GENetic diversity exploitation for Innovative macro-AlGal biorefinery

Deliverable 3.2

Report on yield optimisation including strain selection, coppicing and IMTA

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ALGA+	Helena Abreu, Ana Ribeiro, Madalena Mendes Andreína Azevedo, Bárbara Pitarma, Madalena Mendes	Chapter on strain selection (*Ulva*), section on IMTA for *Ulva* Assistance in field work at ALGAplus farm
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Summary

Cultivation and monitoring of yield and quality of three selected optimised *Ulva* sp. strains identified for their specific value *i.e.*, fast growth, low fouling and high content of valuable components, based on phenotyping data, was compared against wild type or currently used industrial strains.
To optimize yields of *Saccharina latissima* the harvesting windows was defined with a special focus on a possible manipulating factor - the coppicing technique. This is a partial harvest of the sporophytes to stimulate re-growth and yield increase of remaining plants through light improvement and thus enable for multiple harvestings. The technique was tested at both sheltered and exposed sea conditions. Further, the effects of salmon-driven IMTA for *S. latissima* cultivation in exposed sea conditions and seabass-driven IMTA for *Ulva* sp. in land-based cultivation on the production potential and chemical composition was elaborated for different cultivation systems and the benefits of IMTA quantified.

Contents

1 Introduction

This report describes work in mainly Tasks 3.1.2, 3.1.3 and 3.1.4 in GENIALG. Results from this work was also used for a further development of a dynamic growth model described in the report on Deliverable 3.3 – "Simulating growth and composition of *Saccharina latissima* and *Ulva rigida* in IMTA" (2020).

One aim in GENIALG is to design high yielding seaweed cultivation systems producing affordable biomass feedstock of sugar kelp *Saccharina latissima* and sea lettuce *Ulva* sp. for the production of a range of existing and novel products. This includes to improve the cultivation technology and strategy to better take advantage of the environmental premisses in space, light, nutrients, season and genetic resources.

Annual production capacities of *S. latissima* have been estimated from 170 to 340 tons fresh weight (FW) ha^{-1} at sea, with a corresponding dry weight (DW) of 26-33 tons ha^{-1}, respectively (Peteiro & Freire, 2009; Broch et al., 2013; Handå et al., 2013; Wang et al., 2014). Land-based cultivation (integrated with animal production) of *Ulva sp*. has shown effective productivities of 400 to 940 tons FW ha^{-1} $year^{-1}$ (ALGA+ and Bolton et al., 2008 respectively) equivalent to 40 to 90 tons DW/ha/year; other smaller scale trials reached even higher yields with 200 tons DW ha^{-1} $year^{-1}$ (Mata et al. 2010). This illustrates the high biomass yield potentials of these two species, but for upscaling to a commercial scale more knowledge is needed to further enhance the biomass yield as well as the predictability in yield and quality. In this report we present efforts on optimisation of the biomass yields through strain selection, farming in IMTA and oceanic condition, and manipulation of the growth by coppicing.

2 Strain selection (*Ulva* sp.)

Based on the phenotyping results of *Ulva* sp. received from partner NUIG, the three strains that exhibited best growth rate performances (R6, R11 and R13) were selected, all isolated from within ALGAplus facilities, Table 1. Using vegetative propagation techniques, part of the biomass of the three selected strains plus R12 was transferred from the biobank into actively growing cultures.

Table 1. Detailed information about the selected *Ulva* samples collected in 2019-2019 by UAVR and ALGAplus. All strains are kept and maintained in the biobank set at ALGAplus maternity.

Replicate	Algae	Code	Location	Date
R6	*Ulva rigida*	UsAL+	Tank 36, ALGAplus	07/2018
R11	*Ulva rigida*	UsAL+	sedimentation tankpond, Materáqua	07/2018
R12	*Ulva rigida*	UsAL+	Tank 31, ALGAplus	07/2018
R13	*Ulva rigida*	UsAL+	Decant. Tank sedimentation pond, Materáqua	07/2018

Selected strains were initially grown under a short-day photoperiod and later under long-day conditions to accelerate growth. During this period, inside culture chambers, cultures were sequentially transferred into larger culture units with weekly maintenance protocols, until enough biomass was available to transfer to the experimental outdoor tank system (230L capacity) in August 2019. These cultures are still being kept today in the experimental outdoor tank system, with healthy biomass and with no need of restocking, since Time 0, Figure 1.

Figure 1. Detail of selected *Ulva* strains at different steps of the biomass production process: A. Beginning of the biomass production process, indoor. B. During the biomass production process, indoor. C. Outdoor cultivation tanks for *Ulva* at ALGAplus.

In October 2019, the three selected strains (R6; R11; R13) reached cultivation in the commercial size tanks (20 m^3) and were kept until mid-December 2020, with average yields ranging from 0.7-1.1 kg (ww)/m^2/week. In January 2021, these strains were re-upscaled from the stock cultures kept in the experimental outdoor tank system (230L capacity) to commercial size operation tanks, including the new raceway system. At this point, the strains able to withstand the conditions of the commercial size operation with best growth performances are R11 and R6, the latter having reached cultivation in the new raceway system (306 m^2). Besides having obtained better growth rates, strain R6 also displays larger and less perforated sized blades, determined by regular quality controls.

3 Farming in IMTA

3.1 Salmon-driven IMTA with *Saccharina latissima*

Integrated Multi-Trophic Aquaculture (IMTA) is an aquaculture system aiming to mimic an ecosystem by coupling the production of several species with different trophic levels to increase the total biomass production with the same feed input (Buschmann et al., 2001; Chopin et al., 2001) (Figure 2).

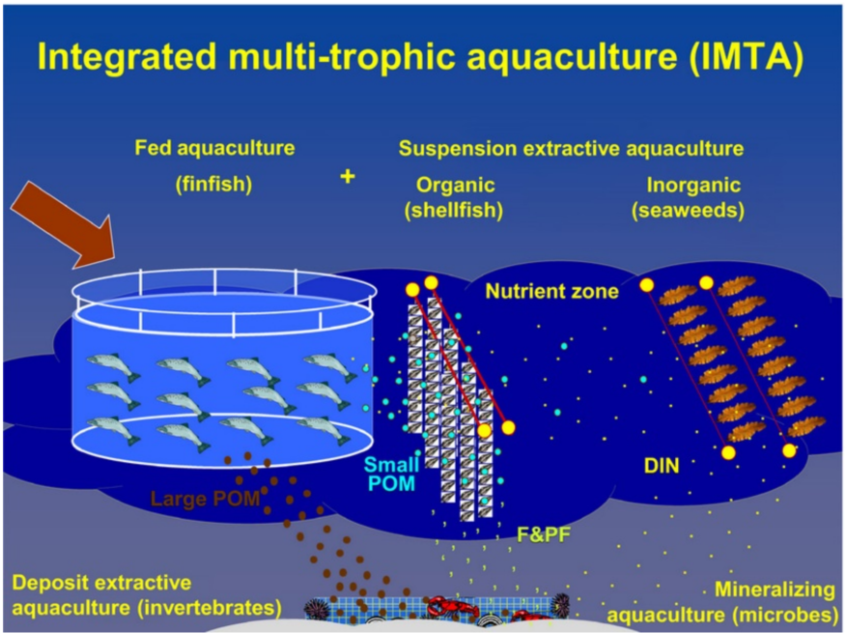

Figure 2. A fish-driven IMTA cultivation system with species of different trophic level: fish, shellfish, seaweed and extractive invertebrates. Illustration copied from Clements & Chopin (2016).

The mean nutrient release to the environment from Norwegian salmon aquaculture is estimated to 70% C, 62% N and 70% P of the total C, N and P input of feed, respectively (Wang et al., 2012). About 44% of the P and 15% of the N in the feed are released in particulate form, while 21% P and 45% N are released in a dissolved inorganic form. In IMTA, at least two or more species of different tropic levels need to be included and the system has to be within the same water mass to be categorized as IMTA. Suitable species should meet the following criteria: Enabled controlled reproduction, enhanced growth in the system, low maintenance, and an economically feasible production (Chopin et al., 2001; Ridler et al., 2007).

Macroalgae assimilate and thereby remove dissolved inorganic nitrogen (DIN, in the form of ammonia) released from fish farms in open-water IMTA systems (Buschmann et al., 2001). With its fast growth and high content of valuable carbohydrates, the brown kelp *Saccharina latissima* is regarded as an attractive species for use in IMTA in Norway (Handå et al. 2013; Fossberg et al. 2018).

Because DIN tends to be low after the phytoplankton bloom in the spring in Norwegian coastal waters and thus a limiting factor for seaweed productivity, macroalgae grown in the vicinity of salmon farms is suggested to take advantage of the nutrients released from the farm (Wang et al., 2012; 2014; Handå et al., 2013). Further, Broch et al. (2013) have shown that in temperate marine waters there is an apparent seasonal mismatch between the fast growth of *S. latissima* from winter to early summer and the highest release rate of nutrients from salmon aquaculture in summer and early autumn. The present experiments were designed to further elaborate on the potential for salmon-driven IMTA with kelp under Norwegian coastal conditions.

In 2018 and 2019 two IMTA-experiments were carried out integrated with a full scale (9000 tons) salmon farm at ACE outside the island of Frøya in Mid-Norway (63°78'N, 08°53'E). Salmon smolts were set out in the cages autumn 2017 and slaughtered out from July to October 2018. A new production cycle was started in March 2019. The salmon biomass and the feed use are shown in Figure 3.

