density, specimen lengths and biomass were tested. Test results show a consistent correlation between the biomass, average length of the plants and the experiences drag forces on the ropes. Indications of a correlation between the total blade area and average lengths of the plants were also shown. The main results are given in Figure 27 for the four ropes tested (denoted n1, n2, n3 and n4). The average biomass per meter, plants per meter, average length of blade and stipe and average width of the blades are (3.97kgm^{-1}, 51, 0.89m, 0.056m, 0.229m) for n1, (4.98kgm^{-1}, 71, 0.774m, 0.115m, 0.271m) for n2, (2.78kgm$^{-1,}$ 205, 0.597m, 0.058m, 0.117m) for n3 and (3.03kgm^{-1}, 182, 0.686m, 0.061m, 0.120m) for n4 (Endresen et al 2019). The ropes with sugar kelp were provided by Seaweed Energy Solutions AS, and collected from their site close to Frøya, Norway. As the sugar kelp was collected from a single site, there was little variation in morphology of the kelp. The shape of the plants will affect the loading.

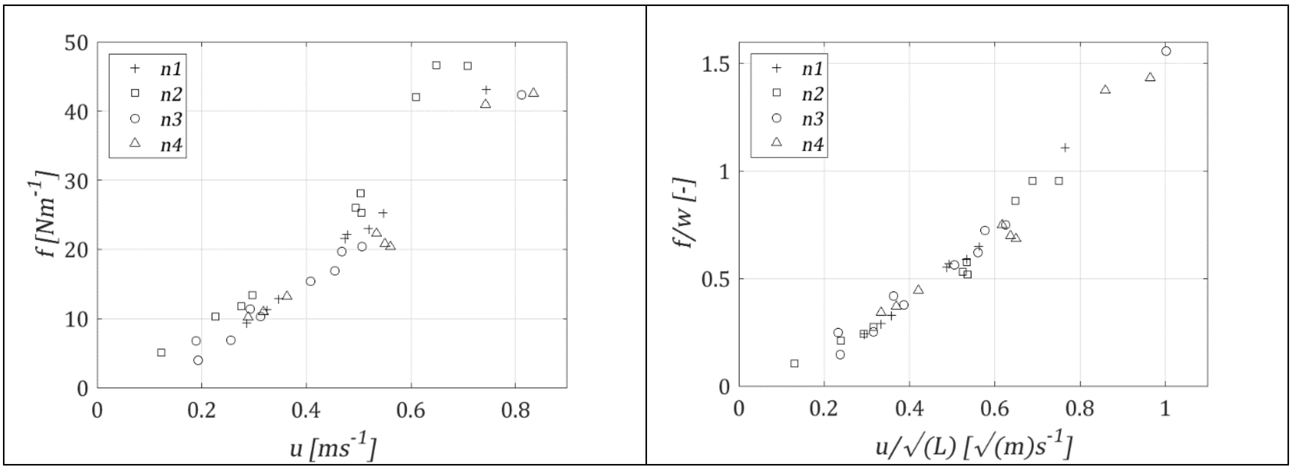


Figure 27. Force per meter of rope for four kelp-ropes as a function of current velocity (left) and forces per meter and biomass as a function of velocity and average specimen length (left). Figure from Endresen et al. (2019).

10.6.2 Numerical tool to simulate forces and deformations of macroalgal sea farms in different sea states.

Results from the experiments will enable more precise modelling and design of seaweed farms in terms of design (farm layout) and dimensioning of components in order to withstand environmental forces (waves, currents). The correlations between kelp parameters (biomass/growth) and current velocities can be implemented into suitable numerical codes for simulations of seaweed farms as has been done by SINTEF Ocean for design of a seaweed farm planned to be deployed on an exposed site in Norway during 2020.

Although the tests were performed in steady flow, the results may to some degree be used for modelling of forces on cultivated sugar kelp in waves. However, for this application uncertainties exists since effects of waves on the kelp (acceleration, mass and added mass) have not been tested. Proposed future work in order to increase knowledge and precision of numerical models is experiments on sugar kelp with waves or with a setup simulating the effect of waves. Experiments with ropes inclined to the current or waves and measurements of the effect from the kelp on the surrounding water velocities will further help to develop methods for design and dimensioning of seaweed farms. The latter effect will also be important for evaluation of growth conditions. Tests including a larger variety in kelp growth and morphology are needed to increase knowledge.

