GENIALG

GENetic diversity exploitation for Innovative macro-ALGal biorefinery

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texto

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Errata 12.05.2021:

in the originally submitted version, Fig.1 had a mistake (too low growth line for Galway), which is coirrected in this version.

Growth characteristics of seaweed strains in relation to culture conditions

Jorunn Skjermo[1], Ole Jacob Broch[1], Silje Forbord[1], Diogo Raposo[2], Frank Neumann[2], Jean-François Arbona[3], Rosa Melo[4], Isabel Sousa Pinto[4], Antoine Fort[5], Jose Maria Fariñas Franco[5], Ronan Sulpice[5], Helena Abreu[6], João Monteiro[7], Elisabete Costa[7], Ricardo Calado[7], Rosario Domingues[7], and Luiza Neves[2]

[1] SINTEF Ocean

[2] Seaweed Energy Solutions

[3] C-Weed

[4] CIIMAR

[5] National University of Ireland Galway and Dingle Bay Seaweeds

[6] ALGAplus

[7] University of Aveiro

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1 Abstract

This report summarizes the monitoring of growth and biomass yield of cultivated *Saccharina latissima* and *Ulva* at the different cultivators in the GENIALG-project over 2 years.

To demonstrate the biomass production and yield at the different cultivation areas included in the project a monitoring program was carried out by the 3 *S. latissima* farmers SES, C-Weed and ALGAPlus over two years. In addition, the two research partners NUIG and CIIMAR did cultivation experiments one year. The partners used a common protocol for measure of the biomass development up to 6 times per cultivation season, including monitoring of the density, wet weight, size (L x W), epiphytes and sampling for chemical compositional analysis. Dry weight and environmental data were also recorded by some of the farmers.

The monitoring revealed big differences between the geographic areas and between years. For instance, C-Weed (Saint-Suliac, France) had a maximum yield of *S. latissima* in June of 14,2 kg/m in 2018 and 20,5 kg/m in 2019, whereas SES (Frøya, Norway) had, respectively, 13,5 and 7,8 kg/m. NUIG (Ventry Harbour, Co.Kerry, Ireland) had some less productivity with 8,3 kg/m (June 2018). CIIMAR did one cultivation trial in 2019 and got 582 g/m at Matosinhos, Leixões harbour and 200 g/m in Aveiro (both Portugal). Chemical characterization of the biomass samples from the *S. latissima* monitoring shows large season- and site dependent variations in protein (5,3-15,5% of DW) and carbohydrates (39,2-53,4% of DW).

A thorough monitoring of the growth and environmental variables was also carried out by ALGAPlus and these data are now being used to develop a mathematical growth model for *Ulva.* A productivity of 442 tons fresh weigh *Ulva* ha^{-1} year^{-1} was demonstrated, with a seasonal variation spending from a quarterly production of 75 tons ha^{-1} during autumn and winter and up to 150 tons ha^{-1} during spring and summer. Low stocking density gave the highest yields, whereas high stocking densities and high water exchange rates gave higher protein content. The lipid content was highest at low stocking densities.

2 Introduction

The production of organisms low in the food chain, like seaweeds was selected as one of the most important strategies to increase mariculture for future sustainable food production by the SAPEA (Science Advice for Policy by European Academies) Review report (2017). Seaweeds constitute a climate-friendly and sustainable biomass resource with a global production of 30 million tons per year, generating a value of 6 billion USD with prospects to increase due to growing food and ingredient industries (FAO, 2018). Asian countries are responsible for 99% of the global seaweed production and have well-developed aquaculture practice, whereas seaweed production in Europe is limited (< 1 %) and despite a growing number of seaweed farmers still relies mostly on harvest from wild populations. European seaweed cultivation is underdeveloped and cannot currently provide the biomass needed for the rapidly developing food industry. Without a guarantee for the requested feedstock amount and quality the process industry hesitates to invest in product development, and without the demand for delivery of the large quantities that the food industry would represent the seaweed farmers on the other hand hesitate to scale up. Improved cultivation technology and a better understanding of the production potential and biomass quality is thus strongly needed to ensure a growth in this industry also in Europe. In this study the production of sugar kelp *Saccharina latissima* and sea lettuce *Ulva sp.* by farmers in North- and South-Europe was monitored over two cultivation seasons, as a baseline for today's production potential and how this should be scaled up.

3 Methods

A 2 years monitoring program was carried out by the two *S. latissima* farmers SES (Frøya, Norway) and C-Weed (Saint-Suliac, France) over 2 years, using a common protocol for measure of the growth and biomass yield. In addition NUIG did a one year registration at the commercial farmer Dingle Bay Seaweeds (Ventry Harbour, Co.Kerry, Ireland) and CIIMAR registrations at two cultivation sites together with ALGAplus (Matosinhos, Leixões harbour and Aveiro, Portugal), using the same monitoring protocol as the farmers. Monitoring of the growth and production of *Ulva* was done by the farmer ALGAplus in Portugal.

For the sugar kelp registrations were done up to 6 times during the cultivation season, from March to June, and all sampling and measurements were done ca 2-3 m below the water surface. Each round was done on 5 individual cultivation ropes in each seaweed farm.

For registration of the wet weight all sporophytes on 1 m cultivation rope was harvested and weighted. The density was measured by counting all sporophytes on 25 cm of the rope, whereas blade length, blade width and stipes length measurements were done on 10 sporophytes. An evaluation of biofouling was also carried out, with special attention to bryozoan coverage.

For the chemical compositional analysis, carried out in GENIALG WP4, samples of 1 kg wet weight was harvested pr rope, drip drained for ca 1 min and packed in plastic bags before freezing at -20°C on arrival at the land base. A scientific paper will be dedicated to the presentation and discussion of the results from the analysis of these samples, and only one example of chemical composition of *S. latissima* biomass is presented in this report.

More detailed descriptions of the monitoring protocol can be found in Annex A.1 – A.5. An example of logging of light and temperature is presented in Annex A.6.

4 Results and discussion

4.1 Production of *Saccharina latissima*

The registration dates and producers of *S. latissima* are summarized in Table 1.

Table 1. The dates for deployment and registrations by the GENIALG seaweed farmers and research partners during the *S. latissima* cultivation season in 2018 and 2019.

