Swansea University
Prifysgol Abertawe

Advancing understanding of kelp aquaculture in Wales: tests across multiple life stages and environmental contexts

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CONSEIL: The food is delicious, isn’t it professor?

PROF ARONNAX: Oh very good, never tasted better. […] May I ask how you are able to set such a table as this Captain?

CAPTAIN NEMO: These dishes come entirely from my ocean kitchen. There is nothing here of the earth.

PROF. ARONNAX: How remarkable.

-20,000 leagues under the sea (1954 film adaptation)

Summary:

Seaweed aquaculture can provide an important food source for a growing world population, without the negative impacts of land-based cultivation, like excessive use of fresh water or pesticides. While seaweed aquaculture is established in Asia, there are still many challenges in the cultivation of seaweeds in Europe.

My research aimed to find solutions to some of the challenges in cultivating three kelp species endemic to Wales: *Laminaria digitata*, *L. hyperborea* and *Saccharina latissima*. I investigated the potential for prediction of optimal sites for seaweed farming through growth modelling, methods for spore acquisition and storage, the effect of nitrate and salinity on kelp growth during microscopic life stages, and changes in kelp morphology throughout the year.

Using a large ria in southwest Wales (Milford Haven) as a test case, model results for *Saccharina latissima* indicated a large section of the nutrient rich, but brackish, part of the ria provides the conditions necessary for good growth and yield.

The effects of salinity and nitrate concentration were similar between kelp species but differed between life stages. Salinity affected germination rate, while nitrate concentration influenced gametophyte growth rate.

Some of the ecosystem services that kelps provide are tied to their morphology. The kelp *L. hyperborea* maintains a large blade and stipe throughout the year, while other species change with the seasons. Meaning some kelps can provide morphology-related ecosystem services throughout year-round, while others cannot.

Kelp spores can be stored using two-step cryopreservation with DMSO as a cryoprotectant, but the survival rate of the spores is too low to be commercially effective. A sori storage time of 24 to 48 hours is recommended for spore acquisition.

Collectively these results show that, while questions remain on some of the details concerning seaweed aquaculture, there is potential for the application of seaweed aquaculture in Wales.

Declarations and statements:

Declaration:

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

[redacted]..

Date.....06-06-2022...

Statement 1

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

[redacted]..

Date....06-06- 2022...

Statement 2

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

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Statement 3

The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

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Contents

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List of figures and tables:

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List of acronyms:

ANOVA	Analysis of Variance
DEB	Dynamic energy budget
DIC	Dissolved inorganic carbon
DIN	Dissolved Inorganic Nitrogen
DMSO	Dimethyl sulfoxide
EMM	Estimated Marginal Mean
GLM	General linear model
LOESS	Locally Estimated Scatterplot Smoothing
PAR	Photosynthetically active radiation
PSU	Practical Salinity Unit
qPCR	quantitative Polymerase Chain Reactions
UV	Ultraviolet light

I. Introduction

I.I The potential of seaweed

The "green revolution" that took place after the second world war was a shift in how we produced food, leading to higher production. This increase in production came at a cost: currently half of all habitable land is used for agriculture (Ritchie and Roser 2013), there is a loss of soil fertility (Rahman 2015), fresh water resources are diminishing (Rahman 2015), and agriculture is contributing to the emission of greenhouse gasses (IPCC. Intergovernmental Panel on Climate Change 2014) and biodiversity loss (Chaudhary et al. 2016). All the while the global population is still growing. By 2050 there will be a projected 9.7 billion people on the planet (United Nations 2019). Feeding this larger, more urbanised, and richer population a healthy diet is projected to require a 25% to 70% increase in food production (Hunter et al. 2017). Future food and feed production will have to increase yield in the long term, maintain biodiversity, conserve fresh water, while not contributing to forms of pollution such as eutrophication and greenhouse gas emissions (Diouf 2009). One such solution could be through an increased production of seaweeds. Seaweed cultivation would not require land or fresh water, and could at the same time improve some of the local water quality parameters (Jiang et al. 2020).

For thousands of years people have used seaweeds as foods, fodder, and fertilizer (Makkar et al. 2016). It has been hypothesized that 13,000 years ago the survival of the first humans in North America was dependent on fish that were plentiful thanks to coastal kelp forests (Erlandson et al. 2015). Today seaweed is used in food, nutraceuticals, pharmaceuticals, biofuels, and a wide range of other applications (Buschmann et al. 2017). Besides their value as a product seaweeds also deliver a range of ecosystem services, including coastal protection and water quality improvement (Dubi and Torum 1997; Rajendran 2009; Bouga and Combet 2015; Pfister et al. 2019). Seaweed forests are also being investigated for their role in capturing carbon, and seaweed aquaculture can provide carbon offsets by replacing products that have a higher carbon footprint (Troell et al. 2022).

According to recent statistics from the Food and Agricultural Organization of the United Nations (FAO), seaweed production has been increasing exponentially. In 2018 the annual world aquaculture production of aquatic algae had more than tripled compared to 2000 (32.4 and 10.6 million tons in 2018 and 2000 respectively) (FAO 2020).

Currently wild seaweed harvesting, and seaweed farming are relatively small niche industries in Europe. Seaweed aquaculture has taken place in mainly 9 East and Southeast Asian countries (Chopin and Tacon 2020). Only 32 countries are harvesting wild seaweeds, for up to a total of 800 000 tonnes for 2018 (Mac Monagail et al. 2017). If we were to expand the harvest of wild seaweed, we would put natural seaweed beds at risk of overexploitation (Thompson et al. 2010; DFO 2013; Ulaski et al. 2020). If we want to use seaweeds as a resource to significantly impact some of the environmental issues we are facing today, we need to produce it in large quantities requiring the use of farming.

I.II Introduction to macroalgae and kelp

Macroalgae, more commonly known as seaweeds, are a diverse group of marine, photo-autotrophic, non-vascular, multicellular organisms that inhabit the coastal regions of ocean waters, usually on intertidal or submerged areas. Unlike microscopic algae, macroalgae are usually attached to rocky substrates. The group consists of three different Phyla: The Rhodophyta (red algae, over 7000 species), Chlorophyta (green algae, about 7000 species) and Phaeophyceae (brown algae, about 2000 species)(Guiry and Guiry 2020).

Of the three groups of seaweed, the individuals of the brown algae species are the largest in size. Unlike the other two groups, there are no known unicellular species of Phaeophyta. Nevertheless, the species within this phylum show a huge range in size and structure, from the microscopically thin strands of *Ectocarpus*, to *Macrocystis pyrifera*, the largest of all seaweeds which can reach lengths of 45m (Guiry and Guiry 2021). Phaeophytes are abundant on the rocky shores of the more temperate regions in the world. The intertidal of these coasts are often dominated by rockweeds (order Fucales) in the upper and middle areas, whereas the lower intertidal and the shallow subtidal area contains members from the kelps (order Laminariales). Kelps are considered ecosystem engineers (Burnaford et al. 2021) and kelp forests can provide a nursing ground and habitat for a variety of marine animals (Bertocci et al. 2015), and act as carbon sinks in coastal areas (Pfister et al. 2019). Besides their ecological functions, the Laminariales are also one of the most economically important group of species, with over 40% (by weight) of aquatic algae production being kelps (FAO 2020).

I.II.I Morphology and tissues

Unlike vascular plants, which are differentiated into true roots, stem and leaves, seaweed tissues are referred to as "thallus". In kelps these tissues are typically differentiated into three distinct segments: the holdfast, a root-like structure that uses tangles of growths called haptera to attach themselves to a hard substrate, the stipe, a stalk structure that supports the blade, which is a leaf like flattened structure where the majority of photosynthesis takes place and is usually the largest part of a kelp (Figure I-1). If light, temperature, and nutrient conditions are right, then the blade will produce a reproductive tissue in the blade called a sorus (plural: sori) (Raven et al. 2005).

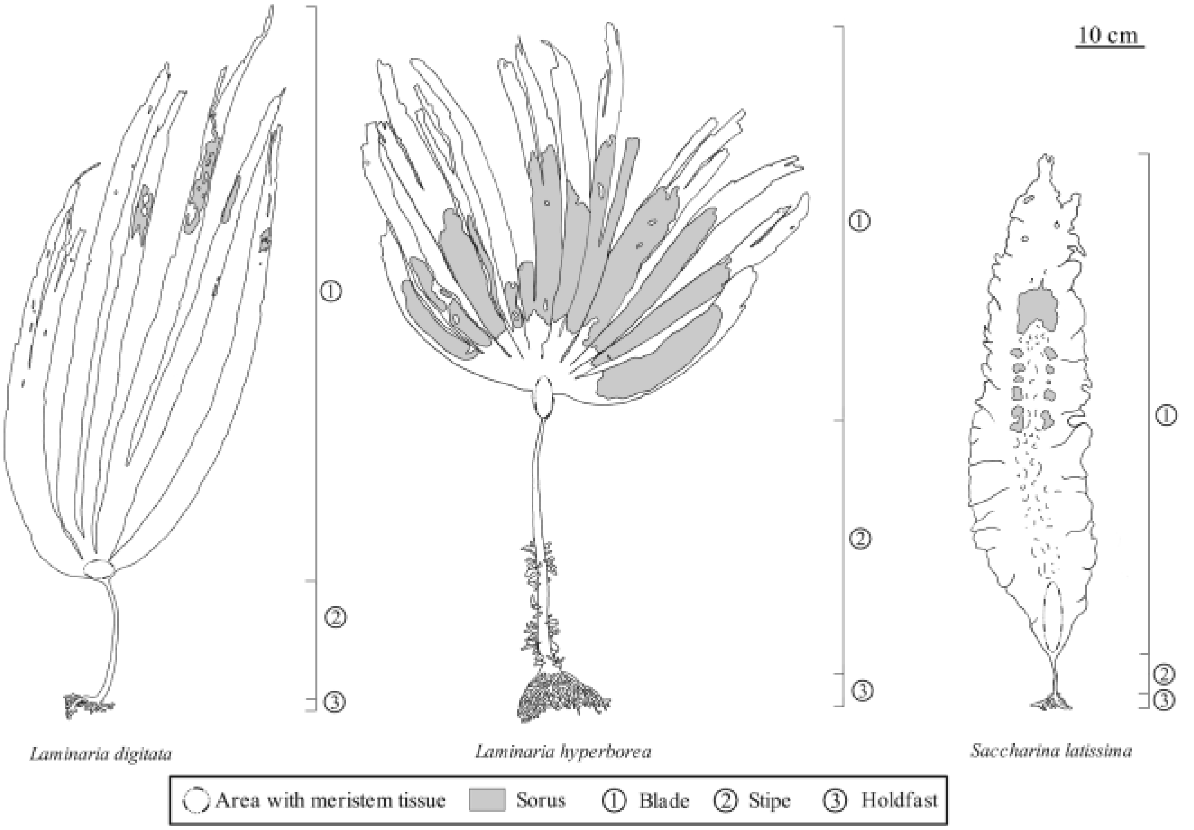


Figure I-1: Drawing of the typical morphology of *Laminaria digitata*, *L. hyperborea*, and *Saccharina latissima*. Holdfast, stipe and blade are indicated.

The Laminariales (kelps) have a heteromorphic alteration of generations (Figure I-2). Diploid sporophytes grow large on the shores. They produce spores, which in this case are microscopic haploid spores with flagella of approximately 5 µm in diameter (Kain 1975). An adult sporophyte of *Laminaria digitata* is estimated to produce 6.6 billion spores in a single year, though these numbers can differ between species and individuals (Chapman 1984). The vast majority of these will not survive, with an estimated 0.31 million recruits getting established per parent plant, and only two sporophytes surviving to visible size per million recruits (Chapman 1984). Spores are produced in sorus that are present on the blades of the kelp (for example in most members of the *Laminariaceae* family) or in a separate blade structure called a sporophyll (in *Alariaceae* family, except *Pleurophycus* genus) (Liu et al. 2017). The spores released are either male or female, and this is determined genetically (Luthringer et al. 2014). Kelp zoospores can disperse up to a few hundred meters depending on local wave and water conditions (Fredriksen et al. 1995; Forrest et al. 2000). Zoospores attach themselves to a hard substrate, after which they produce a germ tube. The cell contents are then moved from the spore into the distal end of the germ tube where the first gametophytic cell has formed (Anderson and Hunt 1988). From this cell, a gametophyte grows, a filamentous microscopic life stage. Under favourable conditions the gametophytes will produce oogonia or antheridium in as little as 8 days (Lüning 1981; Schiel and Foster 2006). But when conditions are not favourable, they can persist, instead growing vegetatively (Schiel and Foster 2006; Destombe and Oppliger 2011). Since gametophytes can persist for a long time they have been hypothesised to function as a sort of gene bank in nature (Barrento et al. 2016; Schoenrock et al. 2021). Induced by pheromones, the spermatozoids are released from the antheridia on the male gametophytes into the seawater where they will swim towards female gametophytes (Bartsch et al. 2008). After the egg cell is fertilised, the zygote will develop into sporophytes while remaining attached to the female gametophyte. The sporophyte will develop over a period of months or years into the macroscopic sporophytes of several meters in size.

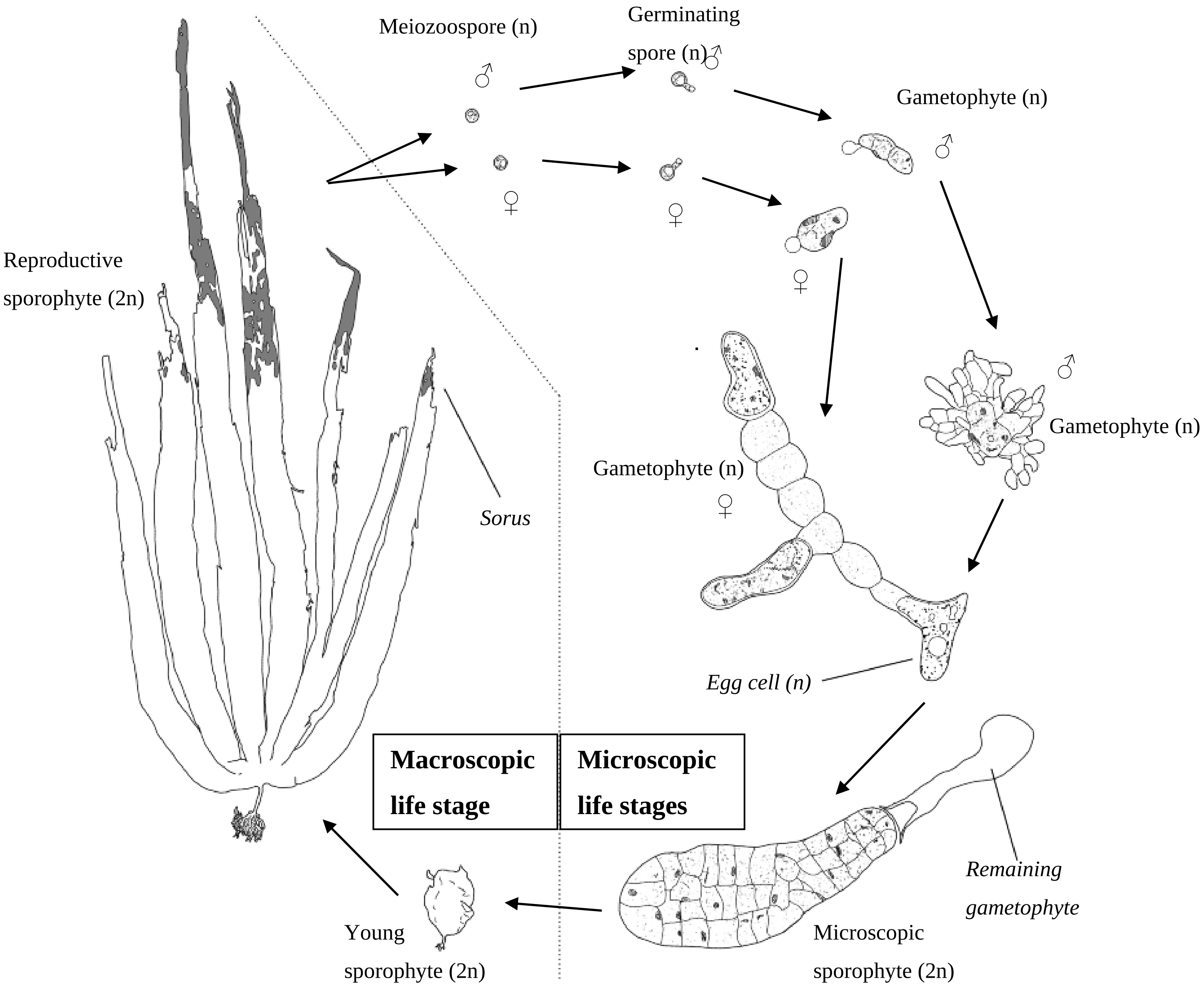


Figure I-2: The life-cycle of *laminaria* sensu lato, based on (Bartsch et al. 2008)

An understanding of the reproductive biology of kelps has enabled the start of kelp cultivation (Yamanaka and Akiyama 1993; FAO 2022). As kelp matures, its blade grows and produces chemically valuable components (Porse and Rudolph 2017), and the kelp itself has value as a source of food (Lindsey Zemke-White and Ohno 1999). The fast growth rates (up to 5% per day) (Stekoll et al. 2021), high bioremediation potential (Grebe et al. 2021), the fact that it does not need to compete for soil with other plants (Vanegas and Bartlett 2013), and variety of uses (food pharmaceuticals, fertilisers, biofuels and more) have led to an increased interest in the aquaculture of kelps (FAO 2020).

I.III Cultivation of Kelp

I.III.I History

Seaweeds have been a part of our diets for thousands of years - records of using seaweeds as a food source date back to 13,000 BC in Chile, 300 BC in China, and 500 AD in Ireland (Nisizawa et al. 1987; Dillehay et al. 2008). In Eastern Asia, vast marine areas have been used as a site for kelp aquaculture for decades now (Xiu-geng et al. 1999). Over the past century an increased understanding in the life histories of several economically valuable seaweeds turned the seaweeds industry from foraging towards farming. The first breakthroughs in research on the cultivation of seaweeds occurred in the 1940s and 50s, when an increased understanding in the life-history of *Porphyra umbilicalis*, *Saccharina japonica* (then *Laminaria japonica*) and *Undaria pinnatifida* allowed for successful cultivation (Drew 1949; Yamanaka and Akiyama 1993; FAO 2022). Since then, dozens of cultivars have been developed to improve quality and yield (Hwang et al. 2019).

In western countries, seaweeds have historically supported communities in times of crises (Mouritsen et al. 2021). The relationship the inhabitants of a country have with seaweed is often based on historical relationships. In Wales the dish laverbread, made by boiling *Porphyra* spp. for several hours, used to be eaten by miners as part of a breakfast (Rees 2019). While the popularity of the dish has waned, its historical tie to the cultural identity of the country has prevented it from disappearing into the history books. In more recent years the development of seaweed aquaculture has started again, for a large part due to interest in the production for biofuels (Azevedo et al. 2019). This has mostly focussed on kelp species and was based on the method developed for *Saccharina japonica* (FAO 2022). Several countries in Europe have set out plans to establish a more biobased economy, which would include the cultivation of seaweeds, not just for biofuel but also as a source of food, pharmaceuticals, hydrocolloids, and other applications (Skjermo et al. 2014; Van Der Molen et al. 2018; Hasselström et al. 2020).

I.III.II Modern day

In 2018, the worlds aquaculture production of seaweeds was over 32 million tonnes (wet weight) valued at $13.3 billion, of which the vast majority was produced in China and Indonesia (57.1 and 28.8% in 2018 respectively) (FAO 2020). Although over 220 species of seaweed were reportedly cultivated, only seven genera of seaweeds make up 96% of the aquaculture production in 2018 (Chopin and Tacon 2020). The brown algae *Saccharina japonica* (known as kombu, formerly *Laminaria japonica*, 35.3%) and *Undaria pinnatifida* (wakame, 7.2%) are used as food and a source of the bio-polymer alginate. The red algae *Eucheuma* (29%) and *Kappaphycus* (4.9%) are used in the production of carrageenan, which is used in cosmetic, food, and industrial applications. *Porphyra & Pyropia* (8.9%) are two morphologically similar genera that are both used to make the dried seaweed food product nori. The red algae *Gracilaria* (10.7%) is used as a source of the hydrocolloid agar, as well as food for humans and shellfish. Indonesia's food production differs from China's in that it is almost exclusively based on the production of *Eucheuma,* of which the majority gets exported (Wright 2017). This leaves Indonesia's seaweed industry largely dependent on the value of carrageenan in the rest of the world. In contrast China exports only a fraction its production (FAO 2018), leaving most in the country where it is part of the local food culture.

Compared to Asia, the North American and European production of seaweeds are still in their early stages. All of Europe's production of aquatic plants (both wild-collected and aquaculture) amount to less than 0.1% of the global production and are mainly based on harvests of wild seaweed (Table I-1 and Table I-2) (Chopin and Tacon 2020). Nevertheless, the interest for increasing this production is high (see for instance (Vincent et al. 2020), and initiatives such as the Safe Seaweed Coalition (safeseaweedcoalition.org) and EU4Algae). Most of the recent increases in production has been in Asia, but Western countries are also having success in increasing their seaweed production (Kim et al. 2019) and it is becoming a part of the local food culture (Bouga and Combet 2015). In the UK, there is interest in expanding seaweed aquaculture as well (Huntington and Cappell 2020). Production in these areas should not only yield a high quantity but should also be produced in a sustainable way without negatively impacting the local environment. For future demands to be met, seaweed aquaculture would have to expand beyond Asia and move into other regions with high production potential while adhering to environmental and sustainability requirements.

Table I-1: Production of aquatic algae globally through aquaculture (grown) in 2018 with their main producers. Total aquatic algae collected from wild sources is indicated at the bottom of the table. (FAO 2020)

Species	**Quantity (tonnes)**	**Top 3 producing countries (% global production)**		
Total grown Phaeophyceae	14929318	China (88.2%)	South Korea (7.5%)	North Korea (3.7%)
Laminaria japonica	11448250	China (89.9%)	South Korea (5%)	North Korea (4.8%)
Undaria pinnatifida	2320430	China (75.6%)	South Korea (22.2 %)	Japan (2.1%)
Unspecified Phaeophyceae	891489	China (99.4%)	Russia (0.5%)	France (0.1%)
Sargassum spp	268910	China (86.4%)	South Korea (13.6%)	
Saccharina latissima	175.239	Norway (99.1%)	Spain (0.9%)	
Alaria esculenta	42	Ireland (95.8%)	Norway (4.2%)	
Nemacystus decipiens	20	Tonga (100%)		
Macrocystis spp	2	Peru (66.2%)	Chile (33.8%)	
Total grown Rhodophyceae	17343783	Indonesia (53.7%)	China (30.8%)	Philippines (8.5%)
Eucheuma spp	9412460	Indonesia (97.8%)	Zanzibar (1.1%)	Philippines (0.8%)
Gracilaria spp	3455595	China (95.6%)	Indonesia (3.3%)	Chile (0.6%)
Porphyra spp	2872793	China (70.2%)	South Korea (19.8%)	Japan (9.9%)
Kappaphycus alvarezii	1597333	Philippines (88%)	Malaysia (10.9%)	Solomon Islands (0.3%)
Unspecified Rhodophyceae	5302	India (100%)		
Meristotheca senegalense	300	Senegal (100%)		
Total grown Chlorophyta	20105	South Korea (85.5%)	South Africa (8.4)	Philippines (6.1)
Capsosiphon fulvescens	7000	South Korea (100%)		
Monostroma nitidum	6800	South Korea (100%)		
Codium fragile	3400	South Korea (100%)		
Ulva spp	1687	South Africa (100%)		
Caulerpa spp	1218	Philippines (100%)		
Total grown Microalgae	70440	China (98.8%)	Chile (0.7%)	Burkina Faso (0.2%)
Spirulina spp	70216	China (98.8%	Chile (0.6%)	Burkina Faso (0.2%)
Haematococcus pluvialis	223	China (89.9%)	Chile (10.1%)	
Chlorella vulgaris	1	Bulgaria (100%)		
Unspecified grown Algae	22543	Japan (99.8%)	Portugal (0.2%)	Spain (0%)
Total grown algae	32386202	China (57.4%)	Indonesia (28.8%)	South Korea (5.3%)
Total collected algae	954979	Chile (25.9%)	China (19.2%)	Norway (17.7%)

Table I-2: Production of aquatic algae in Europe in 2018 from aquaculture (grown) and wild (collected) and top producing countries. source (FAO 2020). No numbers are given for the UK as there are no production estimates available, although seaweed is both harvested and farmed in all four countries of the UK.