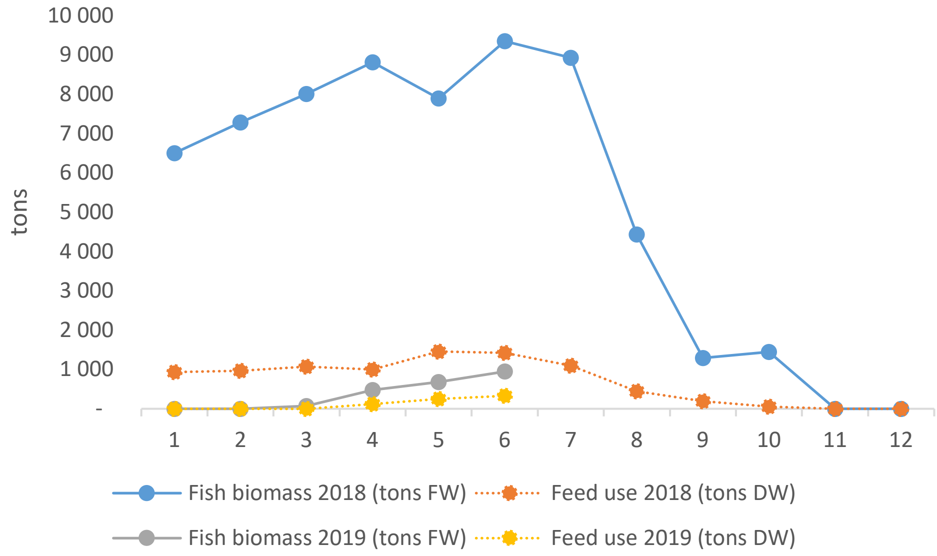

Figure 3. Salmon biomass and feed use (tons) at the ACE salmon farm used for IMTA in 2018 and 2019. The x-axis shows months from January-December.

For farming of sugar kelp in IMTA two cultivation systems were tested, one with vertically hanging droppers for each 10 m on a carrier line in 2018 (Figure 4) and one with a continuous line that was connected to the carrier line for every 5 m in 2019 (Figure 5). For both systems, the kelp was cultivated from 1 to 5 m below the sea surface.

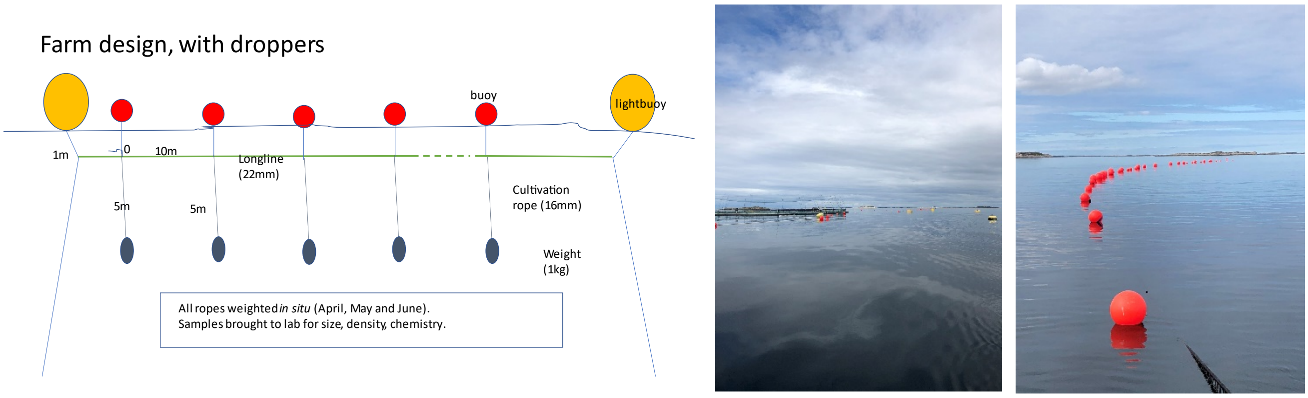

Figure 4. Sketch (left) of the experimental set-up used in 2018 showing the farm design with droppers attached to a 22 mm carrier line. The farm design was used both inside the salmon farm (IMTA) and outside (Ref). Photos (right) show the carrier lines inside the salmon farm and the reference line from 130 to 300 meters from the corner of the salmon farm (Photo: SINTEF).

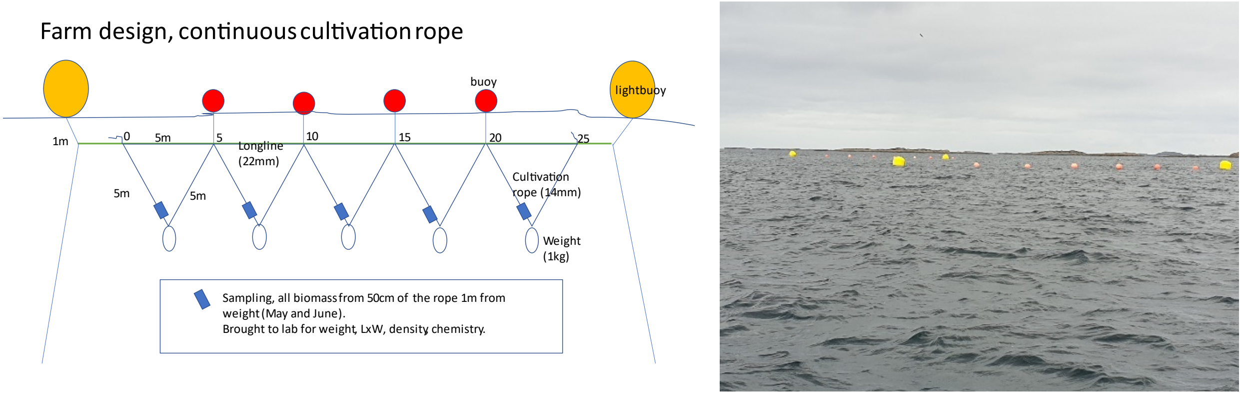

Figure 6. Sketch (left) of the experimental set-up used in 2019 showing the farm design with a continuous cultivation rope attached to a 22 mm carrier line, and the sampling of biomass from 50 cm of rope for biometrics and analysis. The farm design was used both inside the salmon farm (IMTA) and outside (Ref). The photo (right) shows the Ref-farm with the two 30 m long carrier lines, each with 5 buoys (Photo: SINTEF).

Seedlings were produced by seeding spores on 1.2-mm diameter twine coiled around PVC spools and when ready for deployment in the sea (~0.5 mm in length) the twines were machine-transferred to 16 mm ropes and transported to Frøya, according to Forbord et al. 2018. The seedlings were deployed at the 16. February in 2018 and two weeks later in 2019 (1. March). This gave production periods of equal length, 117 and 118 days, respectively.

The carrier lines were placed between salmon cages (IMTA) and as references (Ref) placed between from 50 to 300 m from the edge of the salmon farm. The 16 mm seedlings ropes were deployed at sea by connecting them to the carrier lines. For the vertical 5 m droppers, all biomass per rope was weighted in situ, whereas for the continuous lines sub-sampling of all biomass from 50 cm rope was done at 5 ropes for each site.

The two experiments illustrated well the effects of cultivation in IMTA. The highest biomass yields were obtained by cultivation between the salmon cages, but only in 2018 (Figure 6). In 2019 there were no differences between the kelp biomass production IMTA and at the reference lines (Figure 7). This correlates well with the size of the salmon biomass during the two experimental periods (and thus the N- and P- excretion from the fish), that were high in 2018 and low in 2019. Although some ropes at the reference line were lost in the 2018-experiment, a gradient in biomass could be observed in June, with 12.4 kg m^{-1} on the best ropes in IMTA and 2.7 kg m^{-1} at the most distant rope. The results thus support former studies indicating that IMTA kelp biomass yield is higher in closer proximity to a fish farm.

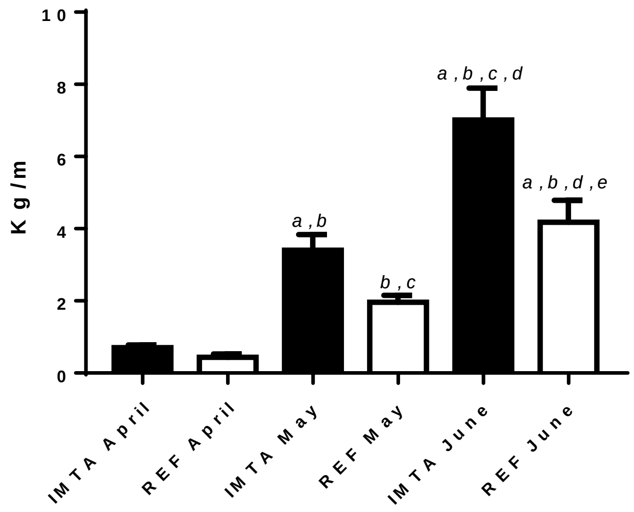

Figure 7. Cultivation trial in IMTA in 2018. Seaweed biomass at the ropes inside the salmon farm (IMTA) and at the reference line (Ref) outside the salmon farm. Source: Monteiro et al., 2021.

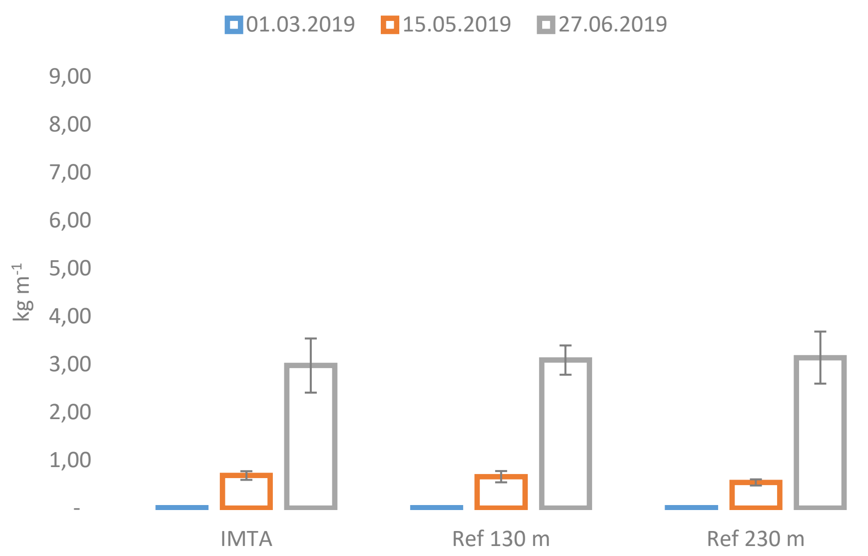

Figure 8. Cultivation trial in IMTA in 2019. Seaweed biomass at the ropes inside the salmon farm (IMTA) and at the two reference lines outside the salmon farm (Ref), weighted at three dates.