Cultivator	**C-Weed**	**SES**	**NUIG/Dingle Bay Seaweed**	**CIMAR/Matosinhos**	**CIIMAR/ALGAplus**
Country	France	Norway	Ireland	Portugal	Portugal
Coordinates	48.585093 N -1.987912 W	63.42193 N 08.52181 E	52.1315233 N -10.3621817 W	41.177355 N -8.702573 W	40.620613 N -8.748006 W
Season 2018					
Deployment	09.01.2018	25.01.2018	04.12.2017		
Registration 1		06.03.2018	08.03.2018		
Registration 2		11.04.2018	12.04.2018		
Registration 3		26.04.2018	26.04.2018		
Registration 4		14.05.2018	24.05.2018		
Registration 5		29.05.2018	22.06.2018		
Registration 6	15.06.2018	12.06.2018			
Season 2019					
Deployment	10.12.2018	23.01.2019		27.02.2019	09.03.2019
Registration 1	18.03.2019	26.03.2019			
Registration 2	17.04.2019	11.04.2019			
Registration 3	15.05.2019	29.04.2019			
Registration 4	25.06.2019	10.05.2019			
Registration 5		27.05.2019		15.05.2019	28.05.2019
Registration 6		11.06.2019			
Sampling depth	1,5m	2m	2m	1m	-

The average biomass production by C-Weed, on the Bretagne peninsula, was stable around 12 kg m^{-1} cultivation rope in both years (Fig.1). In 2019 the yield varied more and was very high at some of the cultivation ropes, with up to 20,5 kg m^{-1}. For the Norwegian farmer SES at Frøya in Mid-Norway the production was highest in 2018, reaching on average 11,2 kg m^{-1}. In mid-May all ropes had > 5 kg m^{-1}. The productivity at this site was much lower the following year, with < 3 kg m-1 in May and an average total yield of 7,2 kg m^{-1} which is only 64% of the yield obtained the previous year. Compared to C-Weed and SES the biomass yield by Dingle Bay Seaweeds was low, only 6 kg m^{-1}.

The two small cultivation trials done in Portugal demonstrate that *S. latissima* can be cultivated also here but that the conditions were very suboptimal compared to the more northern places (Fig. 2). This experiment was carried out as a demonstration of cultivation of sugar kelp at its southernmost limit in Europe. The registration and termination of the trial was done after a cultivation period of only 2,5 months and showed that the growth was between 200-600 g/m (Fig.1). This shows that the production potential may be very low in this area, but a more optimised cultivation system could perhaps enable for a higher productivity, e.g. if the cultivation can be carried out in colder and more nutrients rich water more far from the shore.

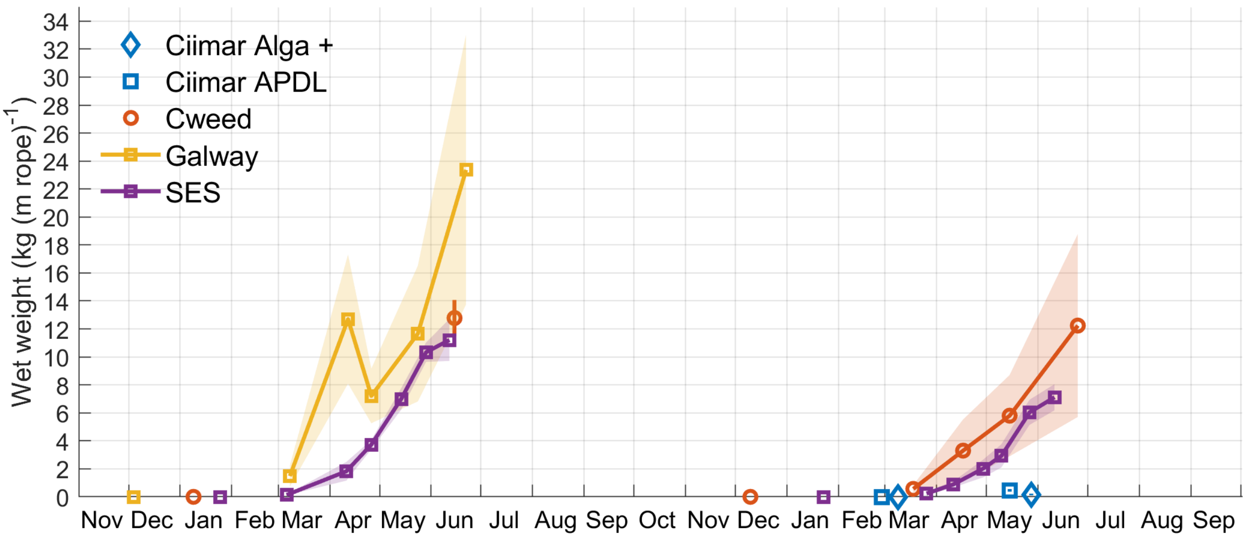

Figure 1. *S. latissima* biomass production as kg wet weight per meter cultivation rope, at different farming sites. CIIMAR and ALGA+ (Aveiro, Portugal), C-Weed (Saint-Suliac, France), Galway (Ventry, Ireland) and SES (Frøya, Norway). Symbols at the x-axix denotes the dates for deployment of seedling-lines in the seafarm.

Figure 2. *S. latissima* cultivated in Aveiro, Portugal by CIIMAR and ALGAplus the spring 2019.

The Figures 3 to 6 show the sizes of the sporophytes at the different cultivation sites, including frond length and width and stipes lengths, and Fig. 7 shows the sporophyte density on the ropes. These data, together with the data for biomass yield and chemical composition, will also be used to model the ecological carrying capacity for the different farms (in GENIALG WP6).