Species	Quantity (tonnes)	Top 3 producing countries (% global production)		
Total grown Phaeophyceae	5256	Russia (86.1%)	France (9.5%)	Norway (3.3%)
Unspecified Phaeophyceae	5039	Russia (89.8%)	France (9.9%)	Denmark (0.2%)
Saccharina latissima	175	Norway (99.1%)	Spain (0.9%)	
Alaria esculenta	41	Ireland (95.8%)	Norway (4.2%)	
Total grown Rodophyceae	1	Spain (100%)		
Gracilaria spp	1	Spain (100%)		
Total grown Microalgae	95.87	Greece (97.8%)	Bulgaria (1.5%)	Spain (0.7%)
Spirulina spp	94.45	Greece (99.3%)	Spain (0.7%)	
Chlorella vulgaris	1.42	Bulgaria (100%)		
Unspecified grown Algae	43	Portugal (81%)	Spain (19%)	
Total grown algae	5396	Russia (83.9%)	France (9.3%)	Norway (3.2%)
Total collected Phaeophyceae	250531	Norway (61.1%)	France (16.3%)	Ireland (11.7%)
Unspecified Phaeophyceae	132593	Norway (94.6%)	Russia (5.3%)	Spain (0.1%)
Ascophyllum nodosum	64533	Ireland (43.4%)	Norway (30.3%)	Iceland (26.3%)
Laminaria digitata	33768	France (94.1%)	Iceland (5.9%)	
Laminaria hyperborea	19435	France (46.2%)	Norway (41.2%)	Ireland (7.2%)
Undaria pinnatifida	202	Spain (100%)		
Total collected Rodophyceae	3026	Portugal (61.1%)	Spain (21.7%)	Italy (13.2%)
Unspecified Rhodophyceae	2382	Portugal (77.6%)	Italy (16.8%)	Ireland (4.2%)
Gelidium spp	641	Spain (100%)		
Porphyra spp	3	Spain (100%)		
Total collected Chlorophyta	800	Italy (100%)		
Unspecified Chlorophyceae	800	Italy (100%)		
Unspecified collected Algae	18655	Norway (87.9%)	Spain (12.1%)	Russia (0%)
Total Collected Algae	273012	Norway (62.1%)	France (14.9%)	Ireland (10.8%)

I.III.III Prospects

The EU and UK are calling for a transformation in their economies, aiming to become more resource efficient with greenhouse gasses being phased out (European Commission 2021). Part of this will be done through the investment in a blue growth strategy. In Europe currently the majority of macroalgae production is still dependant on harvests from wild stocks (FAO 2020; Araújo et al. 2021), but a seaweed cultivation industry is growing. With a coastline of 31,400 km the UK has the natural resources available for a thriving seaweed aquaculture industry, but up till now the production is assumed to have been low (no recent official estimates for seaweed production in the UK are available) (Capuzzo 2022). The species that are being considered for aquaculture production in Europe would be ones that have a high growth rate, contain valuable components, and have a well described life cycle. Since kelps are economically valuable, have a wide variety of uses, and have a history of successful cultivation in aquaculture they are one of the most interesting groups of species for cultivation in the UK. And as such they are the focus of this thesis.

Due to changes in the climate inducing higher seawater temperatures, marine heatwaves have become more common, which is posing a threat to the biodiversity and ecosystem services provided by marine foundational species, including kelps (Smale et al. 2019; Ottersen and Melbourne-Thomas 2019; Arafeh-Dalmau et al. 2020). Future large-scale cultivation efforts will require a better understanding of the interactions between seaweeds and their environment. Among the challenges in seaweed blue economy globally are: 1) the collection and preservation of wild and cultivated kelp germplasm (Hu et al. 2021); 2) The selection of suitable sites under changing environmental conditions (Hu et al. 2021); and 3) the quantification and valuation of kelp ecosystem services (Naylor et al. 2021). Specifically for Europe and the UK, the challenges in seaweed cultivation also encompass the risk of introducing alien species and pathogens, as well as the potential impact of escaped species, in addition to operational optimization, economic and regulatory challenges (Barbier et al. 2019; Vincent et al. 2020; Wilding et al. 2021).

I.III.IV Commercial methods

Kelp cultivation in Western countries generally follows the following process. It starts with acquiring zoospores from wild stocks. The reproductive kelps are then brought to a hatchery where they will be grown until they are young sporophytes. In the hatchery, zoospore release generally involves 3 steps: First a pre-treatment where the sori is cleaned by washing it with water, by brushing it, and/or by rubbing it with clean tissues. Second, the sori are desiccated for about 12h (though times can range from 15 mins to 48h). And third, the sori are placed in seawater to release the spores (Alsuwaiyan et al. 2019). The spores are grown into gametophytes in nutrient enriched seawater, and this culture is developed until the gametophytes become reproductive. Once unfertilised eggs on the female gametophytes, or developing sporophytes are observed the culture is sprayed onto a spool of string. The sporophytes will attach to the culture string and be grown for another one or two months. The sporophytes are transferred from the hatchery to a sea site between October and December, though this can differ depending on location and local temperatures (Peteiro and Freire 2009). Most common practice in Europe here is that the culture string is coiled around a thick rope tied between two buoys. The rope is positioned about 0.5 to 1m below the surface of the water. After 5 to 6 months of growth the kelps will reach their maximum size. If the kelps are left out after May there is a risk of epiphytes growing on them reducing growth and quality of the seaweed. Once harvested the kelps can be processed further. There are a few different cultivation guides detailing the methodology that prospective seaweed farmers can use to set up a kelp farm (Edwards and Watson 2011; Mooney-McAuley et al. 2016). There are also other documents available with more details and different methods to farm seaweeds tested in the UK (e.g. see (Stanley et al. 2019; Wilding et al. 2021)).

I.III.V Environment and site

For the cultivation of seaweeds to be successful the biophysical parameters of their site need to match the seaweeds' requirement for growth. Kerrison et al. (2015) provided a review where the main variables for seaweed growth are discussed. These include temperature, salinity, water motion, nutrients, pH and CO_2-concentration, Photosynthetically Active Radiation (PAR), and ultraviolet (UV) radiation. They also included the biological factors growth density, disease, and grazing loss which will not be discussed here.

Temperature affects the metabolic rate of seaweeds and affects their ability to reproduce. Generally, kelps are tolerant of low winter temperatures, but most of their growth takes place during spring when temperature and light increase, and nutrient concentrations remain high. Temperature is one of the main variables affecting the distribution and survival of kelp forests (Bartsch et al. 2013; Franco et al. 2018; Sudo et al. 2020; Arafeh-Dalmau et al. 2020). In most cases seasonal high temperatures, rather than low temperature, will dictate species geographical ranges and survival success. A persistent rise of temperatures of a few degrees above a species their optimum temperature can destabilise proteins or metabolic pathways and lead to an organism dying (Bolton and Lüning 1982; Kerrison et al. 2015).

Reductions in salinity can affect the osmotic pressure of seaweed cells. *Laminaria* species are known to change their intracellular chemical concentration, in particular mannitol, thus changing their osmolarity (Reed et al. 1985). The level of tolerance to reduced salinities can vary between species (Lind and Konar 2017), life stages (Peteiro and Sánchez 2012), and ecotypes (Buschmann et al. 2004).

Water motion affects growth, morphology, and survival of seaweeds. A boundary layer develops around the thallus of kelp, limiting the uptake of nutrients and CO_2 (Hurd 2000; Vettori and Nikora 2019). The blades of *Saccharina latissima* adjust to both flow conditions as well as nutrient conditions by changing the morphology of their blade (Zhu et al. 2021). High levels of wave impact or current speeds were thought to dislodge kelps, but more recent evidence indicates that dislodgement is not the main source of tissue loss in kelps (de Bettignies et al. 2013). Instead, the kelps adapt to survive these conditions by changing their morphology (Fowler-Walker et al. 2006).

Dissolved inorganic nutrients impact the composition, and growth of seaweeds. Kelps are thought to be season anticipators, storing nutrients in winter and spring to use later in the growth period. Growth can become limited when nutrients concentrations and/or storage is insufficient. Often nitrogen compounds are the limiting factor for growth of seaweeds (Kain 1989; Roleda and Hurd 2019). Locations with higher nutrients levels, for example due to coastal eutrophication, animal aquaculture, or upwelling, could increase seaweed growth (Fossberg et al. 2018; Fan et al. 2019).

With climate change leading to increased concentrations of CO_2 in seawater, the seawater is getting more acidic. An increase in acidity was expected to have a negative effect on seaweed growth. However, in some cases the increased CO_2 concentration ameliorated the negative effects of the lower pH (Roleda et al. 2012; Shukla and Edwards 2017).

Light conditions, specifically Photosynthetically Active Radiation (PAR), can limit the growth of seaweeds. Kelps contain the photopigment fucoxanthin, which transfers on energy to chlorophyll for use in photosystem light harvesting complexes (Owens 1986). Depth and light conditions are a major contributing factor to the spatial distribution of kelps on the shore (Kain 1962; Han and Kain (Jones) 1996). Turbulent water can reduce the amount of light available for photosynthesis. Seaweeds can be photo inhibited by excessive light conditions, but on seaweed farms the amount of light available to the seaweeds can be controlled by using the appropriate depth for specific site conditions (light levels, turbidity). Since light is available at the surface of the water, and in some cases nutrient concentration are higher in deeper water, some researchers have suggested moving farms to deeper water during the night to absorb nutrients and resurfacing them during the day to photosynthesize with the available light (Mortensen 2017a).

While PAR can limit growth, UV can limit the distribution of kelps by damaging the early life stages of macroalgae (meiospores, gametophyte, juvenile sporophyte) (Müller et al. 2008). Exposure to UV-A and UV-B radiation can lead to damage to the proteins in the cells and radical oxygen formation, and can reduce growth and photosynthetic efficiency (Roleda et al. 2007; Müller et al. 2008, 2012).

All these factors can make it difficult to select an optimal site for kelp cultivation. As many of these factors are variable throughout time and change for different kelp life/growth stages, a deep understanding of the effects of biophysical factors on kelps is required, in addition to an understanding of the local conditions of a farm.

I.IV Species selection

On the coast of the United Kingdom there are seven species of kelp: Oarweed (*Laminaria digitata*), Tangle (*Laminaria hyperborea*), Golden kelp (*Laminaria ochroleuca*), Dabberlocks (*Alaria esculenta*), Sugar kelp (*Saccharina latissima*), Wakame (*Undaria pinnatifida*), and although not a true kelp, Furbellows (*Saccorhiza polyschides*) is often included in the list.

In this work I will focus on 3 species of kelp that are interesting for UK based aquaculture as they are endemic, fast growing, and have commercial potential: *Laminaria digitata*, *Laminaria hyperborea* and *Saccharina latissima*. See Table I-3 for an overview of the characteristics of these species. They are all perennial species that grow in the lower intertidal area and/or the sublittoral, provide ecological services, and have a similar life cycle (see text above and Figure I-2). These species can form dense marine forests that function as foundation species for the local marine ecosystems. Kelp species that are also interesting for UK aquaculture, but that fall outside the scope of this thesis due to availability and time constraints, are *Laminaria ochroleuca* (golden kelp), *Alaria esculenta* (dabberlocks), and *Saccorhiza polyschides* (furbellows).

I.IV.I Saccharina latissima

Saccharina latissima (formerly *Laminaria saccharina* (Lane et al. 2006)) occurs in the lower intertidal on the shore. It is predominantly present in sheltered waters (Burrows 2012). It consists of a variety of carbohydrates including laminarin, alginate, cellulose and fucoidan, as well as the sugar alcohol mannitol (Zhang and Thomsen 2019). *S. latissima* gets its common name - sugar kelp - from this last chemical. When it is chewed mannitol gives it a slightly sweet flavour. It is an interesting local variant of kelp endemic to Asia referred to as *kombu*. This similarity has led to this species also being known as *sugar kombu* or *kombu royale*. *S. latissima* consists of one, undivided blade and has a flexible stipe that allows it to align itself with the direction of the current (Buck and Buchholz 2005) (Figure I-1).

I.IV.II Laminaria digitata

Laminaria digitata is a species that grows perennially on the lower intertidal and shallow sublittoral of rocky shores across large areas of the North-Atlantic up to the arctic (Hoek 1982). The sporophyte grows best at 5 to 15 °C (Kerrison et al. 2015). The oval blade is a leathery sheet-like tissue, divided into linear segments, while its stipe is smooth, flexible, and slightly flattened (Bunker et al. 2012). Morphologically its shape, size and chemical composition can depend on local conditions (Nielsen et al. 2016b). *L. digitata* is used as a food, as well as a source of alginate, printer ink, and biodegradable polymer film (Wilding et al. 2021).

Table I-3: Overview of characteristics and information of the species selected for this thesis. Sources - a:(White and Marshall 2007) b:(Hill 2008b) c: (Tyler-Walters 2007). * Reproductive times dependent on local conditions.

	S latissima [a]	***L digitata*** [b]	***L hyperborea*** [c]
Distribution	North Atlantic, as far south as Northern-Portugal	North Atlantic Ocean as far south as Morocco	North-east Atlantic as far south as mid Portugal
Depth	Lower littoral and upper sublittoral (up to 30m)	Lower littoral and upper sublittoral (+1 to -20m)	Lower littoral to upper subtidal (+1 to -36m)
Life span (years)	2-5	6-10	11-20
*Reproductive time**	Year round. Maxima October-April	Year round. Maxima July-August and November-December	September-April
Maximum size (m)	4	2-4	3.5

I.IV.III Laminaria hyperborea

Laminaria hyperborea looks similar to *L. digitata*, but it has a rigid stipe that snaps when bent (Bunker et al. 2012). Since the stipe has a rough exterior, it is also suitable for other species to grow on (Christie et al. 2003). *L. hyperborea* provide a variety of ecosystem functions. The different parts of this seaweed (i.e. blade, stipe and holdfast) also contribute to biodiversity by providing different types of habitat for communities of small invertebrates (Christie et al. 2003).

Generally grows on exposed shores in deeper water than *L. digitata* and *S. latissima* (Kain 1962). *L. hyperborea* is the dominant forest-forming kelp in the UK where it contributes to the local ecosystem (Smale et al. 2013). Commercially it is considered of less importance due to its slower growth rate, but there is potential for its use as a source of alginate, printer ink, or biodegradable polymer film (Wilding et al. 2021). Much of the European production of this species currently comes from trawling in Norway (Vea and Ask 2011; FAO 2020).

I.V Thesis objectives and chapter outlines

For kelp cultivation in Europe to become successful and profitable we need a good understanding of how environmental variables impact kelp growth and reproduction. The broad aim of this thesis was to increase the knowledge base surrounding the cultivation and ecosystem services of kelp species endemic to western Europe. Three of the chapters in this thesis describe how changes in the environment change the survival, growth, and morphology of commercially viable kelp species. The remaining chapter focusses on the protocols surrounding the storage and acquisition of kelp spores. The objectives of this thesis were:

- To develop a tool for the identification of useful sites for a *Saccharina latissima* seaweed farm. This would help potential seaweed farmers in deciding where to set up a new farm even in areas with fluctuating environmental conditions.
- To assess the effects of salinity and nitrate conditions on the settlement, germination, and growth of kelp *Saccharina latissima, Laminaria digitata,* and *Laminaria hyperborea* zoospores. This would aid in understanding of the environmental requirements of these species, which in turn helps understand the spread of seaweeds and their cultivation requirements in environments with a reduced salinity, such as estuaries.
- To describe the morphological changes of *Saccharina latissima, Laminaria digitata,* and *Laminaria hyperborea* throughout the seasons. An additional related objective was to describe how these morphological traits relate to each other. This would aid in the understanding the seasonality of morphology related ecosystem services that kelps provide.
- To test a method for long term preservation of *Laminaria digitata* and *Laminaria hyperborea* zoospores and test the current methodology of acquisition of kelp spores.

This results from this research can help in developing a protocol for a seedbank in which kelp spores can be stored long-term, preserving genetic diversity.

I.V.I Outline chapter 1: Applying a seaweed growth model as a tool to identify suitable sites for Saccharina latissima aquaculture – a case study in the Milford Haven Waterway

The objective of this chapter was to apply a seaweed growth model of *S. latissima* as a tool for the selection of a site for a seaweed farm. While methods exist to select a site based on socioeconomic and legal restriction, the current tools usually do not take the variability of an environment into account. By combining geo-information, data from environmental monitoring systems and a kelp growth model an optimal location for a seaweed farm can be found. This method was then tested on the Milford Haven Waterway, an estuary situated in southwest Wales that is being investigated as a location to set up commercial seaweed farms. Combining environmental data from the waterway and the growth model would provide a deeper understanding of the waterway and indicate sites that could be used for aquaculture purposes.

Research questions:

- What insights can the application of a seaweed growth model provide in site selection for a seaweed farm?
- Which sites in the Milford Haven Waterway are suitable for the cultivation of *S. latissima*?

I hypothesised that a seaweed growth model can be used as a supporting tool in the site selection of a seaweed farm.

I.V.II Outline chapter 2: Establishment and growth of the gametophytes of three kelp species under varied salinity and nitrate conditions

The objective of this chapter was to investigate the effects of salinity and nitrate concentration on the germination and growth of kelp meiospores. The nutrient conditions of a site are one of the most important environmental determinators for growth rate of a seaweed. Estuaries can have a higher-than-average nutrient concentration due to runoffs, but they also have a lower salinity level. There is limited research on the effects of salinity of gametophytes of kelps. Understanding the effects of salinity and nitrate concentration on spore germination and gametophyte growth can help in understanding the requirements these species have for development. Knowing how these two factors affect the haploid life stages of kelps provides insight into the distribution and potential for cultivation of kelps in estuaries. An potential interactive effect might exist because in *S. latissima* sporophytes salinity can affect nitrogen metabolism (Gordillo et al. 2002).

Research questions:

- How do salinity and nitrate concentration affect spore attachment, germination, and early growth of the haploid life stages (meiospore and gametophyte) of *Laminaria digitata*, *L. hyperborea* and *Saccharina latissima*?

I hypothesised that salinity affects spore germination and growth, but not salinity, and that nitrate concentration affected only spore growth.

I.V.III Outline chapter 3: Seasonal changes in morphological traits of three UK kelp species: blade traits vary more than stipe traits.

The objective of this chapter was to evaluate seasonal changes in the morphology of kelps, and to correlate these traits with each other. Kelp composition and growth patterns are known to change throughout the year, but not a lot is known on what these morphological changes are in the field. Since morphological traits influence the ecological role kelp have as a foundation species (in kelp forests) the changes in morphology could impact their ecosystems functions. These morphological traits include blade size and thickness, which directly influences the profitability of wild harvesting activities. In this study functionally relevant morphological traits of the kelp species *Saccharina latissima*, *Laminaria digitata*, and *Laminaria hyperborea* were measured over a two-year period. Changes in morphology were compared with environmental conditions to explain how morphology can be influenced by seasonal changes.

Research question:

- How do morphological traits of kelps change throughout the year?
- What are the differences in traits for these three species on the UK shoreline?

Morphological traits were hypothesised to vary seasonally based on environmental changes and to be different for the three species.

I.V.IV Outline chapter 4: Preservation and cryopreservation of the spores of Laminaria digitata and Laminaria hyperborea

The objective of this chapter was to test a method of preserving kelp spores with cryopreservation, as well as to test the effect of sori storage time on spore release and quality. Developing a method to store kelp spores long term would allow for a seedbank to be established for seaweeds, making it possible to trade in seaweed spores and to make hybridization and the development of cultivars easier.

Research question:

- What is an optimal concentration of cryoprotectant to preserve kelp spores in liquid nitrogen to allow later use?
- What is the effect of sori storage time on spore release and germination *Laminaria digitata*?

I hypothesised that the optimal concentration of the cryoprotectant dimethyl sulfoxide (DMSO) would be between 5 to 20%, and that the optimal storage time for successful spore release would be 24 hours.

I.V.V Outline chapter 5: General discussion

This chapter synthesized the results from previous chapters and discussed outstanding question and challenges in the field of seaweed cultivation and seaweed research.

Chapter 1 - Applying a seaweed growth model as a tool to identify suitable sites for *Saccharina latissima* aquaculture – a case study in the Milford Haven Waterway

Abstract:

Seaweed production through aquaculture is increasingly being looked at as a potential source of food and industrial applications. While farms can produce large quantities of seaweeds the local conditions at the site can have a large effect on the commercial success of the farm, especially nutrient concentrations. Since estuaries are affected by eutrophication these sites have a high nutrient concentration, however their reduced salinity and the variability of the environment make it difficult to predict whether a location in an estuary would be suitable for seaweed cultivation. Only a limited number of tools are available to aid stakeholders looking to start a seaweed farm in site selection. Here I applied a previously developed *Saccharina latissima* growth model as a tool for site selection in a test case for the Milford Haven Waterway, an estuary situated in southwest Wales that is being investigated as a location to set up commercial seaweed farms. The seaweed growth model was adapted to include salinity and blade erosion effects. Data on water temperature, nitrate concentration, salinity, and irradiance in the Milford Haven Waterway was collected through publicly available datasets and local stakeholders. Environmental data was combined to form an average yearly trend for seven sites in the estuary for which *S. latissima* growth was modelled. Model results indicate that five sites could provide the environmental conditions for high growth rates throughout the usual cultivation seasons, with the two remaining sites higher up the rivers having only a relatively small reduction in growth due to salinity effects. Seaweed presence data from the waterway corroborates these findings. These results indicate that the model used can be used as a tool to aid in site selection for seaweed farms. Further development of the model could provide potential entrepreneurs with growth data and give them clear incentives to invest in this kind of sustainable marine food production system.

Introduction:

Kelps are an economically, culturally, and ecologically important group of seaweeds. They have been used as a food, and have a wide variety of other uses in industries (Vásquez et al. 2014; Hynes et al. 2021). Traditionally, seaweeds were harvested from wild stocks, but due to the risk of overharvesting (e.g. (Thompson et al. 2010; Rebours et al. 2014)), and the important ecological role that kelp forests play (Grebe et al. 2019) there has been a shift towards production through aquaculture.

Seaweed farms can be a useful alternative to grow kelp in a cheap and sustainable way, but seaweed growth is influenced by a wide variety of environmental factors that change over time and between locations. For example: sufficient light needs to be available for photosynthesis, temperature affects metabolic rate, salinity can affect osmolarity, and nutrient concentrations help kelp maintain growth (Kerrison et al. 2015). In particular, nitrogen availability is often the limiting factor for seaweed growth (Roleda and Hurd 2019). Since these environmental conditions are linked with local conditions the selection of a site for the cultivation of kelps is an essential part to setting up a successful seaweed farm. With nitrogen availability being one of the main factors to look at when picking a location for a seaweed farm.

Since estuaries are particularly affected by eutrophication (Rabalais et al. 2009), the relatively high nutrient concentration in the estuary could be an indication that this type of location can be a good spot for a seaweed farm. Growing seaweeds in a eutrophied location could be a valuable part of water remediation management (Grebe et al. 2021), could sequester carbon (Kim et al. 2017), and could improve local biodiversity (Visch et al. 2020). Estuaries are also characterised by having brackish water and a very variable environment. Although the reduction in salinity and inconsistency in water quality parameters reduces the survival and reproduction of some kelps (Buschmann et al. 2004), estuaries are still an ecosystem where seaweeds can thrive (Nelson-Smith 1967a; Aldridge and Trimmer 2009). When selecting a site for a seaweed farm these complexities of the physical and chemical parameters, and how they would affect kelp growth need to be considered.

A variety of approaches to site selection for seaweed farms have been applied. Mostly these have focussed on applying geographical information systems to help in spatial decision making (Nath et al. 2000). Biophysical characteristics (e.g. temperature, turbidity, depth) and socio-economic characteristics (e.g. administrative regulations, coastal activities) all need to be considered in the final decision making process. Most studies use a multi-criteria evaluation approach where a selection of biophysical parameters, socioeconomic parameters, or both are used to calculate a suitability score for a location (for examples, see (Radiarta et al. 2011; Silva et al. 2011; De Sousa et al. 2012; Liu et al. 2013; Bagdanavičiūtė et al. 2018)). While a multi-criteria evaluation may work well for the selection of a site, it does not give the stakeholders looking to start a seaweed farm a detailed perspective on how well the seaweeds will grow. This information could be essential in predicting whether a seaweed farm will be profitable (Van Den Burg et al. 2016; Hasselström et al. 2020).

Another method of site selection can be done through the application of seaweed growth models. While there have been studies that combined ecological models with seaweed growth models to simulate the cultivation yields of a seaweed farm (Broch et al. 2013; Van Der Molen et al. 2018). But these studies have mostly focussed on bioremediation (Broch et al. 2013; Fossberg et al. 2018), or were dependent on 3D-hydrodynamic/biogeochemistry models that are not easily accessible to other researchers (Van Der Molen et al. 2018; Broch et al. 2019). Only a single study has used a seaweed growth model as a tool for site selection (Broch et al. 2019).

As properties of the coastal environment can shift based on time of year, location, and other factors a seaweed growth model would need to incorporate these environmental changes. One option for a type of model that includes this are Dynamic energy budget (DEB) models (Kooijman 2009). These models simulate the metabolic processes of an organism throughout its life cycle. Through incorporating environmental measurements with formulas on energy uptake, storage, and utilisation of substances DEB models can be used to make prediction on growth, reproduction, and responses to the environment (Kooijman 2009). These growth predictions can then be used as an indicator for the suitability of the location for a seaweed farm.

A seaweed for which modelling site suitability would help in its cultivation is *Saccharina latissima*. This species is economically valuable, is endemic to Europe and is being investigated for more cultivation projects (FAO 2020; Araújo et al. 2021).This kelp grows according to a seasonal cycle of growth and reproduction. During the winter and the first half of the year the kelp grows its blade (Sjøtun 1993). In the winter months it builds up internal nitrogen reserves while nitrogen remains available (Sjøtun 1993; Zhang and Thomsen 2019). When the light conditions improve in spring and summer, the kelp is no longer limited in growth by light, allowing it to use external nitrogen until concentrations in the water are reduced. And when nitrogen conditions are reduced the kelp uses its reserves to continue growth until internal nitrogen reserves are depleted, the time of which can differ but would usually be around June or July (Sjøtun 1993; Nielsen et al. 2014a). In summer the carbohydrates mannitol and laminarin are produced which play a role in carbon storages. These resources are used to compensate for respiration processes in late autumn and winter when light is limited again (Black 1950; Gevaert et al. 2001). The combination of environmental and metabolic changes could make a DEB model a suitable option for predicting growth.

One seaweed DEB model which could be used as a tool for site selection is the *Saccharina latissima* model calibrated by Venolia et al. (2020). The creators of this model mentioned that their model could be used to predict growth based on environmental inputs and could assist the aquaculture industry by facilitating the choice of site selection. The Venolia model calculates growth based on temperature, irradiance, dissolved inorganic carbon concentration, and nitrogen concentrations. A limitation of the Venolia model is that the effects of salinity and blade erosion were not considered. However, these variables could have a large impact on how seaweeds would grow in a nutrient rich estuary, where the high nutrient load could lead to larger seaweeds prone to erosion, and the reduced salinities could inhibit growth. To date the Venolia model has not yet been applied as a tool for site selection. An additional advantage of this model is that the authors have shared the code for the model. Some of the other models (e.g. (Broch and Slagstad 2012)) are not available, or only available on request. Potential stakeholders interested in starting a seaweed farm would benefit if they were able to model site suitability themselves.