From the experiment in 2018 a minimum of 20 sporophytes were sampled and frozen for chemical characterization. Before chemical analysis the samples were frozen at -80 °C before freeze drying at -40 °C for 48 h, and the dried material was sent to University of Aveiro for chemical characterization of the nutritional quality of the biomass.

For the chemical composition the harvesting period had clearly more impact than cultivation site (Figure 8). Statistically significant differences in the content of ash, protein, lipid and carbohydrate contents were observed exclusively between different harvesting times (Monteiro et al., 2021). There was a trend for a decrease in ash content and an increase in the content in carbohydrates from April to June.

Therefore, the increase in carbon, hydrogen and sulphur with time can be due to sulphated carbohydrates like fucoidan. The lipid content (ranging from 1.30 ± 0.06% in IMTA samples from April to 0.83 ± 0.06% in REF samples from June) was generally higher in IMTA samples, with a lower content of lipids in REF samples in June as compared to earlier months. The protein contents (ranging from 10.51 ± 0.14% in IMTA samples from April to 8.86 ± 1.06% in IMTA samples from June) were not statistically different between IMTA and REF and remained mostly unchanged along harvesting periods.

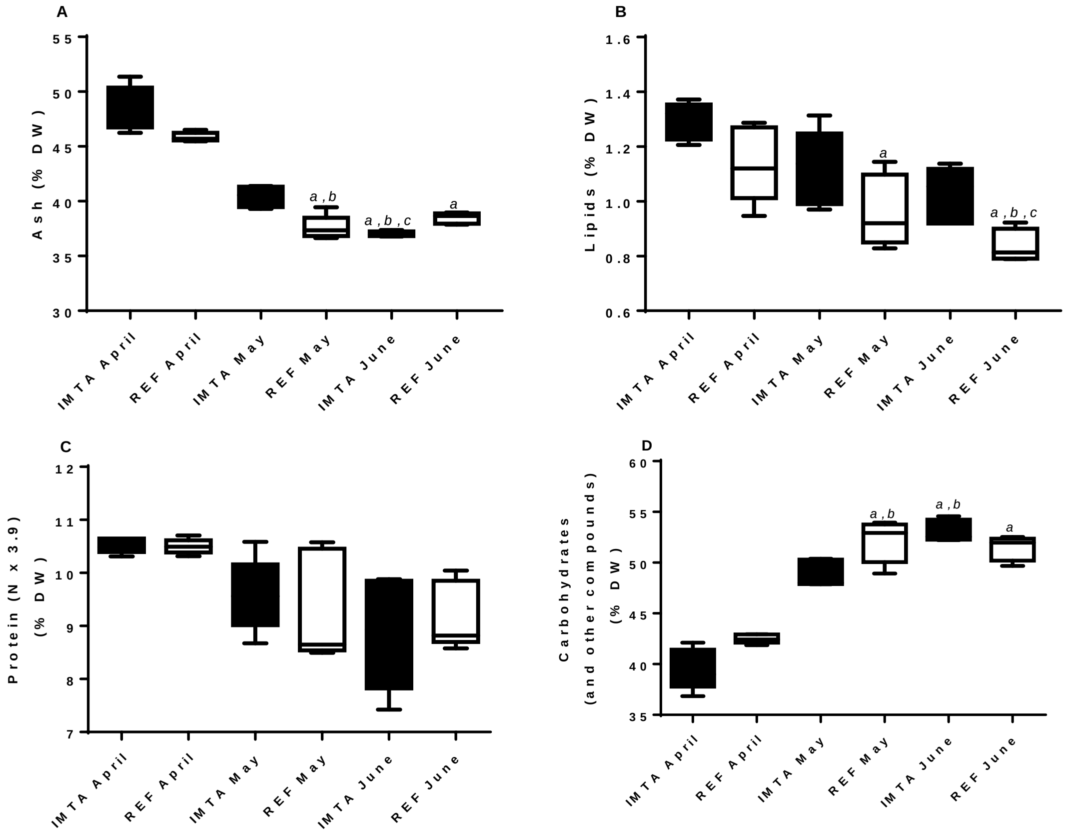

Figure 9. Biochemical compositional analysis of S. latissima samples from different types (REF or IMTA), harvested in different months (April, May and July). Content in ash (A), lipids (B) protein (N*3.9) (C) and carbohydrates and other compounds (D) are presented as percentages of DW. IMTA: integrated multi-trophic aquaculture site; REF: reference site. Values depicted represent means ± standard error of mean for 5 replicates. Statistical significant differences ($q < 0.05$) are presented as follows: a: vs. IMTA April; b: vs. REF April; c: vs. IMTA May. Source: Monteiro et al., 2021.

Since no significant differences in chemical composition was measured between kelp cultivated in IMTA and outside, IMTA farming represents advantages of generating greater biomass yields of similar good quality as in monoculture and in removal and re-use of nutrients effluents from the fish farm.

A more detailed description of the results from this experiment, including an in-depth analysis of the lipids in *S. latissima* samples, is published by Monteiro et al., 2021. See also Rey et al. (2019) and Monteiro et al. (2020) for more reading about lipids analysis of *S. latissima* performed in the GENIALG project. Simulation results on the dispersal of nutrients from the fish farm and the DIN concentrations are presented in Monteiro et al. (2019) and Deliverable 3.3.

3.2 Seabass-driven IMTA with *Ulva* sp.

Selected and current industrial *Ulva* strains were grown in the certified organic land-based production system integrated with seabass, at ALGAplus. The site (14 ha) is located in a Natura 2000 region, Ria de Aveiro (Portugal) with optimal abiotic conditions where *Ulva* (Sea Lettuce) is successfully produced year-round, Figure 9. Water enters the farm at every hight tide, flows into the fish earthen ponds and from there to a decantation pond. Nutrient rich water (nitrate, carbon dioxide (CO_2) and ammonia) from the semi-intensive seabream and seabass farm is then pumped to a filtration system and distributed to the large-scale algae tanks, cleaning a 2400 m^3/day effluent (Figure 10). High biomass yields per surface area can be achieved without adding CO_2 or fertilizers.

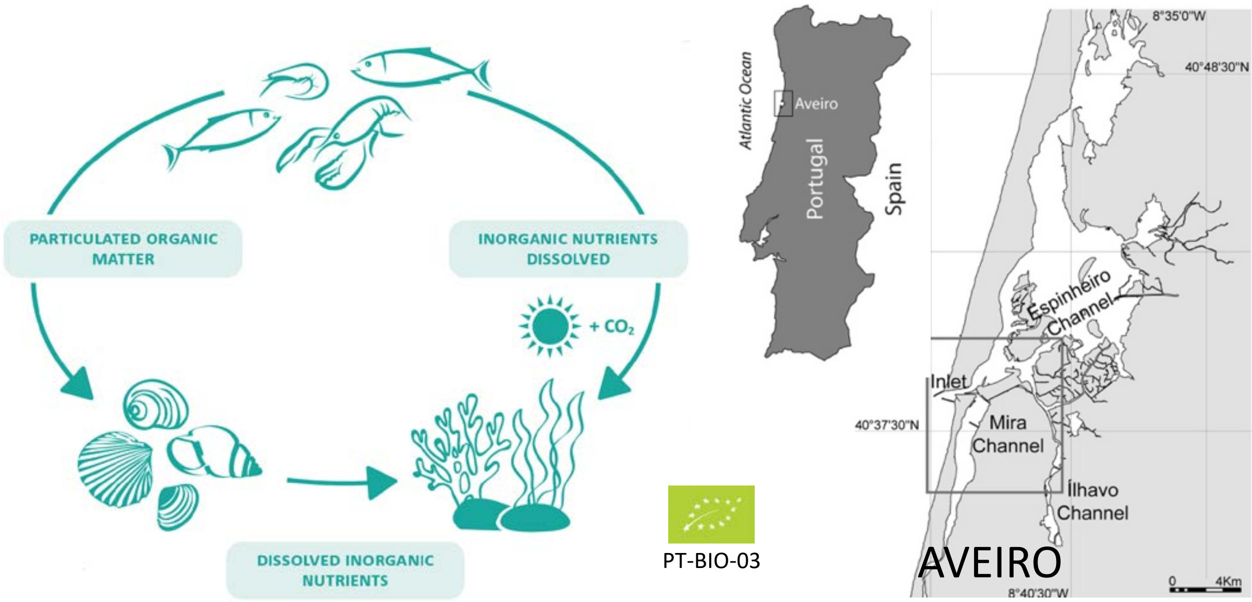

Figure 10. Left - IMTA cultivation scheme demonstration nutrient flow within species of different trophic levels. Illustration by ALGAplus. Right – Location of ALGAplus IMTA farm.