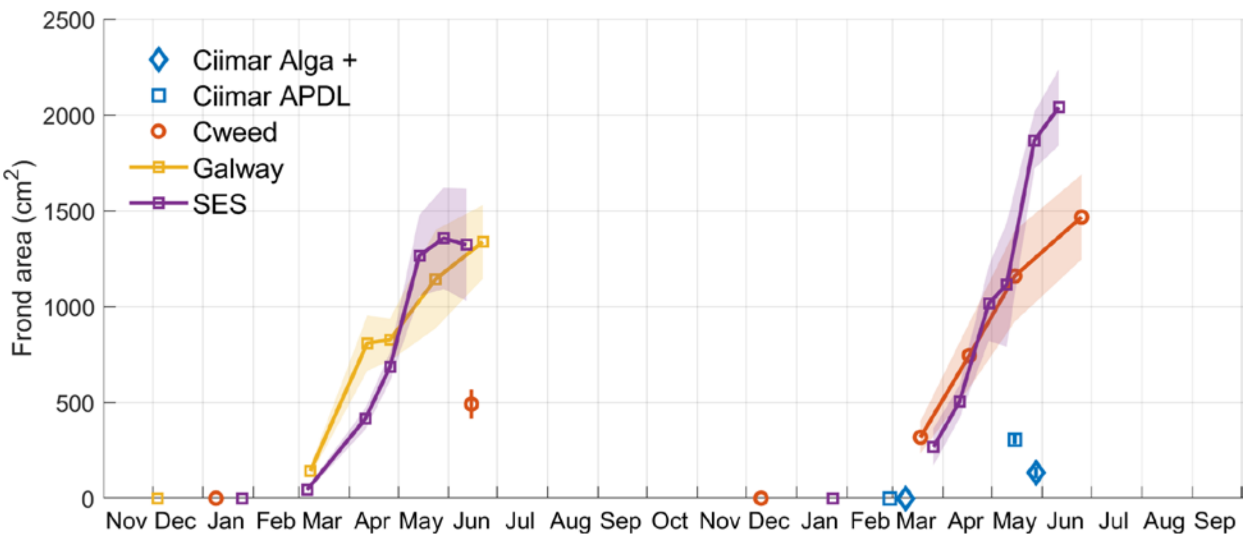

Figure 3. The frond area of *S. latissima* cultivated on ropes. For explanations, see Fig.1.

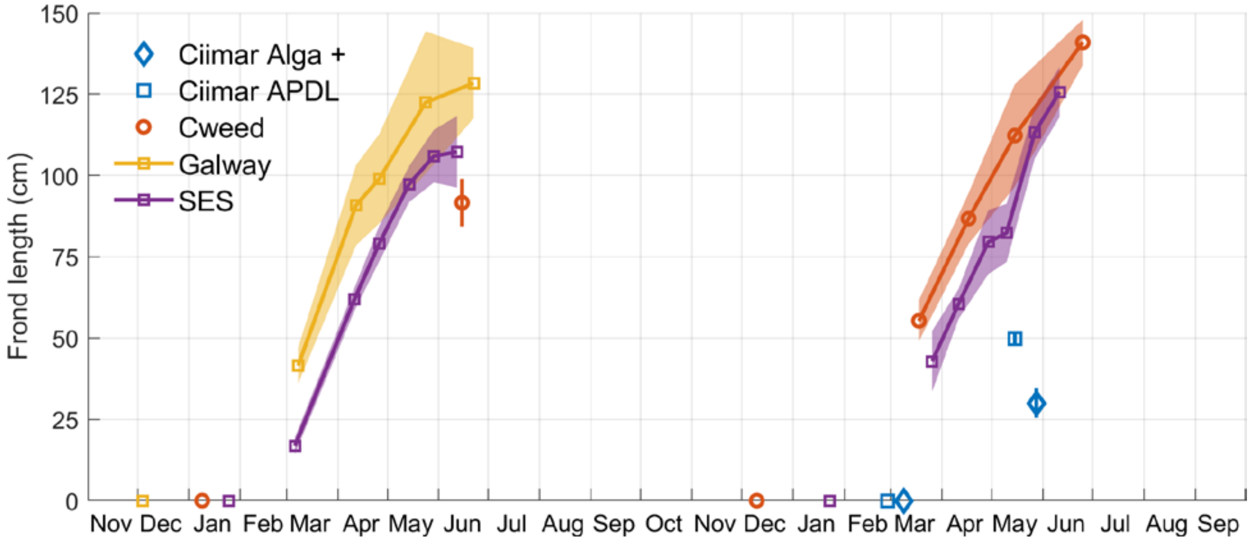

Figure 4. The frond length of the *S. latissima* sporophytes.

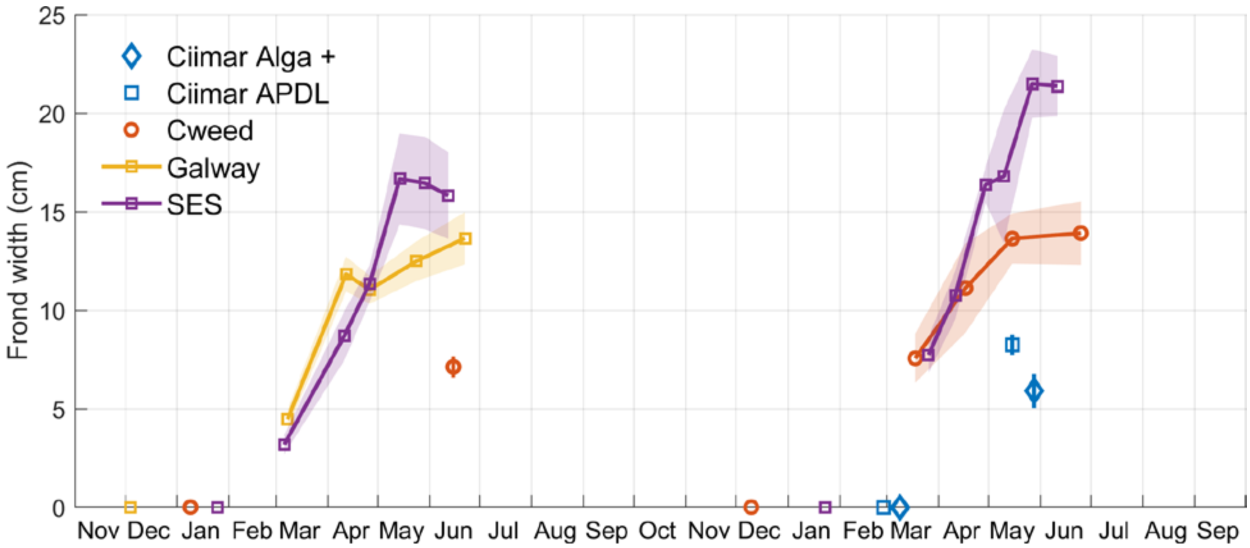

Figure 5. The frond width of the *S. latissima* sporophytes. For explanations, see Fig.1.