My objective in this chapter was to apply a seaweed growth model of *S. latissima* as a tool for checking if the environmental parameters (temperature, nutrients, light) were suitable for a seaweed site. As a case study I applied this model to the Milford Haven Waterway, as this estuary is relatively well studied, has environmental data available, and is being investigated as a location to set up a commercial seaweed farm (Jones 2018). The Venolia model was further developed to include salinity conditions as an effect in predicting growth. The results from the model were then compared to the physiochemical conditions of the area, and presence data of seaweeds in the estuary to give an indication of the reliability of the results.

Method:

Study site description

The Milford Haven Waterway is a natural harbour located in the county of Pembrokeshire in the southwest of Wales and is the country's largest estuary, as well as one of the deepest natural harbours in the world (Carey et al. 2015). The mouth of the estuary is located at the point where the Severn estuary and the Celtic Sea meet. Most of the waterway's freshwater input comes from the Eastern- and Western Cleddau that meet at Picton Point to flow into the Milford Haven Waterway (Gunn and Yenigün 1985). Usually the waterway is differentiated into an upper and lower section, with the upper waterway (sometimes referred to as the Daugleddau) having mostly polyhaline conditions (salinity between 18 to 30 PSU), and the lower haven being mostly euhaline (>30 ppt) (Nelson-Smith 1967b; Nikitik and Robinson 2003). Due to its diversity in habitats and species the waterway is also part of the Pembrokeshire Marine Special Area of Conservations (SAC). It is considered one of the best areas in the UK for inlets and bays, estuaries, reefs, and grey seals (*Halichoerus grypus)* (NRW 2018). The Milford Haven Waterway is subjected to a semi-diurnal co-oscillating tide, which varies the water level by 2 to 8m (Nikitik and Robinson 2003). The Milford Haven waterway is an arm of the sea, enclosing over 110km of coastline with an entrance a mile and a half wide (Nikitik and Robinson 2003). The natural channel has a depth of 15 to 25m at low water spring tides for the first 7 miles and the average width of the haven is 1 mile (Dudley 1976). There is some stratification of both temperature and salinity in the upper levels of the Milford Haven Waterway (Nelson-Smith 1967b). But for the more seaward area where the estuary is wider and deeper, there is a high degree of vertical mixing (Gunn and Yenigün 1985). The monitoring sites in this study are in the area where vertical stratification is assumed to be minimal.

The location and geography of the estuary helped the area develop an economy based around maritime commerce, shipping, and the petrochemical industry. In the 1950s the government decided to develop Milford Haven into Britain's major deep water oil port (Dudley 1976). Till that point the port in the haven was mainly devoted to the fishing industry. In 1960 the first oil terminal was installed (Dicks and Hartley 1982), and since then Milford Haven has quickly grown into the largest oil port in the UK (Foster and Foster 1977). The Milford Haven Waterway is now heavily industrialised and is renowned for its shipping and petrochemical industry. In its capacity as a major oil port, it used to house up to four refineries, including associates jetties and pipelines. Although only one refinery is now in operation (Valero), there have been two new natural gas plants (Dragon LNG and South Hook LNG) that have been constructed (Little et al. 2016).

Its status as a SAC, and its history with oil spills have led to the area being relatively well monitored in comparison to other estuaries (Hiscock and Kimmance 2003; Das and Nassehi 2004)

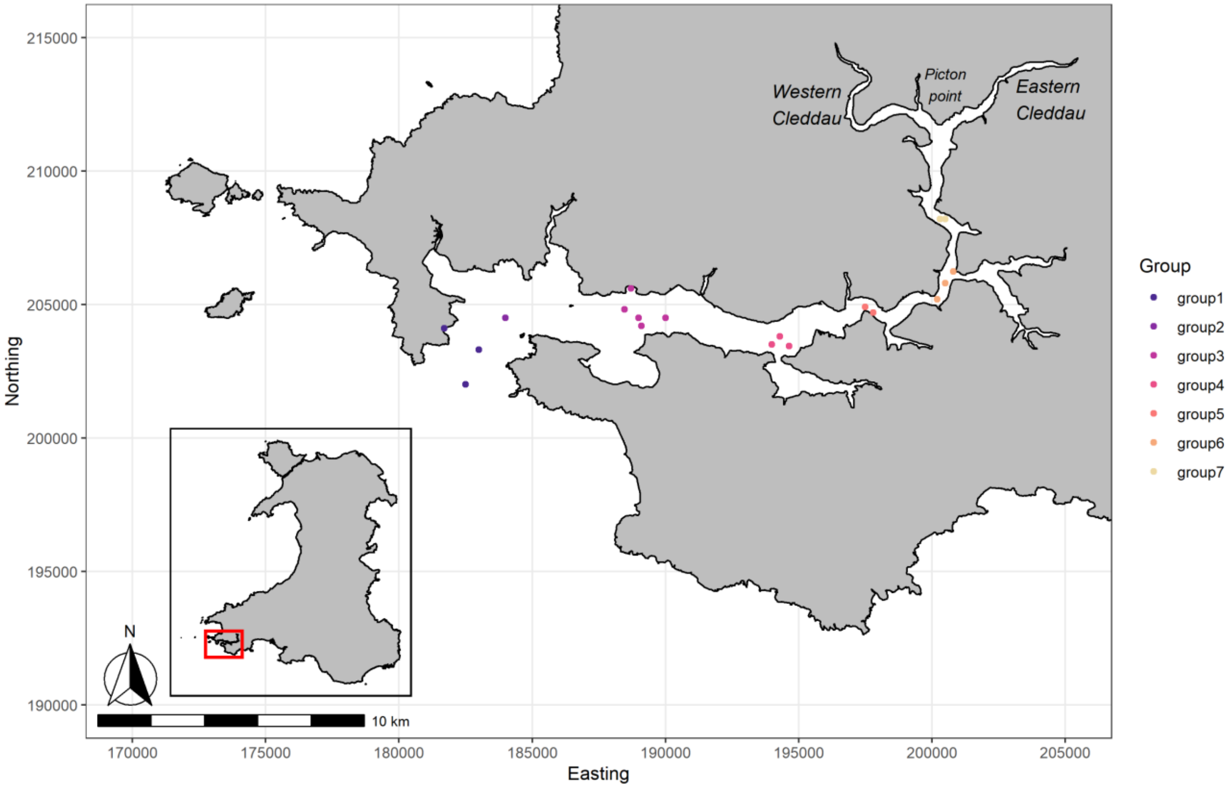


Figure 1-1: Map of environmental monitoring location in the Milford Haven Waterway. Data was grouped together to use as input for model.

Identification of parameters and data acquisition

The main environmental data required for the Venolia model are: temperature, nitrogen concentration, dissolved inorganic carbon concentration (DIC), and irradiance. Salinity was added as an additional variable into the model. Besides these variables the review by Kerrison et al. (2015) also indicated that phosphate, water motion, ultra-violet radiation, growth density, disease and grazers can also determine the growth success in a location. These variables were not considered for the purposes of this study since there is limited data available for these variables or they are not included in the Venolia model.

A round of web search was conducted to obtain data pertinent to the biophysical environment in the Milford Haven waterway. A variety of public hubs for data (e.g. NBN atlas, Natural resources Wales) were found, and people who have contributed to projects monitoring the environment of the Milford Haven Area were contacted with the request if they had relevant data for the Milford Haven Waterway. Six datasets (see Appendices for Chapter 1 on page 132) were found that had relevant data to seaweed growth in the Milford Haven Waterway (Table 1-1). These datasets were merged by combining the nitrate, salinity, and temperature data (see Table S0-1 in the appendix). The locations of the datapoints were plotted on a map and manually grouped into 7 locations in the waterway based on geographical distance (Figure 1-1).

The Venolia model uses hourly data to calculate changes in biomass. Since nitrogen and temperature data was spread over multiple years and did not have the temporal resolution needed to work as inputs for the adapted Venolia model I used a LOESS (Locally Estimated Scatterplot Smoothing) – a non-parametric regression method – to interpolate hourly data from the general annual trends for nitrogen and temperature at these sites. So multiple years were combined to calculate an “average” year for the several sites and the model was run. The results were used as model inputs for Temperature and Nitrate. Irradiance data measured each minute in 2015 and 2016 was averaged per hour and used as irradiance input. A constant DIC was used based on the value from Venolia et al. (2020) since no DIC measurements were available from the Milford Haven Waterway.

Kelps in the UK would usually be grown from October till May (Edwards and Watson 2011). This would mean a maximum of 244 days of culture if this entire timeframe would be used. The model here was run using data from October 1st as day 1 for 305 days (August 1st) to observe how a potential longer growth period would affect growth.

In a paper by Gevaert et al.(2001) the authors presented a formula for the relationship between *S. latissima* dry weight and length:

$$Length = \left(\frac{Dry\ weight}{3.87 * 10^{-3}}\right)^{\frac{1}{1.469}} \tag{1-1}$$

This formula was used by Venolia et al.(2020) as part of their method for calibrating their model. In the discussion and results sections of this chapter it has been used to help reflect on the model results.

To provide an indication of the reliability of the model results I obtained spatial records of *S. latissima* distribution in the Milford Haven Waterway. These spatial records of *S. latissima* distribution were obtained through the databases of the National Biodiversity Network (NBN). Observations in, and near the Milford Haven Waterway from 2000 to 2020 were extracted and plotted onto a map of the area. (NBN Atlas occurrence download at https://nbnatlas.org accessed on Tue Jun 29 2021).

Model results from the adapted model presented here were compared with results generated with the original Venolia model.

Table 1-1: Summary statistics of the measurements used to generate environmental trends in the Milford Haven Area. Presented are the number of measurements (n), the first and ninety-ninth quantile, mean and standard deviation.

Site	**NO_3-N (µmol/L)**					**Salinity (PSU)**					**Temperature (°C)**				
	n	1st quant	mean	99th quant	Std dev	n	1st quant	mean	99th quant	Std dev	n	1st quant	mean	99th quant	Std dev
group1	92	0.04	2.76	23.87	4.76	339	26.13	33.76	35.30	2.75	334	6.9	12.4	17.0	2.81
group2	83	0.03	2.26	11.78	2.95	77	24.22	33.40	35.27	1.82	167	7.32	12.8	17.3	3.01
group3	95	0.03	4.42	27.82	5.74	392	1.75	32.09	35.18	4.69	337	6.5	12.8	18.1	3.32
group4	83	0.03	4.20	14.55	4.35	404	20.58	31.97	34.80	2.92	393	6.25	13.3	18.3	3.44
group5	94	0.03	8.12	67.71	11.86	410	12.88	30.63	42.06	4.57	520	6.35	12.9	18.7	3.58
group6	95	<0.01	10.97	92.65	15.87	352	6.01	28.76	36.67	5.64	347	5.78	13.2	19.7	3.88
group7	83	0.10	11.43	45.69	11.73	460	10.68	27.56	34.52	5.33	429	5.9	13.9	20.0	3.97

Model description

Venolia et al.(2020) calibrated a dynamic energy budget (DEB) model for *S. latissima* based on the macroalgal *Ulva lactuca* DEB model presented by (Lavaud et al. 2020). In their paper they made their R scripts with which to run their model available. The Venolia model uses the environmental variables Temperature, Irradiance, Dissolved inorganic carbon concentration, and Nitrate and Nitrite concentration to calculate growth. This kelp growth model was combined with the environmental data records from the Milford Haven waterway to identify a suitable location for a seaweed farm. Growth predictions were used as a measure for the suitability of the location for an *S. latissima* seaweed farm.

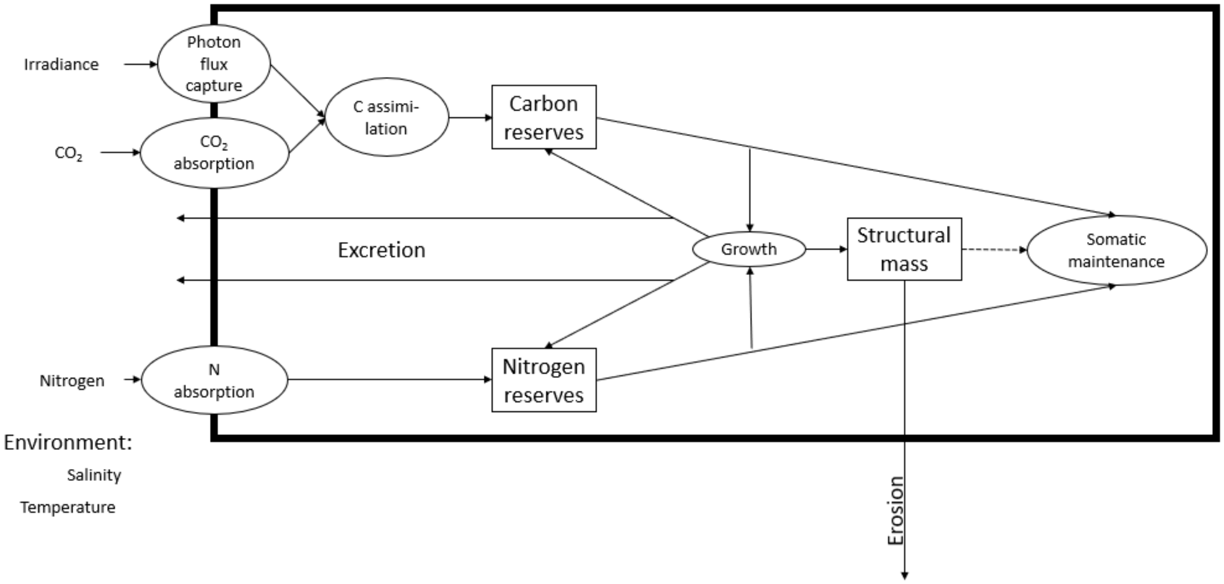


Figure 1-2:Conceptual framework of *Saccharina latissima* (adapted from Lavaud et al. (2020) and Venolia et al.(2020)). The variables Irradiance, Carbon dioxide, Nitrogen, Salinity, Temperature influence the accretion of the state variables Carbon reserves, Nitrogen reserves, and Structural mass. The large square represents the inside of the algae. The variables Salinity and Temperature are included in the corner of the figure to illustrate their influence on the metabolic processes in the algae.

The state variables in the Venolia model are the structural mass of the whole organism (M_V , in mol V, moles of structure), Nitrogen reserve density (m_{EN}, in mol N per mol V), and Carbon reserve density (m_{EC}, in mol C per mol V). Details on the model are given in Venolia et al.(2020), but it is briefly summarised below and in Figure 1-2. Any changes from the Venolia model are discussed, and are in Table 1-2 and Table 1-3.

The Venolia model is based on bioenergetics modelling that can be used to provide production predictions by estimating the flow of mass and energy through an organism, from uptake to usage for maintenance, growth, reproduction, and excretion (Kooijman 2009). The input variables Irradiance, DIC, and Nitrogen were used to calculate how much of the nutrients taken up into the kelp, were converted to reserves and structural mass.

The model assumptions are: that ratios of C, N, H and O within specific reserves or structures remain constant, that surface area remains proportional to volume, that no energy is used for reproduction, and that there is no effect from wave action, biofouling, or photoinhibition.

Table 1-2: Parameters added to the Venolia model for *Saccharina latissima* growth.

Parameter	Parameter description	Parameter Units	Value	Source
eps	Frond erosion parameter	dm^{-2}	0.22	(Broch and Slagstad 2012)
K_a	Structural dry weight per unit area	$g * dm^{-2}$	0.6	(Broch and Slagstad 2012)
Amplitude	Fluctuation in salinity	PSU	10 for group 7 7 for group 6 5 For group 5 1 For group 4 0 for groups 1 to 3	(Das and Nassehi 2004)

Table 1-3: Model equations added to the Venolia model for *Saccharina latissima* growth.

	Equation	Unit	Description	Adapted/based on
1	$r = (J_{VG} * S) - J_V^M$	h^{-1}	Growth factor influenced by salinity	(Broch and Slagstad 2012)
2	$S = \begin{cases} 1 & \text{For } S \geq 25 \\ 1 + \frac{S-25}{18} & \text{for } 16 \leq S < 25 \\ \frac{S}{32} & \text{for } 0 \leq S < 16 \end{cases}$	Unitless	Salinity factor influencing growth	(Broch et al. 2019)
3	$Area = (w_v * M_V)/K_a$	dm^2	Surface area of kelp	(Broch and Slagstad 2012)
4	$S = \begin{cases} 34\,, if\ Salinity \geq 34 \\ Salinity + (Amplitude * \sin\left(\frac{2\pi}{12 + \frac{25}{60}} * t\right)\,, if\ Salinity < 34 \end{cases}$	PSU	Salinity effect incorporating tidal changes in estuary	This study
5	$v(A) = \left(\frac{10^{-6} \exp\left(eps * \frac{W_v * M_v}{k_a}\right)}{(1 + 10^{-6} * (\exp\left(eps * \frac{W_v * M_v}{k_a}\right) - 1)} + 1\right)^{\frac{1}{24}} - 1$	h^{-1}	Erosion rate	Adapted from (Broch et al. 2019) to fit hourly step model
6	$\frac{d}{dt} M_v = (r - v) * M_v$	$mol\ V * h^{-1}$	Change in structural mass	Adapted from (Venolia et al. 2020) to include erosion

Model adaptations

Salinity

Incorporating the response of seaweeds to hyposaline conditions into the Venolia DEB kelp growth model would be complex. It could require calculations involving turgor pressure, carbon reserves, and photosynthetic system while a lot of the variables concerning these processes are not yet understood. So, instead here I opted to take a more simplistic approach based on the salinity effect in the *S. latissima* individual based growth model of Broch et al. (2019). They added in a salinity effect by assuming that salinities below 25 PSU would reduce growth, and salinities below 16 PSU would rapidly reduce growth (Bartsch et al. 2008; Mortensen 2017b). It is described in the following formula:

$$f_{salinity} = \begin{cases} 1, for\ S \geq 25 \\ 1 + \dfrac{S - 25}{18}, for\ 16\ \leq S < 25 \\ \dfrac{S}{32}, for\ 0\ \leq S < 16 \end{cases} \tag{1-2}$$

With S denoting the salinity in PSU.

$$S = \begin{cases} 34\ , if\ Salinity \geq 34 \\ Salinity + (Amplitude * \sin\left(\dfrac{2\pi}{12 + \dfrac{25}{60}} * t\right)\ , if\ Salinity < 34 \end{cases} \tag{1-3}$$

With S being the resulting variable used to calculate the effect of salinity on growth and Amplitude being the change in salinity based on the model from Das & Nassehi (2004) (Figure 1-3). A time of 12 hours and 25 minutes is used as the period of the wave since this is the most common tidal period and matches observations in the Milford Haven Waterway (Gunn and Yenigün 1985). This formula would re-incorporate the effect of the tides into the value for salinity. These short-term fluctuations could affect growth patterns in the model. The relationships modelled using these formulas are illustrated in the supplementary material for this chapter.

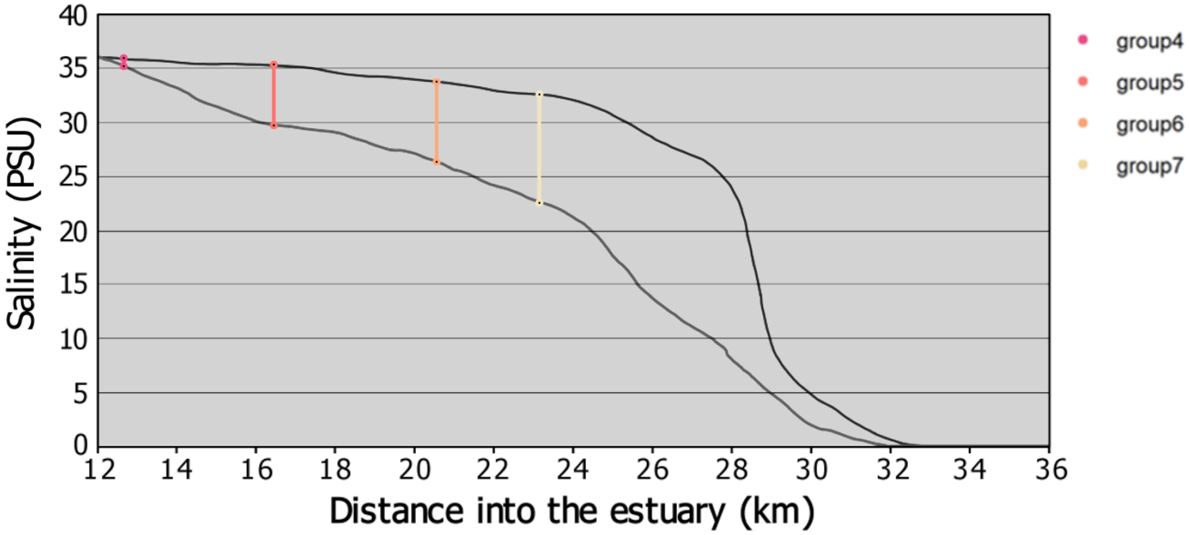


Figure 1-3:Simulated salinity variation in high (upper black line) and low (lower black line) water in the Milford Haven Waterway in a spring tide. Adapted from Das & Nassehi(2004).

Apical frond loss

Due to erosion *S. latissima* loses biomass consistently and this erosion rate is tied to its size (Sjøtun 1993). In the original model by Venolia the authors did not include a tissue loss function. In my version of the model, I believe there would be a benefit to include a tissue loss function as the high nitrate concentration in the Milford haven Waterway would not be limiting for a significant time, leading to a larger size which in turn leads to an increased effect from erosion. Assuming unlimited growth could lead to unrealistically high values if the model were run for long enough, which is why the tissue loss function below was added to the model. The function I am using is based on the size-based tissue loss function from the validated *S. latissima* model of Broch & Slagstad (2012).

The original formula was:

$$v(A) = \left(\frac{10^{-6}\exp(eps * A)}{(1 + 10^{-6} * (\exp(eps * A) - 1)}\right) \qquad (1\text{-}4)$$

With v describing the relative daily rate of frond loss, it being dependent on the area A of the seaweed. The number 10^-6 is the daily rate of frond loss for the theoretical scenario where the A=0. Eps is the rate by which erosion increases with seaweed area A. This formula needed to be converted to an hourly rate to incorporate it into the Venolia model, as well as the input variable Area needed to be converted into the value for structural mass.

$$A = \frac{w_v * M_v}{k_a} \tag{1-5}$$

$$v(A) = \left(\frac{10^{-6} \exp\left(eps * \frac{w_v * M_v}{k_a} \right)}{(1 + 10^{-6} * (\exp\left(eps * \frac{w_v * M_v}{k_a} \right) - 1)} + 1 \right)^{\frac{1}{24}} - 1 \tag{1-6}$$

With w_v describing the molecular weight of structural mass (g mol^{-1}), M_v describing the structural mass in moles, and k_a describing the amount of structural dry weight per area. This function reaches an asymptote at around 0.0293, indicating this as the maximal erosion rate per hour. Over a 24-hour period this would translate to a maximum erosion of 1 (complete erosion) that the original daily formula assumed.

To include erosion in the seaweed growth model I worked under the assumption that only the structural mass of the kelp would contribute to erosion, leaving out other tissue loss factors such as temperature stress, wave action, and mechanical stress from biofouling. Only structural mass M_v would be affected by erosion. This is done by adding erosion as a variable to limit the net growth rate r in the formula:

$$\frac{d}{dt} M_v = (r - v) * M_v \tag{1-7}$$

This formula incorporates size dependent tissue loss into the model.

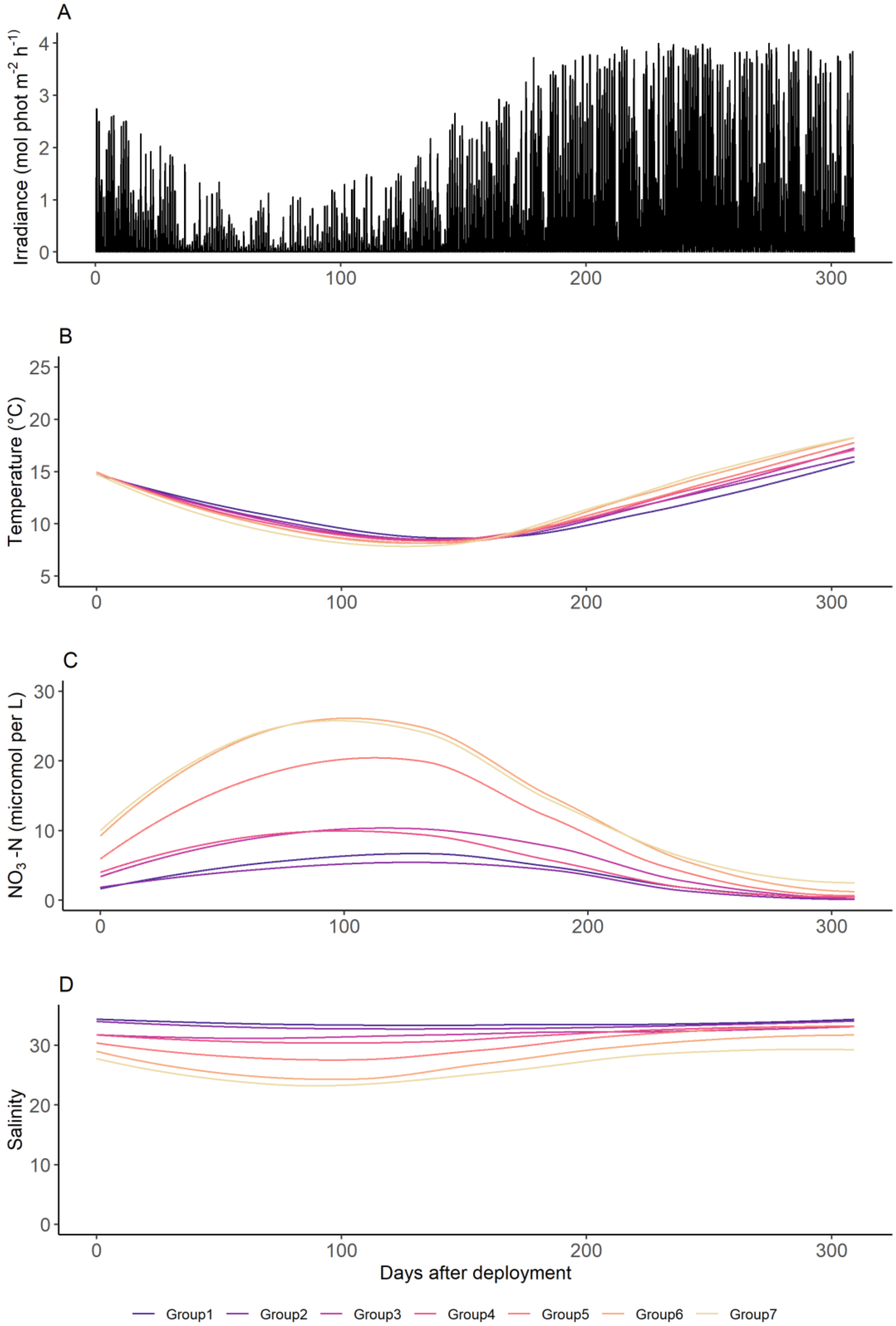


Figure 1-4: Model inputs used for the 7 simulations of observed data in Milford Haven Waterway starting with October 1st as day 1. A) Irradiance forcing used for all sites B) Loess function of temperature C) Nitrate-nitrogen concentration D) Salinity forcing function transformed with a loess function. Note that for salinity the average daily value is shown, but that this was transformed to include tidal effects according to the formulas on page 29.

