Development of Offshore Seaweed Farming: Ecology & Cultivation

Synthesis report 2019

Author(s): M.S. Bernard[1], H. Jansen[1], A. van der Werf[2], I. van der Meer[2], Linda Tonk[1]

Wageningen University & Research report C054/20

1: Wageningen Marine Research

2: Wageningen Plant Research

WAGENINGEN
UNIVERSITY & RESEARCH

Development of Offshore Seaweed Farming: Ecology & Cultivation

Synthesis report 2019

Author(s): M.S. Bernard[1], H. Jansen[1], A. van der Werf[2], I. van der Meer[2], Linda Tonk[1]

1: Wageningen Marine Research

2: Wageningen Plant Research

This research project was carried out by Wageningen Marine Research and Wageningen Plant Research at the request of and with funding from the Noordzeeboerderij and the Ministry of Economic Affairs for the purposes of Policy Support Research Theme 'Maatschappelijk Innovatieprogramma PROSEAWEED' (project number BO-47-001-001).

Wageningen Marine Research
Yerseke, June 2020

Wageningen Marine Research report C054/20

Keywords: Seaweed cultivation, Offshore, Biodiversity, Environmental conditions, North Sea

Client: Stichting Noordzeeboerderij
Zeestraat 84
2518 AD, Den Haag

This report can be downloaded for free from https://doi.org/10.18174/524395
Wageningen Marine Research provides no printed copies of reports

Wageningen Marine Research is ISO 9001:2015 certified.

Wageningen Marine Research, an institute within the legal entity Stichting Wageningen Research (a foundation under Dutch private law) represented by Dr. M.C.Th. Scholten, Managing Director

KvK nr. 09098104,
WMR BTW nr. NL 8113.83.696.B16.
Code BIC/SWIFT address: RABONL2U
IBAN code: NL 73 RABO 0373599285

A_4_3_2 V29 (2019)

Contents

Summary

The upscaling of offshore wind farms in the North Sea creates opportunities for seaweed aquaculture that has the potential to meet part of future resource needs, provided that it is done sustainably. Here a follow-up study of the MIP project in 2018 "Development of Offshore Seaweed Cultivation: food safety, cultivation, ecology and economy" with a focus on ecology and cultivation is presented. In order to ensure a sustainable development of seaweed farming in Dutch offshore and coastal regions in the future, it is essential to collect empirical data on the interaction of seaweed cultivation with marine ecosystems for realistic impact assessments.

In subproject 1 "Ecology – Fauna associated with seaweed aquaculture in the North Sea Farm" ecosystem services and impacts of seaweed farming in the North Sea were investigated on the basis of biodiversity, a key parameter for the functioning of ecosystems. Therefore in 2019 the associated fauna on growing seaweed biomass (*Saccharina latissima*) and cultivation ropes was assessed at the North Sea Farm. A high number of individuals was detected on the seaweeds and cultivation ropes in general (up to 7679 individuals per rope), but species richness was low. Abundance in fauna increased from May to June and all detected species are also known from other hard substrates in the North Sea. Compared to previous assessments of biodiversity with eDNA metabarcoding at the same site, the biodiversity detected in 2019 was very low. However, biodiversity levels may differ from year to year. Moreover, the samples were not taken at the same time points and are therefore not directly comparable and the methodology only included organisms that could be collected by hand (visible to the eye) with a focus on fauna attached to the rope and kelp. It is advised to combine classical morphological biodiversity assessment and eDNA metabarcoding in future assessments to compare results in order to determine the best-suited methodology for biodiversity assessments. Biodiversity in the seaweed farm should be assessed repeatedly every 5 years to check for temporal alterations in fauna composition, especially when cultivation structures, such as anchors, are deployed throughout several years.

In subproject 2 "Cultivation" seasonal variation in biomass productivity and chemical composition of kelp was evaluated in order to determine the optimal time point for harvesting in relation to the desired end product. Biomass production at the test-farm was very low in 2019, compared to previous years and a seaweed farm test location near Helgoland in the North Sea. Below 4m environmental conditions for growth were unfavourable (mainly light limitation) for *Saccharina latissima*. Both in 2018 and 2019 large differences in standing crops over time and depth were observed. Contrary, true protein levels varied only slightly over time. If protein is the target product, final biomass yield of *S. latissima* will determine the profitability of the mariculture. A combination of economic analyses and growth experiments may assist in determining the optimal cultivation technique.

The 2018 experiments performed at the North Sea Farm showed large seasonal variability in the chemical composition of seaweed tissue, and high amounts of nitrogen-containing compounds besides proteins variations. Therefore in 2019, nitrogen, starch and nitrate content in the seaweed tissue were analysed. Nitrate content in *S. latissima* varied throughout the season and could not fully explain the difference between N measured by Dumas and true protein content in the 2019 samples. Therefore, other seaweed components containing nitrogen must explain this variation, e.g. its accumulation in cellular nitrate pools. As a final note, in order to improve the understanding of environmental conditions in the farm it is recommended that nitrate and phosphate concentrations, two essential macronutrients for growth in seaweeds, should be assessed in the water column at different depths and time points.

1 Introduction

In 2018, the MIP project "Development of Offshore Seaweed Cultivation: food safety, cultivation, ecology and economy" by the Noordzeeboerderij and several institutes of Wageningen Research investigated a broad range of aspects regarding offshore seaweed production in the North Sea (see synthesis report by Jansen et al. 2019). The focus of the follow-up project in 2019 lay on ecology (subproject 1) and cultivation (subproject 2).

1.1 Subproject 1: Ecology – Fauna associated with seaweed aquaculture in the North Sea Farm

Biodiversity is a key parameter for the functioning of ecosystems and an important parameter in several marine policies, e.g. in water framework directives. In 2018 a factsheet was published describing positive and negative effects of seaweed aquaculture on biodiversity based on a literature review (Jansen and Tonk 2018). However, empirical and quantitative data is still largely lacking.

In 2019 we assessed the associated fauna on growing seaweed biomass (*Saccharina latissima*) and cultivation ropes at two time points, in May and June. At each time point five individual cultivation lines were collected by divers. The fauna was removed manually from the seaweed biomass and the cultivation ropes and identified morphologically to the lowest possible taxonomic level. Temporal differences in biodiversity are highlighted by comparing the results obtained in May and June. Moreover, a comparison was made with fauna associated to other hard substrates in the North sea and to results of a previous study, where the biodiversity in the North Sea farm was assessed by means of DNA metabarcoding of settlement plates (Bernard et al. 2019).

1.2 Subproject 2: Cultivation

The experiments performed in 2018 showed a strong seasonal variation in biomass productivity and chemical composition of kelps. Therefore, this topic was further evaluated in 2019 in order to determine the optimal time point for harvesting in relation to the desired end product. Independent of the final application, a key requirement for the production of seaweeds on a commercial scale, is to ensure a continuous supply of uniform biomass, since high variation in productivity and biochemical composition poses difficulties for the processing industry.

1.2.1 Seasonal variation in standing crop and depth dependence

Biomass productivity of kelps showed not only a strong seasonal variation but also a strong depth gradient in previous field studies. In 2019, biomass production was determined at three time points: in May, June and July. At each time point five individual cultivation lines were collected. Biomass was analysed by dividing each line into seven sub-samples of one meter each over the vertical profile. For each section the fresh and dry weight of the harvested biomass were determined. A subset of these samples were used for the analyses in 2.2.

1.2.2 Seasonal variation in nitrogen and starch content and amino acid composition

When it comes to the valorisation of kelp biomass, not only quantity but also quality is of importance. The previous experiments performed at the Noordzeeboerderij in 2018 indicated large seasonal variability in the chemical composition of seaweed tissue, and high amounts of nitrogen-containing compounds besides proteins, which were observed specifically in May. As it is not known whether this represents a re-occurring phenomenon, nitrogen and starch content in the seaweed tissue were analysed

including the nitrate levels in five individual samples, taken at three time points. Furthermore, the results on the total amount of amino acids were compared between 2018 and 2019 in a subset (in duplo) of samples, also taken at three time points: in May, June and July. These six samples were selected for an analysis of total amino acid composition in order to validate the phenomenon that was observed in 2018: a high accumulation of nitrogen-containing 'non-protein' compounds in May that might increase the commercial value of the kelp biomass. The nitrate concentrations were also determined in 11 samples, collected in 2018: at two timepoints and two depths of the ropes.

1.2.3 Environmental conditions in the North Sea Farm

The observed seasonal variation and depth gradient in biomass production and biochemical composition is likely to be related to depth-dependent changes in the environmental conditions. In order to obtain a better understanding of the abiotic environment at the North Sea Farm, light irradiance and temperature were measured continuously during the entire cultivation period by data- loggers (Onset Computer Corporation, HOBO®, USA) deployed at multiple depths. Additionally, measurements were performed in June providing information about turbidity, salinity, temperature and conductivity at depths from 0 to 20m.

2 Fauna associated with seaweed aquaculture in the North Sea Farm

2.1 Introduction

In order to ensure a sustainable development of seaweed farming in Dutch offshore and coastal regions in the future, it is essential to collect empirical data on the interaction of seaweed cultivation with marine ecosystems for realistic impact assessments. Here we investigated ecosystem services and impacts of seaweed farming in the North Sea on the basis of biodiversity, a key parameter for the functioning of ecosystems.

Natural kelp forests are among the most diverse and productive ecosystems in the world (Steneck et al. 2002). Their three-dimensional structures support complex food webs and provide food, habitat and breeding areas for a variety of associated organisms (Bartsch et al. 2008; Christie et al. 2009). Natural seaweed beds usually occur on rocky substrate, providing a versatile habitat consisting of both soft and hard substrate which attracts a high number of different organisms.

While the biodiversity in natural seaweed populations has been well-studied over decades (Dayton 1985; Steneck et al. 2002), only few studies have addressed the biodiversity in seaweed farms (Walls et al. 2016; Wood et al. 2017; Bernard et al. 2019). The hard substrate in seaweed farms is limited to the anchors and cultivation structures and seaweeds are usually suspended in the water column. Thus, seaweed cultivated in (offshore) farms may not be as easily accessible to benthic invertebrates as natural (coastal) seaweed beds. On the other hand, seaweed farming could also act as a stepping stone for invasive species or as a reservoir for diseases and pests (Loureiro et al. 2015; Bernard 2018; Campbell et al. 2019). For instance, epiphytic algae are a major concern for seaweed aquaculture, since the coverage reduces yields and quality of the cultivated seaweed (Potin et al. 2002).