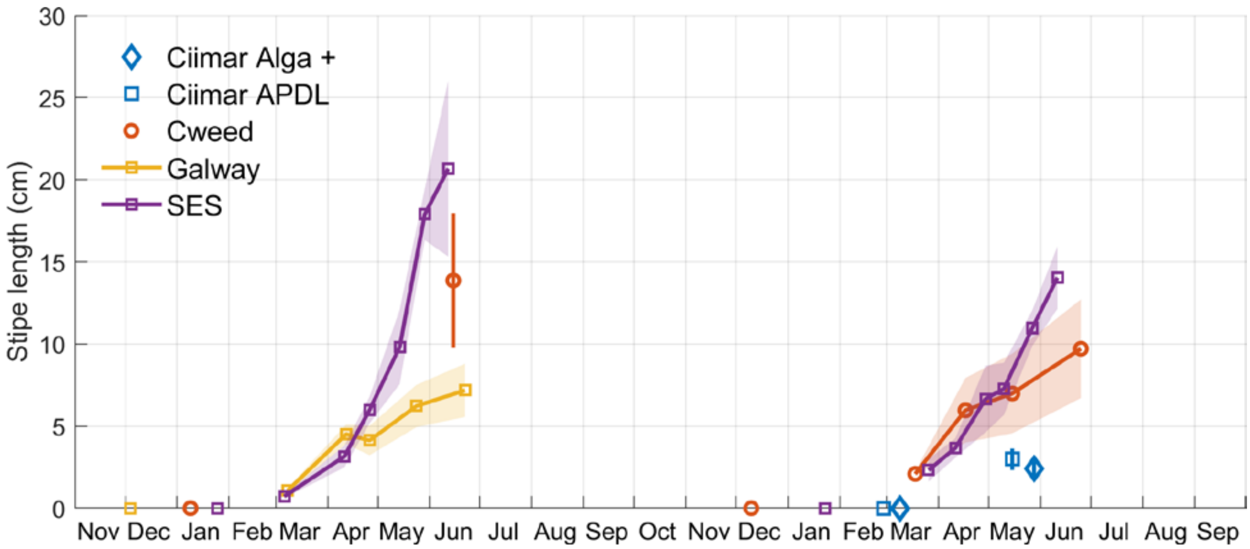

Figure 6. Stipes length of the *S. latissima* sporophytes.

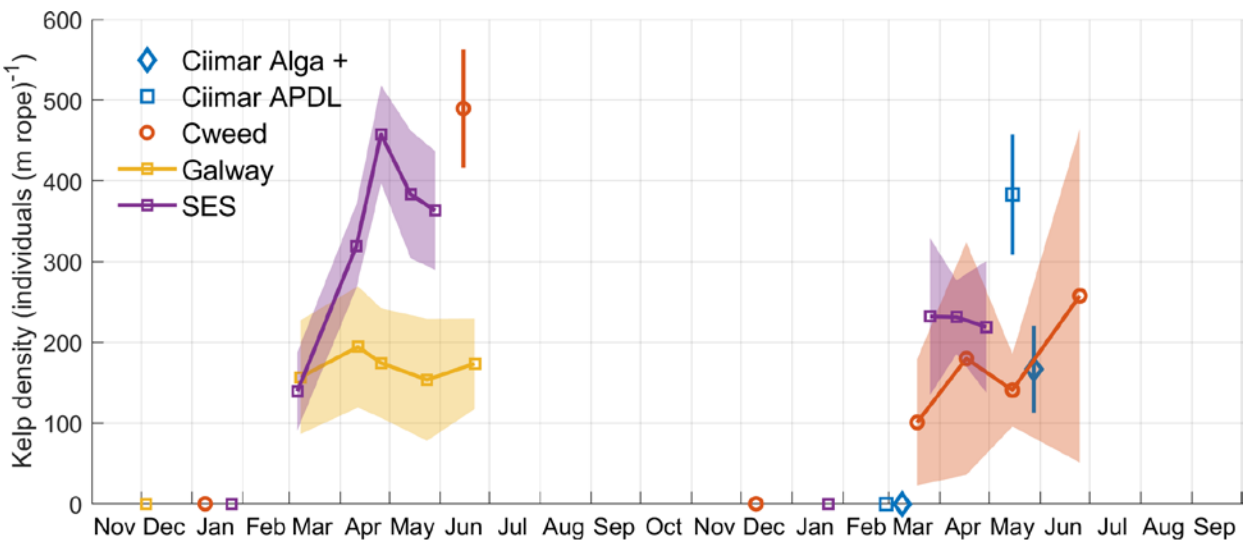

Figure 7. Density of the *S. latissima* sporophytes on the cultivation rope. For explanations, see Fig.1.

The chemical composition of the *S. latissima* samples is now being analysed and will be finalised and summarised during the last project year. Results for the most relevant months for harvesting is presented in Fig.8 and show that there are large variations in the protein and carbohydrate content between the different cultivation sites.

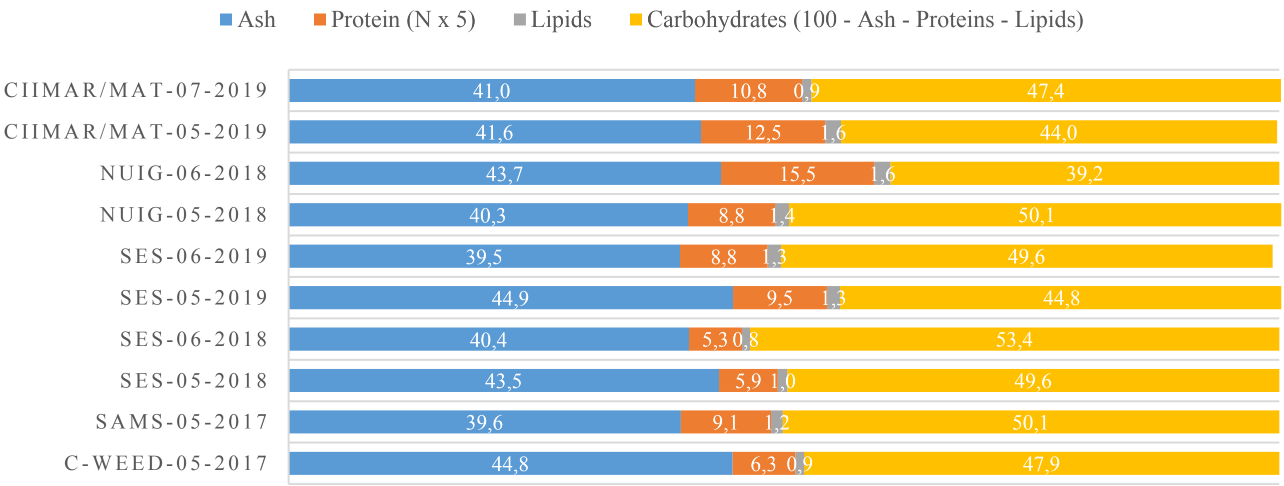

Figure 8. The chemical composition of *S. latissima* cultivated by GENIALG-partners at different locations in Europe from 2017-2019.