10.6.3 Publications

- Endresen PC, Norvik C, Kristiansen D, Birkevold J, Volent Z (2019). Current Induced Drag Forces on Endresen, Per Christian, Norvik, Carina, Kristiansen, David, Birkevold, Jens, and Volent, Zsolt. "Current Induced Drag Forces on Cultivated Sugar Kelp." *Proceedings of the ASME 2019 38th International Conference on Ocean, Offshore and Arctic Engineering*. *Volume 6: Ocean Space Utilization*. Glasgow, Scotland, UK. June 9–14, 2019. V006T05A007. ASME. https://doi.org/10.1115/OMAE2019-96375
- Norvik C (2017). Design of Artificial Seaweeds for Assessment of Hydrodynamic Properties of Seaweed Farms. MSc-thesis, Norwegian University of Science and Technology (NTNU).
- Norvik C (2016) "Design of artificial seaweeds for assessment of hydrodynamic properties of seaweed farms". SINTEF summer student report.

11 Research Needs

11.1 Seedling Biology

- Cultivation technology to enable mass production of *P. palmata* seedlings on substrate including a program for controlled sorus induction, spore release, seeding, seedlings incubation condition, contamination control and cryopreservation.
- Open sea aquaculture of *P.palmata* for evaluation of environmental conditions and seasonal effects.
- Better understanding of the seasonal and geographic impact for fertility in *A. esculenta* at different sites along the Norwegian coast, better methods for sorus disinfection and for contamination control of gametophyte cultures.

11.2 Sea Cultivation

- Due to local variations, pilot investigations should be undertaken to determine the suitability of a given potential farm location, by generating knowledge on suitable cultivation depths and the best deployment and harvesting windows.
- Registration of environmental data (salinity, currents, nutrients) during field cultivation experiments to support the growth and biochemical results.
- Field cultivation experiments further north than 69 °N to look at the impact of high latitude on chemical content, growth and biofouling.
- Controlled laboratory experiments with environmental factors influencing biofouling.
- Laboratory experiments testing effect of environmental factors and genes on seaweed defense and/or resistance to biofouling.
- Chemical content on main fouling species as well as product development of end- products including biofouling.
- Large scale sea cultivation of *P. palmata* with subsequent chemical analysis.

11.3 Genetics

- Baseline studies of population genetics of all macroalgae under consideration for cultivation.
- Studies of intrinsic and extrinsic factors influencing gene spreading in macroalgae.
- Population genetic studies linking functional traits to genotypes.
- In situ studies of “crop-to-wild” spread of genes from sea farms to local populations.

11.4 Marine Modelling

- Development of more detailed models for the content of interesting/valuable compounds (e.g. proteins, polyphenols, pigments, carbohydrates) in kelps and how these depend on and arise as interactions between external conditions, metabolism, and growth.
- Development of more detailed models for the content and accumulation of potentially unwanted components (e.g. iodine, heavy metals, environmental toxins) in kelps.
- Linking model parameters to genetics studies.
- More detailed modelling of fouling on kelps (also including other organisms such as hydroids). In particular, the spatiotemporal patterns of first appearance of fouling and dispersal of the larval stages of the fouling organisms.

11.5 Seedling, Deployment and Harvest Technology

- Seeding: Direct seeding is limited today to a few vendors using protected methods and materials to attach seedlings or sporophytes to the substrate. Knowledge sharing on the methods may speed up automation and further development of optimal techniques, materials and equipment.
- Further development of instrumentation methods for quantification of spore density and gametophyte biomass are feasible and use of machine learning (ML) should be investigated. Using thresholding on the saturation color plane proved a robust method, but ML might improve the identification of kelp from other features in the image further. Many ML-techniques depend on the structural features of the objects, which might be an advantage in this case. Teaching a ML-network for object detection the difference between healthy kelp, unwanted growth and other categories may be a step forward in the use of machine vision for kelp measurements.
- SPOKe-specific: Principles of control and selection of mechanisms and actuation for the gantry robot and its movement around the submerged modules is of essence. Solutions for relative positioning measurements between the robot and the modules as it moves and deploys from the vessel.
- Optimal substrate geometry. This has a great impact on lab and farm design, and also deployment and harvesting equipment. In the proposed concept SPOKe, vector seeding is the basis, but the concept is not limited to 1D substrates. In order to select a geometry, the whole production line must be considered.
- Harvesting: The work within MACROSEA produced concepts for harvesting but did not encompass development and automation on equipment. This task is heavily dependent on substrate geometry and farm design, and has to be developed in detail subsequently of these tasks.