Results:

Environmental variables used as model inputs

The environmental parameters used as inputs for the model all followed a seasonal pattern (Figure 1-4): Irradiance was higher in summer, when the days were longer and it would've been less cloudy on average (Figure 1-4A). Temperatures upstream in the estuary reached lower levels in the middle of the winter, and higher levels in midsummer than those further downstream (Figure 1-4B). Nitrate was generally higher upstream in midwinter, and consistently much lower in summer (Figure 1-4C). The nitrate concentrations were highest the farthest upstream. Salinity near the ocean was relatively constant around 33-34 PSU (Figure 1-4D). Further upstream the tidal effects in the estuary led to a mixing between the freshwater from the river and the saltwater from the sea.

Model results

The modelled kelp lengths under all seven growth scenarios are shown in Figure 1-5. The results indicate a good, consistent growth of *S. latissima* throughout the Milford Haven Waterway (Figure 1-5A and B). The weight of the kelp remains low for most of the early growth period regardless of how far upstream the estuary the kelp would be located. After about 150 days into the growth period the growth of the modelled kelps nearest to the mouth of the estuary outpaces the ones further upstream. Once the kelps reach a weight of about 40g the structural mass of the kelp starts eroding faster than new structural mass can be formed (Figure 1-5C). The maximum total dry weight reached 39.8g in group 2, corresponding to a length of over 5m based on the formula by Gevaert et al.(2001). Group 7 had the lowest maximum dry weight of 33.1g.

The growth rates of the kelps in groups 1 to 5 were relatively high until June when nitrogen storages would run out (Figure 1-5B and Figure 1-6). After June, the structural weight of the kelps decreased, but there was still an increase in weight due to the increase in carbon storage. For groups 1 to 4 these carbon reserves were much higher than those of the locations downstream. Contrarily nitrogen reserves appear similar in all groups. Salinity in the estuary fluctuates widely during the day due to the tidal effects, and this has a noticeable impact on the growth of the kelps upstream in the estuary. For group 7 there is a time period of over 100 days where the kelp growth would be halved, at least part of the day, due to the detrimental salinity effects (Figure 1-5D). Some further model results are presented in the supplementary material for this chapter in the appendix.

Comparison model results between original model and adapted model

For the locations unaffected by salinity (groups 1 through 4) the original Venolia model provided similar results to the adapted model for most of the time modelled (Figure 1-7A). After 215 days of growth the erosion rate starts to increase in the adapted model, limiting growth in the adapted model. In the original model growth is not incorporated. In locations affected by salinity (groups 5, 6 and 7) growth rate is reduced compared to the original model.

The growth factor *r*, indicating positive growth (excluding the negative effects on growth factor due to erosion in the adapted model) is compared for the two models in Figure 1-7B. In the locations corresponding to groups 1 to 4 growth factor *r* for the adapted and original remain identical, while in groups 5 to 7 there is a fluctuation in the adapted model where it is reduced compared to the original model.

Comparing model results with species observations

S. latissima have been observed through a large part of the estuary in 2000-2021 (Figure 1-8). The kelps were present both outside and inside the estuary. There were 64 prescence observations in the dataset, with the furthest upstream observation slightly south of the monitoring sites from group 7.

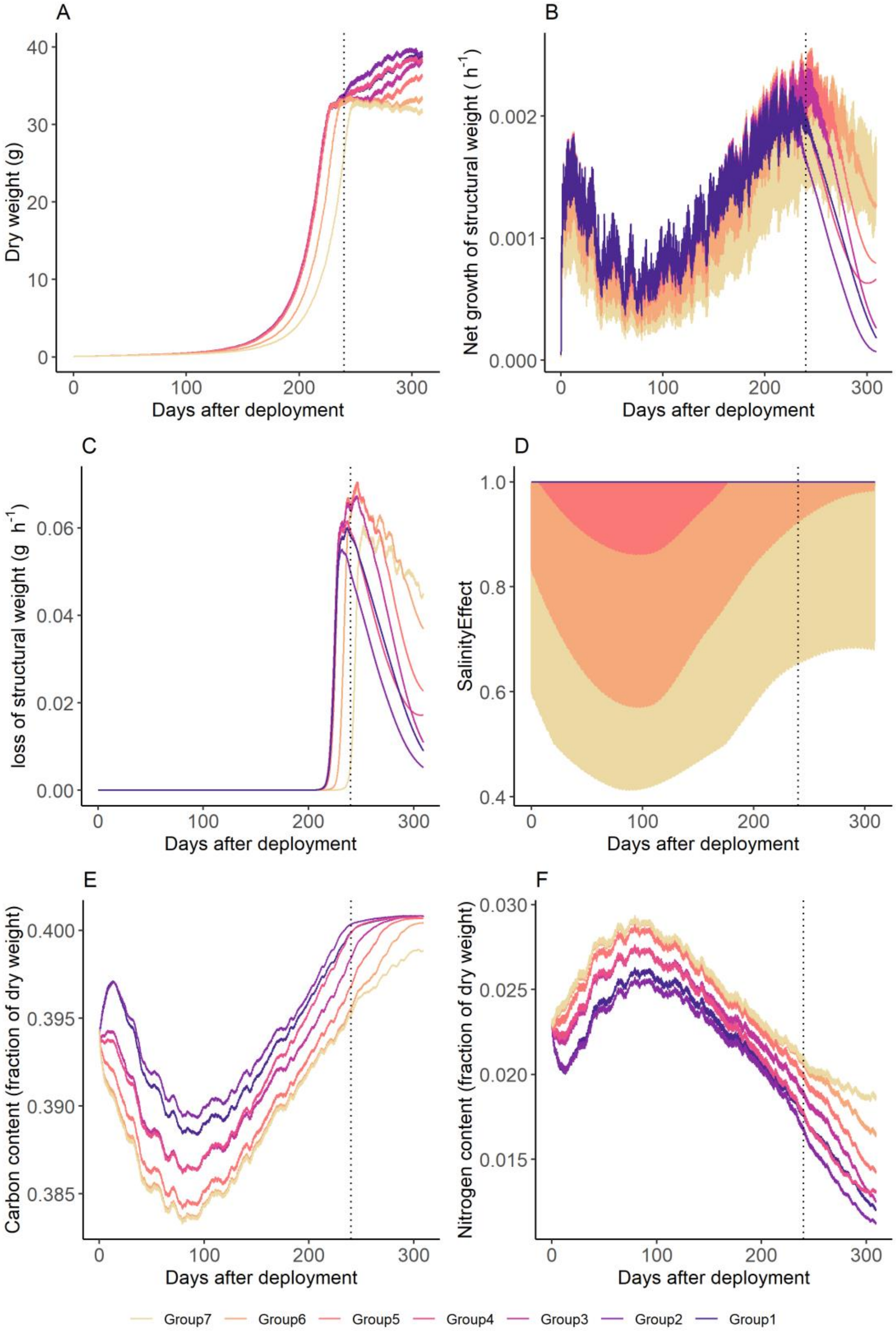


Figure 1-5: Model results (A) weight (B) net growth, r, per hour (C) Carbon content as fraction of dry weight (D) Nitrogen content as fraction of the kelps dry weight (E) Photon flux (F) salinity effect on growth. The dotted line indicates May 31st. Cultivated kelps would usually be harvested by that date.

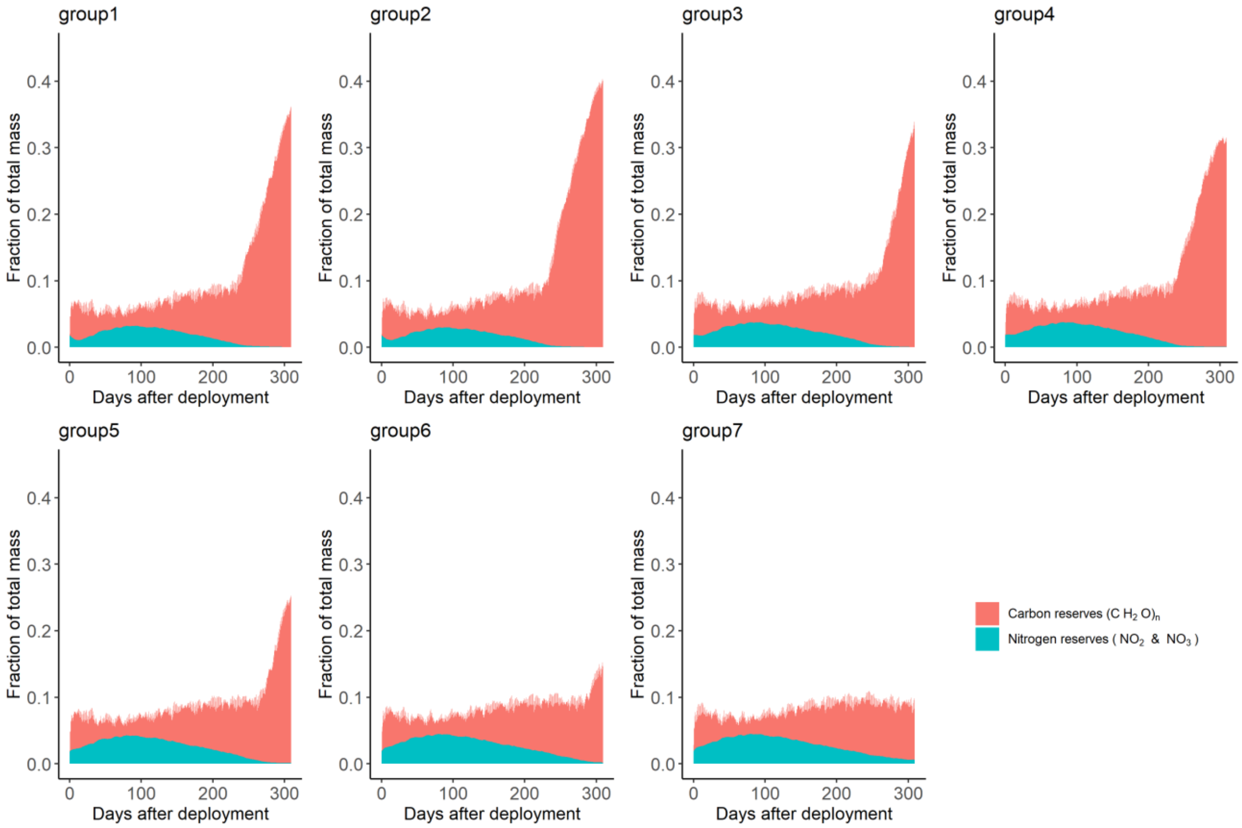


Figure 1-6: Carbon and nitrogen reserves over time in modelled *Saccharina latissima* in seven locations in the Milford Haven Waterway.

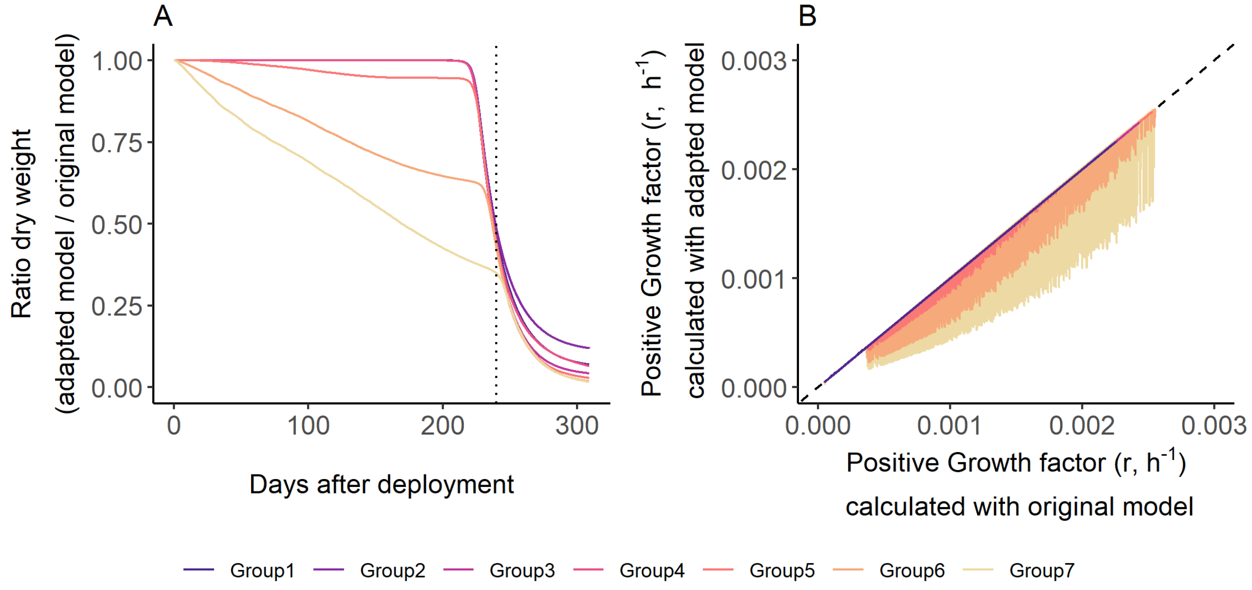


Figure 1-7: Comparison for dry weight and growth factor model results between the original Venolia model and the adapted model presented in this chapter. A) the ratio between dry weight calculated through the original model and the adapted model over time. The black dotted line in figure A indicates when cultivated kelps would usually be harvested. B) the correlation between growth factor (r) for the two models, the line of equality (where y=x) is shown as a black dotted line. Note that figure B shows the gross positive growth without having incorporated the effects of erosion.

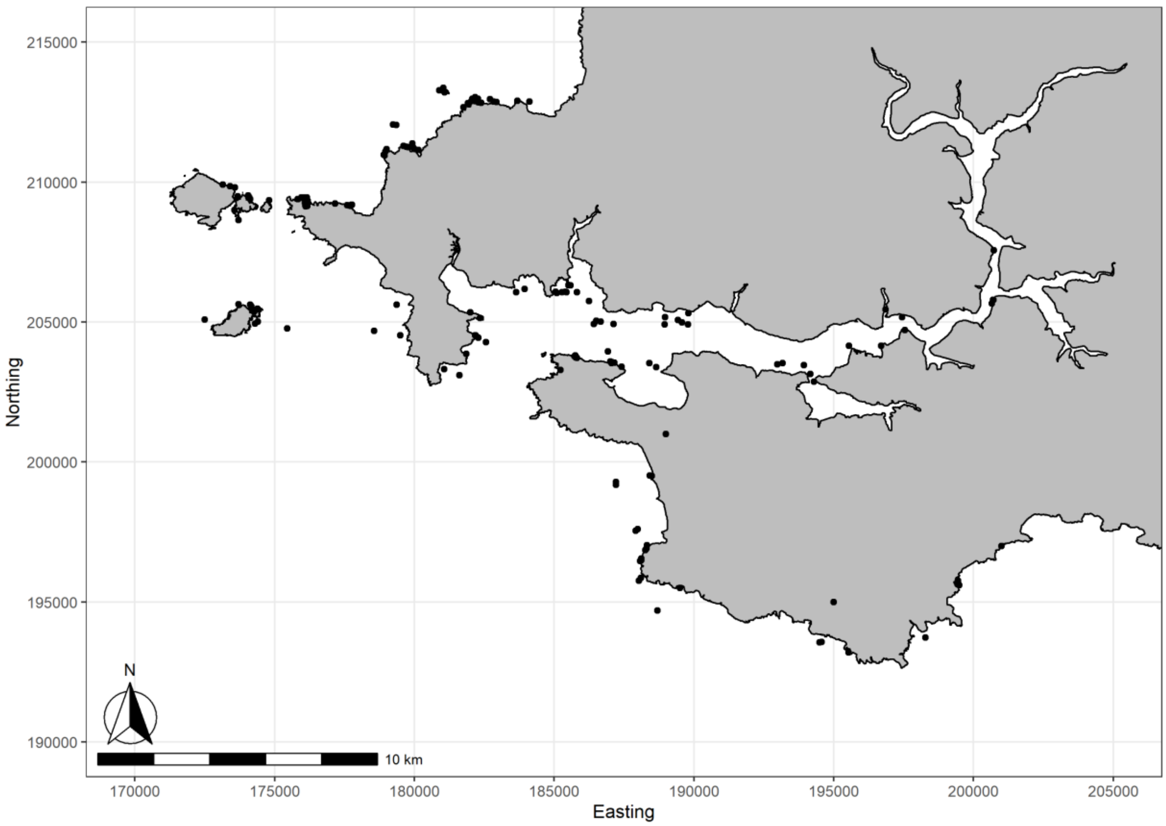


Figure 1-8: Locations where *S. latissima* was observed in the Milford Haven from 2000-2020. Data accessed from the NBN Gateway on 29/06/2021.

Discussion:

I found that seaweed growth models can be successfully used to predict whether the general environmental conditions of a site are suitable for seaweed growth, though there are major limitations to using this method. This methodology can be used to indicate that a site is suitable for a seaweed farm, but other environmental and technical constraints (e.g. water depth, conflict with other uses) would need to be taken into account as well. Model results indicate that a large section of the Milford Haven Waterway would be suitable to set up a seaweed farm. These results are confirmed by presence data and optimal growth parameters for *Saccharina latissima* from previous studies (Kerrison et al. 2015). Most of the area has enough nitrate available to support kelp growth during the growing season. The model presented here has significant limitations that would need to be addressed before this model can be used to predict quantitative growth results accurately. These limitations are discussed in detail below but include a dependency on large amounts of available data, a focus on biophysical parameters only, and limited calibration and verification of the adapted model. Once the model can accurately predict kelp growth it could aid in the calculations of a seaweed farm's revenues, providing potential investors/farmers with clear incentives to set up a farm.

Patterns in development of carbon and nitrogen content are similar to those found in the model of Broch & Slagstad (2012). Carbon content is higher at the start and end of the harvest period, and nitrogen content was higher in January when it is more readily available. However the model differed from Broch & Slagstad in the simulations of the size of the kelp. The results from the model presented in this chapter did not run into limitations for growth, leading to a large seaweed that would be unlikely to reach that size in reality due to factors that were not part of the model (e.g. wave action, grazing). In the study by Aldridge et al. (2021) (which applied the Broch & Slagstad model to a different situation) a similar growth pattern was found where growth was high until April when nitrogen reserves started to become limiting. However, in this study nutrient levels remained suitable for growth, and the reduction in net growth was due to loss of structural weight.

The existing literature on the suitability of the Milford Haven Waterway for seaweed farming is limited, with only one previous study found that included this location in their research (Welsh Government 2022). However, this study had limitations, as it assumed no light availability for seaweeds in the entire estuary and did not consider important environmental factors such as temperature, salinity, and nutrient concentrations. Therefore, this present study represents the first attempt to comprehensively investigate the environmental suitability of the Milford Haven Waterway for seaweed aquaculture.

The carbon and nitrate content of seaweeds can both influence the economic success of a farm. Carbon and nitrogen are both key components for seaweed growth and metabolic processes. *S. latissima* stores energy in the form of carbohydrates such as mannitol and laminarin, with concentrations varying throughout the year and peaking in the second half of the year. For use as a biofuel, it is necessary that seaweeds would have a high carbon content(Adams et al. 2011b). Nitrate is stored in cellular storages and will function as a reserve for when nitrate concentration in the water is lower and will influence growth (leading to a difference in total biomass) (Chapman et al. 1978; Schiener et al. 2014). Model results for carbon content in this study was lowest just after irradiance was at its annual minimum (around day 80, corresponding to late December). The minimal carbon content is earlier here than in other studies which reported a minima in March (Gevaert et al. 2001; Nielsen et al. 2014a). In the same studies nitrogen content peaked in March instead of December (Gevaert et al. 2001). However the high nitrogen content in December and January, and the lower carbon reserves are similar to other model results (Broch and Slagstad 2012; Aldridge et al. 2021)

One of the challenges of growing seaweeds in an estuary relates to salinity levels. Growth, biomass yield, and photosynthesis are reduced at lower salinities (Karsten 2007; Forbord et al. 2020a), but the precise effects of salinity on seaweed growth are not fully understood. For juvenile *S. latissima* a reduction in growth of up to 25% was observed when were cultured at a salinity of 21 (Gerard et al. 1987). The main process that seaweeds are thought to adapt to hyposaline environments is through maintaining a consistent osmotic flow of water. If a seaweed were placed in a freshwater environment the relatively higher osmotic value in the cells of the seaweed will increase their uptake of water into the cells, in turn increasing the turgor pressure in the cells to the point that they could rupture (Kirst 1990). To reduce the turgor pressure in the cells, they will release metabolites into the water. *S. latissima* will release a sugary liquid (presumably mannitol) after being immersed in freshwater (Vettori et al. 2020). There have been no studies that have specifically looked at turgor pressure in *S. latissima*. The blades of *S. latissima* will show changes within one hour. The blades bleach, and blisters filled with water can develop under the outer cell layers in the distal regions (Vettori et al. 2020).

The experiments that have looked at the effects of salinity tend to focus on either natural settings where salinity was consistently low (e.g. (Nielsen et al. 2016b; Forbord et al. 2020a)), or on controlled settings where they exposed seaweeds to a constant lowered salinity in a lab (e.g. (Fredersdorf et al. 2009; Lind and Konar 2017)). There have not been experiments that tested the effect of daily fluctuating salinity levels on kelp survival and growth. These fluctuations would be a twice-daily phenomenon in estuaries that are heavily affected by tides. This lack of data on how the changing conditions in an estuary would affect the growth and development of *S. latissima* and other kelps in general makes it difficult to predict how this would affect kelp metabolism. However, the distribution data suggests that at least some individuals can survive in these areas with high fluctuations.

Although applying a kelp growth model to several locations can be a useful tool for site selection the applicability of the model has some limitations: Firstly, the Milford Haven Waterway is a highly studied area with a lot of historical data available on how the environment in it has changed over time (Nelson-Smith 1967b; Langston et al. 2012; Little et al. 2016; NRW 2016). Most seaweed models require either daily or hourly data as inputs to calculate growth (Broch and Slagstad 2012; Lavaud et al. 2020; Venolia et al. 2020). Without a detailed dataset, as well as a detailed understanding of the biophysical properties of a location it would not be possible to model seaweed growth, nor make a prediction on the suitability of the location for a seaweed farm. Other studies have used biogeochemistry models of the environment to provide input variables for a seaweed growth model (Van Der Molen et al. 2018). In this study this issue was solved by combining data from several nearby sites and interpolating an "average year" from that data. But this approach may not be suitable for sites where there is not enough quantitative data on water quality. And secondly, another limitation in this study is that I only looked at a selection of biophysical parameters for site selection. There are a variety of other biophysical parameters, such as grazing, wave action, phosphate, micronutrients, disease, pollution, substratum (which can affect the type of anchoring system), and more. Pollution in particular could be an important parameter due to the history of the Milford Haven Waterway. Besides biophysical parameters there are also socio-economic factors, such as shipping lanes, local infrastructure and perceptions of local residents, that would need to be considered in the site selection process (e.g. (De Sousa et al. 2012; Liu et al. 2013; Thomas et al. 2019; Billing et al. 2021).

Another issue concerns the calibration of the original Venolia model. The model was calibrated in Venoila et al. (2020) with observations of length data, which had been converted from mass using a formula for *S. latissima* from Gevaert et al. (2001). The formula for this relationship between size and weight was based on seaweeds up to 150 cm in length and had a corresponding mass of 7g. It is not clear if this formula would provide accurate weight estimates for *S. latissima* with lengths longer than 150cm due to non-linearity in the length-mass relationship (Choi et al. 2018; Campbell and Starko 2021). In the application of the model in the present study the predicted dry weight of the kelps reached up to 40g, which the formula would convert to a length of more than 5 meters. This length would likely be unrealistic for this area. Either the formula from Gevaert et al. (2001) could be unrepresentative of the seaweeds grown in this area, or the calculation itself may not provide good estimates at lengths longer than 150cm.

Most numerical models are quantitatively verified to ensure that the results are sufficiently robust and reliable (Jakeman et al. 2006). The model used by Venolia was also verified using field-based growth experiments, but to apply the model to the selection of a location for a seaweed farm in an estuary the model was adapted to include erosion and salinity effects. The values of the results due to these changes were not validated as part of this research. However, comparisons between the original and the adapted model indicated that for a large part of the growth period the model results were similar (Figure 1-7A and B). This was the case if neither salinity nor erosion have a strong effect on the results, which in the adapted model used here is when salinity is below 25 and when *S. latissima* dry weight is above 20g. An additional qualitative validation was done by comparing growth results with *S. latissima* presence data in the Milford Haven Waterway. The apparent distribution of *S. latissima* observed in the field is consistent with predictions of growth made by the model. The model predictions for growth in the adapted model match those of the verified Venolia model, as well as apparent distribution in the port. This further supports the conclusion that those sites would be suitable for growing *S. latissima*.