In order to assess the impact of seaweed farming on marine environments for a sustainable future development of the Dutch seaweed sector, reliable assessments and empirical data on the biodiversity in seaweed farms are needed.

2.2 Material and Methods

Five seaweed cultivation ropes of 7m length with *Saccharina latissima* - installed vertically from 0m down to 7m below sea level - were harvested by divers from the North Sea Farm on 16th of May 2019 and the 19th of June 2019. All ropes were transferred directly into a barrel and transported to Yerseke immediately after the harvest.
Fauna on the seaweeds and cultivation ropes was assessed morphologically. All fauna was removed manually from the seaweed and the ropes, preserved in ethanol (96%) and identified to the lowest possible taxonomic level based on their morphology with the help of identification keys and a microscope. Due to the harvesting technique (the entire rope was collected in one barrel and it is likely that animals have moved during the transport), no information on biodiversity according to cultivation depth could be made. All fauna found in the barrel was identified, counted and the average number of animals added to the total fauna found on each rope.

2.3 Results

A high number of individuals was detected on the seaweeds and cultivation ropes in general (up to 7679 in June, Fig. 1), but species richness was low. A tripling in the total number of individuals within one month was observed and numbers increased from 2287 ± 385 individuals/rope in May to 7258 ± 1645 individuals/rope in June (Fig. 1).

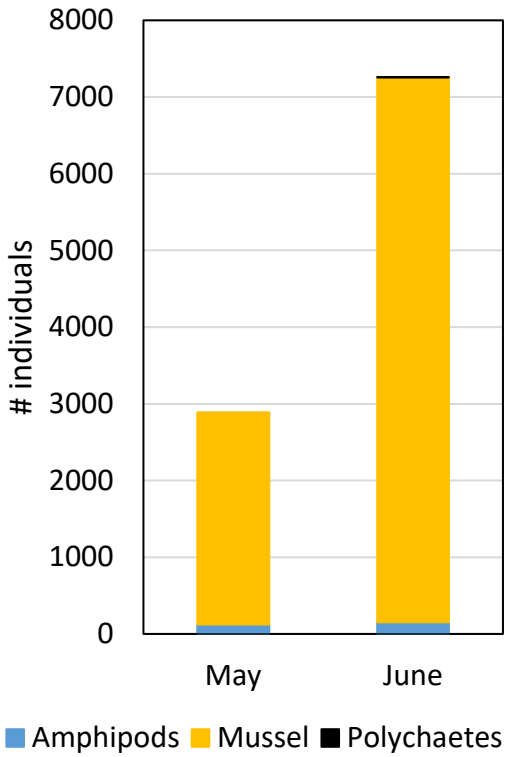


Figure 1 *Total number of individuals on seaweeds and cultivation ropes per rope in May and June 2019 (N=5).*

Amphipods, mussels and polychaetes were found on the seaweeds and ropes. Most of the fauna was located on the ropes whereas the seaweeds were generally less populated (Fig. 2A). More mussels were found on the bottom end of the rope (Fig. 2B) than on the end that was fastened on the surface.

Figure 2 *A. S. latissima harvested in June. B. Bottom end of a rope harvested in June heavily overgrown by mussels.*

The amphipods found on the seaweeds and cultivation ropes consisted mainly of one species, i.e. *Jassa* sp. (Fig. 3). In May, a second species – *Gammarus* sp. – was found on one of the ropes (Fig. 4). Amphipods are an order of the Crustaceans (Arthropoda). They are an important component of marine ecosystems and often act as grazers (Duffy 1990). Amphipods are well-known to be associated to wild and cultivated seaweed (James et al. 1986; Knip and Scheibling 2007) and both of the identified species have been previously observed on the cultivated kelps *Alaria esculenta* and *Laminaria digitata* on the west coast of Ireland (Walls et al. 2016, 2017). Furthermore, *Jassa* sp. was also detected by DNA metabarcoding on settlement plates deployed in the North Sea Farm in 2018 (Bernard et al. 2019).

Figure 3 *Jassa herdmani. Photo by Hans Hillewaert (wikicommons)*

Amphipod numbers increased slightly during the cultivation season from 120 ± 38 individuals/rope in May to 154 ± 41 individuals/rope in June (Fig. 4).

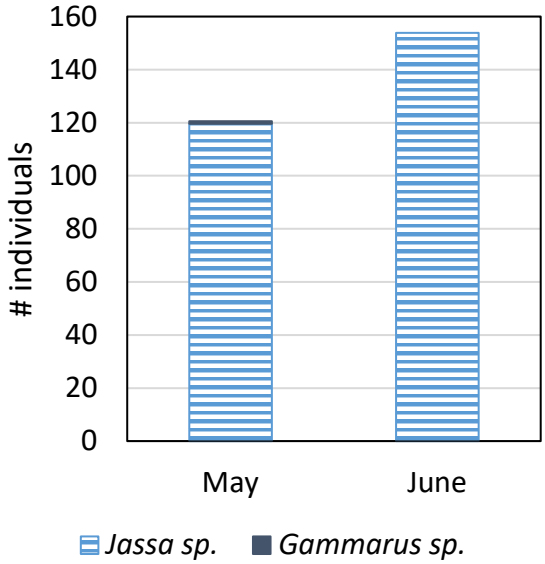


Figure 4 *Total number of amphipods found on the seaweeds and cultivation ropes per rope in May and June 2019 (N=5). Two species of amphipods were found: Jassa sp. and Gammarus sp.*

The number of mussels on the seaweed and cultivation ropes increased 2.6 fold from 2768 ± 405 individuals/rope in May to 7100 ± 1614 individuals/rope in June (Fig. 5). Two different mussel species - *Mytilus edulis* and *Mytilus trossulus* - have been detected by DNA metabarcoding in a previous study at the North Sea Farm (Bernard et al. 2019). These two species are cryptic species, i.e. they are not distinguishable based on morphological characteristics. They co-occur at the Atlantic and North Sea coasts and are counted to the same species complex of "blue mussel" (Väinölä and Strelkov 2011; Mathiesen et al. 2017). Since a species identification based on the morphology was not possible both species are reported here as *Mytilus* sp.. Molecular techniques, such as DNA barcoding, are necessary for an identification

The mussels were highly abundant on the cultivation ropes, especially on the lower part of the ropes that were suspended to a depth of approx. 7m (Fig. 2). From May to June the mussels increased in numbers and also in size.

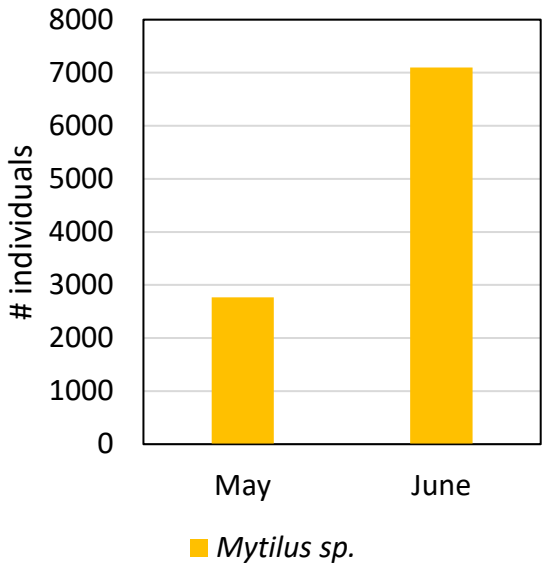


Figure 5 *Total number of blue mussel found on the seaweeds and cultivation ropes per rope in May and June 2019 (N=5).*

Two species of polychaetes were found on the seaweeds in very low numbers. Polychaetes are a class of annelid worms and are also known as bristle worms. Similar to amphipods, polychaetes are frequently found on wild and cultivated seaweed (James et al. 1986; Knip and Scheibling 2007; Walls et al. 2016). However, no polychaetes were identified by DNA metabarcoding on the settlement plates that were deployed in the North Sea Farm in 2018 (Bernard et al. 2019).

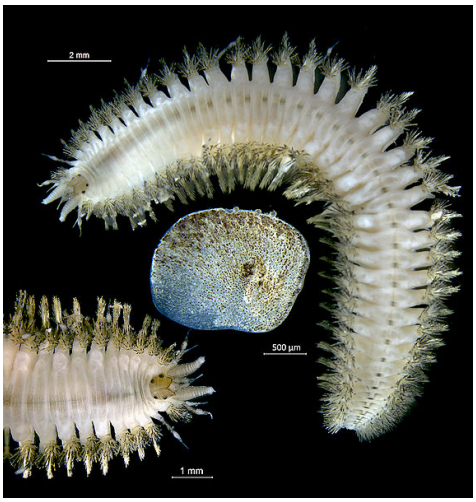


Figure 6 *Lepidonotus squamatus. Photo by Hans Hillewaert (wikicommons).*

Lepidonotus squamatus (Fig. 6) and an unidentified polychaete species were detected on the seaweeds *S. latissima* in June. No polychaetes were detected in May (Fig. 7).

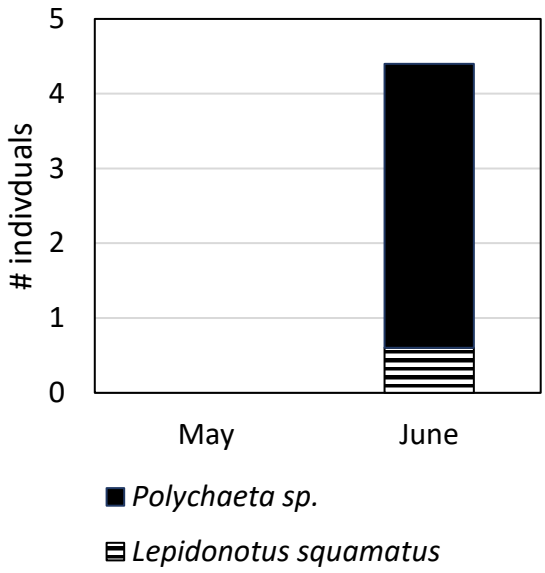


Figure 7 *Total number of polychaetes found on S. latissima and cultivation ropes per rope in May and June 2019 (N=5). Two species were found: Lepidonotus squamatus and an un-identified polychaete species.*

2.4 Discussion

Methodology

In this study, biodiversity has been assessed based on morphological characteristics. The advantage of this methodology is that it provides a reliable quantification of fauna. On the other hand, this technique needs highly trained staff and has some more limitations, such as the detection of cryptic, small, and rare species, as well as juvenile life stages, which are difficult to identify based on morphological characteristics only (Leray and Knowlton 2015; Pavan-Kumar et al. 2015; Thomsen and Willerslev 2015). Furthermore, part of the microscopic fauna (such as diatoms, the most abundant taxon in a previous metabarcoding analysis) cannot be analysed using this method, as only animals were analysed that could be collected manually. Biodiversity can also be assessed using DNA metabarcoding, but eDNA samples can be troublesome in regard to determining the appropriate spatial scale. When eDNA is detected in a farm, it doesn't necessarily mean that the animal has actually been associated to the ropes or the seaweed. Another method to investigate biodiversity is by visual surveys either by SCUBA diving or underwater camera systems. These methods are mainly fit for larger mobile species, such as fish.