11.6 Sea Farms

- Experiments yielding data for a larger variety in growth density, specimen growth and morphology.
- Experiments with growth ropes inclined to the loads (current and waves).
- Experiments to determine forces on sugar kelp ropes when exposed to waves.
- Experiments measuring the effect on the surrounding water flow from the kelp.

11.7 A National Technology Action Platform for Automated Seaweed Production

In order to accelerate a successful upscaling of large scale macroalgae cultivation and create a new bio-marine industry in Norway, a national research infrastructure (Figure 28) should be established to serve as a hub for innovative industry and research groups targeting the new, blue bioeconomy and a climate friendly low-carbon society.

With a successful industrialization of macroalgae cultivation Norway can establish a future, marine feedstock bypassing competition with land-based agricultural resources and at the same time contribute to the replacement of fossil resources. Industrialization of a nearly carbon-neutral, non-fed cultivation of macroalgae will contribute to an environmentally friendly development of the Norwegian aquaculture sector and to a sustainable exploitation of the ocean.

Figure 28. The knowledge basis created in MACROSEA suggests that a national research infrastructure on automation and standardisation of production equipment could accelerate the development of industrial macroalgae cultivation in Norway.

12 Scientific Project Participants

Table 2. Scientific project participants in the MACROSEA-project.

Scientific Project Participants			
1	Jorunn Skjermo	SINTEF Ocean	**WP1 leader** Co-supervisor Silje Forbord
2	Stein Fredriksen	University of Oslo	WP1 partner
3	Yngvar Olsen	NTNU	**WP2 leader** Supervisor Silje Forbord and Saifullah Co-supervisor Siv Anina Etter
4	Anette Brun	Aarhus University	WP2 partner
5	Kristine Steinhovden	SINTEF Ocean	WP1 and WP2
6	Kjersti Sjøtun	UiB	**WP3 leader** Co-supervisor Alexander Thompson
7	Shaojune Pang	IOCAS	WP3 partner
8	Ole Jacob Broch	SINTEF Ocean	**WP4 leader**
9	Shane Rogers	Clarkson University	WP4 partner
10 11	Morten Alver and Torfinn Solvang	SINTEF Ocean	**WP5 leaders**
12 13	Andreas M. Lien and Per Christian Endresen	SINTEF Ocean	**WP6 leaders**
14	Silje Forbord	NTNU	PhD WP2 (WP1)
15	Sanna Matsson	APN and UiT	PhD WP2
16	Saifullah	NTNU	PhD WP2
17	Alexander Thompson	SAMS	PhD WP3
18	Peter Søndergaard Schmedes	DTU Aqua	PhD DTU-NTNU cooperation connected to MACROSEA WP1 and WP2
19	Siv Anina Etter	NTNU	PhD DTU-NTNU cooperation connected to MACROSEA WP1 and WP2
20	Michele Stanley	SAMS	Supervisor Alexander Thompson
21	Bodil Bluhm	UiT	Supervisor Sanna Matsson
22	Kjell Inge Reitan	NTNU	Supervisor Siv Anina Etter Co-supervisor Peter Søndergaard Schmedes
23	Mette Møller	DTU Aqua	Co-supervisor Peter Søndergaard Schmedes
24	Hartvig Christie	NIVA	Co-supervisor Sanna Matsson
25	Anna Metaxas	Dalhousie University	Co-supervisor Sanna Matsson
26	Klaas Timmermans	Royal Neth Inst for Sea Res	Scientific advisory board
27	Susan Holdt	DTU Food	Scientific advisory board
28	Bénédicte Charrier	CNRS Station Biologique de Roscoff	Scientific advisory board
29	Reinhold Fieler	APN	Partner, host Annual meeting 2018/2019
30	Aleksander Handå	SINTEF Ocean	**Project leader**

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