Potential stakeholders interested in starting a seaweed farm in the Milford Haven Waterway should consider how the environmental conditions are going to change in the future. Environmental changes are likely going to be brought about by shifts in local policy and by climate change. In 2015 the Milford Haven water framework directive indicated the Dissolved Inorganic Nitrogen (DIN) concentration was too high and as a consequence classified its water quality as “moderate” (NRW 2016). The Milford Haven Waterway, under the guidelines of the Urban Waste Water Treatment Directive and Water Framework Directive, has a target of reaching ‘good’ status by 2027 (NRW 2016). Climate change is estimated to increase temperature, sea level, intensity of storm events, and change river flow speeds (Robins et al. 2016). These factors could change what location in the estuary would be optimal for the placement of a seaweed farm, or which seaweed species would be suitable to grow there (Bartsch et al. 2013).

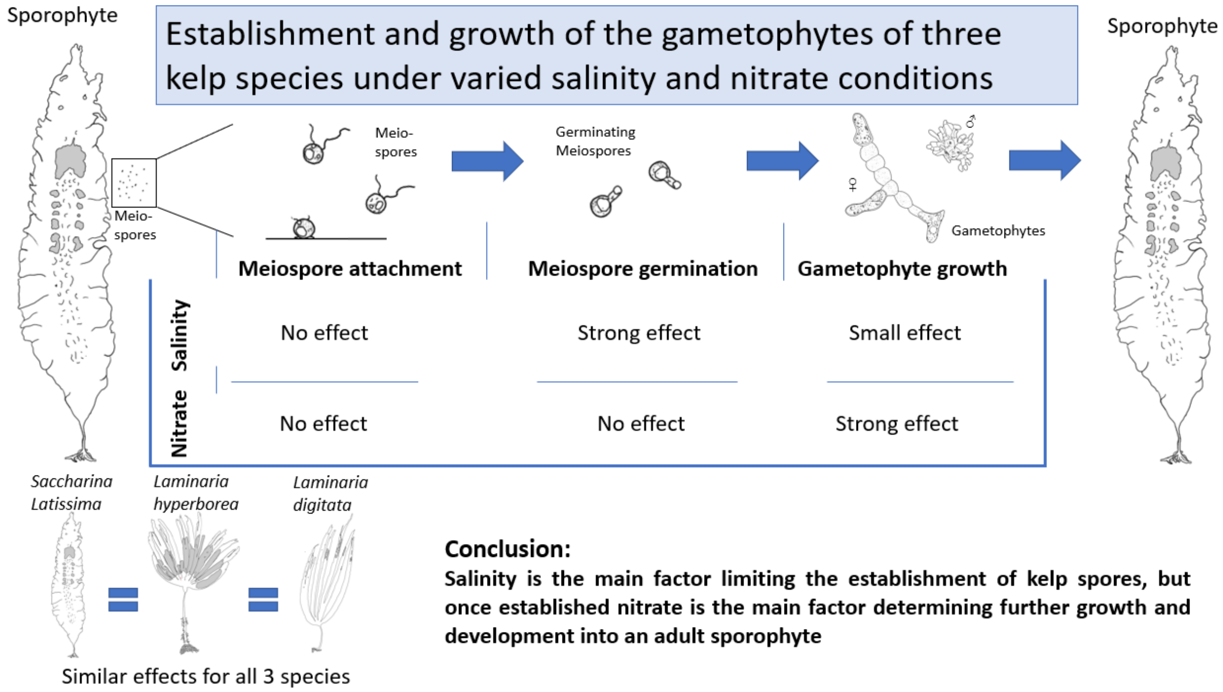
Conclusion:

Seaweed production in the UK is currently still in its infancy. However, the data shown in this chapter indicate that there are areas in the UK that have the potential to be used as a location for seaweed aquaculture. The method of applying a DEB kelp growth model can be used to indicate a suitable site for a seaweed farm. And while there are significant limitations and requirement to the use of a seaweed growth model for site selection, this method has the potential to be developed to predict not only which site is suitable, but also how much revenue a farm in such a location could make. This could provide potential entrepreneurs with clear incentives to invest into this form of sustainable agriculture.

Chapter 2 - Establishment and growth of the gametophytes of three kelp species under varied salinity and nitrate conditions

Abstract:

There is increasing interest in European seaweed production for food, bioremediation and phycocolloid production. Selection of a suitable site to cultivate seaweeds is one of the most important decisions for the success of a seaweed farm, with nutrient concentrations being essential to maintain high growth rates. Estuaries could provide a suitable location for seaweed farms because of their increased nitrate concentrations due to runoff from terrestrial systems. However, the reduced salinity in estuaries can cause stress in kelps by disrupting their internal osmotic pressure. There has been limited research on how reductions in salinity affect the growth of kelp gametophytes. In this study I tested the tolerance of the gametophytes of three key kelp species (*Laminaria digitata, L. hyperborea* and *Saccharina latissima*) to salinities and nitrate conditions typical for an estuary. Spores of these three species of kelp were released and settled on glass slips in a multifactorial experiment with 4 salinities (16, 22, 28, 34 ppt) and 2 nitrate concentrations (1.6 and 9.7 µM) (values representative of those in the Milford Haven Waterway), as well as a control with F/2 medium. Five replicates of gametophyte solutions per treatment were grown at 12 °C at an irradiation of 50 µmol m^{-2} s^{-1} with a photoperiod of 12L:12D. Settlement and germination were measured after one, and seven days respectively. Gametophyte growth was quantified by measuring their two-dimensional surface area under a microscope every 10 days for 30 days. Spore settlement remained high regardless of salinity and nitrate conditions, but germination rates were reduced in all species when salinity was at 16 ppt (6 to 23% compared to control germination rate). Growth rates of gametophytes were reduced in lower nitrate concentrations, as well as reduced salinities, but the effect of nitrate was much stronger. These results indicate that salinity is the main factor influencing the germination of spores in the species tested, but once a gametophyte has been attached to a substrate growth is mostly influenced by nutrient conditions. This means that estuaries could be suitable for kelp farming, but more research is needed into how fluctuations in salinity impact kelp growth.



	Meiospore attachment	Meiospore germination	Gametophyte growth
Salinity	No effect	Strong effect	Small effect
Nitrate	No effect	No effect	Strong effect

Conclusion:

Salinity is the main factor limiting the establishment of kelp spores, but once established nitrate is the main factor determining further growth and development into an adult sporophyte

Figure 2-1: Graphical abstract

Introduction:

Cultivation of brown algae, in particular kelps, is becoming established in western countries with several countries planning to transition towards a more bio-based economy involving seaweed cultivation (Skjermo et al. 2014; Van Der Molen et al. 2018; Hasselström et al. 2020). With this increase in interest in algae cultivation it is likely that more farms for kelp aquaculture will be set up over the coming years. Kelp species (order *Laminariales*) contain some of the largest, most fast-growing species. Kelp cultivation can help in the provision of food, pharmaceuticals, biofuels, and a wide range of other products and services (Buschmann et al. 2017). The selection of a suitable site for a kelp farm is essential to the production potential and profitability of the farm (Nath et al. 2000; Kerrison et al. 2015). Since often nitrate concentration is the limiting factor for growth of kelps (Roleda and Hurd 2019) a good location for a seaweed farm would be one where the nitrate concentration is relatively high. Establishing a seaweed farm in an area with higher concentrations of nutrients would not only benefit the growth of the seaweeds on this farm, but also reduce the amount of eutrophying nutrients washing out to sea (Kim et al. 2015; Jiang et al. 2020; Grebe et al. 2021). In addition, there is also a preference to establish a kelp farm relatively close to the shore since that would reduce building costs and costs of transportation.

Estuaries provide a protected location that can be used to cultivate macroalgae. Agricultural runoffs can lead to a general increase in nutrient levels at these sites, resulting in a nutrient-rich, brackish transition zone between river and maritime environments. Nitrate and ammonium are present in high concentrations in coastal waters across all the UK, correlating strongly with catchment area size (Nedwell et al. 2002). These high concentrations have led to prolific growth of macroalgae (Tubbs and Tubbs 1983; Lavery et al. 1991; den Hartog 1994). Besides nutrition, cultivation in lowered salinities is also associated with a delayed onset of biofouling on the kelps (Forbord et al. 2020a), as well as leading to a different composition of the mature sporophyte (Nielsen et al. 2016b; Bruhn et al. 2017). This difference in composition would allow for other uses than that same species grown in standard salinity (Nielsen et al. 2016b). However, due to the lowered salinities questions remain on how suitable estuaries are as a site for seaweed cultivation.

The lifecycle of members in the order *Laminariales* (kelps) consist of an alternating microscopic and macroscopic life stage. The microscopic meiospores (haploid zoospores resulting from meiosis) settle and develop into male or female gametophytes (Figure I-2). After an ovum is fertilised, the diploid embryo grows into the sporophyte in their adult phase (Bartsch et al. 2008). The studies that researched salinity tolerance of kelps have generally focussed on the sporophyte stage and recommends sites with a salinity of 30-35 (Kerrison et al. 2015), which is standard value for seawater. The tolerance level of kelps to reduced salinity can vary between species and can also depend on other environmental factors (Karsten 2007; Diehl et al. 2020). In western Europe three species that are considered for cultivation and their value to the local ecosystems are *Saccharina latissima, Laminaria digitata,* and *Laminaria hyperborea* (Wilding et al. 2021). The most studied species of these three is *S. latissima*, which showed no reduction in photosynthesis or growth rate at salinities as low as 20 PSU (Druehl 1967; Gerard et al. 1987; Monteiro et al. 2021) and can survive a salinity as low as 10 PSU for several days (Karsten 2007; Spurkland and Iken 2011; Peteiro and Sánchez 2012; Mortensen 2017b). *L. digitata* has an even higher tolerance as they can tolerate a five day time period at 5 PSU and experience a reduction in photosynthetic performance of 40% (Karsten 2007). In comparison, less is known about the salinity tolerance of *L. hyperborea*. Growth of young sporophytes stopped between 16 and 6 PSU depending on level of pollution in the water (Hopkin and Kain 1978). These tolerances indicate that a wider range of an estuary could be used for seaweed cultivation.

While most of the research on environmental effects has focussed on the sporophyte stage, the microscopic life stages are considered the most sensitive life stages (Coelho et al. 2000; Nielsen et al. 2014b). Most of the studies on the haploid life stages of kelps have focussed on the effects of temperature and salinity (Fredersdorf et al. 2009; Lind and Konar 2017), but have rarely focussed on the interaction between salinity and nitrate (Gordillo et al. 2002). Earlier research has shown the importance of checking for interactive variables (Zacher et al. 2016; Fernández et al. 2020; Schmid et al. 2020; Diehl et al. 2020; Blain and Shears 2020). In a *S. latissima* sporophytes salinity can substantially affect their nitrogen metabolism (Gordillo et al. 2002), and in the kelp *Macrocystis pyrifera*, nitrogen ameliorated the negative effects from temperature (Fernández et al. 2020). In the chlorophyte seaweed *Ulva* nitrogen can offset the negative effects of salinity (Kamer and Fong 2001). Nitrate is also thought to be one of the cellular osmolytes in *L. digitata* that help regulate its cellular osmotic pressure (Davison and Reed 1985).

Understanding the effect of both salinity and nitrate on spore germination and gametophyte growth can help in understanding the species requirements for environmental factors, thus helping to understand the spread of seaweeds and the cultivation of seaweeds in locations that have reduced salinity, such as estuaries.

The aim of this study was to determine how salinity and nitrate concentration affect the spore attachment, germination, and early growth of the haploid life stage (meiospore and gametophyte) of *L. digitata, L. hyperborea* and *S. latissima*. To investigate this, meiospores of these three species were settled on glass cover slides and cultivated under laboratory conditions in four salinities (16, 22, 28, 34 ppt) and two nitrate concentration (1.6 and 9.7 µM) multifactorially for 30 days. Spore attachment success, germination rate, and growth were measured. These salinities and nitrate concentrations were chosen based on environmental conditions in the Milford Haven Waterway (NRW 2016), a UK estuary in southwest Wales where there are initiatives to start a seaweed farm.

Materials and methods:

Fertile sporophytes of *L. digitata* were collected on the 30th of September 2019 from Langland Bay near Swansea, UK. Sporophytes of *L. hyperborea* and *S. latissima* were collected on the 11th of February 2020. The procedure used to obtain spores was based on Edwards and Watson (2011). Sporophytes were taken to the lab where all reproductive tissue was cut out of the sporophytes and cleaned of epiphytes using paper towels and filtered autoclaved seawater (Whatman^{TM} Grade 1 qualitative filter paper). Reproductive tissue was dried with paper towels and kept overnight at 4 ^{o}C in clean paper towels. All reproductive tissue (61, 86, and 7.5 g for *L. digitata*, *L. hyperborea* and *S. latissima,* respectively) of the *L. digitata* and *L. hyperborea* sporophytes was put in 200 ml autoclaved seawater to allow sporulation. Since only two reproductive sporophytes of *S. latissima* were found spore release for this species was done in 100 mL autoclaved seawater. The reproductive tissues were removed after 1h by filtering the solution through a 41µm nylon mesh and the resulting spore concentration was counted with a haemocytometer. The spore density of the *L. hyperborea* spore solution was too high, so it was diluted to 800 ml with additional autoclaved seawater before counting.

Culture media were made prior to the start of the experiment (Figure 2-3). Artificial seawater was made by mixing Instant Ocean® Sea salt with half a litre of Milli-Q water to obtain the salinities 16, 22, 28 and 34. F/2 nutrient media without nitrogen compounds (Lin 2005, p. 507) was used to enrich the salt solutions, and a nitrate solution was made by mixing 0.206g of $NaNO_3$ with 500mL Milli-Q water. By mixing these solutions, culture media with four different salinities (34, 28, 22 and 16) and two different nitrate concentrations of 1.61 and 9.68 µM (equals 0.1 and 0.6 mg NO_3 $*L^{-1}$) were created, resulting in a total of 8 different treatments. These nitrate concentration were chosen as they are indicative of the winter and summer concentrations at the border of the transitional and coastal waterbodies in the Milford Haven Waterway (NRW 2016). One control treatment with 34 salinity and 882 µM NO_3 (equals 54.7 mg NO_3 $*L^{-1}$), the nitrate concentration of standard f/2 medium (Guillard 1975) was used in the experiment as well.

Three sets of repli-dishes (10 x 10 cm; 25 wells) per kelp species were used to culture the spores, one set for measuring attachment success, one for germination and the last for growth. Each well of the repli-dishes used to measure attachment or germination success had one 15 mm diameter circular borosilicate glass slide at the bottom. An amount of aliquot containing approximately 100,000 spores (in 99.5, 113 and 168 µL for *L. digitata*, *L. hyperborea* and *S. latissima*, respectively) was pipetted into each of the 25 wells per repli-dish with 3ml of each treatment solution. Each treatment was randomly assigned to a well with 5 replicates per treatment. Afterward, the repli-dishes were placed in an incubation cabinet at 12 ^{o}C at an irradiation of 50 µmol m^{-2} s^{-1} with a photoperiod of 12L:12D. A summary of these actions is presented in Figure 2-2 and a summary of the treatments is presented in Figure 2-3.

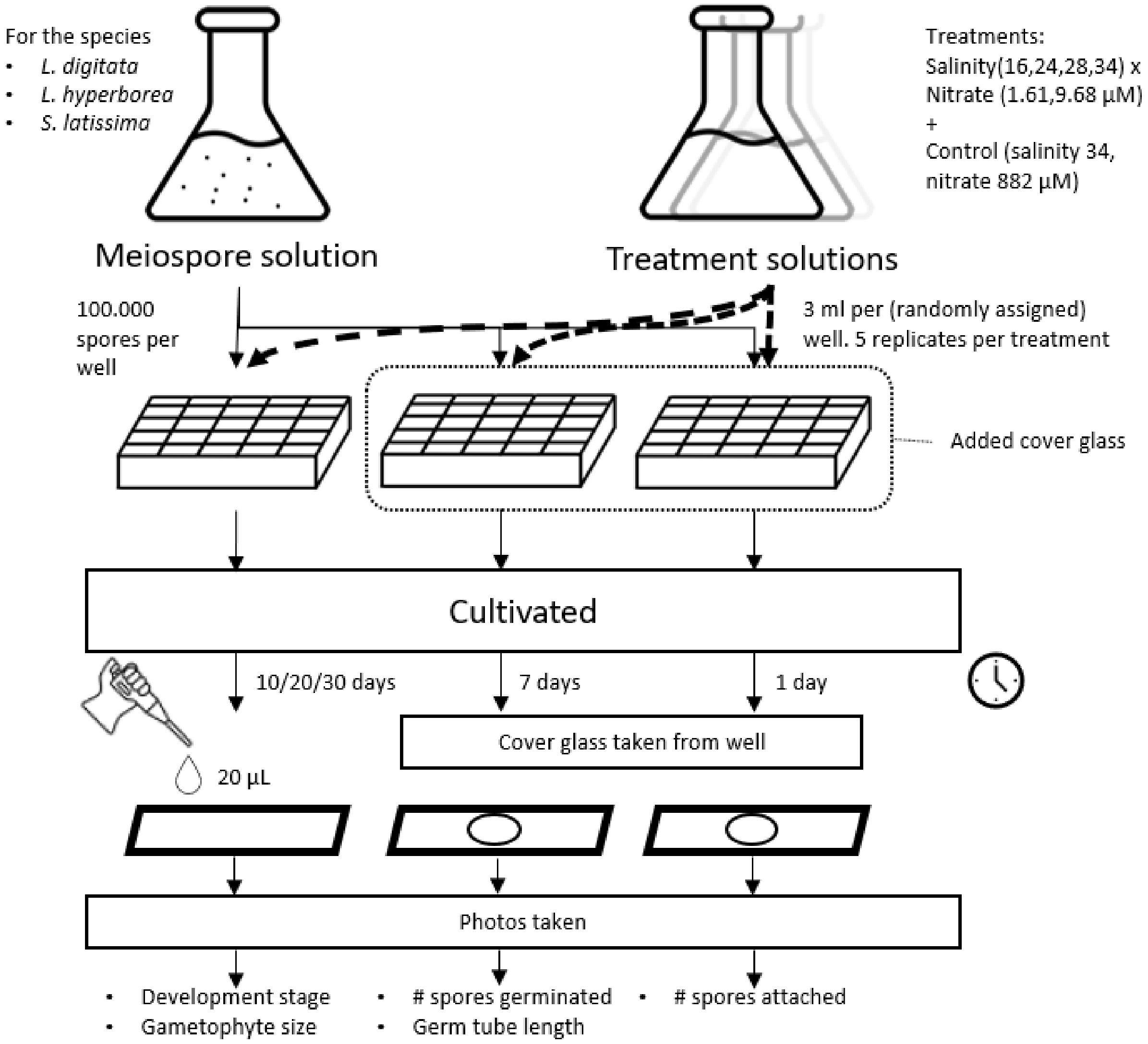


Figure 2-2: schematic overview of the methodology for this study.

For the measurement of attachment success, the spores were incubated and allowed to settle for 1 day. The circular glass slide from each well were taken out and gently dipped into filtered, autoclaved seawater to remove any unattached spores. Attachment success was then quantified using a light microscope at 400x magnification by haphazardly taking pictures of 10 fields of view (0.094 mm^2 per view) and counting the number of attached spores. The same was done after 7 days (post spore release) to measure germination success. Germination success was expressed as the number of germinated spores divided by the average number of attached spores for that treatment. For the growth measurements, a 20 µL aliquot was taken from each well after mixing the medium. The sample was studied under a light microscope and images of the developing gametophytes were taken with an Olympus UC30 camera every 10 days for 30 days. Where possible pictures of at least 10 gametophytes per replicate were taken. Half of the medium was replaced every 10 days by carefully removing the medium from the top to remove as little of the settled gametophytes as possible. The resulting images were analysed with ImageJ and surface area was used as a measurement for gametophyte growth. The developmental stages of the measured gametophytes were categorised into seven groups based on their developmental characteristics and reproductive structures according to a method used by Oppliger et al. (2012) for *Lessonia nigrescens*. these stages were: 1) settled meiospores, 2) germinated spore (in possession of a germ tube), 3) gametophyte of one or two cells, 4) female gametophytes of more than two cells, 5) male gametophytes of more than two cells, 6) reproductive female gametophyte (bearing oogonia), and 7) fertilized ovum growing as a microscopic sporophyte(Figure 2-4).

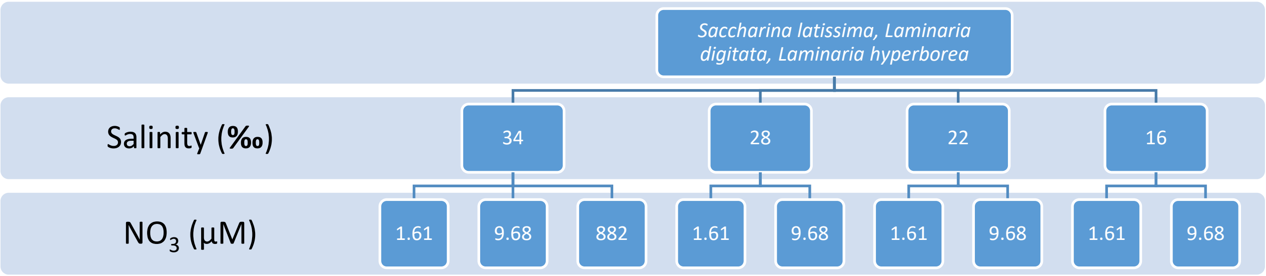
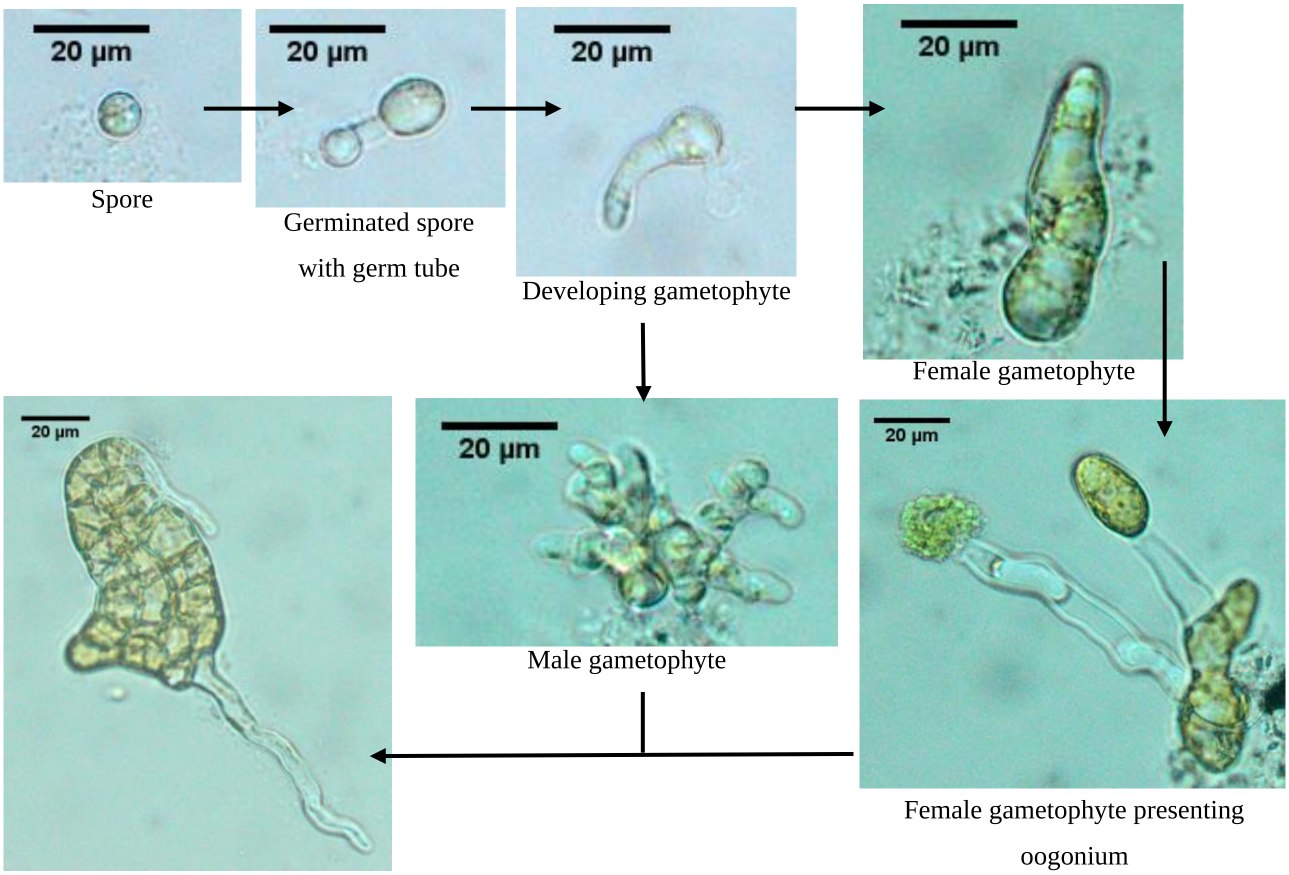


Figure 2-3: A schematic of the factorial design of the Gametophyte cultivation experiments. It illustrates the experimental treatment combinations that zoospores of the three species were subjected to (n=5). Salinities of 16 to 33 are present in the Milford Haven estuary and NO_3 concentrations represent summer and winter conditions.