Comparison to previous experiments and other hard substrates in the North Sea

In 2018, settlement plates were deployed in the North Sea and analysed by DNA metabarcoding after 6 months exposure (Bernard et al. 2019). In total, 134 different taxa were identified, but the reliability of this method is still unclear and it does not provide a quantification of fauna. Furthermore, the settlement plates were harvested later in the year, therefore the results cannot be compared directly to the results from 2019. Furthermore, in 2018 seaweed (*S. latissima*) growth was much higher, up to 3.8 kg m^{-1}, which may also have attracted more species. For the future, it would be interesting to directly compare the two different methods (classical analysis and DNA metabarcoding) to define which method leads to more reliable results.

Biodiversity has also been assessed at other hard substrates, such as the offshore wind park? Egmond an Zee, located 10-18km off the coast of Egmond an Zee (Bouma and Lengkeek 2012). Different methods were combined by the authors to assess the biodiversity on the monopiles and the scour

protection layer, including video footage and photos, scrape and benthos samples including subsequent lab analysis. Experiments were performed in 2008 and 2011. In total 55 species were recorded on the monopiles in 2008, while 23 additional species were recorded in 2011. This stresses that repetitive experiments in subsequent years can provide additional information and are crucial for an ecological survey. Similarly, in the present study, a high amount of mussels was detected on the monopiles. In February, the mussels were distributed in patches covering up to 60% of the monopiles' surface, whereas in September the entire zone was almost fully covered by mussels. Especially the deeper subtidal zone from 3 to 12m was characterised by a thick layer of mussels, covering 90-100% of the surface. Mussels reached densities of up to 4000/m², but also other species, such as the amphipods *Jassa* sp. and *Lepidonotus squamatus* were identified in lower numbers in the present study at the North Sea Farm. At a lower depth of 12 to 15m crustaceans, anemones and tubularia were found on monopiles.

Schrieken et al. (2013) investigated the biodiversity on a ship wreck at the Dogger Bank by SCUBA diving and recorded 61 species. Among the observed species were all species identified in the present study, but also several others, such as nine fish species that could not be detected using the methodology used in the present study.

Invasive species

No invasive species were detected on the seaweeds or ropes in this study, whereas two invasive species were found on the settlement plates in 2018: the bay barnacle *Amphibalanus improvisus* and the tropical red alga *Kappaphycus* sp. However, eDNA records are not sufficient for confirmation because the origin of the DNA of these species is unclear. To confirm the presence of invasive species in the farm, it is necessary to detect them morphologically.

Implications for seaweed farming

Biodiversity on seaweed or cultivation structures is not only of interest for seaweed farmers in order to assess the impact of their farm on the marine environment, it can also be of more practical interest. For instance, cultivation lines that are overgrown with mussels are difficult to harvest and a contamination with mussels may also affect product quality (Tonk et al. 2018). At the North Sea Farm, only few mussels were found on the seaweed itself and therefore no effect on the quality of the biomass is expected. However, a large amount of mussels on the cultivation lines may interfere with automated harvesting systems. This may not be relevant early in the year, if seaweed is harvested in April or May, since fewer and smaller mussels were found on the cultivation ropes during May. If seaweed is harvested later in the year in June or July, on the other hand, harvesting (both biomass and harvesting equipment) may suffer from extensive mussel growth on the lines. This has to be taken into account in management strategies for seaweed harvest.

Another recommendation for seaweed farming in the North Sea is the use of shorter vertical ropes since mussels were mainly found at lower depth. It is also recommended to repeat the biodiversity assessment in a few years to investigate temporal changes, similar to what has been done for other hard substrates in the North Sea (Bouma and Lengkeek 2012). In that case, we recommend to combine different methodologies, such as classical biodiversity assessment, DNA metabarcoding of settlement plates and video or photo footage to cover a broader spectrum.

3 Seasonal variation in kelp biomass and biochemical composition

3.1 Introduction

Commercial cultivation of seaweeds is still in its infancy in Europe, both in terms of management and in terms of productivity. For further development of the sector, seaweed crops with predictable yields are essential. Yet, previous experiments at the North Sea Farm (Jansen et al. 2019) and literature indicate substantial seasonal variation in productivity. Further insight into these seasonal variations are necessary to match requirements of the processing industry (demand) and the farm management (supply), and thereby to define the optimal harvest moment.

3.2 Material and Methods

Five seaweed cultivation ropes of 7m length - installed vertically from 0m up to 7m below sea level - were harvested by divers from the North Sea Farm on 16th of May 2019, 19th of June 2019 and 9th of July 2019. Each rope was divided into 7 parts of 1m each. Fresh weight (FW) per part was determined immediately after harvest.

Samples collected in May and June were kept in seawater at 4°C for two days, before they were transferred to Wageningen for the determination of dry weight (DW). In Wageningen, the samples were centrifuged to remove excess water and dried afterwards in an oven at 60°C.

Samples collected in July were frozen after determination of FW and transferred to the laboratory in Wageningen, where the frozen samples were dried in an oven at 60°C.

In total 15 samples of *S. latissima* were harvested in 2019 and analysed for crude protein/ total nitrogen concentrations (Dumas method), NO_3^- and starch content. In addition six samples were analysed for protein and amino acid content. In 2018, 11 samples collected in May and June were solely analysed for nitrate content.

3.3 Results

In the top water layer from zero to one meter below sea level, seaweed crop increased from May to July and decreased with increasing depth (Fig. 8). In the water column 1–3 m below sea level, the average seaweed crop in June was slightly higher than in July. In July, two lines out of five did not contain any biomass in the layers 1-3 m below sea level, explaining the lower standing crop.

In 2018, problems were encountered during the growing season due to entanglement of ropes, which may have caused physical damage of the seaweed thalli (Jansen et al. 2019). This was not the case in 2019 and all seaweed thalli looked healthy and unharmed (Fig. 2A). The average standing crop over 7 meters of line in 2019 represented only one fifth (20%) of the crop in 2018, suggesting large year to year variation. The three highest standing crop values observed in 2019 were ~ 10 times lower compared to observations in 2018. Standing crop below 4 meters was very low or absent in both years, 2018 and 2019.

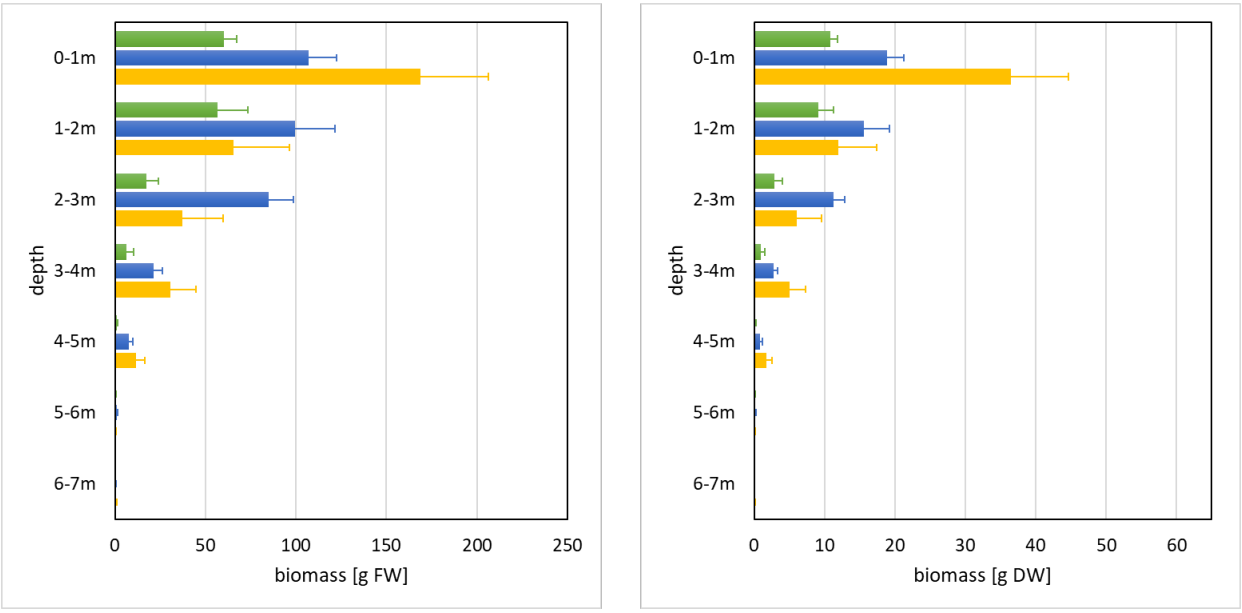


Figure 8 Average standing crop *(fresh and dry weight with standard error bars) at different depths in May (green), June (blue) and July (yellow).*

Only a few seaweed thalli were found in the lower parts (6-7m) of the ropes (Fig. 9). Since the ropes were heavily overgrown with mussels, it is likely that competition for space between mussels and seaweed occurred.

Figure 9 *Mussels growing on the lower part (6-7m depth) of a rope harvested in July. Although some seaweeds were found (indicated by red arrows), almost the entire substrate of the rope was occupied by mussels.*

Biochemical analyses

The *S. latissima* samples as shown in Table 3.1, taken at three timepoints (in May, June and July 2019) in five-fold, have been analysed for biochemical composition. Crude protein was analysed by means of the Dumas method (*6.25, a conversion factor used to convert N content to protein content), carbohydrates or starch via the AGS method (http://www.norfor.info/Files/pdf-dokumenter/pdf_lab/Analyses/Starch_Method_Spectrophoto.pdf) and nitrate (https://archive.epa.gov/water/archive/web/html/vms57.html). Six samples were analysed for total protein (protein and free amino acids) amino acids levels marked with the symbol 'x' in Table 3.1. Crude protein concentration was calculated as total nitrogen concentration (DUMAS-method; https://onlinelibrary.wiley.com/doi/full/10.1111/j.1541-4337.2010.00114.x) times 6.25. To determine the true protein concentration, the concentration of all 20 protein amino acids (bound in

proteins and free amino acids) was determined on the same sample. True protein/ amino acid concentration was calculated as sum of all protein amino acids, after correction for H_20 that binds to the individual amino acids after protein hydrolysis.