Commercial size farming of the selected *Ulva* strains (R6; R11; R13), through vegetative propagation has been in operation since October 2019, including the new raceway system in 2021. Stocking density and water renewal rates are the same used for routine production operations at the farm, monitoring is done every two weeks. Our experiments have demonstrated that R6 and R11 displayed better growth performances, with an average yield of 1 kg (ww)/m^2/week, higher than the average yield at ALGAplus currently at 3 kg/m^2/month (3 years measurements). Two raceways are now fully operational (306 m^2 area each) for the cultivation of *Ulva* and another 13 are projected, with a total area of 3750 m^2 and a minimum production capacity of 135 tons (ww)/year.

Figure 11. Land-based IMTA system with seabass and *Ulva* production, at ALGAplus.

Water quality data from several collection and discharge points (farm entry, fish and *Ulva* outflows) was tested for NH_4^+ concentration at different times of the day (pre-dawn; zenith and pre-dusk) illustrating well the effects from cultivation in an IMTA system, Figure 11. Average *Ulva* yield in cultivation is 420 tons (ww)/ha/year, total N 5% and N removal 4.2 tons/ha/year.

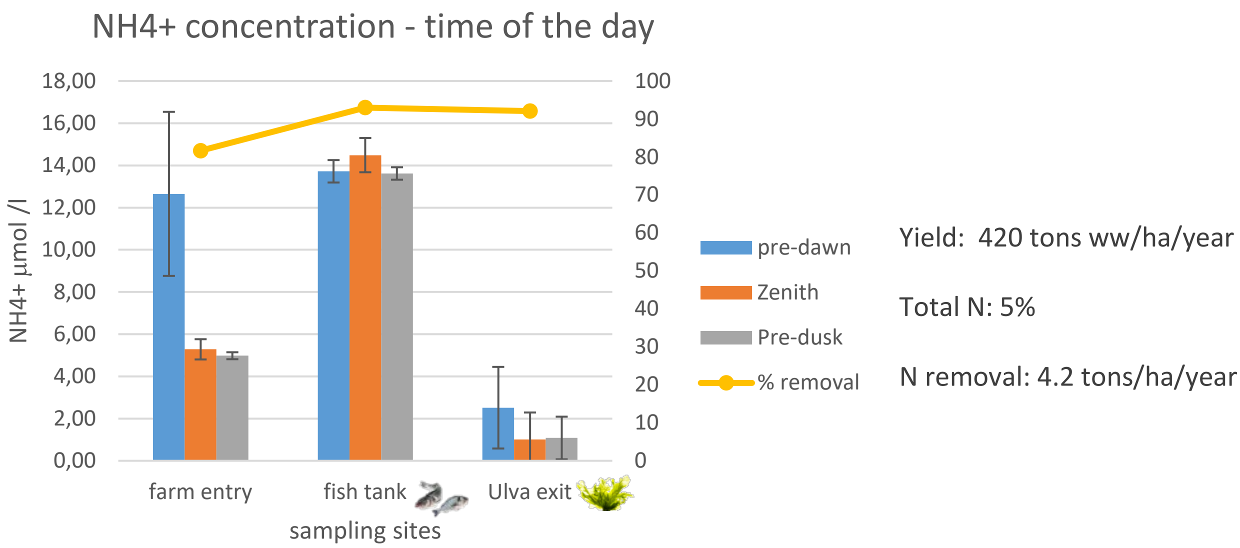

Figure 12. NH_4^+ concentration at different times of the day in three locations, at farm entry, fish tanks and *Ulva* tanks exit.

On the follow up of this work, representative samples of the selected strains and control samples (commercial strains) from the tank system (230L capacity) and commercial size operation tanks (20 m^3) were collected between July and December of 2020 and sent to UAVR partner for elemental analysis. Before chemical analysis, the samples were frozen at –80 °C and then freeze dried. The dried material analyzed at the University of Aveiro for elemental analysis of C and N. Data set of C, N, C:N was plotted and is depicted in Figure 12, Figure 13 and Figure 14.

For C and N elemental composition and C:N ratio of R11, a similar pattern was observed. Both patterns are dependent on harvesting period (Figure 12). Comparing these features to control, the amplitude of the fluctuation between maximum and minimum is of same order and generally has the same trend along the harvesting period. In terms of C and N, representative samples of the strains and control samples (commercial strains) from the tank system (230L capacity) and commercial size operation 20 m^3 tanks follow similar trends along the sampling period.

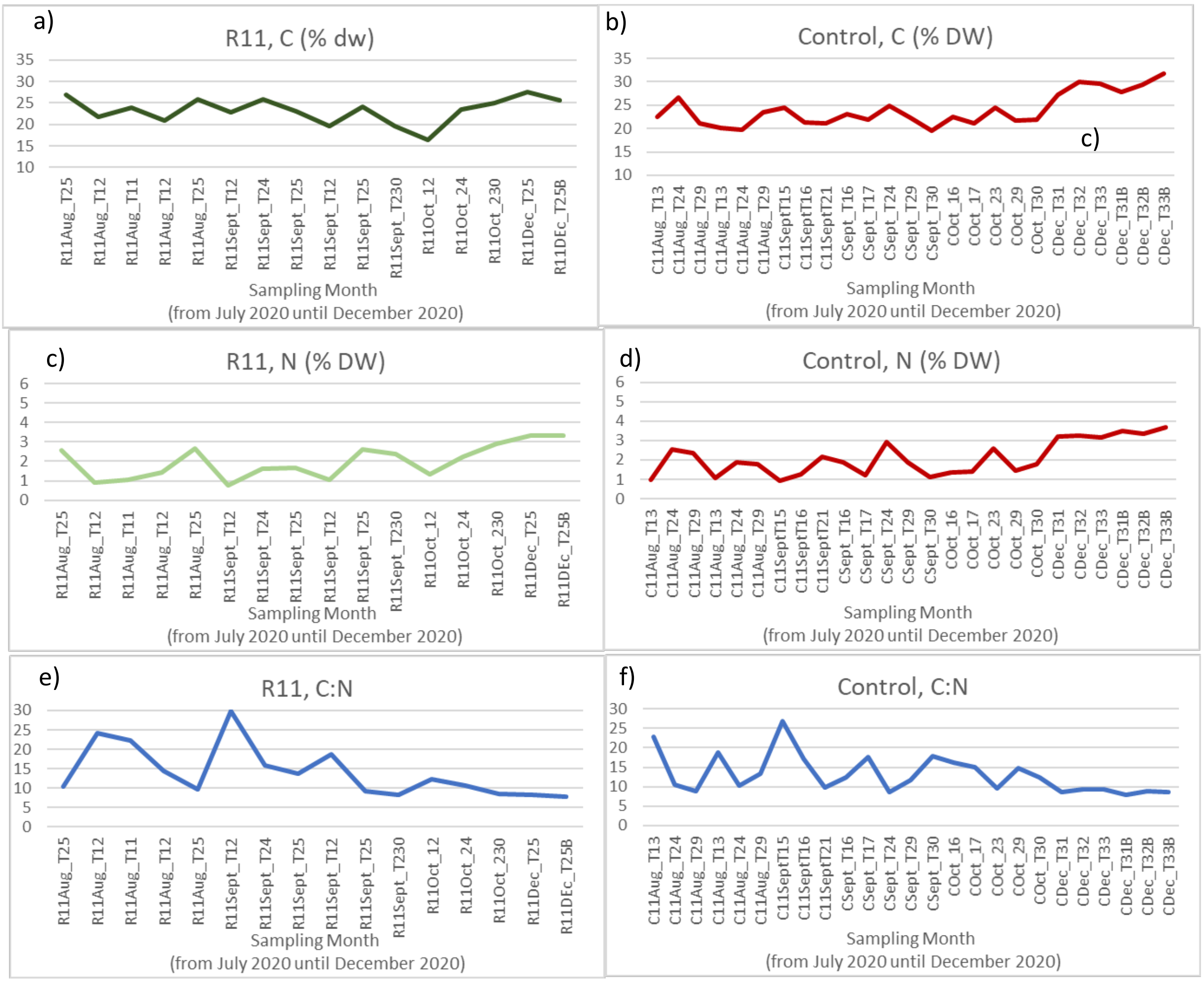

Figure 13. Elemental composition (C, N), C:N ratio analysis of *Ulva* spp. samples from selected strains (R11) and commercial strain (ALGAplus, control C), harvested in different months (July, August, September, October, December 2020). Samples S11 and S13 were collected from the maternity in November 2020. C and N contents are presented as percentages of DW. Values depicted represent means ± standard deviation for 3 replicates (data for publication).

For C and N, the elemental composition has a similar pattern for R13, and both patterns are dependent on harvesting period (Figure 13). Comparing these features to control, the lowest order amplitude of the fluctuation between maximum and minimum was observed in control samples. An irregular up and down is observed in samples from September, both in R13 strains from commercial size operation 20 m^3 tanks but also in the control samples. Moreover, a minimum in C% was achieved for the strains from tank system (230L capacity) collected in September. In regard to C:N ratio, a maximum value in *Ulva* was observed in *samples* collected from Tank 34 (20 m^3) harvest in September but follows a constant pattern on harvesting period for the other samples - ranging between 5 to 10. Excluding R13 from Tank 34, a constant ratio C:N ratio was detected in samples along the harvesting period that contain higher amount of N% than control samples. In fact, the plot of C:N ratio and N% showed a typical behaviour of C:N upon N% content (Corzo, 1991), however, the ratios obtained for R11 are in accordance with obtained with controls pattern (Figure 14).