Statistics

To test the effects of salinity and nitrate concentration on spore attachment and germination success a General Linear Model (GLM) was used with a quasi-poisson distribution to account for overdispersion. The resulting model was then used to perform a Tukey post-hoc test on the treatment variable using the 'glht' function from the 'multcomp' package. I computed estimated marginal means (EMMs) for each level of the treatment variable using the 'emmeans' function from the 'emmeans' package. Finally, I used the 'cld' function from the 'multcomp' package to show which treatment groups were significantly different from each other based on the Tukey-adjusted p-values ($p<0.05$). I tested for normality of the germ tube length variable using the Shapiro-Wilk test. Since the germ tube length data was found to be non-normal, I used the non-parametric Kruskal-Wallis test to test for differences in germ tube length among treatment groups. To further explore differences among treatment groups, I used a pairwise wilcox test to perform pairwise comparisons, with the Benjamini-Hochberg procedure to adjust for multiple comparisons. The resulting pairwise comparisons of p-values were used to identify statistically different groups using letters and are presented in the figures below.

The mean gametophyte surface area was not normally distributed so it could not be evaluated with an ANOVA (with fixed factors: salinity, nitrate concentration and day of measurement). Instead, a Kruskal-Wallis rank sum test was used to see if treatments had an effect on surface area. Pairwise Wilcoxon rank sum tests were used as post hoc tests to detect significant differences between treatments after Kruskal-Wallis ANOVAs in the same way as described above. All statistical test were performed in R, version 4.1.0 (R Core Team 2021) through RStudio version 1.4.1106 (RStudio Team 2021).



Spore

Germinated spore with germ tube

Developing gametophyte

Female gametophyte

Male gametophyte

Female gametophyte presenting oogonium

Early sporophyte

Figure 2-4: Developmental categories used in this study based on Oppliger et al. 2012. Showing the categories: 1. settled meiospore, 2. germinated spores, 3. (developing) gametophytes of 1 or two cells, 4. female gametophytes of more than two cells, 5. male gametophyte of more than two cells, 6. mature female with oogonium but no sporophytes, and 7. Microscopic sporophytes.

Results:

Laminaria digitata spore attachment success seemed to be mostly unaffected by salinity and nitrate conditions (Figure 2-5). Spore attachment varied from 8 to 36 per 0.094mm^2 and average spore density per field of view was 10.93 ± 4.37 µm^2, with it only being significantly different for two treatments, the low nitrate treatments (1.16 µM**)** at salinities of 22 and 34. The spore attachment for *Laminaria hyperborea* showed a similar pattern with no differences among treatments, except for the treatment with a salinity of 16 and nitrate concentration of 9.68 µM compared to the treatment with a salinity of 34 and nitrate concentration of 882 µM (Top figures in Figure 2-6 and Figure 2-5). For *Saccharina latissima* some of the high salinity and nitrate concentrations led to a reduced spore attachment (Top figure in Figure 2-7).

The general pattern for spore germination was similar across the three species tested. Low salinities led to a reduced level of spore germination with spores incubated at a salinity of 16 having the lowest germination rates for all species. Increased nitrate conditions resulted in a generally positive effect on germination rates, though this was not statistically significant across all treatments (Middle figures in Figure 2-5, Figure 2-6, and Figure 2-7. Growth of germ tubes after 7 days showed a similar pattern where salinities below 34 and lower nitrate concentrations led to reduced growth across all three species tested, though this effect was less pronounced for nitrate concentration than salinity. The salinity and nutrient treatments both played a statistically significant role in shaping spore germination and germ tube growth ($p<0.001$).

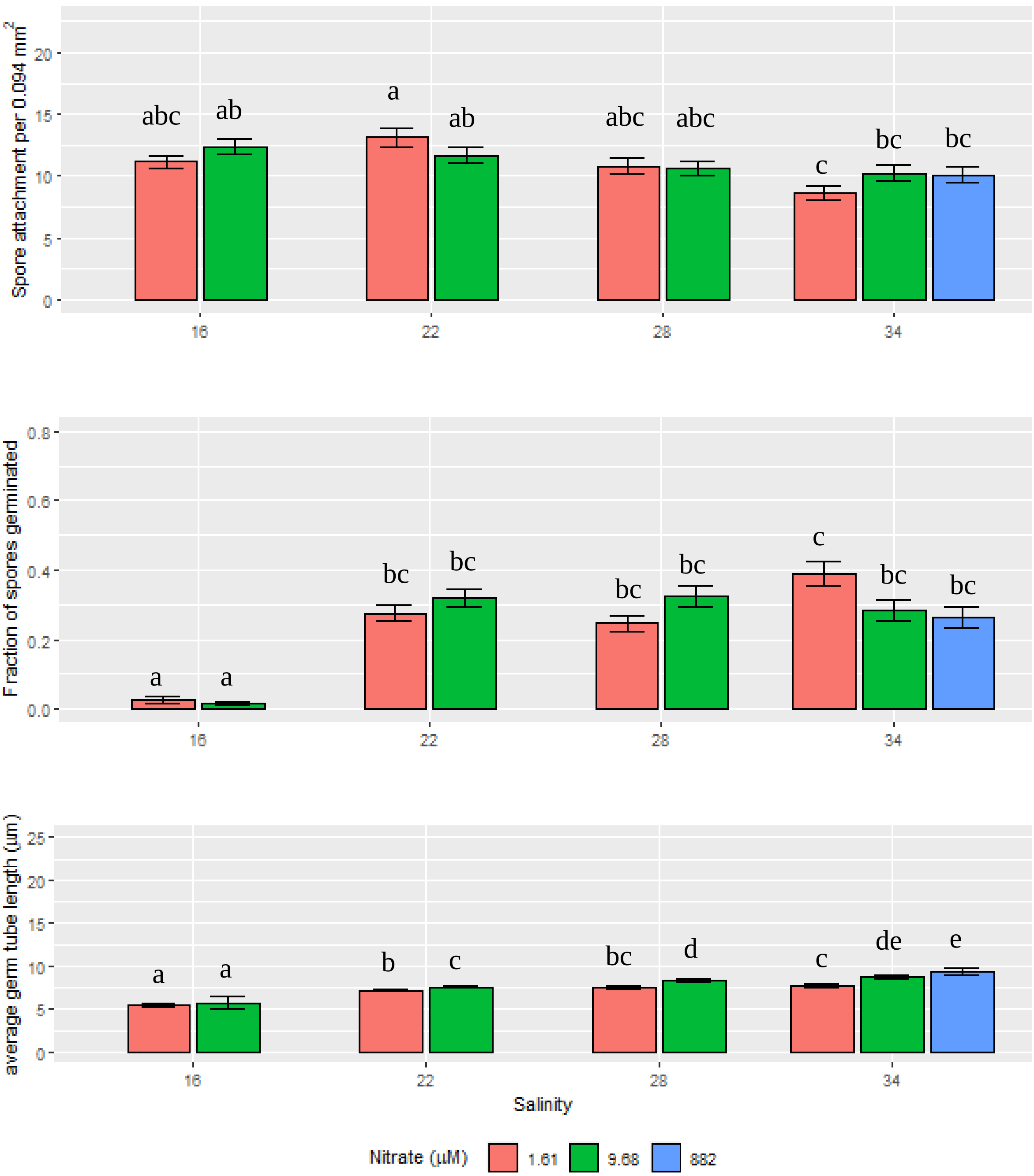


Figure 2-5: The effects of salinity (16,22,28,34) and nitrate (1.61, 9.68 and 882 µM) media concentration on spore attachment success (after 1d), germination rates and germ tube growth (after 7d) for *Laminaria digitata* spores. Mean values ± standard errors are presented. Different letter groups indicate statistically significant differences among treatment groups (determined using a Tukey post hoc test for the GLM with quasipoisson distribution in the top and middle figures and using paired Wilcoxon rank sum tests in the bottom figure). Treatments with the same letter are not significantly different from each other. A p-value of < 0.05 was considered statistically significant for all tests.

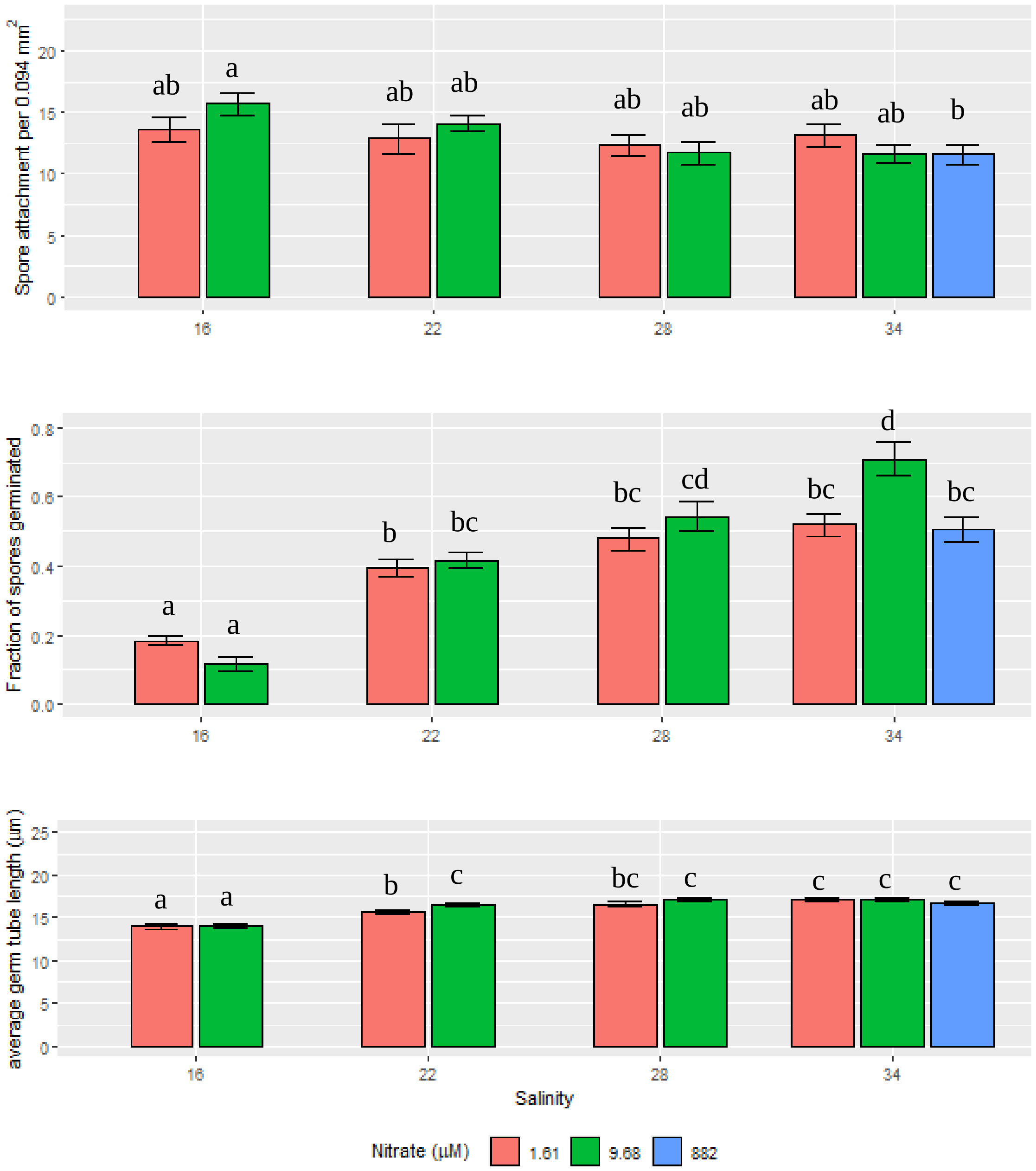


Figure 2-6: The effects of salinity (16,22,28,34) and nitrate (1.61, 9.68 and 882 µM) media concentration on spore attachment success (after 1d), germination rates and germ tube growth (after 7d) for *Laminaria hyperborea* spores. . Mean values ± standard errors are presented. Different letter groups indicate statistically significant differences among treatment groups (determined using a Tukey post hoc test for the GLM with quasipoisson distribution in the top and middle figures, and using paired Wilcoxon rank sum tests in the bottom figure). Treatments with the same letter are not significantly different from each other. A p-value of < 0.05 was considered statistically significant for all tests.

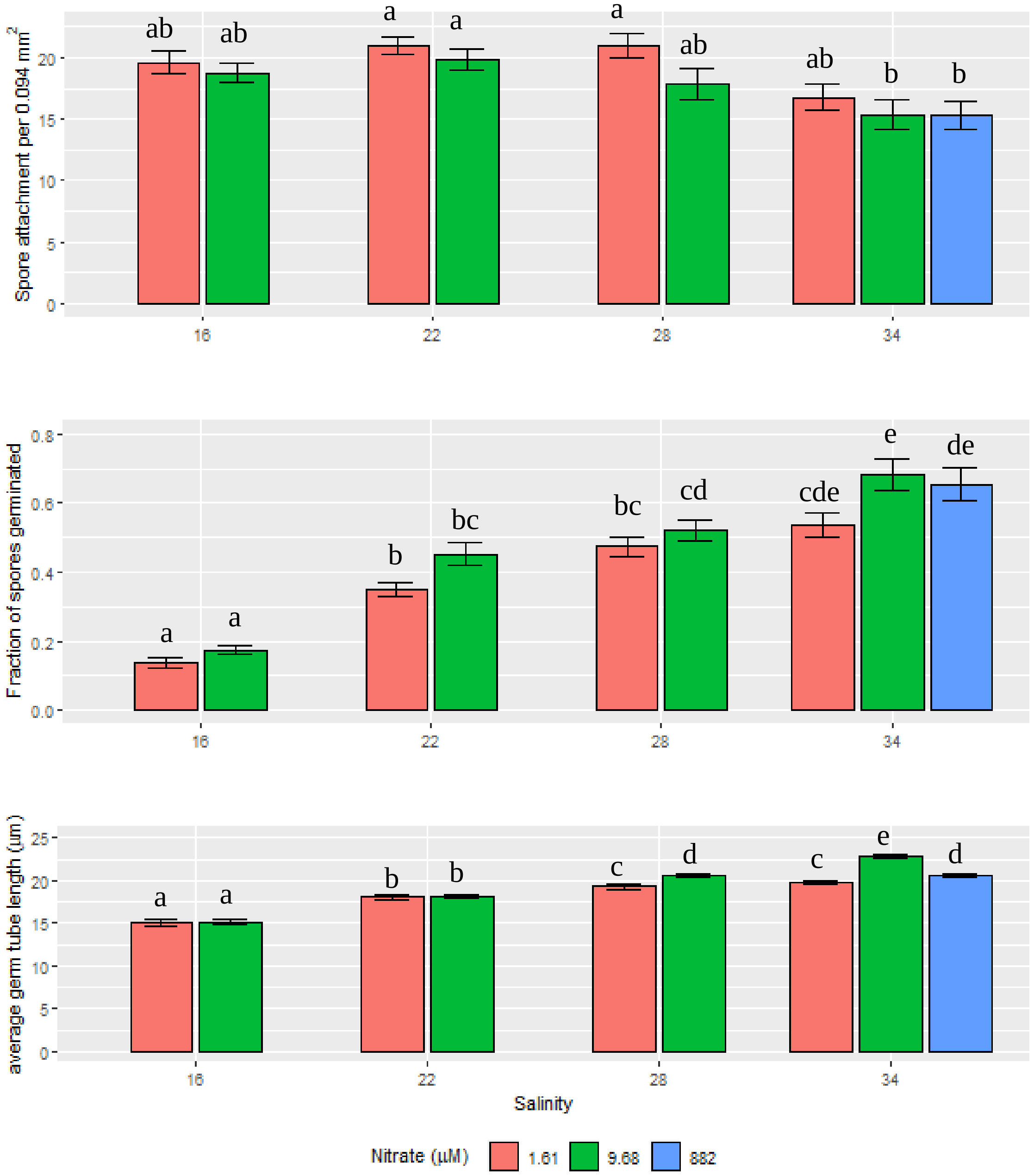


Figure 2-7: The effects of salinity (16,22,28,34) and nitrate (1.61, 9.68 and 882 µM) media concentration on spore attachment success (after 1d), germination rates and germ tube growth (after 7d) for *Saccharina latissima* spores. . Mean values ± standard errors are presented. Different letter groups indicate statistically significant differences among treatment groups (determined using a Tukey post hoc test for the GLM with quasipoisson distribution in the top and middle figures, and using paired Wilcoxon rank sum tests in the bottom figure). Treatments with the same letter are not significantly different from each other. A p-value of < 0.05 was considered statistically significant for all tests.

Results of the experiment showed that the surface area of gametophytes surface area started to differ between treatments as early as 10 days into the experiment for all three species (Figure 2-8, Figure 2-9, Figure 2-10). Higher nitrate concentrations led to statistically significant higher gametophyte size (Table 2-1), and there was a slight trend that higher salinities had the same effect, although much less pronounced, and not consistently statistically significant.

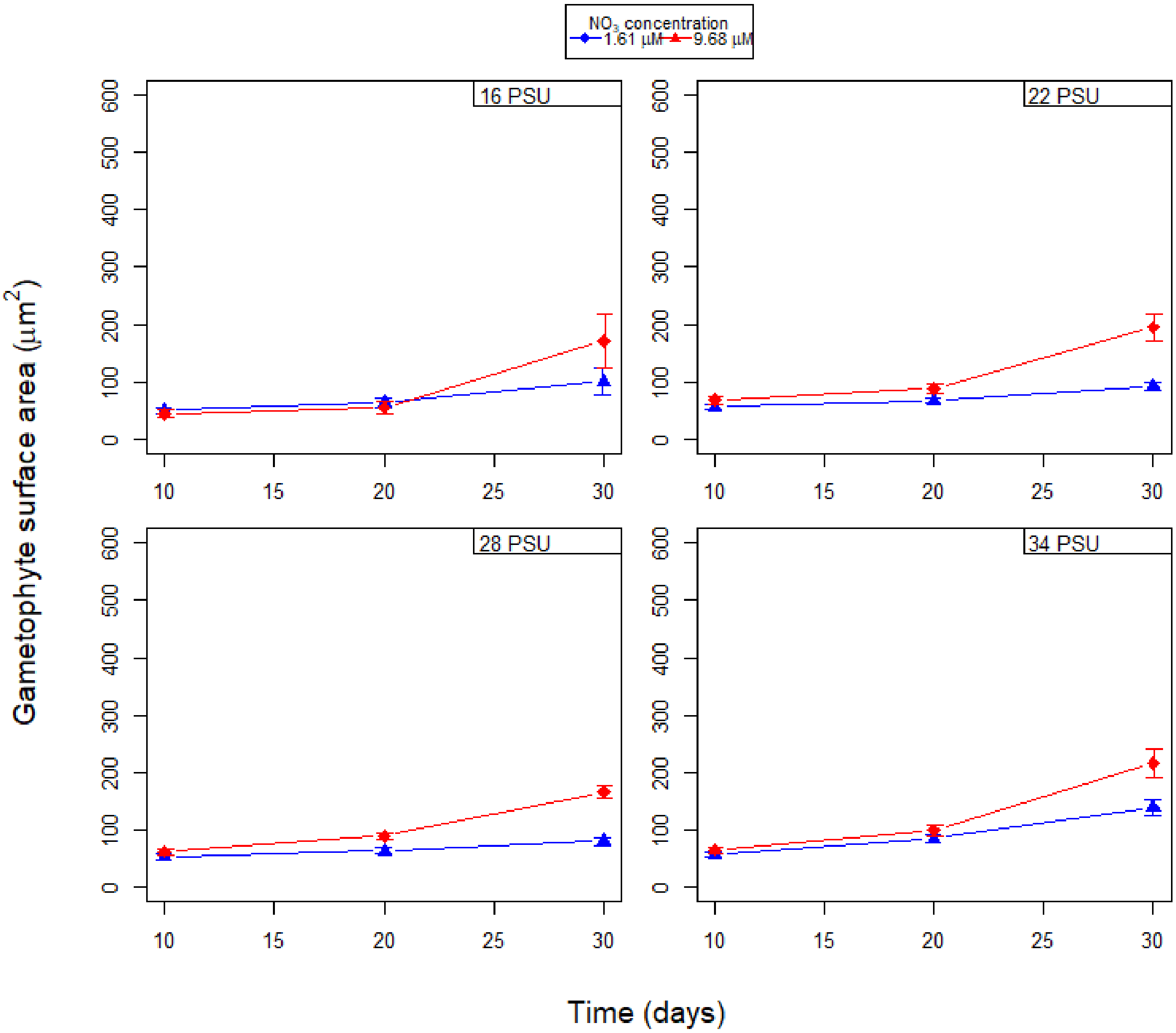


Figure 2-8: *Laminaria digitata* gametophyte growth in relation to time and nitrate concentration. Size is represented in mean surface area ± 2 standard errors.

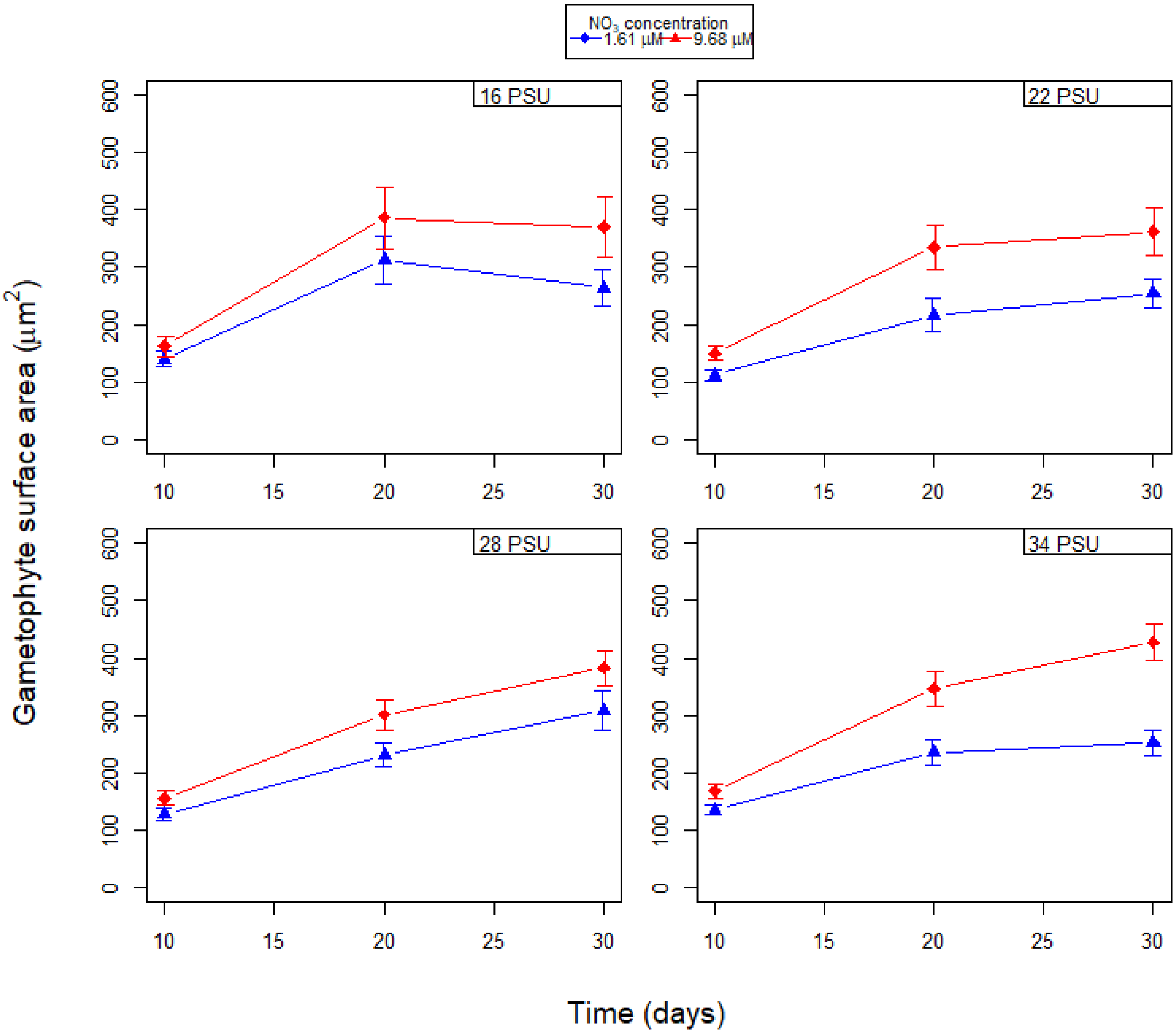


Figure 2-9: *Laminaria hyperborea* gametophyte growth in relation to time and nitrate concentration. Size is represented in mean surface area ± 2 standard errors.