Table 3.1 *Samples of S. latissima were analysed for nitrogen, nitrate and starch concentration and a subset was analysed for total protein amino acid content*

Code	date	DW, g	crude protein (Dumas), starch, nitrate	total pAA
1	16/05/2019	10.06	x	x
2	19/06/2019	16.56	x	x
3	09/07/2019	31.8	x	x
4	16/05/2019	9.58	x	
5	19/06/2019	18.9	x	
6	09/07/2019	61.3	x	
7	16/05/2019	14.83	x	x
8	19/06/2019	21.84	x	x
9	09/07/2019	43.1	x	x
10	16/05/2019	10.77	x	
11	19/06/2019	11.44	x	
12	09/07/2019	35	x	
13	16/05/2019	8.86	x	
14	19/06/2019	25.75	x	
15	09/07/2019	11.4	x	

Table 3.2 *Mean values (± SD) of five-fold samples taken at three timepoints for crude protein concentration in percentage per dry weight as analysed by Dumas method * 6.25, true protein as total protein amino acids concentration, nitrate (NO3-) concentration (in mg/kg) and starch (% DW).*

	Crude protein (Dumas*6.25)	**% True protein (total pAA)**	**Nitrate (mg/kg)**	**% Starch**
May	19.88 (±3.45)	12.7	2800 (±2503)	0.8 (±0.4)
June	17.7 (±2.04)	11.7	1280 (±275)	0.2 (+0.2)
July	18.2 (±0.98)	13.8	290 (±181)	0.6 (±0.2)

The crude protein concentrations based on nitrogen analysis varied between 18 and 20% DW throughout the season from May to July 2019, and slightly decreased (see Table 3.2 and Figure 10). The same holds for the true protein content, varying between 12 and 14%. There is a correlation between crude protein and true protein concentrations, as was seen in the analysis of *Ulva* sp. in relation to seasonal and annual variation. The true protein levels in *Saccharina* were comparable to the results from 2018 (between 10-11%).

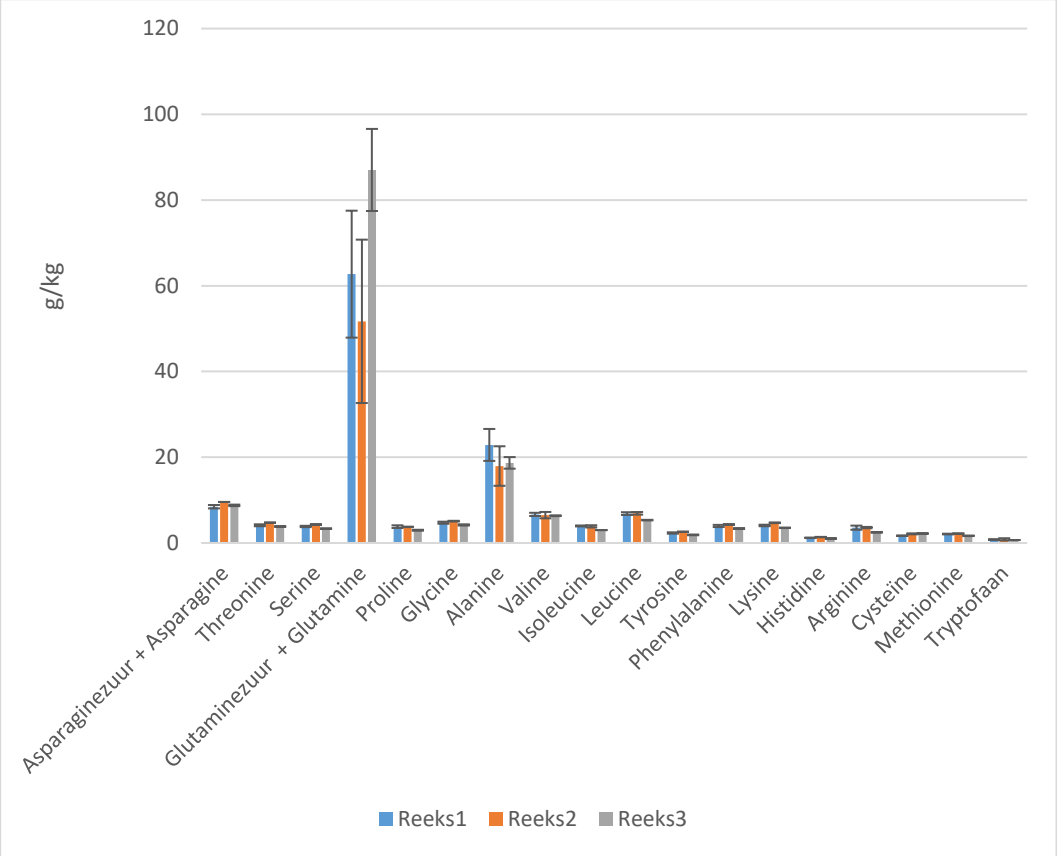


Figure 10 Average individual amino acid concentration for two samples per time point (blue bars May, orange bars June and grey bars July).

Starch content in *S. latissima* collected in June was relatively low, but comparable to results of 2018 (between 1.2 and 0.5%).

Nitrate was analysed because of the unexpected discrepancy between crude protein levels (actual N levels) and true protein content of the samples collected in May compared to the samples collected in June2018. The high nitrogen levels are possibly explained by the presence of metabolites that contain N, such as alkaloids. High nitrogen levels may also be caused by an accumulation of nitrate in seaweed tissue and therefore the nitrate (NO_3^-) levels were determined. Observed nitrate concentrations were ~~is~~ higher in May, than in June and July. A comparable discrepancy between crude protein based on Dumas versus true protein levels was not observed in 2019.

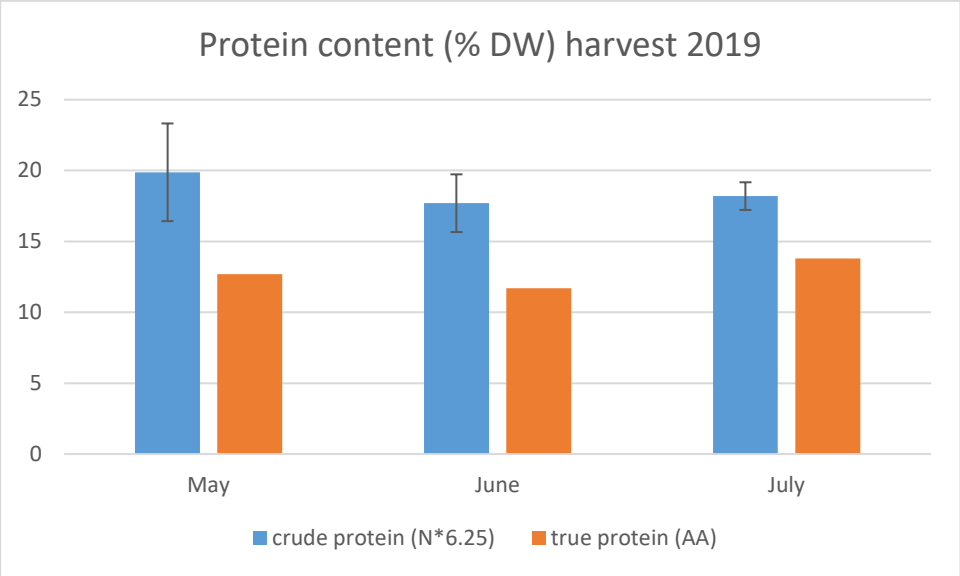


*Figure 11 Protein content as percentage of DW as analysed by total nitrogen using Dumas method*6.25 (blue bars) and via accumulation of total protein amino acids levels (true protein) as represented by the orange bars for the 2019 samples taken in five-fold at three harvesting timepoints.*

We also analysed a subset of 2018 samples for NO3- concentration, which is shown in Table 3.3 and Figure 12.

*Table 3. Mean values of two-fold 2018 samples taken at two timepoints for crude protein concentration in percentage per dry weight as analysed by Dumas method * 6.25, true protein as total protein amino acids concentration, starch and nitrate (NO3-) concentration (in mg/kg). D1: depth 0-2.3 meter, D2: depth 2.3-4.6 meter.*

2018	% crude protein (N*6.25)	% true protein	Nitrate (mg/kg)	% starch
May D1	27.8	11.2	69800	1.2
May D2	29.8	10.2	74200	0.5
June D1	13.7	10.5	4500	1.1
June D2	13.6	10.1	5600	0.6

In the 2018 samples, there was a clear discrepancy between the crude protein concentration and the true protein concentration in the samples taken at two depths in May versus June. These samples were analysed for nitrate concentration, revealing an extremely high nitrate concentration in May, which was 10-times higher, compared to June 2018 and 20-30 times higher in 2019. The high nitrate concentrations observed in samples retrieved in May 2018 compared to samples collected in June 2018 partly explains the difference between crude protein versus true protein levels in 2018. However, the reason for this high nitrate accumulation in *S. latissima* in May 2018 remains unclear.