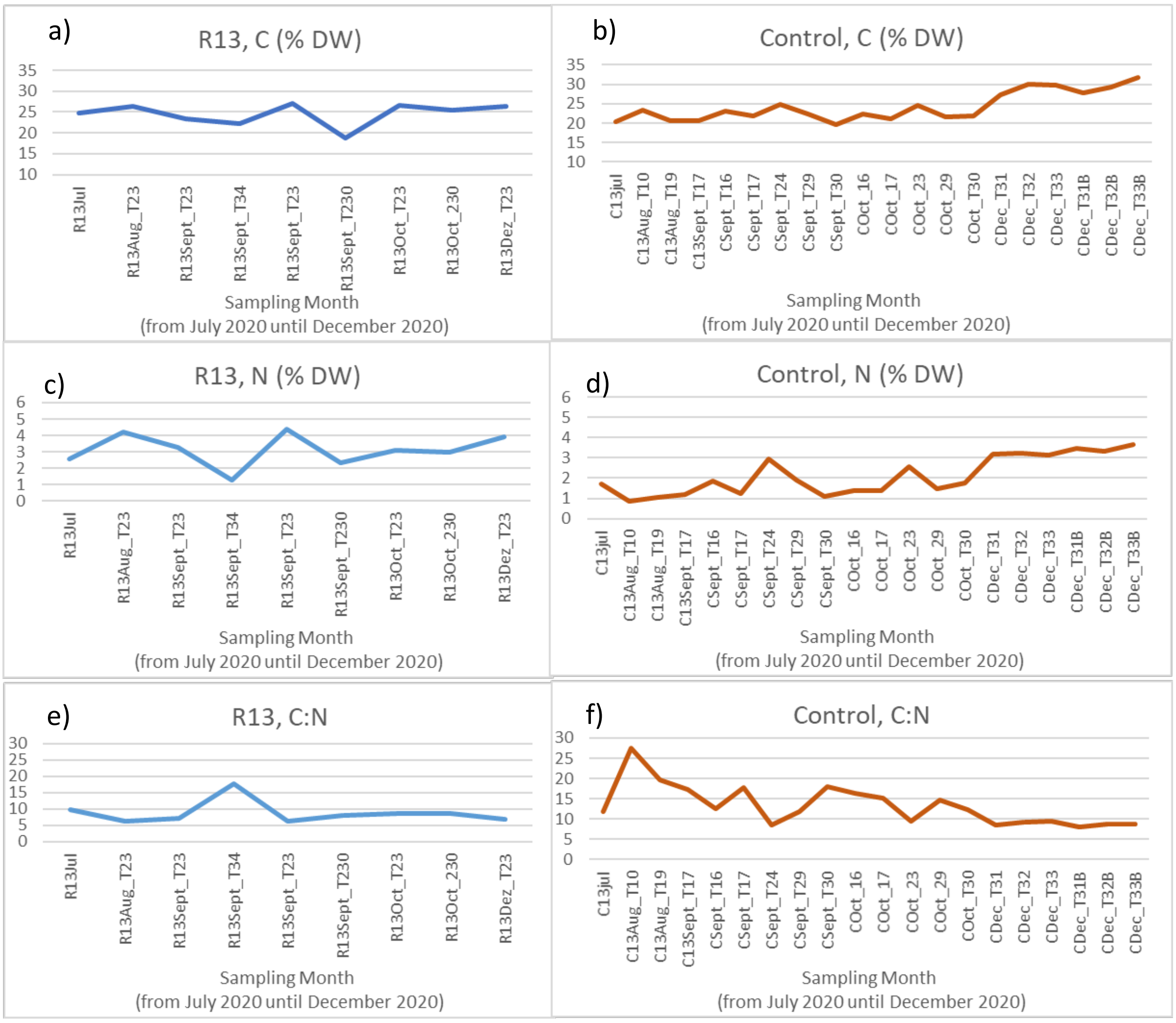

Figure 14. Elemental composition (C, N), C:N ratio analysis of *Ulva* spp. samples from selected strains (R13) and commercial strain (ALGAplus, control C), harvested in different months (July, August, September, October, December 2020). Samples S11 and S13 were collected from maternity in November 2020. C and N contents are presented as percentages of DW. Values depicted represent means for 3 replicates.

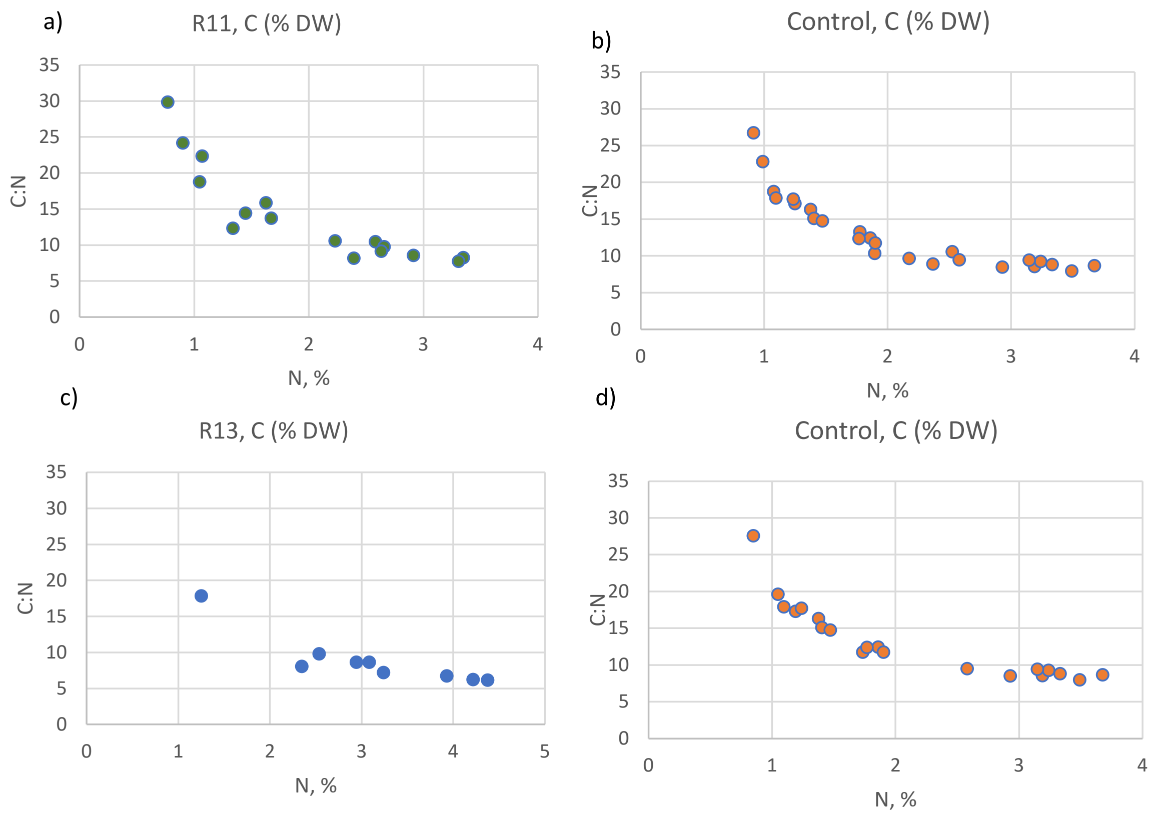

Figure 154. C:N ratio and nitrogen content in *Ulva* strains and controls: (a) R11, (b) control of R11; (c) R13; (d) control of R 13. Values depicted represent means for 3 replicates.

See also Lopes et al. (2019), Moreira et al (2020, 2021) and da Costa et al. (2019) for more reading about elemental analysis and protein content of *Ulva* species performed in the GENIALG project.

4 Farming in exposed conditions (*Saccharina latissima*)

A comprehensive study by Forbord et al. (2020) of *S. latissima* cultivated along the Norwegian coast demonstrated great variations in the yield, chemical composition and biofouling of the harvestable biomass and that the site selection is of outmost importance for the seaweed farmer. Further, by using the mathematical ocean model SINMOD, Broch et al. (2019) concluded that larger and not at least more productive areas can be accessed by moving the seaweed farming to more offshore sites in open, exposed oceanic waters, on and outside the continental shelf. Here the growth is higher due to more stable nutrients availability and temperature, and there are fewer conflicting activities (less occupied areas).

For *S. latissima*, a species that grows naturally in sheltered conditions, it is not obvious that the strong currents and high waves in open ocean conditions will favour fast growth and high biomass production. To answer this question a cultivation experiment was carried out in exposed oceanic sea conditions, partly as verification of the theoretical considerations from the mathematical model but also to disclose whether *S. latissima* can be cultivated in extreme wave- and currents exposed conditions, thus enabling offshore farming. This is highly relevant for the development of a new industry based on low-trophic aquaculture along the Norwegian coast and that also is of high relevance for other countries with interest for seaweed farming in more open oceanic areas.

The GENIALG project got access to this experiment through a collaboration with two projects financed by the Møre and Romsdal County: TAREAL2 on the biological experiment and AKVALAB on the design and construction of the sea-farm. The ocean areas outside the town Kristiansund at the Western coast of Mid-Norway were evaluated for a suitable site and a new license for cultivation at the very/highly exposed location "Grip" was applied for and granted in 2019 (Figure 15).

In winter 2020 a sea-farm was constructed and deployed at the site, and sugar kelp seedlings on ropes were deployed at the farm on the 3. February. At this farm, the average water current velocity from March to June was 12 – 13.7 cm/sec, but currents over 40 cm/sec was measured 15 days during the measurement period, enabling for a real evaluation of how *S. latissima* tolerate oceanic growth conditions. Seedlings were also deployed at a reference farm placed in sheltered fjord conditions at the location "Or" in the Freifjorden, about 15 km from Grip.