Table 2. Epiphytic scores on the *S. latissima* sporophytes and biomass dry weights as registered by the GENIALG farmers. Score from 0 (no) to 5 (complete coverage). See Annex A.3 and A.5 for more details.

Seaweed farmer	Year, reg.no.	Biofouling organisms (see Annex A.3)					Biomass dry weight %
		Membrani-phora	Electra	Snails	Filamentous algae	Other organisms	
C-Weed	2018	0	2	Y	Y	N	15,9
	2019-1	0	0	N	N	N	13
	2019-2	0	0	N	ectocarpus	N	11,4
	2019-3	0	0	N	ectocarpus	copepods	14,5
	2019-4	0	0	N	ectocarpus	copepods, sponges	16,4
SES	2018-1	0	0	N	N	N	11,1
	2018-2	0	0	N	N	N	8,1
	2018-3	0	0	N	some diatoms	N	8,9
	2018-4	0	0	N	Y	N	8,9
	2018-5	2	2	N	Y	N	8,7
	2018-6	3	3	N	Y, porphyra	ghost shrimps	10,3
	2019-1	0	0	N	N	N	n.a.
	2019-2	0	0	N	Y	N	n.a.
	2019-3	0	0	Y	Y	N	9,2
	2019-4	0	0	N	Y (lots), porphyra	N	10,7

	2019-5	3	3	N	Y (lots), porphyra	N	9,3
	2019-6	4	4	N	Y (lots), porphyra	hydroids	9,6
NUIG - Dingle Bay Seaweed	2018-1	1	1	N	N	N	n.a.
	2018-2	1	1	Y	Y	blisters	n.a.
	2018-3	1	1	N	Y	N	n.a.
	2018-4	3,6	1	N	Y	lumpsuckers, amphipods, obelia	n.a.
	2018-5	5	5	N	Y	hydroids, lumpsuckers	n.a.
CIIMAR - Matosinhos	2019	0	0	N	Y	N	14,3
CIIMAR - ALGAplus	2019	0	0	N	Y	N	13,6

Biofouling by epiphytic organisms induce lower quality and decides the harvesting time and thus the length of the cultivation season. Regular registrations of different types of epiphytes on the sporophytes blade and stipes was done by the farmers. A specific attention was paid to the incidence and proliferation of bryozoan as this restricts the application of the seaweed for fine food products, at an early stage mainly due to the less delicate look and later due to possible impact on the food quality like taste and consistency. The incidence of crustaceans, like copepods and skeleton shrimps (Caprella), may be very critical for use as food or in food products due to the risk of hyper allergic persons. Table 2 gives an overview of the biofouling state at the different registration points. The dry weight of the biomass was also measured and varied between 8,1-11,1 in the northernmost farm (SES) and 11,4-16,4 in the southern farms.

4.2 Production of *Ulva*

Ulva is cultivated only by one of the farmers in GENIALG, ALGAplus in Portugal, in a land-based integrated multi-tropic aquaculture (IMTA) system ahere the algae farm receives nutrients rich effluent water from fish aquaculture ponds in Aveiro. The yearly production is summarized in Table 3 and the effects of high or low stocking density and high or low water exchange rates on productivity and growth rates are presented in Fig. 9 and 10, and on the content of carbohydrates, protein, lipids and ash in Fig.11.

Table 3. Production of Ulva at ALGAplus' farm in Aveiro.

	Production of Ulva				
	Summer	Autumn	Winter	Spring	Total, per ha per year
kg fw m^{-2} $month^{-1}$	5,0	2,5	2,5	4,7	
kg fw ha^{-1} $month^{-1}$	50 231	25 314	24 582	47 233	
ton fw ha^{-1} $month^{-1}$	50,2	25,3	24,6	47,2	
Cumulative, tons fw ha^{-1}	150,7	75,9	73,7	141,7	442,1

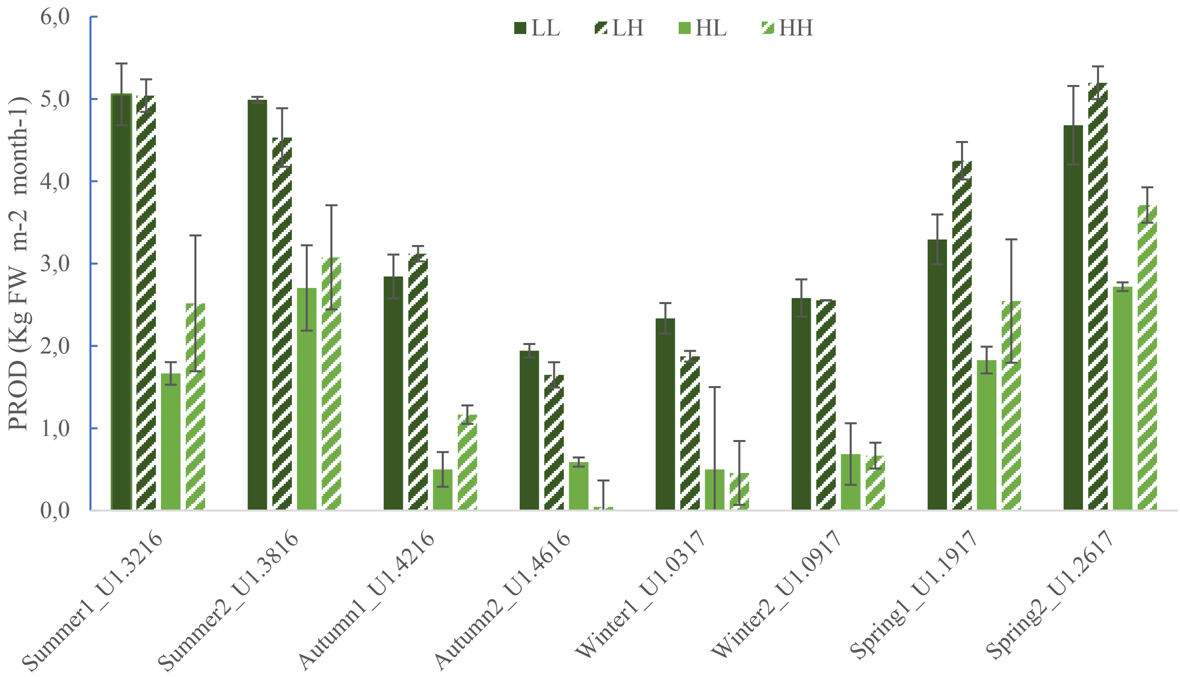

Figure 9. Effect of stocking density and water flux at the productivity of *Ulva* as kg fresh weight per m^2 per month at different seasons at ALGAplus' farm in Aveiro. LL = Low stocking density, low water flux; LH = Low stocking density, high water flux; HL = High stocking density, low water flux; HH = High stocking density, high water flux.