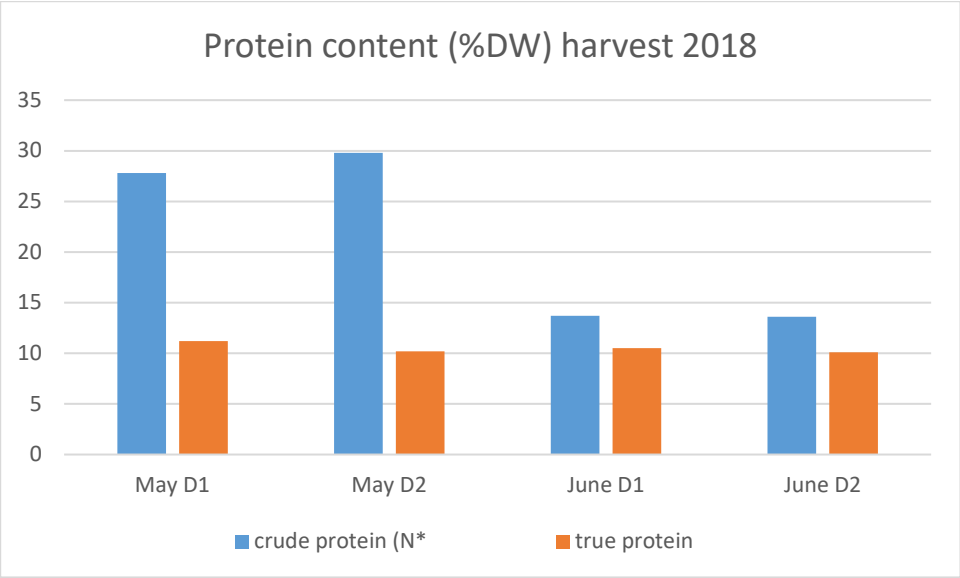


*Figure 12 Protein content as percentage of DW as analysed by total nitrogen using Dumas method*6.25 (blue bars; crude protein) and via accumulation of total protein amino acids levels (orange bars; true protein) for the 2018 samples taken in three-fold at two harvesting timepoints at two depths.*

Figure 13 shows the difference in nitrate concentration in *S. latissima* samples in 2018 and 2019. The nitrate concentrations are extremely high in May 2018, compared to *S. latissima* collected in June 2018, as well as all samples collected in 2019.

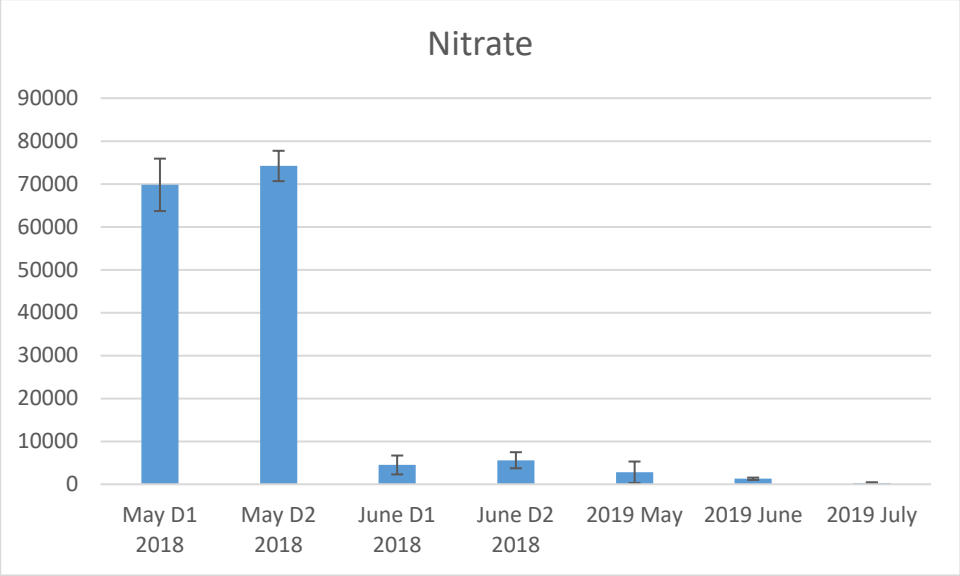


Figure 13. Nitrate (NO3-) concentrations (unit) in S. latissima collected at (location) and at different depths (define depth again) in May to July 2018 and 2019.

3.4 Discussion

In 2018, on average approx. 1.2 kg FW in the first 2.3 meters below sea level were observed, whereas in 2019 the average FW over the first 2 meters was 0.19 kg, approx. 16% of the FW in 2018 (Jansen et al. 2019). Far greater *S. latissima* standing crops of 4 kg FW/m for the North Sea have been reported (Buck and Buchholz 2004). We have no underlying data and or information available explaining these differences. Several factors need to be addressed for future experiments: year to year variation in growth, seeding densities, distance of cultivation ropes to each other and different genetic strains. Investigating the effects of these factors may provide insight the differences found in FW in this study compared to other studies of *S. latissima* in the North Sea. Both in 2018 and 2019, negligible standing crops were observed below 4 meters of depth. A combination of economic analysis (annual exploitation costs expressed as Euro per kg FW) and ecological results can be used to determine the optimal cultivation depth.

Biochemical composition

Based on Dumas calculations the crude protein level in 2018 samples was two times higher in May compared to June whereas the true protein content was comparable between these months. It was discussed that Dumas measures nitrogen, including nitrogen present in non-protein compounds present in the plant, such as alkaloids and other nitrogen-containing metabolites. Seaweeds can also store nitrate (NO_3^-) in in cellular nitrate pools (Naldi & Viaroli 2001). Nitrate analyses from 2018 and 2019 samples were therefore included in this study.

However, the two-fold difference between crude protein content and true protein content that was seen in 2018 was not observed in 2019. There is generally a difference between crude protein and true protein content when measured with the Dumas calculation (N content times 6.25 = protein content) but this does not vary over the season as seen in the 2018 samples. When focussing on true protein content, which is between 12-14% of DW for *S. latissima* in May to June, seasonality is not a determining factor in choosing when to harvest. Growth of biomass should be considered a priority scheduling the harvest.

Nitrate content varied throughout from May to July and could not fully explain the difference between N measured calculated? by Dumas and measured? true protein content in the 2019 samples. Metabolomics analysis can identify and detect (high-value) nitrogen-containing metabolites that may explain this variation. However these analyses were not in the scope of this research.

4 Environmental conditions in the North Sea Farm

4.1 Introduction

Both growth rates and biochemical composition of kelps are affected by environmental conditions (Bruhn et al. 2016). Unfavourable environmental conditions can also increase biofouling (Dean and Jacobsen 1984).

Abiotic factors such as light availability, temperature and nutrient availability are important for kelp growth. Light intensity is often seen as the most important abiotic factor for kelp growth (Bruhn et al. 2016, Dean & Jacobsen 1984). Kelps are photoautotroph organisms which convert light and CO_2 to carbon-rich biomass via photosynthesis. Depending on the strain, light saturation in kelp sporophytes is usually reached at irradiances of 20-100 µmol photons m^{-2} s^{-1} (reviewed by Bartsch et al. 2008). If irradiance is too low photosynthesis cannot take place efficiently. Similarly, too high light intensity of more than 250 µmol photons m^{-2} s^{-1} can also inhibit kelp growth (Bartsch et al. 2008).Nutrient availability is another important abiotic factor, especially in a seaweed farm where nutrients are more easily depleted depending on the density of the cultivated seaweed and the ecosystem it is grown in (nutrient rich inshore versus offshore areas). Optimal nitrate conditions for *S. latissima* are described as high as 10 µM NO3- (Kerrison et al. 2015), respectively 20 umol/L for nitrate and 1.5 umol/L for phosphate (Lubsch & Timmermans 2019). In addition temperature has also been shown to affect kelp growth (Davison et al. 1991). Cold-temperate North Atlantic kelp species can grow in temperature from 0 to 20°C with optimal temperatures between 5 and 15°C, dependent on the strain (Bolton and Lüning 1982; tom Dieck (Bartsch) 1992; Wiencke et al. 1994). Above 17 ºC growth becomes limited and at 23 ºC the kelp dies. Other factors, such as salinity or water motion may also have an effect on the kelp growth. A precise description of environmental factors could help to improve the understanding of growth and health of seaweeds cultivated in the North Sea.

4.2 Material and Methods

HOBO data loggers (brand: Onset Computer Corporation, MA, USA) to measure average temperature and light intensity every 30 min., were deployed on site for continuous-data at seven depths between 0m and 7m. Light intensities measured in Lux were converted to µmol photons m^{-2} s^{-1} using the conversion factor of 0.0185 for sunlight conditions (Borowitzka and Moheimani 2013). Weekly and monthly averages were calculated for temperature and light intensity. Data loggers were exchanged for clean ones by divers on the 01.02.2019, 19.04.2019 and 16.05.2019.

Additionally, a CTD measurement (YSI Inc., Xylem Group, OH, USA) was performed on the 19.06.2019. The measured parameters were temperature, salinity, conductivity and turbidity.

4.3 Results

4.3.1 Temperature

Over the entire cultivation period from the 1st of February until the 26th of June, temperature ranged from 5.0°C to 21.2°C. Throughout the first half of the cultivation period until April, no temperature differences were detected between depths (Fig. 10). However, from week 15 onwards slight differences in temperature occurred, for instance in weeks 14 and 18, with slightly higher temperatures (ranging from approx. 1 to 3 °C) at a depth of 0-2m than at lower depths (below 2m).

The HOBO data- logger that was deployed at a depth between 3 and 4m broke during the deployment and was filled with water when it was taken out on the 1st of February. Therefore, no temperature values from the aforementioned time and depth exist (see Fig. 10).

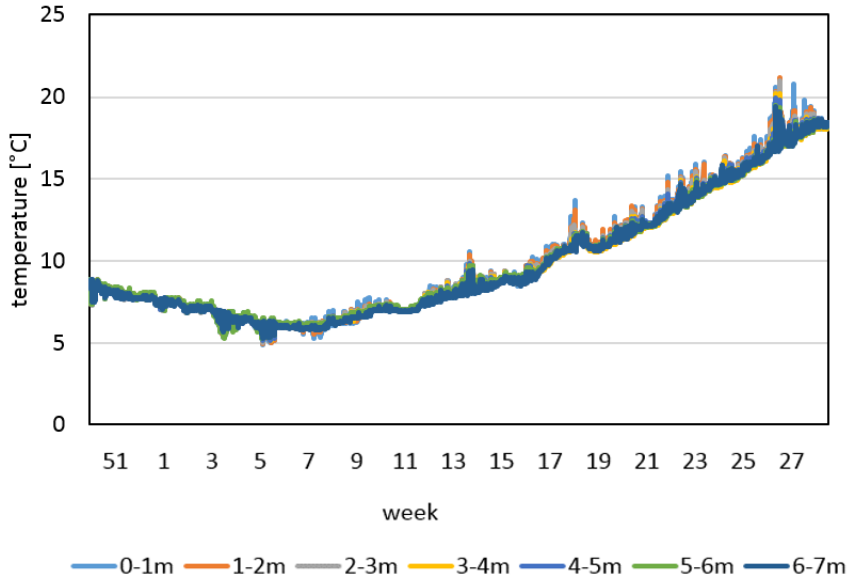


Figure 10 *Temperature profile between a depth of 0 and 7m, measured continuously (average of 30 min) with dataloggers (HOBO Onset Computer Corporation) throughout the cultivation period from December to July.*

Temperature measured by a CTD probe in shallow water (down to 5m depth) was up to 1.5°C higher than in deeper water down to 20m depth (Fig. 11). Differences in temperature up to 1°C were also detected in shallow water during upwards and downwards measurements (Fig. 11, grey and black line), whereas no differences between the two measurements (upwards and downwards) were found at 7.5m and lower. It is unknown why this occurred, as normally upward and downward measurements provide similar readings.