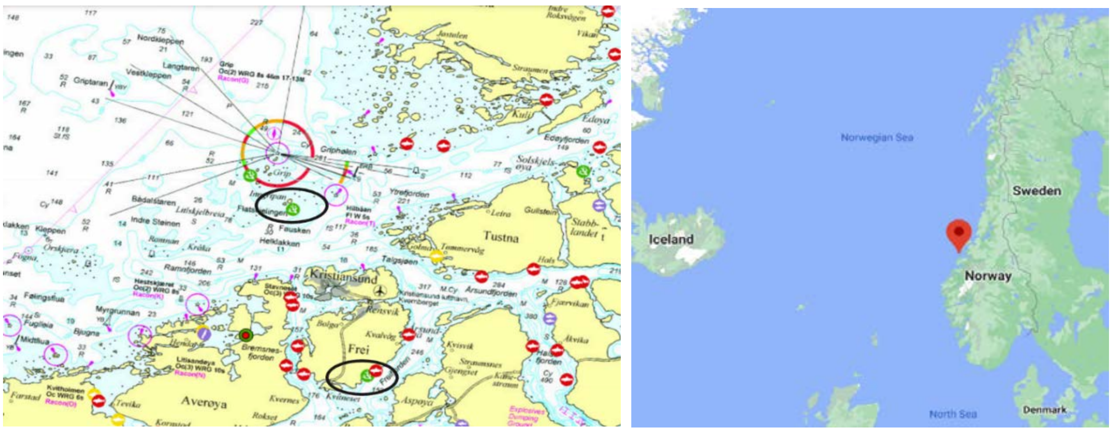

Figure 16. Maps showing the positions for a cultivation experiment with *S. latissima* at an exposed (Grip) and a sheltered (Or) location at the coast of Mid-Norway. At the left map Grip is the black circle at the middle and Or the black circle.

Registrations on the 23. April, 19. May, and 8./12. June showed an average biomass development from 0,9 kg m^{-1} in April to 5 kg m^{-1} in June at the fjord site Or, and from 0,3 kg m^{-1} in April to 5,7 kg m^{-1} at the ocean site Grip in June (Figure 16). The growth was slow in the beginning at the ocean site but during a 20-day period, from 19. May to 8. June, the biomass increased by a factor of 7. At the fjord site the weight doubled during this period.

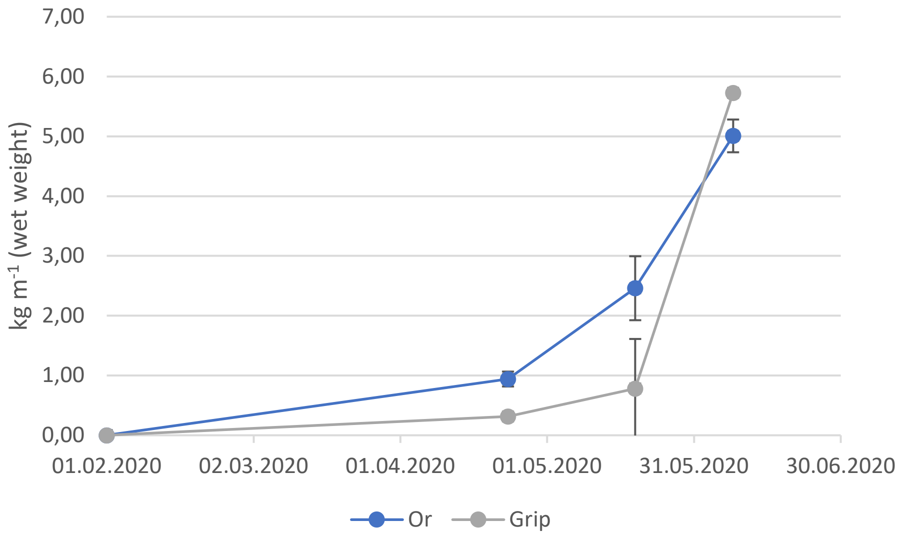

Figure 17. *S. latissima* biomass at the two cultivation sites Or (fjord) and Grip (ocean) in 2020 (AVG ± SEM). This experiment was part of the project TAREAL2 financed by Møre and Romsdal County.

Higher weight at Grip was due to larger individual sporophytes. It was measured that the sporophytes at the ocean site got thicker and heavier than at the fjord site (Figure 17), possibly to tolerate the stronger hydrodynamic exposure.

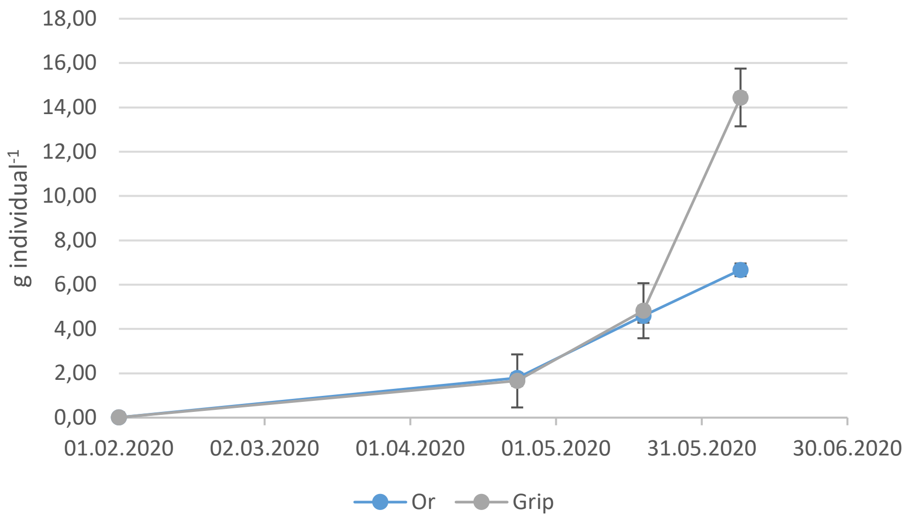

Figure 18. Individual weight of *S. latissima* sporophytes cultivated at Or (fjord) and Grip (ocean) in 2020 (AVG ± SEM). This experiment was part of the project TAREAL2 financed by Møre and Romsdal County.

The water nitrate concentration (Figure 18) and high growth rate at Grip suggest that the kelp was still in a phase of fast growth at the time of the last registration, suggesting a potentially bigger harvestable biomass after some more days or weeks. However, careful monitoring would be needed in this period to avoid detrimental biofouling of the biomass. Colonies of bryozoans was visible at both sites (Figure 19) and although still at neglectable numbers in mid-June, especially at the ocean site, these may develop quickly during the summer weeks (Forbord et al. 2020).

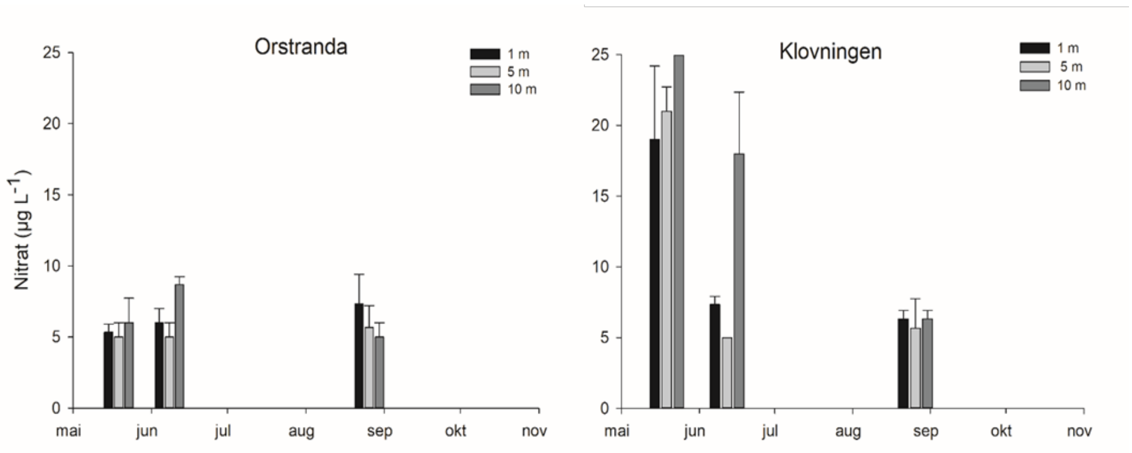

Figure 19. Water nitrate concentration at three depths in the two test farms at Or (Orstranda, fjord site) and Grip (Klovningen, ocean site).

Figure 20. Bryozoan colonies on the *S. latissima* sporophytes at the fjord site Or (left) and the ocean site Grip (right) in June (Photo: SINTEF).

The study demonstrated that sugar kelp can be cultivated in extreme wave- and currents exposed conditions, enabling access to large cultivation areas farther from the coast and thus in less conflict with other kinds of utilisation of the sea areas at the near coast. Although the growth rate was low during the first weeks the biomass yield at the end of the trial was equal to the one at the sheltered site and it is expected that an even higher yield could have been obtained after a few more weeks. Thus, at more exposed oceanic conditions the cultivation season probably can be prolonged for an enhanced exploitation of the biomass production potential of this species.

5 Increase of the harvesting window through coppicing (*Saccharina latissima*)

As biofouling may destroy much of the *S. latissima* biomass if not harvested in due time, GENIALG aimed to study alternative cultivation strategies to possibly mitigate this challenge and to prolong the harvesting window. Coppicing, *i.e.*, partly harvesting of the biomass before it is damaged by epiphytes but leaving the meristem for re-growth for one or several more harvestings, is one strategy that has been successful at the Faroe Islands (Bak et al., 2018) and that was tested in two trials in GENIALG.

The first coppicing trial was carried out at SES' farm outside the island of Frøya in Mid-Norway (63°78'N, 08°53'E) in 2018. Partial (70%; leaving 15 cm of the blade) and total (100%) harvesting of the sporophyte tissue was carried out on five 5 m ropes in May and combined with a final harvest of the fully grown sporophytes in August, both un-coppiced and coppiced, after a 3.5-month period of re-growth.