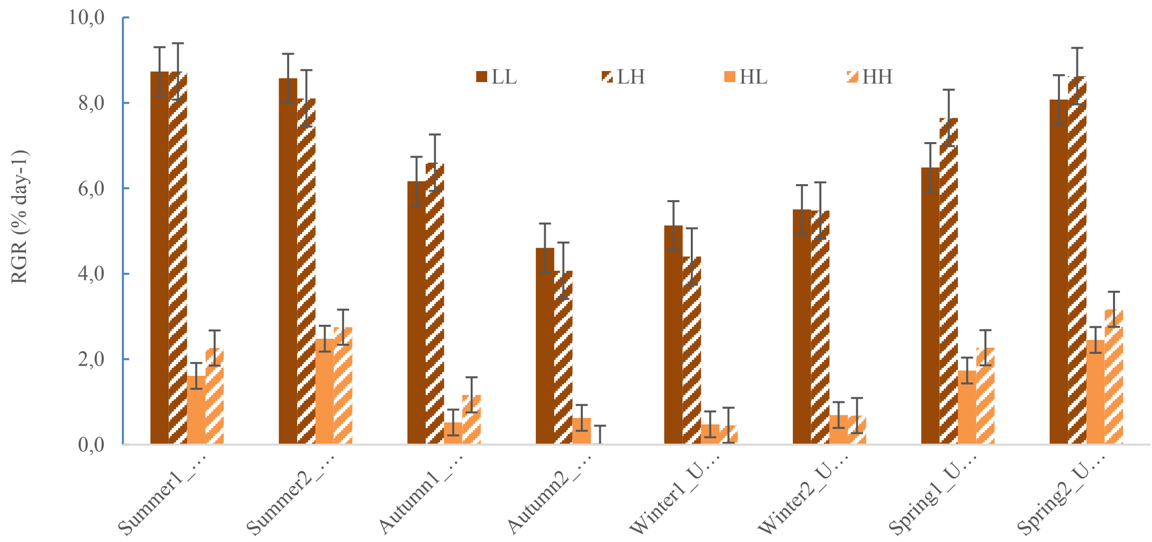

Figure 10. Relative growth rate (% growth per day) of *Ulva*. LL = Low stocking density, low water flux; LH = Low stocking density, high water flux; HL = High stocking density, low water flux; HH = High stocking density, high water flux.

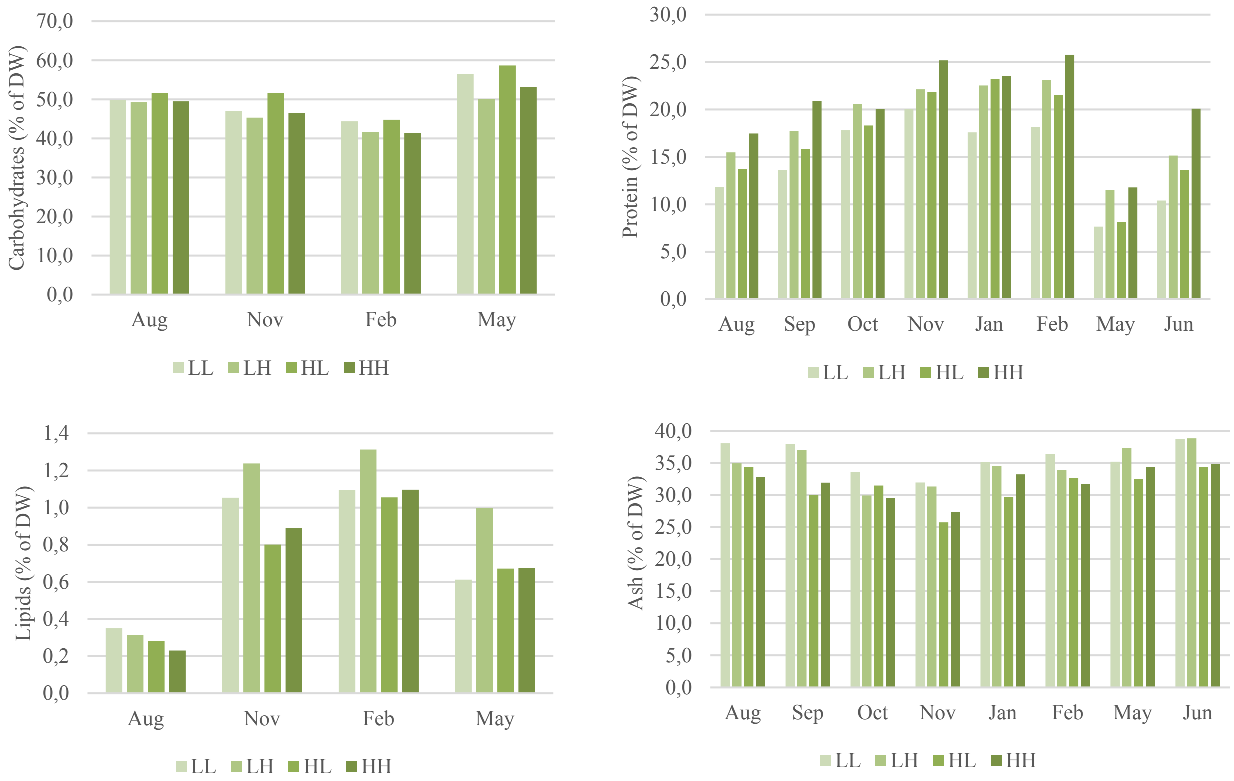

Figure 11. Seasonal variation in the chemical composition of cultivated *Ulva* at different stocking densities and water exchange rates. LL = Low stocking density, low water flux; LH = Low stocking density, high water flux; HL = High stocking density, low water flux; HH = High stocking density, high water flux.