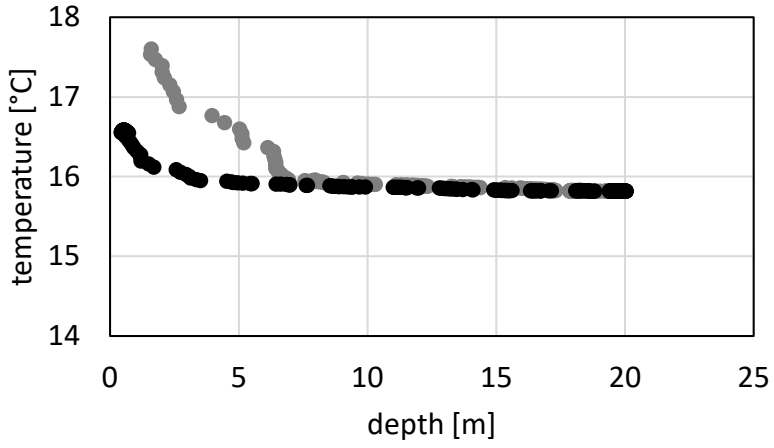


Figure 11 *Temperature measured at different depths between 0 and 20m by means of a CTD - probe. Grey = downward measurement on 19.06.2019 (week 25), black = upward measurement.*

4.3.2 Light intensity

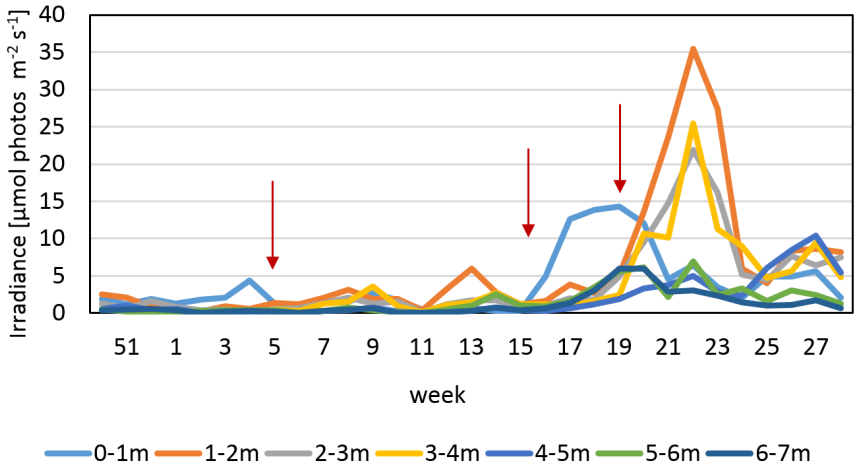


Figure 12 *Light intensity (µmol photons m^{-2} s^{-1}) measured with a data logger (HOBO Onset Computer Corporation, MA, USA) between 0 and 7m throughout the cultivation period December 2018 until July 2019. Red arrows indicate the time points when loggers were exchanged, and clean loggers were deployed again.*

Light conditions varied at the different depths during the entire cultivation period. A highest light intensity was expected at shallow depths of 0-1m which was confirmed during winter (see Fig. 12, week 3-5, blue curve). However, from week 7 onwards higher light intensity was detected at 1-2m and 3-4m depth. When the loggers were changed in week 16 (Fig. 12, red arrow), the highest light intensity was detected just below the surface. After one week, this changed again and highest light intensity was detected at 1-2,m 2-3m 3-4m depth, likely due to biofouling. Since the changes are congruent with the exchange of the loggers, it can be expected that the differences in the values measured at 0-1m are caused by biofouling and overgrowth of the sensors. While this is not a problem in winter, with increasing temperatures and light intensities biofouling organisms settle on the sensors more quickly and in higher numbers during late spring and summer (Watson and Barnes 2004).

In December, January and April light intensity followed a logical depth-dependent pattern (Fig. 13) with higher light intensity detected at upper depths (0-3m) than at lower depth (3-7m). During the other months however, more light was detected at 1-2m depth than just below the surface. In February and March light intensity at 4-7m depth was very low from 0.1 to 1 µmol photons m^{-2} s^{-1}. Values in these depths increased from May onwards to up to 9.3 µmol photons m^{-2} s^{-1} in 4-5m depth in July. As described before, the fluctuation in shallow depths may be due to biofouling.

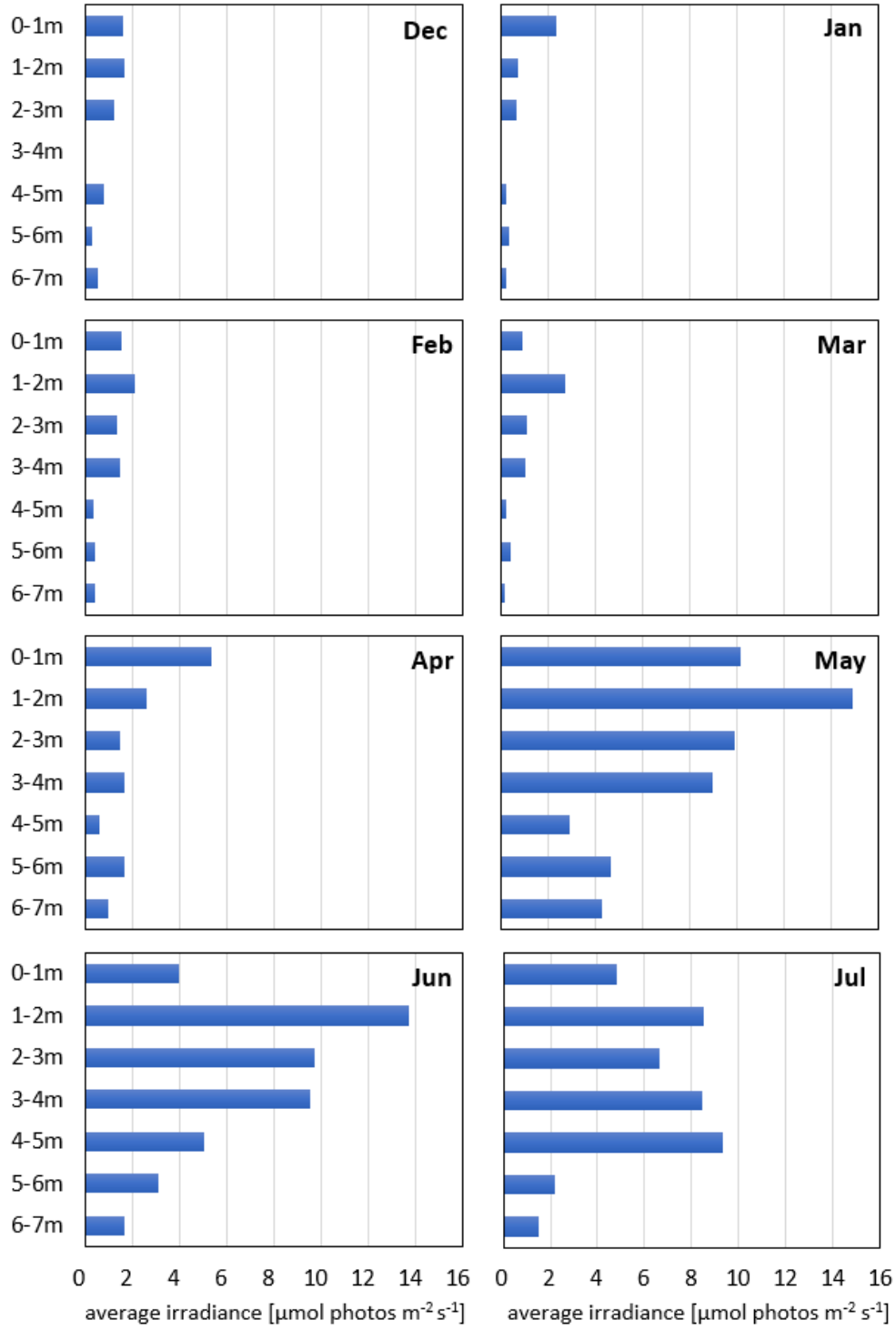


Figure 13 *Average light intensity per month measured by HOBO loggers at different depths from 0-7m. Note that all data has been used to build this figure, although sensors may have been overgrown at certain times.*

4.3.3 Other parameters

Turbidity is defined as the reduction of transparency of a water body caused by the presence of suspended particulate matter (ISO 7027). In the open ocean, turbidity is often led back to phytoplankton growth, but it can also be caused by sediment entering the water body through human activities. High turbidity levels decrease the light penetration into the water column which may limit the growth of photoautotroph organisms such as seaweed (Oliveira et al. 2012). The turbidity measured at the North Sea Farm differed significantly with varying depth. There was a steep decrease in turbidity between 0 and 2.5 from 4-5.5FNU (Formazin Nephelometric Unit, a unit used for turbidity) to 0.5FNU (Fig. 14).

Turbidity was low from 2.5 to 12.5m and increased between 12.5 to 20m (bottom depth) to values similar to the surface.

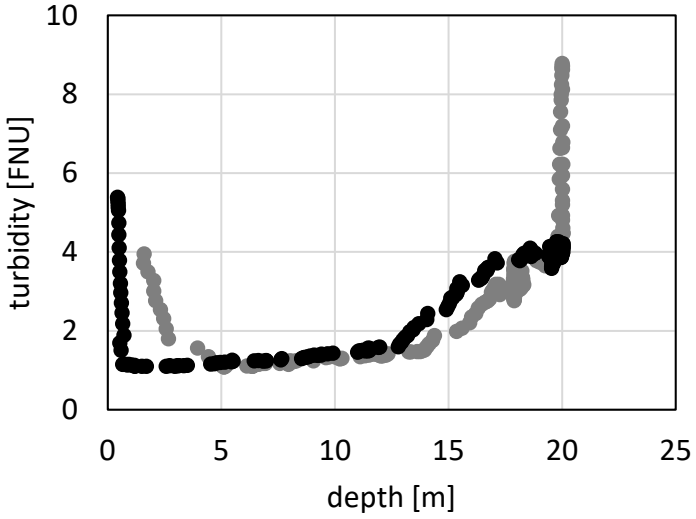


Figure 14 *Turbidity (FNU) measured at different depths between 0 and 20m by a CTD. Grey = downward measurement, black = upward measurement.*

Similarly to turbidity, salinity can affect kelp growth significantly (Spurkland and Iken 2011). The optimal salinity range for kelp growth has been reported from 27 to 33 psu (Gerard et al. 1987; Nielsen et al. 2014). All values measured in depths from 0 to 20m at the North Sea farm were lying within the optimal range (Fig. 15). Salinity increased from 28-29 psu to 32 from 0 to 5m and stayed constant at 32 PSU between 5 and 20m. The large difference in salinity at 0 and 5m depth, however, shows that the water body is not as well-mixed as previously assumed (Tonk et al. 2018). This is also evident from the measured thermocline in Fig. 11.