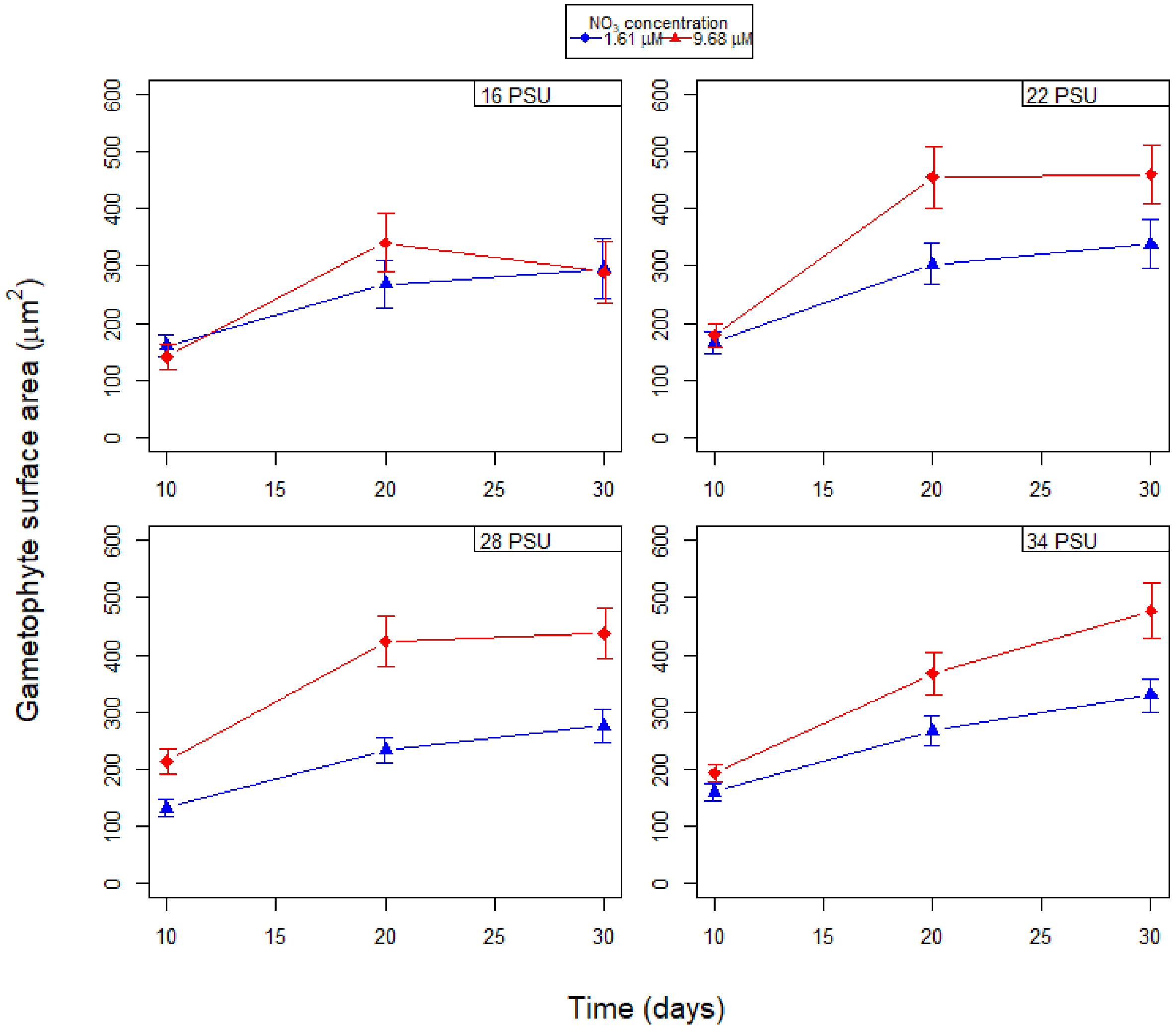


Figure 2-10: *Saccharina latissima* gametophyte growth in relation to time and nitrate concentration. Size is represented in mean surface area ± 2 standard errors.

Table 2-1: Mean gametophyte surface area after 30 days of growth in different salinities and nitrate concentrations +- s.e. (number of measurements), Different letter groups indicate statistically significant differences among treatment groups within species (determined using using paired Wilcoxon rank sum tests). Treatments with the same letter are not significantly different from each other. A p-value of < 0.05 was considered statistically significant for all tests.

	Salinity			
Nitrate (µM)	**16**	**22**	**28**	**34**
Laminaria digitata				
1.16	101.5 ± 11.9 (27) **AB**	92.8 ± 3.6 (74) **A**	80.5 ± 3.3 (57) **B**	138.8 ± 6.9 (81) **D**
9.68	171.5 ± 23.7 (27) **CD**	195.4 ± 11.8 (84) **CE**	166.4 ± 5.9(89) **C**	216.3 ± 12.3 (66) **E**
882				2667.7 ± 252.1 (86) **F**
Laminaria hyperborea				
1.16	*264.6±15.6 (62)* ***AB***	*254.6±12.5 (94)* ***A***	*309.4±16.9 (101)* ***BD***	*252.1±11.2 (107)* ***A***
9.68	*370.3±26.1 (71)* ***CD***	*361.8±21.0 (86)* ***C***	*382.8±15.5 (101)* ***CE***	*427.2±15.9 (144)* ***E***
882				*2172.2±183.7 (105)* ***F***
Saccharina latissima				
1.16	*295.6±26.7 (100)* ***A***	*338.3±21.2 (113)* ***AB***	*275.6±14.6 (101)* ***A***	*329.2±14.5 (119)* ***B***
9.68	*288.7±26.9 (76)* ***A***	*460.3±25.5 (163)* ***C***	*437.2±22.5 (132)* ***C***	*477.1±24.3 (130)* ***C***
882				*3365.0±510.7 (97)* ***D***

Figure 2-11 shows an overview of the fractions of gametophyte development stages found in the experiment for the three species researched. In *Laminaria digitata* the development of the gametophytes stalled at the spore stage when salinity was 18 ppt, and in the germination stage when salinity was higher. In *L. hyperborea* and *S. latissima* most gametophytes had passed that stage in within 10 days in the same conditions and had grown into developing gametophyte consisting of 2-3 cells. More multicellular gametophytes (consisting of more than three cells) were found when nitrate concentrations in the culture medium were higher, and when salinity was closer to that of seawater (34 ppt). Generally multicellular male gametophytes were found in greater numbers than female gametophytes in all three species.

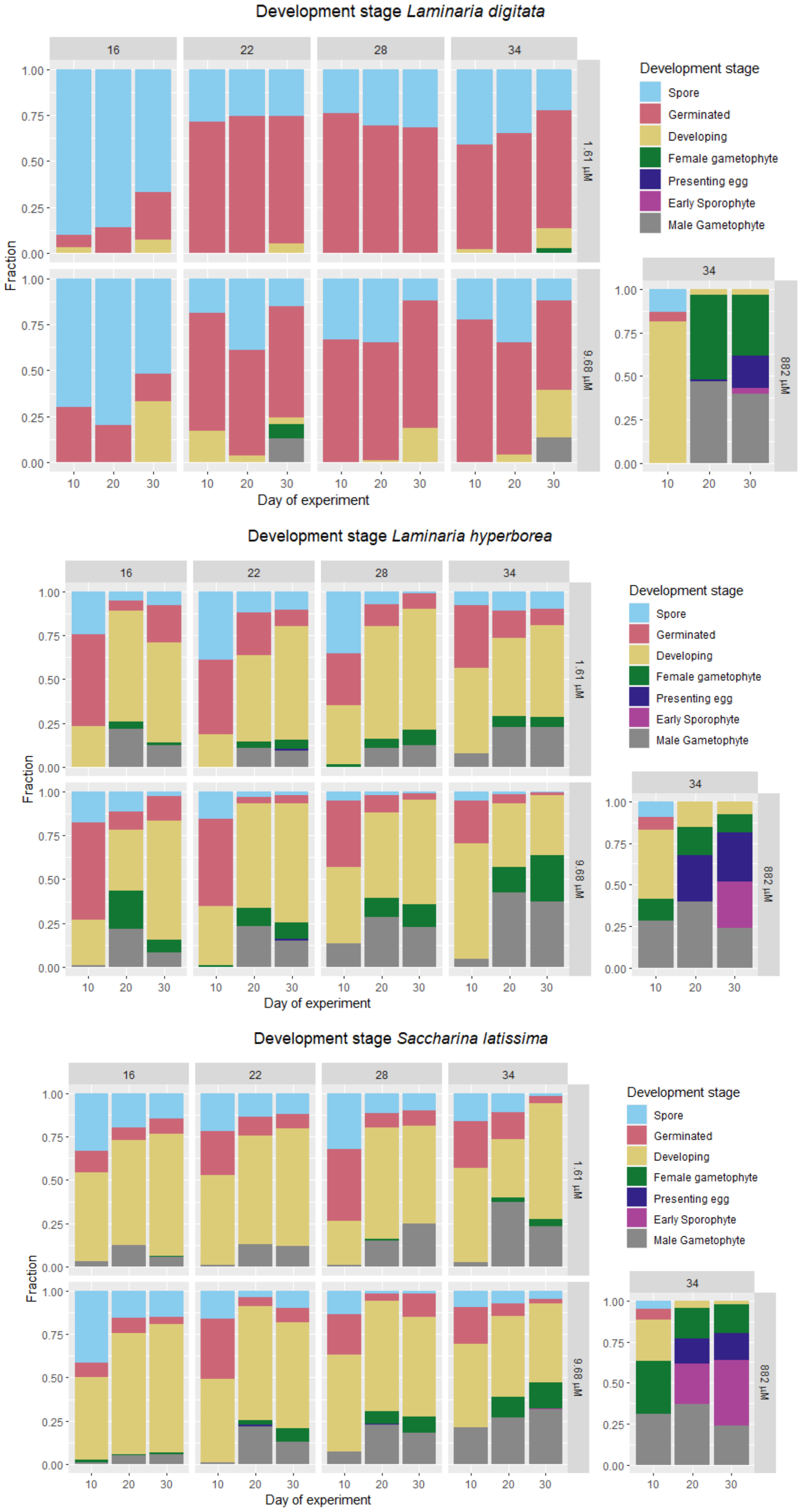


Figure 2-11: Microscopic life stages of gametophytes of three species of kelp observed after 10, 20 and 30 days of development in different salinities and nitrate concentrations.

Discussion:

In this study, the earliest life stages of *Laminaria digitata*, *Laminaria hyperborea,* and *Saccharina latissima* were shown to be affected by both salinity and nitrate concentration, but the effects of these factors were dependent on their life stage (spore or gametophyte). No interactive effect between salinity and nitrogen concentration was found. Spore settlement was largely unaffected by nitrate and salinity conditions, germination was heavily reduced in low salinities, germ tube growth was slightly affected by both salinity and nitrate, and longer term (vegetative) growth was affected by nitrate conditions and much less by salinities. This is the first study that has tested whether there is an interactive effect of salinity and nitrogen in the gametophytic stage of these kelp species, and the first study to test salinity tolerance of the haploid life stages of *L. hyperborea*.

Reductions in germination rates due to lowered salinities could be an important contributing factor for why kelps are not found further upstream in estuaries (Schoch and Chenelot 2004). Germination rates for another kelp species, *Alaria esculenta*, are similarly affected by salinity (Fredersdorf et al. 2009). This indicates a bottleneck that is limiting the establishment of kelp species in brackish water. Since millions of meiospores are produced by a single sporophyte (Chapman 1984), this abiotic factor alone heavily reduces the number of gametophytes that will become established on the shore. A relatively high density of gametophytes need to be present for egg cells produced by female gametophytes to be fertilised (Reed 1990; Schiel and Foster 2006). These findings indicate that when correlating environmental factors with species distribution, like is standard practice with current species distribution models (Elith et al. 2011; Assis et al. 2018), tolerance levels found in research on sporophytes would not match those found with field observations of presence of a species. This further emphasizes that when making conclusions on an area’s suitability for the natural establishment of a species the entire life history needs to be considered.

These findings could have important implications for seaweed aquaculture, as researchers need to focus on sporophyte environmental tolerances and optima rather than focusing on gametophyte or spore requirements when assessing a site's suitability. Depending on tolerance information based on presence/absence models alone may lead to the incorrect assumption that a site is unsuitable due to unfavourable germination conditions, when in reality the adult sporophyte could thrive there. Therefore, it is crucial for seaweed aquaculture site selection to consider the entire life history of kelp species to maximize successful growth and yield in a given location. In seaweed aquaculture sensitivity of the spore stage is mitigated as the seaweeds are allowed to germinate in the lab under suitable conditions and are moved to the field, usually as sporophytes (Edwards and Watson 2011; Flavin et al. 2013; Kerrison et al. 2018).

Hyposaline conditions require macroalgae to regulate their internal osmotic pressure to prevent the loss of water from the cell. The exact mechanisms behind this have not been studied in the gametophytic stage, but in adult sporophytes this is done by changing the intracellular concentrations of mannitol (Reed et al. 1985; Wright et al. 1989; Thomas and Kirst 1991). Brown algae are thought to have a vacuole with osmolytes for short term acclimation in the cytoplasm (Hurd et al. 2014, p. 327). Since meiospores do not have this vacuole (Henry and Cole 1982) they are unlikely to be able to quickly adapt to changes in osmolarity, yet spore attachment was largely unaffected by salinity in this study. This indicates that the meiospores can tolerate low salinities for at least 24 hours without it impacting their settlement. These results are supported by the findings of Lind & Konar (2017) who observed a mild effect of salinity on spore settlement with salinities of 31 and 26 for *Eualaria fistulosa*, but not on *S. latissima*.

This is the first study that has looked at longer term gametophyte growth at low salinities. Most studies that have investigated gametophyte growth of kelps at low salinities have only checked germ tube elongation. Germ tube elongation of *S. japonica* (Han et al. 2011), *Eualaria fistulosa, Nereocystis luetkeana, S. latissima* (Lind and Konar 2017) was reduced at salinities of 26 and below. In this study germ tube length was reduced at salinities of 22, but not at 28 (Figure 2-5, Figure 2-6, and Figure 2-7). Results for later gametophyte size (Table 2-1) show a reduced influence of salinity on growth, indicating that gametophytes might have a method to adapt to the reduced salinities. The gametophyte stages most likely have a method to adapt their osmotic pressure through osmolytes. In sporophytes osmotic pressure is mainly controlled through mannitol, K^+, Na^+, Cl^- and NO_3^- (Davison and Reed 1985; Reed et al. 1985). This also highlights the importance of measuring longer term growth when studying the effects of environmental variables. Different life stages, long term effects, and acclimation can all influence a species' tolerance to environmental stressors.

Inorganic nutrients are essential for the growth of phototrophic organisms, yet they did not have a measurable effect on spore settlement in this study. Earlier research on the settlement of the kelps *Macrocystis pyrifera* and *Pterygophora californica* indicated that spore settlement rate increases with higher nutrients concentrations (Amsler and Neushul 1990), and Kerrison et al. (2016) found increased spore settlement for *L. digitata* when seawater was enriched with F/2 culture medium, but that was not found here. Potentially the lowest nutrients level in this study were sufficient for spores to settle.

In some of the treatments in this study spore settlement was affected by salinities closer to those of normal seawater (Top figure in Figure 2-7). This could be an unintended side effect from the artificial sea salt used in this study. This salt failed to dissolve completely, leaving a small amount of precipitate on the slides the meiospores would settle on. When the slides were carefully rinsed some of the spores that had attached themselves to the precipitate instead of the slide would be washed off, leading to a reduction of spores counted when determining spore attachment success for the treatments with higher salinity. This could have led to the mild reduction in spore attachment in the higher treatments with higher salinities.

This study has investigated the effects of a constant reduction in salinity, while estuaries have a constant fluctuation in salinity and nutrient conditions. Fluctuations in salinity would require kelps to change their osmotic concentration often, which could work as a different stressor than a consistently low salinity. Future research should investigate the effects of constant changes in environmental factors, like those seen in estuaries, and how these fluctuations would affect the growth and survival of kelps. How salinity fluctuations affect kelp gametophytes has not been studied, but the young sporophytes of *Sargassum thunbergii* still showed survival of over 90% when exposed to salinities of 12 ppt twice a day (for 8 hours each time), and similar relative growth rates to the control (32 ppt) (Chu et al. 2012), indicating that this species could at least tolerate salinity fluctuations like those seen in estuaries. Whether kelp gametophytes can grow and develop under similar conditions would require further research, but the presence of kelp sporophytes in estuaries would indicate that they can tolerate fluctuations in salinity to a certain degree.

In this study, environmental conditions like those found in Milford Haven Waterway were used. With a surface area of 54 km^2 and a catchment area of 424 km^2 providing nutrient runoffs this location could provide a potential site for seaweed aquaculture (Nedwell et al. 2002). The experimental results from this study indicate that a large portion of this estuary would be suitable for the aquaculture of the species used here.

Conclusion:

Understanding the sensitivity of kelp spores to environmental factors is important to predict the successful establishment of kelp forests. And in addition, it can provide insights into the possibility of using areas with brackish water to set up kelp farms. My results provide evidence that the effect of salinity on germination rates of spores is the first barrier to successful establishment of kelps in a brackish environment, such as an estuary. However, once a gametophyte has settled and is growing, the nitrate conditions are a bigger influence on its development and growth. This indicates that lines seeded with kelp gametophytes could be an option for establishing a kelp farm in an estuary, where the nutrient concentrations are generally higher, and salinities are lower. This would not only help in the production of a product for commercial use but would also mitigate the runoff from eutrophying nutrients into the local ecosystem.

Chapter 3 - Seasonal changes in morphological traits of three UK kelp species: blade traits vary more than stipe traits.

Abstract:

Kelp forests provide essential ecosystem services to marine flora and fauna, as well as people that are valued in the range of billions of pounds annually. Some of these services (habitat provision, carbon storage, coastal protection and more) are tied to their morphological traits (blade length, stipe surface area). Both kelp chemical composition and growth pattern are known to change depending on environmental conditions, but little is known about how kelp morphology changes in the field through the seasons. In this observational study a suite of functionally relevant morphological traits of kelp species abundant and widespread on UK and north-eastern Atlantic coasts - *Saccharina latissima*, *Laminaria digitata*, and *Laminaria hyperborea* - were measured over a two-year period. Changes in morphology were compared with environmental conditions to explain how morphology can be influenced by seasonal changes. Traits related to different kelp parts were correlated, such that a large degree of overall variation could be summarized by extracting indicative traits from each part/organ. Further analyses were done on dry mass on the different parts of the kelps. Blade dry mass was higher in summer for all species. No difference in stipe dry weight was found for *L. hyperborea* through the year, but for *L. digitata* it was higher in autumn than in winter, while *S. latissima* had a higher stipe weight in summer. *L hyperborea* had thicker tissue in winter, but for the other two species no seasonal difference was detected in blade thickness. These results support earlier studies indicating that kelps reduce their blade size to prevent tearing and dislodgement due to increased wave action, though there are more factors that could influence morphology. This could also be relevant for aquaculture practices since farming activities in high-energy environments could also result in smaller blades. This study further demonstrated that there are traits in some species that remain relatively constant despite seasonal changes in the coastal environment, and as a result these species can provide certain ecosystem functions year-round, such as the stipe of *L. hyperborea* providing a habitat for other species.

Introduction:

Kelps (Laminariales, Phaeophyceae) are a vital component of coastal ecosystems around the world (Harley et al. 2012; Pessarrodona et al. 2019). Kelp forests can provide a wide variety of ecosystem services, including habitat creation, primary production, carbon storage, eutrophic mitigation, coastal defence, and cultural services (Gundersen et al. 2017; Mortensen 2017a; Hasselström et al. 2018; Bayley et al. 2021). These services are estimated to be worth billions of pounds annually (Beaumont et al. 2008). By altering light levels (Wernberg et al. 2005), water flow (Gaylord et al. 2007), and sedimentation rates (Eckman et al. 1989; Connell 2003), kelps adapt the local environment as ecosystem engineers (Jones et al. 1994). The 3-dimentional seascape that is provided by kelp forests is key to many of the ecosystem services that kelp species provide (Gaylord et al. 2007; Bustamante et al. 2017; Wernberg and Filbee-Dexter 2019). Additionally, kelp economic value as an aquaculture product is also influenced by their morphological traits, for example with thinner, more delicate blades being preferred for culinary purposes. Kelp morphology also influences their ability to withstand physical stressors such as wave action and storms (de Bettignies et al. 2013).

The morphology of the kelps forming this habitat are heavily influenced by the environment they inhabit (Table 3-1 and (Coppin et al. 2020)). For instance, blade shape can be affected by hydrology, blade size by nutrients, and stipe length by light conditions (Kain and Jones 1963; Spurkland and Iken 2012; Millar et al. 2020; Blain et al. 2020; Zhu et al. 2021). Since the morphological traits of kelps are tied to some of the ecosystems functions they provide (Table 3-2), such as the blades of kelps functioning as habitat and food source for other marine life, these seasonal changes would impact the ecosystem services that they provide (Christie et al. 2003; Smale et al. 2013). Seasonal shifts in environmental conditions can then induce a change in the morphology of kelps (de Bettignies et al. 2013; Henry 2018; Hereward et al. 2018). In terrestrial ecosystems many deciduous trees lose their leaves in autumn, and in a similar way some kelp species show changes in their morphology in different seasons. In *Laminaria digitata* this is expressed by the species having a period of blade elongation in spring and blade erosion in autumn (Hereward et al. 2018). In *Ecklonia radiata* there is a similar peak in erosion in autumn (de Bettignies et al. 2013).

Three kelp species that are important foundation species along the North-East Atlantic coastlines are *Saccharina latissima*, *Laminaria digitata* and *Laminaria hyperborea*. *S. latissima* has one long blade and is generally situated in sheltered areas in the lower littoral where it can live up to 4 years (White and Marshall 2007; Bunker et al. 2012). It has a shorter, flexible stipe which allows it to quickly reorient an align with the direction of the currents. *L. digitata* has a segmented blade that expands gradually from a smooth, flexible, oval stipe. It grows in more exposed areas in the lower littoral (Bunker et al. 2012). Its reproductive period spans the entire year, but it is most proliferous in August and September and has a lifespan of 6 to 10 years (Hill 2008a). *L. hyperborea* is situated lower on the shore, and would usually remain sublittoral (Kain 1962). This species has a rigid stipe, providing an extra habitat for other species (Christie et al. 2003). It can live up to 20 years (Tyler-Walters 2007). Figure 3-2A shows an overview of the reproductive times and the times of year with maximal erosion and production for these species.

Most *Laminaria* species are considered “Season anticipators”: species that adapt their growth and reproductive strategies based on an annual rhythm controlled by environmental triggers, instead of environmental conditions in the moment (Kain 1989; Lüning and tom Dieck 1989). *L. digitata* grows throughout the summer while *S. latissima* and *L. hyperborea* reduce growth in midsummer and build up carbon reserves to continue growth later, resulting in a different growth pattern (Lüning 1979). Both kelp chemical composition and growth patterns are known to follow an annual cycle (Kain 1989; Gevaert et al. 2001; Krumhansl and Scheibling 2011). *L. digitata* and *L. hyperborea* exhibit a peak reproductive period that is followed by a period with a lot of erosion (Figure 3-2A). In *L. hyperborea* the old tissue is immediately replaced with new tissue. These differences in growth patterns could lead to morphological differences in kelp, both between species and between seasons. There is however a lack of studies describing variation in morphological traits for these kelp species between seasons.

In this chapter I evaluated the changes in morphological traits of *S. latissima, L. digitata* and *L. hyperborea* through the year. I conducted surveys and screened individuals for their traits through several seasons at a single location. I specifically investigated: (1) how kelp species traits differ throughout the year, (2) what the differences in traits are for these three species on the UK shoreline. After which I also discuss what effects the differences in morphology between species and time in the year could have on the kelps’ ecosystem functions.

Table 3-1: Kelp traits, their functional relevance and ecosystem services that individual traits underpin. 1: Trait, plant analogue and function from (Cappelatti et al. 2019) and references therin.

Trait	Plant analogue	Part	Function	Environmental variable affecting trait
Stipe length	Stem	Stipe	Bring kelp closer to water surface.	Light conditions (Kain and Jones 1963; Blain et al. 2020), hydrodynamics (Zhu et al. 2021), age (Kain and Jones 1963; Teagle et al. 2017)
Stipe surface area	Stem	Stipe	Bring kelp closer to water surface.	Light conditions (Kain and Jones 1963; Blain et al. 2020), hydrodynamics (Zhu et al. 2021), age (Kain and Jones 1963; Teagle et al. 2017)
Holdfast	Roots	Holdfast	Attachment to substrate	Adaptation to hydrodynamics (Sjotun and Fredriksen 1995)
Blade length	Leaf size	Blade	Tied to blade size. More photosynthetic area	Trade-off between light capture (King and Schramm 1976) and hydrodynamics (Pereira et al. 2019; Millar et al. 2020)
Blade width	Leaf width	Blade	Tied to blade size. More photosynthetic area.	Relates to current speed (Gerard 1982; Hurd et al. 1996; Fowler-Walker et al. 2006)
Volumes	Volume	All		All factors affecting growth (Kerrison et al. 2015)
Biomass	Biomass	All	Tied to size. Influences production	All factors affecting growth (Kerrison et al. 2015)
Density	*Not applicable*	Whole	Buoyancy	None known (Schiel and Foster 2006; Bartsch et al. 2008; Henry 2018)
Specific thallus area[1]	Specific leaf area	Blade	Light capture. Slows water loss	Factors affecting blade size.

Trait	Plant analogue	Part	Function	Environmental variable affecting trait
Thickness[1]	Leaf thickness	Blade	Physical structure. Slows water loss/wave impact/ reduced photosynthesis	Hydrodynamics (Fowler-Walker et al. 2006; Millar et al. 2020), Age (Starko et al. 2018).
Surface area to volume ratio[1]	Leaf SAV	Whole	Nutrient capture, Slows water loss	Factors affecting blade thickness, length, and width.
Thallus dry matter content[1]	Dry matter content	Whole	Physical structure, Increases tolerance to desiccation	Seasonal variation (Black 1950)
Total length[1]	Total length/height	Whole	Competition for light	Factors affecting stipe length and blade length.
Holdfast ratio[1]	Total	Whole	Reduced requirement for strong attachment	Factors affecting holdfast, stipe, and blade weight.

Table 3-2: A selection of ecosystem services provided by kelp and their associated traits. Note that many services are associated with one or more morphological trait.

Category of ecosystem service	**Ecosystem service**	**Associated trait(s)**	**Mechanism**
Regulating service	CO_2-drawdown	Blade area Blade thickness	Through photosynthesis. The blade is the main photosynthetic structure (Bartsch et al. 2008), and a thinner blade tends to be more photosynthetically efficient (Millar et al. 2020)
	Direct water quality remediation	Blade size	Through photosynthesis and absorption of nutrients a kelp forest can reduce the effects of ocean acidification and eutrophication (Gundersen et al. 2017; Pfister et al. 2019). These processes mostly take place in the blade.
	Coastal protection	Blade size and shape. Blade and stipe flexibility.	Large, rigid kelps which produce a lot of drag attenuate waves, while small flexible kelps move with the waves and provide a low or negligible effect on wave attenuation. (Morris et al. 2020) (Hondolero and Edwards 2017)
Provisioning service	Human/animal food	Blade weight and shape	Kelps are sold based on weight and their quality for human consumption is influenced by shape (Peteiro and Freire 2009).
	Raw materials	Weight and composition	Sold by weight. Differences in composition of the kelp can influence their usability in certain industries (e.g. (Adams et al. 2011a; Abraham et al. 2019)).
Supporting service	Habitat provision	Blade, stipe, and holdfast size and structure	Kelp forests provide a three-dimensional habitat structure providing substratum (Christie et al. 2003) and shelter (Bertocci et al. 2015; Leclerc et al. 2016) for marine organisms.
	Food supply	Blade and stipe	Kelp detritus can be an important source of carbon and nitrogen for subtidal and intertidal organisms. In addition some marine organisms feed directly on kelp blades, and less frequently on kelp stipes (Teagle et al. 2017).
Cultural service	Recreational and educational value	Unknown, but potentially tied to species, size, and forest density	Diving and fishing in kelp forests can provide recreational and education value (Menzel et al. 2013; Vásquez et al. 2014).