The growth rates and productivity of *Ulva* were highest with a low stocking density. At low stocking densities the water flux has little effects as the nutrients supply is sufficiently high also by the low rate, however at high stocking rates a higher water flux ensures some higher production. The protein content was on average 17,7(4,9) % of the dry weight. A high water exchange rate gave higher protein content in the *Ulva* biomass, due to the higher supply of nitrogen from the fish farm. The lipid content is very low in Ulva, especially in the summer, but the increased water exchange rate gave slightly higher content.

5 Conclusions

This two-year baseline study was carried out to demonstrate the production potentials by different seaweed farmers in Europe. The *S. latissima* productivity varied strongly between the partners and between years. A yield of minimum 6 kg m^{-1} should be expected as a minimum at all the three commercial farms that participated in the project and an amount that the farmers can promise to deliver if they postpone all the harvesting until June. For delivery of biomass for chemical processing and biorefinery this is the most relevant time, as the quality of the sporophytes still can be fine for that purpose, but probably not for use in fine food any longer. Despite that most farmers start harvesting in April the farms should probably be dimensioned for 3 x the April weight on their lines, as there can still be a very good growth the following weeks.

The ALGAplus seaweed farm demonstrated a capacity to produce 442 tons fresh weigh *Ulva* ha^{-1} $year^{-1}$, with a seasonal variation spending from a quarterly production of 75 tons ha^{-1} during autumn and winter and up to 150 tons ha^{-1} during spring and summer. Low stocking density gives the highest biomass growth, whereas high stocking densities and high water exchange rates gives the highest protein content in *Ulva* whereas the lipid content is highest at low stocking densities.

6 References

FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO

Science Advice for Policy by European Academies (SAPEA), 2017. Food from the oceans: how can more food and biomass be obtained from the oceans in a way that does not deprive future generations of their benefits? Berlin: SAPEA. doi:10.26356/foodfromtheoceans

7 Appendix

A.1 Registrations during cultivation of *S. latissima*.

Sample no.	GENIALG Measurements S.latissima	Size/Number	Comments	On board	On land	On lab
	Number of sampling dates	6	In month 3, 4, 5 and 6			
	Replicates per date	5	Replicate sampling points: Droppers or positions in the farm *			
	Depth for sampling	2-3m	From 2 to 3m depth on droppers; current cultivation depth in case of horisontal line			
	Segment length to sample	1m	From 2 to 3m depth on droppers	x		
1	**Density (count no. individuals)**	25cm	From center of segment, count all individuals >10cm		x	
2	**Wet weight (productivity)**	1m	Drain for 1 min, cut all individuals (blade, stipe and holdfast) and weight in net	x		
	Morphometrics		From center of segment			
3	Length blade	10			x	
4	Width blade	10			x	
5	Length stipe	10			x	
6	**Photo**	2x	One overview with full size plants and one close up on epiphytes		x	
7	**Reproductive status**	Yes/No			x	
8	**Epiphytes**		From center of segment			
	Bryozoans	Scale 1 to 5	Membranipora and Electra on a scale from 1 to 5 (SES' protocol)		x	
	Other fouling organisms	Yes/No	Snails, filamentous algae, hydroids, mussels, on plant and rope		x	
9	**Dry:wet weight ratio**	5-10 individuals	If plants are large, take 5 individuals for dry weight estimation. Wipe surface with paper, (grind), take 3 sub-samples or replicas, weight before and after drying at 90˚C. Weigh until constant weight.			x
10	**Biomass sampling for analysis**	2 kg	All plants from 1-2 m depth at (or close to, in case of small biomass) each replicate point. Drain for 1 min, cut all individuals (blade, stipe, holdfast), put in plastic bags, chilled storage during transport before freezing	x		
			*Can be 5 x 6 ropes used only for the GENIALG-samples (destructive sampling each time), or the 5 sampling segments can be on defined positions in the farm and after each sampling (no. 1-10) the ropes are left for further use by the farmer.			

A.2 Registration scheme

The scheme is available as excel-document.

GENIALG WP3, Task 1.1 Registrations of growth characteristics for S. latissima (monitoring of phenotypic and growth parameters)

Cultivator (name)	
Locality	
GPS coordinates	
Date for deployment (DD.MM.YYYY)	
Depth for sampling (m below surface)	
Registration number (1-6)	
Registered by (name)	
Registration date (DD.MM.YYYY)	

	R1 *Replicate/rope 1*			R2 *Replicate/rope 2*			R3 *Replicate/rope 3*			R4 *Replicate/rope 4*			R5 *Replicate/rope 5*		
Density (#ind at 25 cm rope)															
Wet weight (all ind at 1 m rope)															
Morphometrics (cm)	Blade length	Blade width	Stipe length	Blade length	Blade width	Stipe length	Blade length	Blade width	Stipe length	Blade length	Blade width	Stipe length	Blade length	Blade width	Stipe length
Sporophyte 1															
Sporophyte 2															
Sporophyte 3															
Sporophyte 4															
Sporophyte 5															
Sporophyte 6															
Sporophyte 7															
Sporophyte 8															
Sporophyte 9															
Sporophyte 10															
Photo taken (X)															
Reproductive status (Y/N)															
Epiphytes															
Membraniphora (1-5)															
Electra (1-5)															
Snails (Y/N)															
Filamentous algae (Y/N)															
Other (Y/N)															
Biomass samples 2kg (X)															

Dry : wet weight ratio (Av. % dry weight)		%

A.3 Comments to scheme

Epiphytes fouling scores - bryozoans:

Score:

1	Negligible fouling
2	Very small colonies start to appear (dots)
3	Small colonies visible and starting to grow (<1cm)
4	Mid-large colonies (>1cm)
5	"Take over" of significant parts of the blades

Density:

All sporophytes on 25cm rope are counted, ignore smallest (<10 cm).

Wet weight:

Harvest biomass from one (1) meter rope and measure weight in a calibrated net.

A.4 Biomass sampling for analysis and processing

For the small scale processing trials and *dry:wet* weight ratio:

2 kg or all plants from 1m rope at each replicate point.

Make sure to keep 5-10 individuals for the dry:wet-ratio (next paragraph) before freezing the rest. If plants are very large, use 5 individuals. NB: Only need one sample for dry weight per date.

1. Drip drain rope for 1 min
2. Cut off the sporophytes and wipe excess water off the surface with paper
3. Put in code-marked plastic bags. CODE example: SLSES15042018-R1 (Saccharina latissima, Seaweed Energy Solutions, date, replicate 1).
4. Keep at chilled storage during transport
5. Freeze at -20°C.
6. Samples are shipped to receiver as frozen or freeze dried (to be decided in agreement).