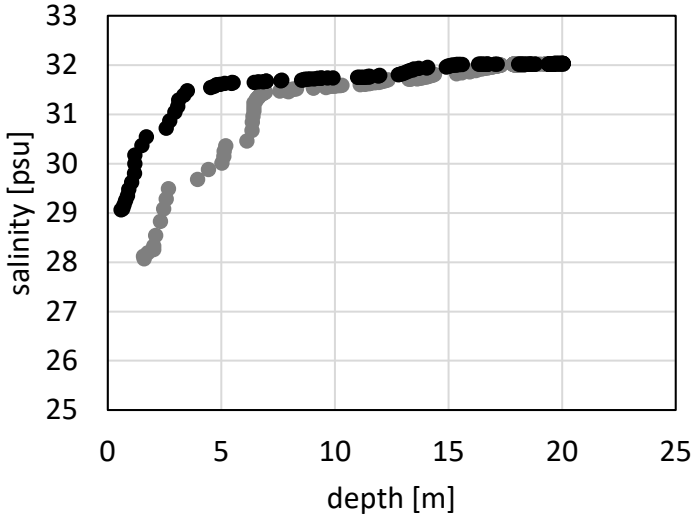


Figure 15 *Salinity (psu) measured at different depths between 0 and 20m by a CTD. Grey = downward measurement, black = upward measurement.*

4.4 Discussion

Methodology

The temperature measured with the data-loggers showed consistency with point measurements conducted with CTD -probe and therefore seem reliable. The loggers can be recommended for continuous temperature measurements.

The light values measured with the data-loggers, on the other hand, were not consistent. The loggers were overgrown with a biofilm and cleaned during each site-visit. The cleaning led to substantial changes in the detected light concentrations. Therefore, as soon as the loggers are overgrown with a biofilm the results are no longer trustworthy. As the shift in data usually follows a continuous slowly decreasing pattern as fouling progressively increases, detecting a drift towards un-reliable measurements can be difficult. In order to obtain reliable results, loggers should be cleaned regularly and at least every 2 weeks (Lehaitre and Compère 2008). Since this corresponds to a high amount of work, the data loggers in their present form are not recommended for continuous measurements of light conditions in the North Sea. A solution could be the use of automatic wipers or scrapers attached to the logger to keep it clean. Wipers are already included in a number of oceanographic instruments and can be custom-adapted to other instruments. Other possibilities to keep the loggers clean include the use of ultrasonic waves or UV light antifouling devices (Lamers Systems Care, NL and Seabed BV, NL, respectively). Chemical biocides, such as bistributylinoxide (TBT), which has been applied as an agent against biofouling in the past, can have deleterious effects on the marine environment and should therefore not be used.

An alternative to continuous measurements of light intensity could be a series of point measurements. However, point measurements exclude light conditions at bad weather conditions because boat trips cannot be arranged then and will therefore not represent the entire spectrum of environmental conditions at the farm. An~~other~~ alternative is an installed and automated (remote controlled) CTD at the cultivation site.

Depth-dependent variation of environmental conditions and recommendations

Except for temperature, all measured environmental factors showed depth-dependent variation. At the same time, it was shown that biomass decreased with increasing depth. The results therefore suggest that the environmental conditions for seaweed growth in the North Sea Farm may be better in shallow water down to 4 metres, depending on turbidity and how far light penetrates the water column. At the North Sea Farm light was potentially an important factor influencing kelp productivity (Bruhn et al. 2016). Due to high turbidity and resulting low irradiance in deeper waters, the amount of light that reached the lower part of the lines was too little to assure efficient photosynthesis in the seaweed tissue. Subsequently, growth was slowed down and even inhibited under low light conditions at depths between 5 and 7m. Based on these results, we recommend that cultivation of *Saccharina latissima* in the North Sea Farm should be restricted to 0 to 5m depths. Instead of vertical cultivation ropes, horizontal ropes could be deployed which would allow the kelp along the entire rope to receive sufficient light to enable growth.

Another factor that strongly affects seaweed growth is nutrient availability (Nielsen et al. 2014; Boderskov et al. 2016). A feasibility study into the suitability of offshore windfarm locations for mariculture indicated that, as a result of the N and P from the Delta region, locations in the southern part of the North Sea, including locations close to the North Sea Farm, seemed more suitable than northern locations (van den Bogaart et al. 2019). This is primarily based on N and P fluxes that strongly determine the growth potential of seaweed. In all cases, the scale, cost and productivity will determine the economic feasibility. Nutrient concentrations were not measured in this study, but we recommend to perform depth-dependent nutrient measurements (for instance at two or three depths along the 7m line) during the entire cultivation period in the upcoming years in order to obtain a better understanding on the environmental conditions at the North Sea Farm and to be able to define all factors that may affect biomass production.

5 Conclusions and recommendations

Biomass production at the test-farm was very low in 2019, compared to previous years and a seaweed farm test location near Helgoland in the North Sea (Buck and Buchholz 2004, Jansen et al. 2019). This was especially the case at depths lower than 4m, where environmental conditions for growth were unfavourable for *Saccharina latissima* light limitations hindered photosynthesis. Both in 2018 and 2019 large differences in standing crops over time and depth were observed. Contrary, true protein levels varied only slightly over time. If protein is the target product, final biomass yield of *S. latissima* will determine the profitability of the mariculture.

Biochemical analyses showed that true protein content was relatively constant, varying between 12-14% DW from May to July. The biomass production levels should be highest when choosing a specific moment for harvesting if the focus is on protein production. In contrast to the biochemical results obtained in 2018, there was no discrepancy between crude protein measurements and true protein levels throughout the season. Crude protein analysis via Dumas method generally over-estimating true protein content, however the extreme discrepancy found in the 2018 samples from May remain unexplained. Nitrate content in *S. latissima* varied throughout the season and could not fully explain the difference between N measured by Dumas and true protein content in the 2019 samples. Therefore, other seaweed components containing nitrogen must explain this variation, e.g. its accumulation in cellular nitrate pools. For the 2018 samples, the huge discrepancy between crude protein as measured by Dumas and true protein can partly be explained by high nitrate concentrations detected in *Saccharina latissima* in May 2018 (two depths).

Great abundances in fauna (up to 7679 individuals per rope) were found on the seaweed lines. Biodiversity, however, was low and the fauna consisted mainly of blue mussels. Abundance in fauna increased from May to June and all detected species are also known from other hard substrates in the North Sea. Compared to previous assessments of biodiversity with eDNA metabarcoding at the same site, the biodiversity detected in 2019 was very low. In addition, biodiversity levels may differ from year to year. However, the samples were not taken at the same time points and are therefore not directly comparable. Moreover, the methodology only included organisms that could be collected by hand and were visible to the eye. In addition, the methodology focussed on the fauna attached to the rope and kelp and therefore did not include organisms that are attracted to the seaweed and cultivation structure but remain in the water column.

Recommendations for cultivation of *Saccharina latissima* in the North Sea Farm:

1. Based on the results from 2018/2019, cultivation should be restricted to a depth down to 4m since environmental conditions (mainly light limitation) seem to be unfavourable for seaweed growth below 4m. As an alternative to vertical lines, cultivation on horizontal lines could be considered. A combination of economic analyses and growth experiments may assist in determining the optimal cultivation technique.
2. HOBO loggers should only be applied for temperature measurements. Due to biofouling, they are not suitable for continuous light measurements unless they are cleaned regularly, e.g. every two weeks or by means of anti-biofouling devices.
3. The data-loggers deployed for measurements in the North Sea Farm measured continuously. Only one logger broke during the experimental period from December to the 1st of February. Therefore, it is recommended to deploy loggers at all depths in duplicates throughout the entire experimental period.
4. Nutrient concentrations for nitrate and phosphate, two essential macronutrients for growth in seaweeds should be assessed at different depths and time points in order to improve the understanding of environmental conditions in the farm.
5. Similar to ecological surveys on coastal areas and hard substrates, biodiversity in the seaweed farm should be assessed repeatedly every 5 years to check for temporal alterations in fauna composition, especially when cultivation structures, such as anchors, are deployed throughout

several years. Since the results obtained by classical morphological assessment and DNA metabarcoding differed significantly, it is advised to combine both methods in future assessments and to compare the results in order to determine the best-suited methodology for biodiversity assessments. Additionally, a baited-camera system that has previously been used to monitor diversity of mobile fauna in a seaweed farm in the Eastern Scheldt (Tonk et al. 2019) could be tested in the North Sea Farm, as both settlement and the classical biodiversity assessment on seaweed cultivation ropes are focussed on sessile fauna.

6. The best, but very expensive, way to analyse true protein content is via protein amino acid analysis. Crude protein analysis via Dumas method can result in an overestimation of protein content, especially when seaweeds accumulate nitrate (NO3). On top of that, there are still other N-containing compounds (not proteins) that are accumulating and add to N concentration as analysed by the Dumas method. It is of interest to identify potential N-containing metabolites in *S. latissima*, as they may represent high-value compounds.

6 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. This certificate is valid until 15 December 2021. The organisation has been certified since 27 February 2001. The certification was issued by DNV GL.

Furthermore, the chemical laboratory at IJmuiden has EN-ISO/IEC 17025:2017 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2021 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation. The chemical laboratory at IJmuiden has thus demonstrated its ability to provide valid results according a technically competent manner and to work according to the ISO 17025 standard. The scope (L097) of de accredited analytical methods can be found at the website of the Council for Accreditation (www.rva.nl).

On the basis of this accreditation, the quality characteristic Q is awarded to the results of those components which are incorporated in the scope, provided they comply with all quality requirements. The quality characteristic Q is stated in the tables with the results. If, the quality characteristic Q is not mentioned, the reason why is explained.