The highest biomass yield was obtained through the combined harvesting strategy and the results also suggest that the growth was *stimulated* by the coppicing (Table 2). However, the biomass in August was very damaged by bryozoan biofouling which restricts the application of it. It is thus suggested to look more thoroughly for the actual optimum harvesting window when using the coppicing, which in the case of SES' farm probably is much earlier than the end of August. Further, as the growth rate of the sugar kelp is very high in April as there still are ambient nutrients available (Forbord et al. 2020), an earlier coppicing period than May should also be tested. In this way it should be possible to increase the yield from the farm and also to harvest biomass with different qualities for several possible end products.

Table 2. The *S. latissima* biomass yield (kg m^{-1}, SEM in brackets) obtained either by a full harvest in May (100%) or by a partial harvest by coppicing of the sporophytes in May (70%) and a new full harvest in August. The trial was conducted in 2018 at SES' farm at Frøya, Norway.

	Biomass yield (kg m^{-1})		
Harvested in May	May	August	Total
0 %		7.7 (1.3)	7.70
70 %	2.0 (0.1)	8.6 (1.1)	10.60
100 %	2.8 (0.1)		2.80

A repetition of the trial, but with an earlier coppicing (April) and full harvest (July) was planned at SES' farm the spring 2020 but was abrupted due to the Covid-pandemic. As an alternative the project got access to the TAREAL2 "Ocean farming"-experiment described in Chapter 4. This trial included two sites, one at exposed, oceanic conditions and one at sheltered fjord condition. Here, partial (60%) and total (100%) harvesting of the sporophyte tissue was carried out on 1 m of all the ropes in mid-June and the re-growth of the coppiced sporophytes during the summer measured 2,5 months later, as a final harvest in August.

The possible biomass harvest was site dependent, with only stipes left on the ropes at the fjord site whereas a little biomass could be harvested at the ocean site in August (Table 3). No positive effects from coppicing could be observed on the biomass growth or the biofouling resistance. The sporophytes were completely covered by bryozoans and tunicates and the use of this biomass would therefore be restricted to non-human application (Figure 20). However, it should be noted that at the time of harvesting in June the sporophytes were still in good growth, suggesting that a final harvest in *e.g.*, mid-

July would have given a better outcome than the harvest in August, both of coppiced and un-coppiced sporophytes.

Table 3. The *S. latissima* biomass yield (kg m^{-1}, SEM in brackets) obtained either by a full harvest in June (100%) or by a partial harvest by coppicing of the sporophytes in June (60%) and a new full harvest in August. The trial was carried out in 2020 at two different cultivation sites, one exposed (ocean) and one sheltered (fjord) close to Kristiansund, Norway, in collaboration with the TAREAL2-project.

	Biomass yield (kg m^{-1})					
	Ocean (Grip)			**Fjord (Or)**		
Harvested in June	June	August	Total	June	August	Total
0 %	-	1.52 (0.42)	1.52	-	> 0.5	-
60 %	2.69 (0.81)	1.61 (0.45)	4.30	3.31 (0.04)	> 0.5	3.31
100 %	4.37 (1.31)	-	4.37	5.39 (0.06)	-	5.39

Figure 21. *S. latissima* on ropes in August, completely damaged by epiphytic biofouling by bryozoans, tunicates and hydroids. The kelp was cultivated at a sheltered fjord site (3 photos left) and at an exposed oceanic site (3 photos right) near Kristiansund, Norway, in collaboration with the TAREAL2-project in 2020 (Photo: SINTEF).

Samples from June and from coppiced/un-coppiced sporophytes in August were analysed for content of carbon (C), nitrogen (N), polyphenols, fucoxanthin, and astaxanthin. Several other carotenoids were found (adonixanthin, echinone, carotene or lycopene, and zeaxanthin or lutein), but without analytical standards their identities are not confirmed, and their concentrations are presented in arbitrary units and should be interpreted semi-quantitatively. The C and N content increased from June to August, and there was an indication that the coppicing also induced higher C and N content in the tissue (Figure 21). The increased N content in August indicates an enhanced protein level, probably be due to the biofouling organisms, as also seen in Forbord et al. 2020.

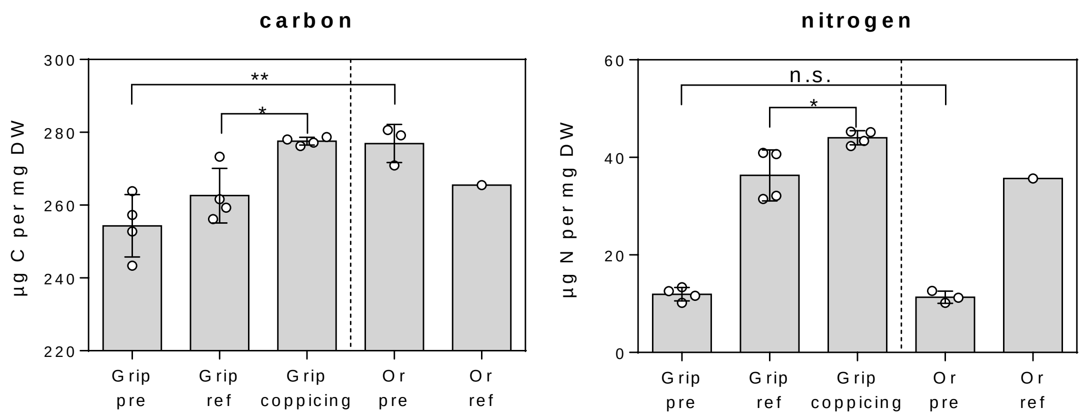

Figure 22. Carbon and nitrogen in *S. latissima* samples from the ocean site Grip and the fjord site Or in 2020. "Pre" are samples before coppicing, in June. "Ref" are samples from non-coppiced sporophytes at the final harvest in August. "Coppicing" are samples of the re-grown sporophytes at the final harvest in August. Both August samples were heavily affected by biofouling. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, n.s. indicates not significant.

The polyphenol content did not vary between the two sites nor the seasons for harvesting whereas the fucoxanthin was slightly higher at the ocean site (not significant), and substantially higher in June than in August (Figure 22). Again, due to the biofouling it must be expected that the composition in August not only reflects the kelp, but also other species. Therefore, the biomass at this stage should have other applications than for pure kelp biomass.

The analysis of carotenoids other than fucoxanthin indicates very low levels (<0.5% of dry weight) (Figure 22) and despite high market prices for these it is not known whether kelp-based pigments can be made at a competitive cost. For instance, astaxanthin has a world marked price exceeding ~6000 Eur/kg but the production costs for astaxanthin extracted from kelp need to be less than the one for astaxanthin rich microalgae like *e.g., Haematococcus* to be regarded as a potential good commercial source. With less than 5 ng astaxanthin per g DW sugar kelp this is doubtful, especially since it is unknown whether the astaxanthin is derived from the kelp itself of the biofouling. Regardless, even fucoxanthin levels are likely too low to add to the lucrative potential of kelp biomass.

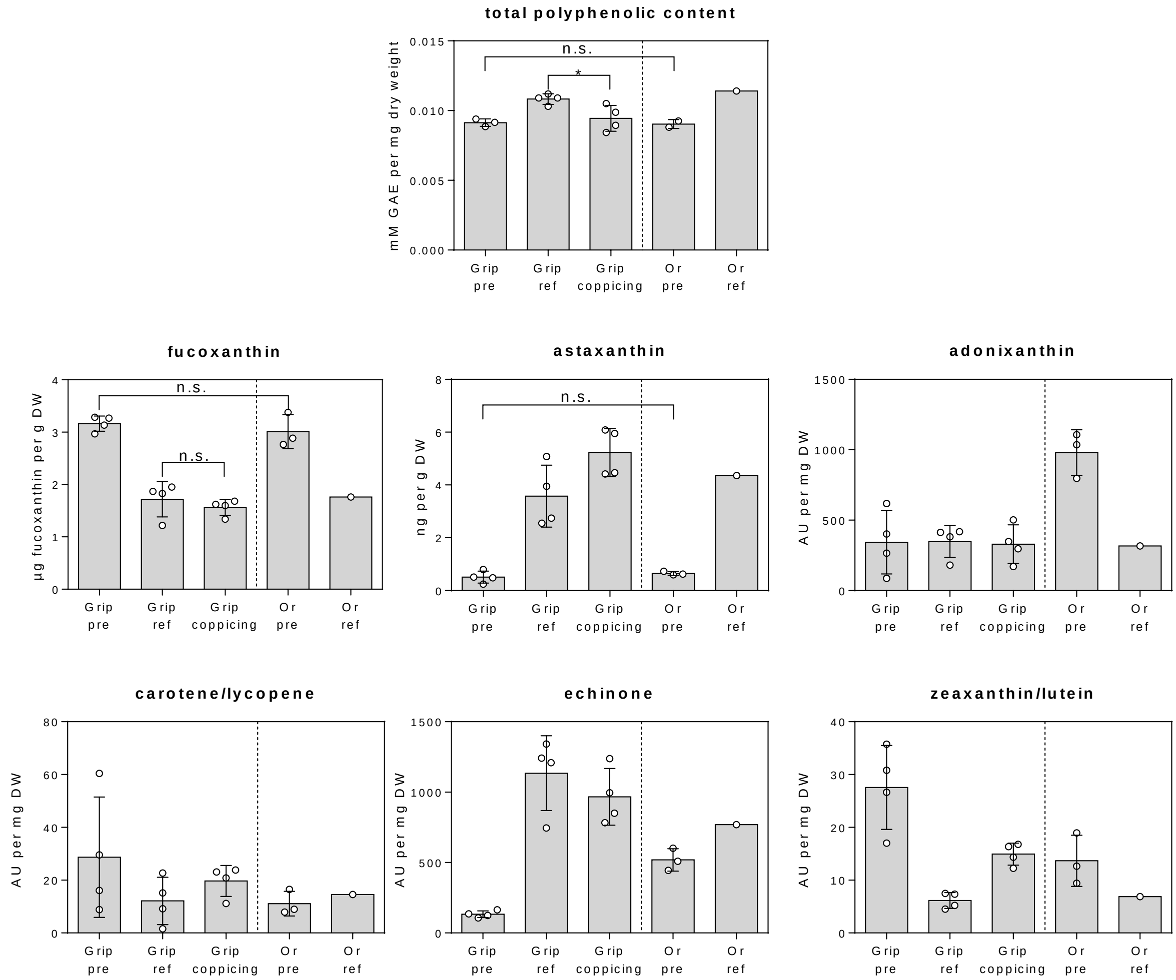

Figure 23. Total polyphenolic content and carotenoids in *S. latissima* samples from the ocean site Grip and the fjord site Or in 2020. "Pre" are samples before coppicing, in June. "Ref" are samples from non-coppiced sporophytes at the final harvest in August. "Coppicing" are samples of the re-grown sporophytes at the final harvest in August. Total polyphenolic content is reported in gallic acid equivalents (GAE). Only fucoxanthin and astaxanthin were quantified using analytical standards, so the concentrations of the other carotenoids are reported as arbitrary units (AU). Both August samples were heavily affected by biofouling. * indicates $p \leq 0.05$, n.s. indicates not significant.