A.5 Protocol for dry weight : wet weight ratio

From **one** replicate point:

1. Minimum 5 individuals, wipe plant surface with paper gently
2. Grind or use whole individuals
3. Distribute biomass for 3 replicas on pre-weighed Al-foil
4. Weigh with a balance with 0.00 g accuracy to have wet weight
5. Dry at 90°C for 24 hours and weigh to have dry weight. Weigh until constant weight (may repeat after 2h to check if stable weight).
6. Use final weight in calculation of % dw (remember to subtract weight of Al-foil).

NB: Large sporophytes may require longer drying time. Grinding and taking well mixed sub-samples can be done instead of using whole individuals.

A.6 Logging of light and temperature

An example of logging of temperature and light with a HOBO-logger is presented below and shows how the temperature varies between 10 and 21°C the first season and from 6 to 21°C the second year at the cultivation site of C-Weed on the Bretagne peninsula. It also demonstrates the fast biofouling of the light sensor and thus the uncertainty of logging of light unless the sensors are regularly cleaned, maybe as often as every/every second week in certain periods of the year.

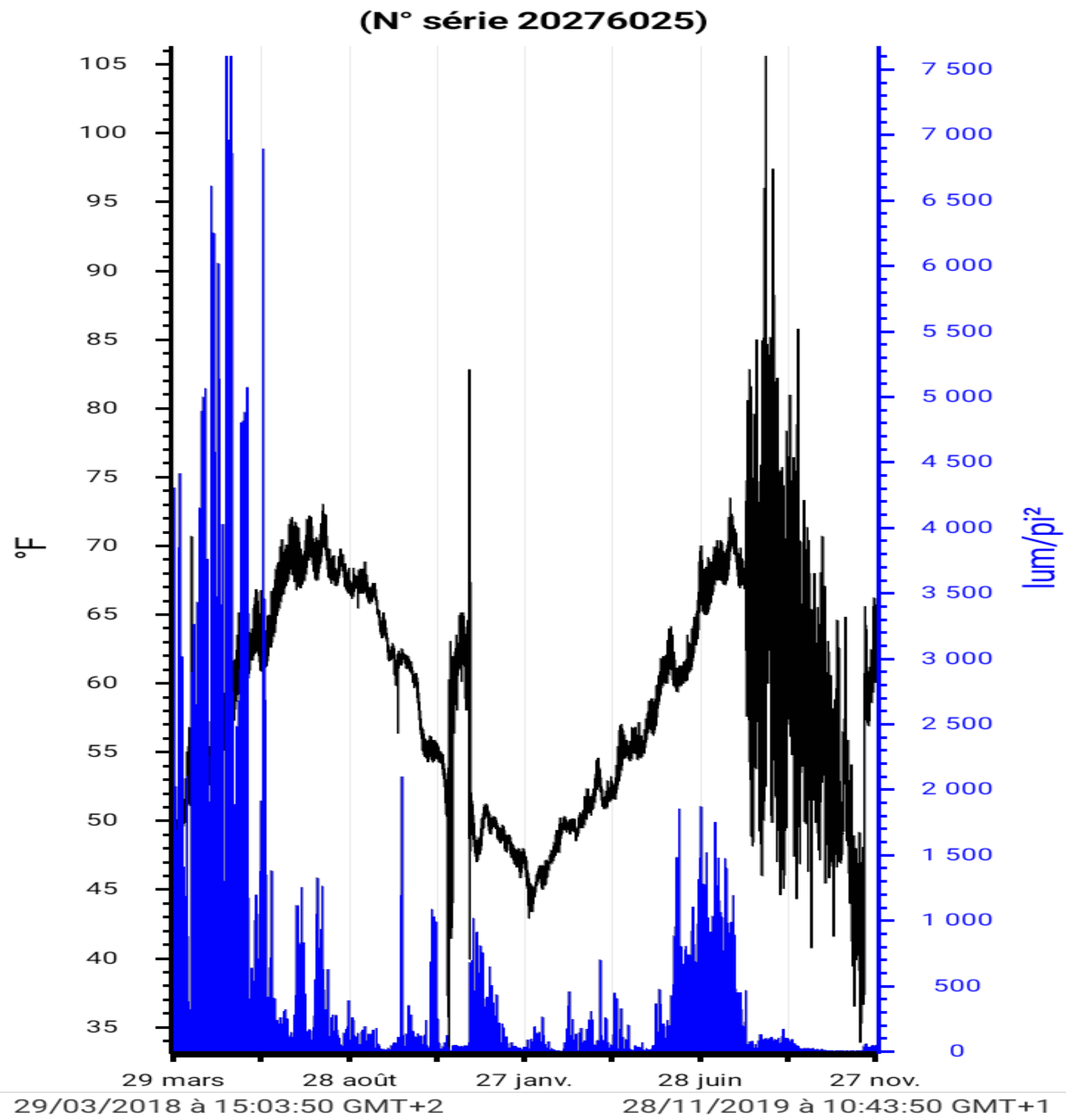

Figure 12. Temperature (black; left axis) and light (blue; right axis) in the seaweed farm of C-Weed in 2018-2019.

A.7 Monitoring protocol for quality control of Ulva

Monitoring protocol for quality control of Ulva produced in the commercial production tanks

- Protocol being followed since Feb 2019 – previous data were random/not accurate.
- All the parameters (Table 1) as well as associated information (below) are registered in our production management software.
    - Associated Information:
        - Origin of the batch
        - Time in culture
        - Tank size
        - Yield and RGR

- Pictures are taken for every sample
- Sampling frequency and replicates:
    - 1 tank daily (from the group of tanks that is being harvested that day)
    - 6 individuals per tank, randomly chosen from a wider sample collected by the production staff

Registered parameters:

Tank #							
Nº individual / sample ref.	**Frond size** <5 cm 6-10 cm 11-20 cm >20 cm		Perforated Vs regular frond	Reproductive status (sporulation vs vegetative)	Fouling (Y/N)	Picture of most abundant epiphytes (Y/N)	Colour*
	Largest axis	Shortest axis					
1							
2							
3							
4							
5							
6							

NOTES:

First trial with 10 individuals/per tank took more than 45 min. Thus, the decision was to adjust to 6 individuals per day (from the same tank).