The quality of the test methods is ensured in various ways. The accuracy of the analysis is regularly assessed by participation in inter-laboratory performance studies including those organized by QUASIMEME. If no inter-laboratory study is available, a second-level control is performed. In addition, a first-level control is performed for each series of measurements.
In addition to the line controls the following general quality controls are carried out:

- Blank research.
- Recovery.
- Internal standard
- Injection standard.
- Sensitivity.

The above controls are described in Wageningen Marine Research working instruction ISW 2.10.2.105. If desired, information regarding the performance characteristics of the analytical methods is available at the chemical laboratory at IJmuiden.

If the quality cannot be guaranteed, appropriate measures are taken.

References

Bartsch I, Wiencke C, Bischof K, et al (2008) The genus Laminaria sensu lato : recent insights and developments. Eur J Phycol 43:1–86. doi: 10.1080/09670260701711376

Bernard MS, Tonk L, de Groot GA, et al (2019) Biodiversity monitoring in seaweed farms by DNA metabarcoding using settlement plates and water samples

Boderskov T, Schmedes PS, Bruhn A, et al (2016) The effect of light and nutrient availability on growth, nitrogen, and pigment contents of Saccharina latissima (Phaeophyceae) grown in outdoor tanks, under natural variation of sunlight and temperature, during autumn and early winter in Denmark. J Appl Phycol 28:1153–1165. doi: 10.1007/s10811-015-0673-7

Bolton JJ, Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic Laminaria species (Phaeophyta) in culture. Mar Biol 66:89–94. doi: 10.1007/BF00397259

Borowitzka MA, Moheimani NR (2013) Algae for Biofuels and Energy

Bouma S, Lengkeek W (2012) Benthic communities on hard substrates of the offshore wind farm Egmond aan Zee (OWEZ)

Bruhn A, Tørring D, Thomsen M, et al (2016) Impact of environmental conditions on biomass yield, quality, and bio-mitigation capacity of Saccharina latissima. Aquac Environ Interact 8:619–636. doi: 10.3354/aei00200

Buck BH, Buchholz CM (2004) The offshore-ring: A new system design for the open ocean aquaculture of macroalgae. J Appl Phycol 16:355–368. doi: 10.1023/B:JAPH.0000047947.96231.ea

Campbell I, Macleod A, Sahlmann C, et al (2019) The Environmental Risks Associated With the Development of Seaweed Farming in Europe - Prioritizing Key Knowledge Gaps. Front Mar Sci 6:107. doi: 10.3389/FMARS.2019.00107

Christie H, Norderhaug KM, Fredriksen S (2009) Macrophytes as habitat for fauna. Mar Ecol Prog Ser 396:221–233. doi: 10.3354/meps08351

Davison IR, Greene RM, Podolak EJ (1991) Temperature acclimation of respiration and photosynthesis in the brown alga Laminaria saccharina. Mar Biol 110:449–454. doi: 10.1007/BF01344363

Dayton PK (1985) Ecology of Kelp Communities. Annu Rev Ecol Syst 16:215–245

Dean TA, Jacobsen FR (1984) Growth of juvenile Macrocystis pyrifera (Laminariales) in relation to environmental factors. Mar Biol 83:301–311. doi: 10.1007/BF00397463

Duffy JE (1990) Amphipods on seaweed: Partners or pests? Oecologia 83:267–276

Gerard VA, DuBois K, Greene R (1987) Growth responses of two Laminaria saccharina populations to environmental variation. Hydrobiologia 151–152:229–232. doi: 10.1007/BF00046134

James PSBR, Krishnamurty C, Rodrigo JXR (1986) Studies on the fauna associated with the cultured seaweed Gracilaria edulis. Proc Symp Coast Aquac 4:1176–1182

Jansen H, Tonk L (2018) Zeewierproductie en biodiversiteit

Jansen HM, Tonk L, v d Werf A, et al (2019) Development of offshore seaweed cultivation: food safety, cultivation, ecology and economy

Kerrison, P. D., Stanley, M. S., Edwards, M. D., Black, K. D. & Hughes, A. D. 2015. The cultivation of European kelp for bioenergy: Site and species selection. Biomass & Bioenergy 80:229-42.

Knip DM, Scheibling RE (2007) Invertebrate fauna associated with kelp enhances reproductive output of the green sea urchin Strongylocentrotus droebachiensis. J Exp Mar Bio Ecol 351:150–159. doi: 10.1016/j.jembe.2007.06.011

Lehaitre M, Compère C (2008) BIOFOULING and UNDERWATER MEASUREMENTS. Prot sensors against fouling 463–493

Leray M, Knowlton N (2015) DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. Proc Natl Acad Sci 2014:201424997. doi: 10.1073/pnas.1424997112

Loureiro R, Gachon CMM, Rebours C (2015) Seaweed cultivation: Potential and challenges of crop domestication at an unprecedented pace. New Phytol 206:489–492. doi: 10.1111/nph.13278

Lubsch & Timmermans 2019. Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding dissolved inorganic nitrate uptake in Saccharina latissima and Laminaria digitata (Phaeophyceae). J. Phycol. 55:637-650.

Mathiesen SS, Thyrring J, Hemmer-Hansen J, et al (2017) Genetic diversity and connectivity within Mytilus spp. in the subarctic and Arctic. Evol Appl 10:39–55. doi: 10.1111/eva.12415

Nielsen MM, Krause-Jensen D, Olesen B, et al (2014) Growth dynamics of Saccharina latissima (Laminariales, Phaeophyceae) in Aarhus Bay, Denmark, and along the species' distribution range. Mar Biol 161:2011–2022. doi: 10.1007/s00227-014-2482-y

Oliveira VP, Freire FA de M, Soriano EM (2012) Influence of depth on the growth of the seaweed Gracilaria

birdiae (Rhodophyta) in a shrimp pond. Brazilian J Aquat Sci Technol 16:33. doi: 10.14210/bjast.v16n1.p33-39
Pavan-Kumar A, Gireesh-Babu P, Lakra WS (2015) DNA Metabarcoding: A New Approach for Rapid Biodiversity Assessment. Cell Sci Mol Biol 2:
Potin P, Bouarab K, Salaün JP, et al (2002) Biotic interactions of marine algae. CurrOpinPlant Biol 5:308–317. doi: 10.1016/S1369-5266(02)00273-X
Schrieken N, Gittenberger A, Coolen J, Lengkeek W (2013) Marine fauna of hard substrata of the cleaver bank and dogger bank. Ned Faun Meded 41:69–78
Spurkland T, Iken K (2011) Salinity and irradiance effects on growth and maximum photosynthetic quantum yield in subarctic Saccharina latissima (Laminariales, Laminariaceae). Bot Mar 54:355–365. doi: 10.1515/BOT.2011.042
Steneck RS, Graham MH, Bourque BJ, et al (2002) Kelp forest ecosystems: Biodiversity, stability, resilience and future. Environ Conserv 29:436–459. doi: 10.1017/S0376892902000322
Thomsen PF, Willerslev E (2015) Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. Biol Conserv 183:4–18. doi: 10.1016/j.biocon.2014.11.019
Tom Dieck (Bartsch) I (1992) North Pacific and North Atlantic digitate Laminaria species (Phaeophyta): hybridization experiments and temperature responses. Phycologia 31:147–163. doi: 10.2216/i0031-8884-31-2-147.1
Tonk L, Bernard M, Jansen H (2019) The use of video-techniques for monitoring and quantification of mobile fauna in marine cultivation systems
Tonk L, van Dalen P, Jansen H (2018) Bepaling van de larvendynamiek en mossel broedval bij de Noordzeeboerderij ten behoeve van optimalisatie oogstmoment zeewier
van den Bogaart L, Poelman M, Tonk L, Neitzel S, van der Wal J, Coolen J, Machiels M (2019) Geschiktheid zeewindparken voor maricultuur en passieve visserij: Een kwalitatieve beoordeling van geschiktheid van windparklocaties voor voedselproductie. Wageningen Marine Research rapport C044/19
Väinölä R, Strelkov P (2011) Mytilus trossulus in Northern Europe. Mar Biol 158:817–833. doi: 10.1007/s00227-010-1609-z
Walls AM, Edwards MD, Firth LB, Johnson MP (2017) Successional changes of epibiont fouling communities of the cultivated kelp Alaria esculenta: predictability and influences. Aquac Environ Interact 9:57–71. doi: 10.3354/aei00215
Walls AM, Kennedy R, Fitzgerald RD, et al (2016) Potential novel habitat created by holdfasts from cultivated Laminaria digitata: Assessing the macroinvertebrate assemblages. Aquac Environ Interact 8:157–169. doi: 10.3354/aei00170
Watson DI, Barnes DKA (2004) Temporal and spatial components of variability in benthic recruitment, a 5-year temperate example. Mar Biol 145:201–214. doi: 10.1007/s00227-003-1291-5
Wiencke C, Bischoff B, Bartsch I, et al (1994) Temperature Requirements and Biogeography of Antarctic, Arctic and Amphiequatorial Seaweeds. Bot Mar 37:247–260. doi: 10.1515/botm.1994.37.3.247
Wood D, Capuzzo E, Kirby D, et al (2017) UK macroalgae aquaculture: What are the key environmental and licensing considerations ? Mar Policy 83:29–39. doi: 10.1016/j.marpol.2017.05.021

Justification

Report C054/20
Project Number: BO-47-001-001

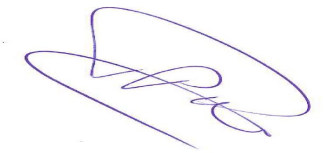
The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved: Dr. A. Lubsch
Researcher

Signature: 

Date: June 17th, 2020

Approved: Dr. Ir. T.P. Bult
Director

Signature: 

Date: June 17th, 2020

Wageningen Marine Research
T +31 (0)317 48 09 00
E: marine-research@wur.nl
www.wur.eu/marine-research

Visitors' address
- Ankerpark 27 1781 AG Den Helder
- Korringaweg 7, 4401 NT Yerseke
- Haringkade 1, 1976 CP IJmuiden

With knowledge, independent scientific research and advice, **Wageningen Marine Research** substantially contributes to more sustainable and more careful management, use and protection of natural riches in marine, coastal and freshwater areas.

Wageningen Marine Research is part of Wageningen University & Research. Wageningen University & Research is the collaboration between Wageningen University and the Wageningen Research Foundation and its mission is: 'To explore the potential for improving the quality of life'