6 Recommendations

Strain selection (*Ulva* sp).

The considerable genetic, growth and metabolic differences observed in the diverse panel of seaweed strains analysed by GENIALG (link with WP2 – Selection and biobanking) indicate a strong potential for strain selection to increase productivity in aquaculture setups. Once highly productive and quality strains are identified, they must be preserved as seedstock, particularly important for commercially large-scale seaweed cultivation. The selected strains are being successfully kept both in the biobank and in cultivation at ALGAplus, with good yielding results and biomass quality, in comparison to the current commercial strains. However, to fully realize the potential of selected strains, the viability for commercial uses should be assessed through repeated, long-term trials. Additionally, further studies should aim to continue increasing the biobank collection and gene pool, with special focus on growth, disease resistance and biochemical composition traits.

Farming in IMTA (*Saccharina latissima*)

Significant effects on biomass increment in IMTA are seen only during periods when the fish biomass was high, and not during periods of low fish biomass due to the low nutrient excretion. The kelp biomass must be cultivated in close proximity to the farm to directly utilize the nutrient loads from the fish. Harvesting period has proven to be more crucial for the biochemical composition than the cultivation location (IMTA vs reference site), but IMTA farming represent the advantage of generating greater biomass yields and should be considered during site selection.

Farming in IMTA (*Ulva* sp.)

IMTA systems are a sustainable form of producing seaweed which represents to be highly valuable in terms of biomass production. *Ulva* sp. can be used as "biofilters" to remove inorganic matter from seabream farm effluents, recycling of the nutrients included in the water-waste generated by fish farming (uneaten food and excretion products) to enhance seaweed production. This kind of production has beneficial impacts on the economic, environmental, and social sustainability of this activity

Exposed conditions (*Saccharina latissima*)

To fully realize the great potential in offshore seaweed farming the development of seaweed farms suitable for larger depths, stronger water currents, higher waves and longer transportation distances is required, as well as tools for remote monitoring of the farm structures and the biomass development at these environmental conditions.

Coppicing (*Saccharina latissima*)

The studies on coppicing of sugar kelp partly illustrate the potential in coppicing and suggest that the growth of the sporophytes is stimulated by this manipulation. However, the best timing for the coppicing and second harvest was not revealed from these two experiments and further studies should aim for identification of the optimum time window for the coppicing and the second and possible third harvests. These optimum dates are site dependent and will probably also vary between years, but better knowledge about the productivity and quality at the sites may open for several partial harvestings for dedicated applications, *e.g.*, for fine food in early spring, food and feed components and high-value compounds from the more fully grown biomass some later in the spring, and finally a fully grown biomass for use in biorefinery or for fertilizers, biofuel like biogas or for carbon dioxide removal products like biochar.

7 References

Bak, U., A. Mols-Mortensen, Ó. Gregersen (2018). Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting. *Algal Research* 33 (2018) 36-47.

Bolton JJ, Robertson-Andersson DV, Shuuluka D, Kandjengo L (2009) Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. J Appl Phycol 21: 575–583.

Broch OJ, Alver MO, Bekkby T, Gundersen H, Forbord S, Handå A, Skjermo J, Hancke K (2019). The kelp cultivation potential in coastal and offshore regions of Norway. Front Mar Sci 5:529.

Broch, O. J., Ellingsen, I. H., Forbord, S., Wang, X., Volent, Z., Alver, M. O., Handå, A., Andresen, K., Slagstad, D., Reitan, K. I. et al. (2013). Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway, Aquaculture Environment Interactions 4(2), 187-206.

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

Chopin, T., Buschmann, A. H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G. P., Zertuche-Gonz_alez, J. A., Yarish, C. and Neefus, C. (2001), Integrating seaweeds into marine aquaculture systems: a key toward sustainability, Journal of Phycology 37(6), 975-986.

Clements, J & Chopin, T. (2016) Ocean acidification and marine aquaculture in North America: Potential impacts and mitigation strategies. Reviews in Aquaculture 0, 1–16.

Corzo, A (1991). C/N ratio in response to nitrogen supply and light quality in *Ulva rigida* C. Agardh. Scientia Marina 55, 405-411

da Costa, E.; Ricardo, F.; Melo, T.; Mamede, R.; Abreu, M.H.; Domingues, P.; Domingues, M.R.; Calado, R. (2020). Site-specific lipidomic signatures of sea lettuce (*Ulva* spp., Chlorophyta) hold the potential to trace their geographic origin. *Biomolecules*, *10*, 489.

Forbord, S., Matsson, S., Brodahl, G.E. et al. (2020), Latitudinal, seasonal and depth-dependent variation in growth, chemical composition and biofouling of cultivated *Saccharina latissima* (Phaeophyceae) along the Norwegian coast. J Appl Phycol 32, 2215–2232 (2020).

Forbord, S., Steinhovden, K. B., Rød, K. K., Handå, A. and Skjermo, J. (2018), Cultivation protocol for *Saccharina latissima*, Protocols for Macroalgae Research, 1st Edn, eds B. Charrier, T. Wichard, and CRK Reddy (Boca Raton, FL pp. 37-59.

Fossberg, J., Forbord, S., Broch, O. J., Malzahn, A. M., Jansen, H., Handå, A., Førde, H., Bergvik, M., Fleddum, A. L. and Skjermo, J. (2018), The potential for upscaling kelp (*Saccharina latissima*) cultivation in salmon-driven integrated multi-trophic aquaculture (imta), Frontiers in Marine Science 5.51

Handå, A., Forbord, S., Wang, X., Broch, O. J., Dahle, S. W., Størseth, T. R., Reitan, K. I., Olsen, Y. and Skjermo, J. (2013), Seasonal-and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway, Aquaculture 414, 191-201.

Lopes, D., Moreira, A.S.P., Rey, F., da Costa, E., Melo, T., Maciel, E., Rego, A., Abreu, M.H., Domingues, P.; Calado, R., Domingues, M.R. (2019). Lipidomic signature of the green macroalgae *Ulva rigida* farmed in a sustainable integrated multi-trophic aquaculture. *J. Appl. Phycol.*, *31*, 1369–1381.

Mata, L., Schuenhoff, A, Santos, R. (2010). A direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva rigida*. J Appl Phycol (2010) 22:639–644

Monteiro J. P., Melo T., Skjermo J., Forbord S., Broch O. J., Domingues P., Calado R., Domingues M.R.(2021). Effect of harvesting month and proximity to fish farm sea cages on the biochemical and lipid profile of cultivated *Saccharina latissima*. Algal Research 54:102201

Monteiro, JP, F. Rey, T. Melo, A.S.P. Moreira, J.-F. Arbona, J. Skjermo, S. Forbord, J. Funderud, D. Raposo, P.D. Kerrison, M.-M. Perrineau, C. Gachon, P. Domingues, R. Calado, M.R. Domingues. (2020), The unique lipidomic signatures of *Saccharina latissima* can be used to pinpoint their geographic origin, Biomolecules, 10: 107.

Moreira, A.S.P., da Costa, E., Melo, T., Sulpice, R., Cardoso, S.M., Pitarma, B., Pereira, R., Abreu, M.H., Domingues, P., Calado, R., Domingues, M.R. (2020). Seasonal plasticity of the polar lipidome of *Ulva rigida* cultivated in a sustainable integrated multi-trophic aquaculture. *Algal Res.*, *49*, 101958.

Moreira, A.S.P., da Costa, E., Melo, T., Lopes, D., Pais, A.C.S., Santos, S. Pitarma, B., Mendes,M.,, Abreu, M.H., Collén, P. N., Domingues, P., Domingues, M.R. (2021). Polar Lipids of Commercial *Ulva* spp. of Different Origins: Profiling and Relevance for Seaweed Valorization. Foods, 10(5), 914.

Peteiro, C., Freire, Ó. (2009). Effect of outplanting time on commercial cultivation of kelp *Laminaria saccharina* at the southern limit in the Atlantic coast, N.W. Spain. Chin. J. Ocean. Limnol. 27, 54

Rey, F., D. Lopes, E. Maciel, J. Monteiro, J. Skjermo, J. Funderud, D. Raposo, P. Domingues, R. Calado, M.R. Domingues (2019), Polar lipid profile of *Saccharina latissima*, a functional food from the sea, Algal Research, 39, 101473.

Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S., Page, F., Reid, G., Szemerda, M., Sewuster, J. et al. (2007), Integrated multi- trophic aquaculture (imta): A potential strategic choice for farmers, Aquaculture Economics & Management 11(1), 99-110.

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

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