Materials and methods:

Seaweed collection

The morphological traits of the kelps *S. latissima, L. digitata,* and *L. hyperborea* were quantified from individuals taken from Langland Bay, Wales (Figure 3-1). Langland bay is located in the relatively sheltered southern part of the Gower peninsula and the western part of the bay is characterized by a gently sloping boulder field with a large tidal range of up to 8m. Sampling took place on 16 dates between March 2018 and March 2020 (Table S0-3). During low spring tides at least 5 individuals of each species were haphazardly chosen and were harvested using a sharp knife, including as much of the holdfast as possible. Since the amount of time that individuals could be sampled was limited (due to tidal conditions) it was not possible to use quadrants or grids to select individuals. As such, they were sampled by manually selecting them in an attempted haphazard manner. Since individuals were identified by eye, there was likely a sampling bias towards larger individuals. Samples were taken with at least 3m distance on the shore between them. The sampled seaweeds were stored in plastic bags and transported to the lab for further analysis. Samples were kept at 4˚C until analysis for a maximum of 2 weeks.

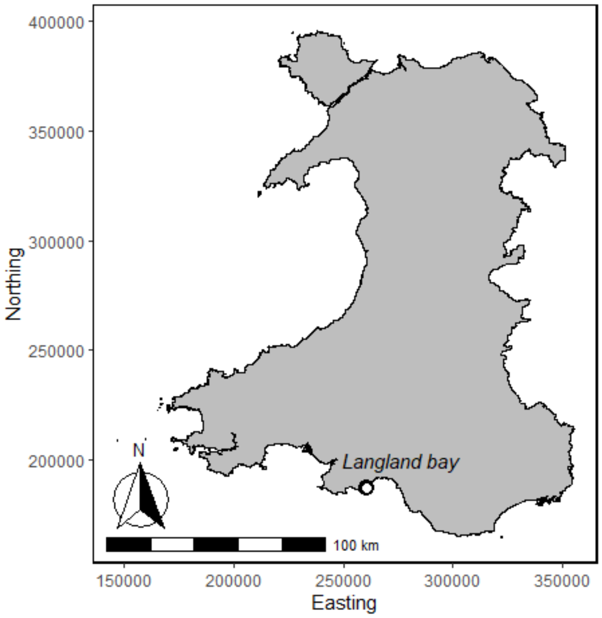


Figure 3-1: A map of Wales with in the southern part of the country the sampling site "Langland bay" indicated.

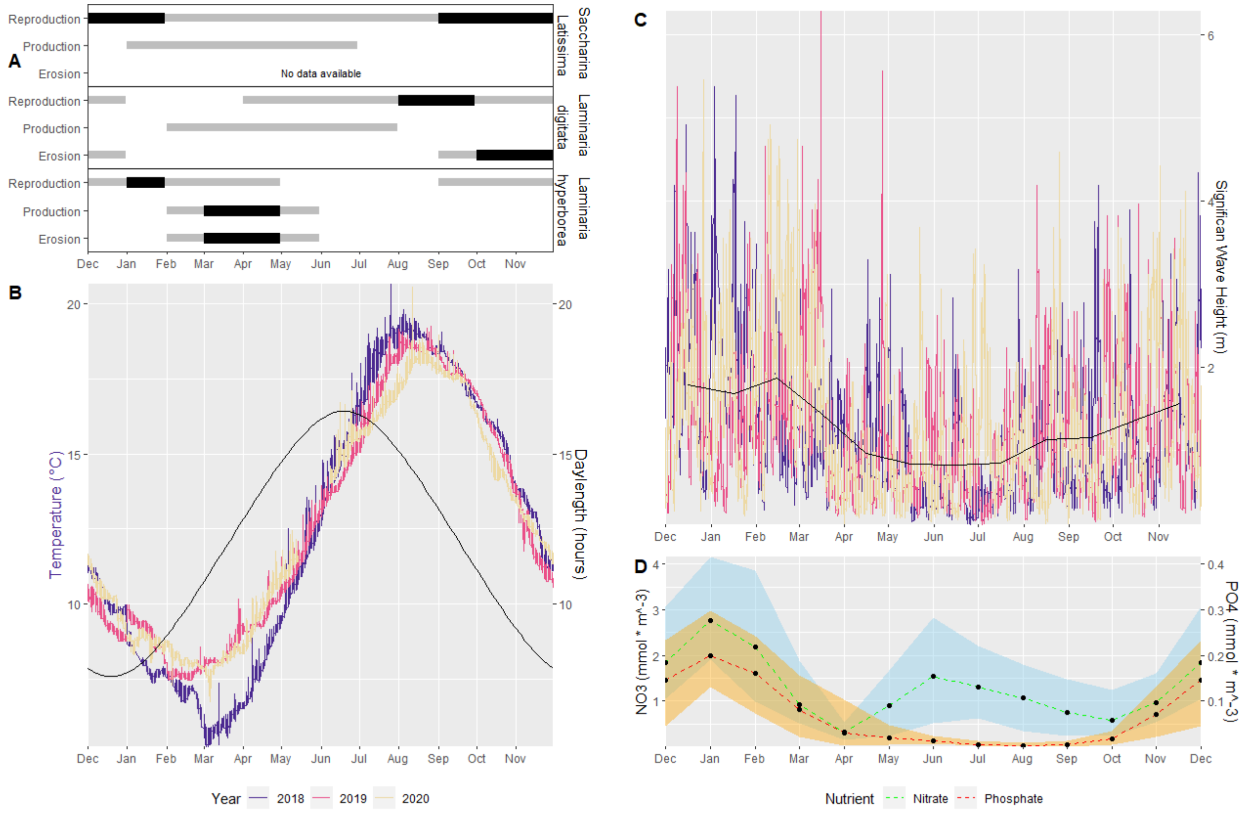


Figure 3-2: A: Times of reproduction, production of biomass, and erosion of biomass in the UK for three kelp species in the bar chart in grey. Times with most reproduction/production/erosion in black. (Kain and Jones 1963; Kain 1975; Lüning 1979; Bartsch et al. 2008, 2013; Forbord et al. 2012; Hereward et al. 2018; Pessarrodona et al. 2019). B: Temperature (colours, in degrees Celsius) and daylength (black, in hours) from 2018 to 2020 near sampling site (Langland Bay, Wales; Based on Scarweather buoy data from Cefas WaveNet). C: Significant wave height in meters in 2018 to 2020 (same colors for year as 3-1B). Monthly mean in black. D: Range of monthly means of nitrate and phosphate concentrations from 1993-2019 near Langland. (based on data from E.U. Copernicus Marine Service Information (http://marine. copernicus.eu)). Nitrogen concentrations of more than 5 mmol/m^3 and phosphate concentrations of over 0.3 mmol/m^3 are considered ideal (Kerrison et al. 2015).

Environmental data

Environmental data was gathered from public datasets. Temperature and significant wave height data (H_{m0}, four times the standard deviation of the surface elevation) were acquired from the Scarweather buoy from the WaveNet wave monitoring network at a resolution of twice an hour from the start of 2018 to end 2020 (Cefas, https://wavenet.cefas.co.uk/). This buoy is located 25km south of the sampling site. Nutrient data at the water surface were acquired from the nearest site available (20km southwest) in the Global Ocean Biogeochemistry Hindcast dataset (which uses PISCES biogeochemical model) from the EU Copernicus Marine Environment Monitoring

Service (Copernicus Marine and Environment Monitoring Service, http://marine.copernicus.eu). Nutrient data is presented per month based on daily data.

Morphological measurements

Morphological traits were measured in the lab. Methodology for trait measurements was based on Cappelatti et al. (2019). Each individual sampled from the field was placed on a light pad (MiniSun, Manchester, UK) and an image was taken (180 dpi resolution). When an individual did not fit on the light pad pieces of the blade were cut and photographed either in a separate picture or on the side of the stipe if room was available. Thallus thickness was averaged for each individual seaweed with a digital vernier calliper from measurements of 10 haphazardly selected spots on the thallus (read to two places after the decimal point with an estimated inaccuracy of ± 0.02mm). The length of the stipe was measured in centimetres with a ruler. The volume of stipe, thallus, and holdfast were measured separately by placing them in a graduated cylinder partially filled with tap water and observing the change in water level. Weights of the seaweed parts were measured before placing them in a drying oven at 70 ˚C. Dry weights of the parts were measured when a constant dry weight was achieved (after at least 3 days).

The photographs of samples on the light pad were analysed with the scientific image analysis software ImageJ (version 1.51k) (Schneider et al. 2012). The length, width, and surface area were measured for the thallus, stipe and holdfast of all seaweed samples. The number of digits for the *Laminaria* species were also noted. Obtained data was used to calculate the following variables: Photosynthetic area (surface area of blade and stipe visible in photo * 2), Specific thallus area (blade surface area/dry mass), blade surface area to blade volume ratio (Surface area/volume), Total dry mass content (Dry mass/fresh mass), Aspect ratio (length/width), frond/stipe weight ratio and Holdfast/rest weight ratio. See Table 3-1 for explanation of the functional relevance of the traits.

Statistics

All statistical analyses were performed in R, version 4.1.0 (R Core Team 2021) through RStudio version 1.4.1106 (RStudio Team 2021). Correlation matrices were constructed to summarise associations between the measurements conducted on kelp morphological traits using a Pearson parametric correlation test.

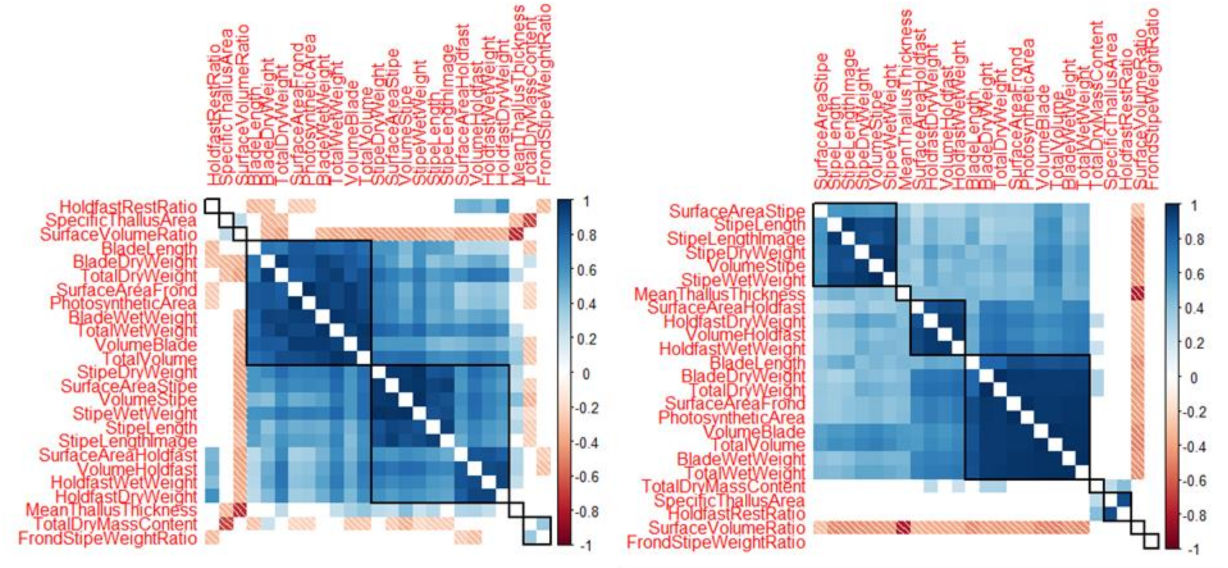
Since significant correlations were found between many of the measured parameters the further analyses were focussed on dry weight measurements of blade and stipe, as well as blade thickness. These three traits were chosen because they were representative of patterns seen in some of the other traits measured and because dry weight is a commonly measured trait in many studies on kelp. As the data for dry-weights was not normally distributed (Shapiro-Wilk test), a non-parametric Kruskall-Wallis test was applied to test the differences between species and seasons. Measurements taken over the monitoring period were aggregated by season when analysed. Meteorological seasons (Autumn: September till November, Winter: December till February, Spring: March till May, and for Summer: June till August) were used to test for seasonal effects.

Results:

Environmental conditions

The seawater temperature changes in Langland Bay followed a predictable seasonal pattern where the temperature fluctuated from 7.5 °C in February or March to 18-19 °C in early to mid-August. In 2018 the seawater temperatures were lower by 5 °C at the end of winter, and 1.5 °C higher in early Autumn than the other two years of the study. The changes in temperature follow an approximate sinusoid pattern that is two months delayed from the sinusoid for daylength.

Significant wave height (H_{m0}) was higher in winter than in summer, with spring and autumn acting as transitional stages (Figure 3-2B). Phosphate concentrations were high in winter, but lower throughout the rest of the year (Figure 3-2C). Phosphate was still available in spring (especially early spring), but was almost entirely gone during summer. Nitrate concentrations were highest in winter as well but were also relatively high through a large part of the rest of the year. Mean nitrate concentration only fell below 1 mmol*m^{-3} in Spring (March, April, May) and autumn (September, October, November).



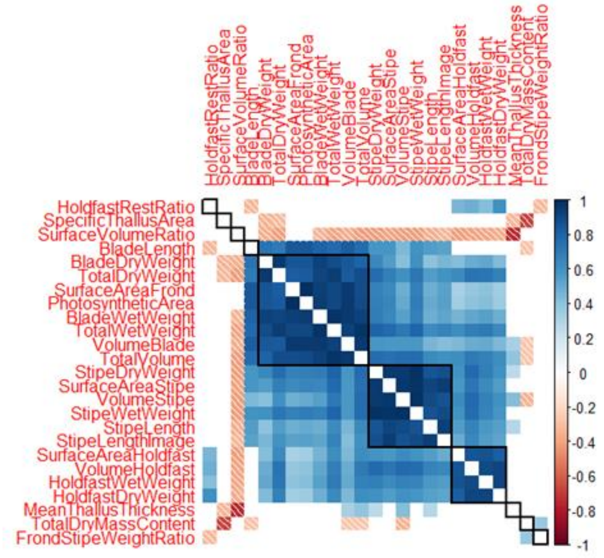


Figure 3-3: correlation matrixes between kelp traits for *Saccharina latissima*, *Laminaria digitata*, and *Laminaria hyperborea*. Black boxes indicate stronger correlations between the traits. Correlations along the diagonal and correlations with a significance below p=0.05 (meaning they were not statistically significant in a Pearson parametric correlation test) are left blank

Correlations between traits

Correlations between the measured traits showed a similar patten in the three species (Figure 3-3). Trait measurements on the segments of the kelp (blade, stipe, holdfast) tended to have a strong positive correlation with other traits on that same segment (Figure 3-3). As a result, further analyses were focussed on the dry weights of these segments.

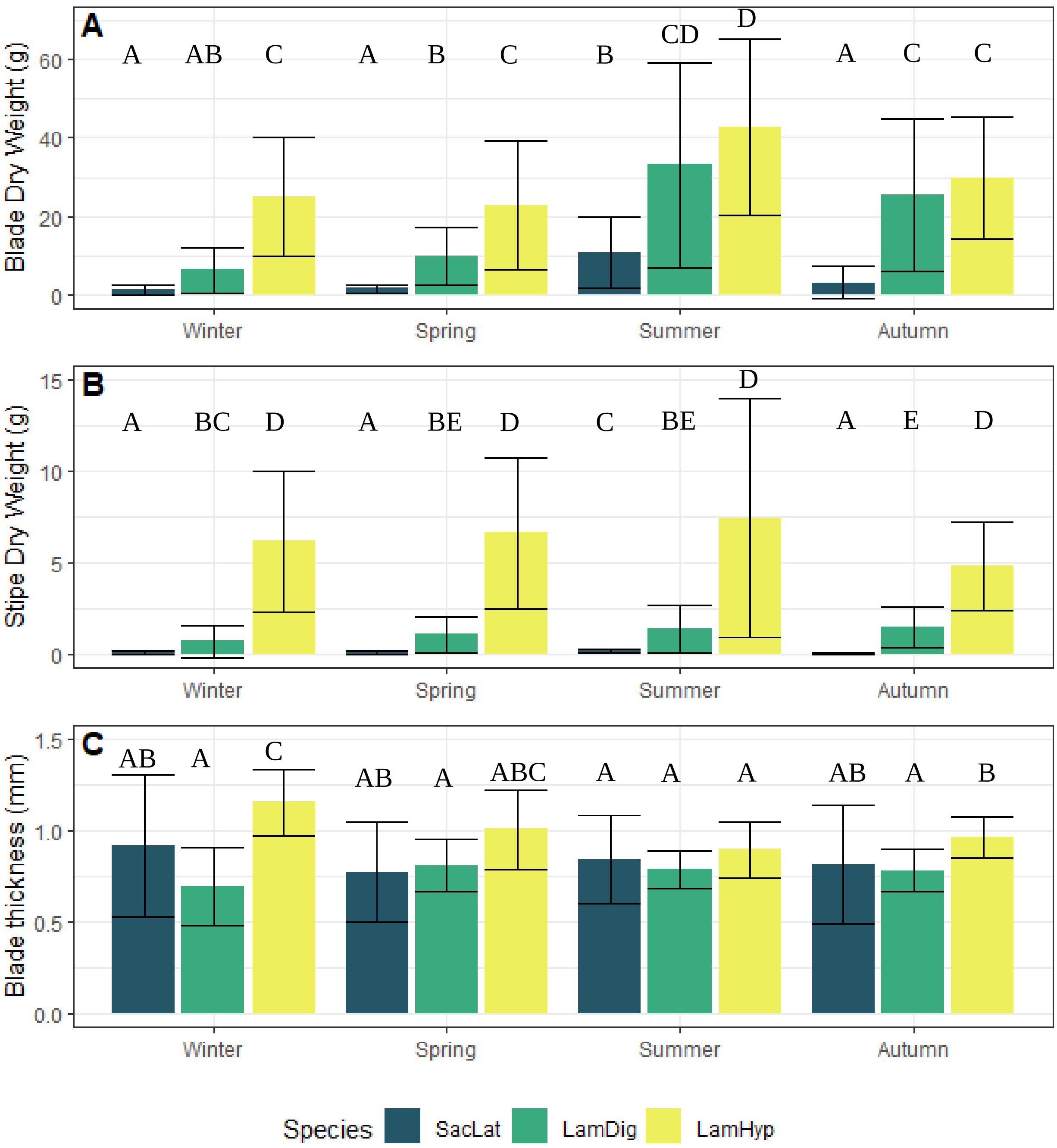


Figure 3-4: Mean dry weights of *Saccharina latissima*, *Laminaria digitata*, and *Laminaria hyperborea* blades (A) and stipes(B). As well as mean blade thickness (C) in four seasons on the shore of Langland Bay (Wales) from 2018 to 2020. +- 1 standard deviation. Different letter groups indicate statistically significant differences among treatment groups within species (determined using using paired Wilcoxon rank sum tests). Treatments with the same letter are not significantly different from each other. A p-value of < 0.05 was considered statistically significant for all tests.. number of individuals measures ranged from 14 to 37 per season/species combination.

Seasonal changes in morphology weight and blade thickness

Throughout all seasons and species there was a large variation for the measured traits. Results for Kruskal-Wallis Rank sum tests yielded significant differences between most traits and seasons for the measured species (Table 3-3)..For all species blade dry weight was at its highest in summer, and at a lower level in the rest of the year (Figure 3-4A), though the seasonal difference was not statistically significant in all species. Stipe weights were more consistent over the seasons. There were no significant differences in stipe dry weight for *L. hyperborea* between seasons (Figure 3-4B). For *L. digitata* the stipe weight was less in winter than in autumn. *S. latissima* stipe dry weight was higher in summer than the rest of the year.

Table 3-3:an overview of the results for Kruskal-Wallis Rank sum tests checking differences between seasons for three traits of three species of kelp.

	S. latissima			***L. digitata***			***L. hyperborea***		
Trait	**χ^2**	**df**	**p**	**χ^2**	**df**	**p**	**χ^2**	**df**	**p**
Blade dry weight (g)	36.09	3	<0.001	29.94	3	<0.001	13.07	3	<0.05
Stipe dry weight (g)	21.06	3	<0.001	7.98	3	<0.05	2.59	3	0.46
Blade thickness (mm)	1.48	3	0.69	2.12	3	0.54	28.72	3	<0.001

L. hyperborea had both the heaviest mean dry weights of blade and stipe of the species measured, followed by *L. digitata*, and *S. latissima* having the lightest. The difference in stipe dry weights were high between the species, for instance in summer the stipe dry weight of *L. hyperborea* was 29 times the weight of the *S. latissima* stipe. No differences were found in the blade thickness of *L. digitata* and *S. latissima* between the seasons. In *L. hyperborea* the blades were thicker in winter than in autumn, and blade thickness in autumn was in turn thicker than in summer (p<0.001). In spring the variation in blade thickness was larger and no statistically significant differences were found with the other season (Figure 3-4C).

Morphology changes over time

The seasonal change in morphology is shown in Figure 3-5. The drawings are of representative individuals found on the shore. The kelps reach their largest size in summer and are at their smallest in winter. Growth in winter and spring pushes out the previous year’s remaining tissue to a more distal part of the blade. The older tissue can be distinguished from the fresh tissue as it feels more rigid, tougher, and is generally a shade darker. During spring this tissue erodes or is torn off the blade.

For *S. latissima* the older tissue is present at the distal end of the single blade. For *L. digitata* and *L. hyperborea* the older tissue can be present at either the end of an individual digitate blade – like in the *L. digitata* individual from winter-, or at the end of two or three central digitate blades like the individual shown from spring.

Figure 3-5: Morphology of representative samples of *Saccharina latissima* (top), *Laminaria digitata* (middle), and *Laminaria hyperborea* (bottom) in Langland Bay, Wales in winter, spring, summer, and autumn (left to right). Older tissue from the previous year is shown in grey, sori on blades is dark grey. Bar in the top left corner is 60cm. All individuals are to scale.

Discussion:

The main results of this study show that size and biomass of *L. digitata, L. hyperborea,* and *S. latissima* blades change depending on seasonality, but the stipe size and weight are generally more consistent. The traits of the kelp species in this study also differed, with *L. hyperborea* being the largest and heaviest, followed by *L. digitata* which in turn is still heavier than *S. latissima*. These factors influence the ecosystem services provided by kelps (Villegas et al. 2008; Smale et al. 2015; Morris et al. 2020; Gundersen et al. 2021). The size and shape of kelps influence the coastal protections and habitats provided by seaweeds (Hondolero and Edwards 2017; Morris et al. 2020). While there have been studies presenting a detailed look at the morphology of *L. hyperborea* (Kain and Jones 1963), there has been limited research on the seasonal changes in morphology of these species. I also demonstrated that the morphological traits (properties of stipe, blade) of *S. latissima, L. digitata* and *L. hyperborea* are strongly correlated with one another, indicating that future morphological studies do not need to measure all traits measured here but can focus on traits such as dry weight or size.

A wide variety of factors influence the morphology of kelps, including waves and currents (Sjotun and Fredriksen 1995; Roberson and Coyer 2004; Millar et al. 2020; Zhu et al. 2021), light (Kain and Jones 1963; Blain et al. 2020), nutrients (Blain et al. 2020), age (Sjotun and Fredriksen 1995; Kim et al. 2018), seasonal anticipation (Kain 1989; Lüning and tom Dieck 1989), pollution (Oyarzo-Miranda et al. 2020), salinity (Vettori et al. 2020), and genetics (Roberson and Coyer 2004; Spurkland and Iken 2012). Disentangling the effects of a single variable in an observational study is difficult. There were no changes in salinity measured. The most likely factors for the seasonal changes in morphology, were changes in the hydrodynamics, light- and nutrient-conditions, and seasonal anticipation. Genetics of the population, or effects by pollution were assumed not to have an effect in this study.

Decreased light conditions, both from turbidity, depth and seasonal changes, can lead to lower stipe weights, and thinner thallus morphology (Kain and Jones 1963; Azevedo et al. 2019; Blain et al. 2020). Continuous reductions in the amount of light reaching the kelp most likely lead to shorter stipes. Potentially due to reduced total growth, or changed resource allocation prioritizing the main photosynthetic tissue - the blade - over the stipe (Blain et al. 2020). Since the stipes of kelps are structures that remain for several years the differences in stipe weights found in this study between seasons were minimal. The differences in stipe weight here could be due to age effects. Samples were taken from a population on the coast that would have both recruitment and mortality, and since new recruits would become noticeable on the shore in late winter these individuals would have a reduced stipe weight lowering mean stipe weight. This study did not include age measurement of kelps. For some kelp species it is possible to measure age based on growth rings in the stipe (Kain and Jones 1963). Future morphological studies should include this as a measurement.

Another trait related to light conditions is blade thickness. Thicker blades have a lower photosynthetic efficiency (Millar et al. 2020), so if kelps prioritised photosynthetic efficiency during times of year with low light conditions the blades would be thinner in the winter months. However, in this study the blades of the kelps were either just as thick, or thicker, in seasons with short daylengths as in seasons with long daylengths. This means that light levels are unlikely to explain the morphological differences in blade thickness observed in this study.

The increased weights of the kelp blades in summer is most likely due to the availability of both nutrients and light. While the effects of nutrients on morphology have not been widely studied, seasonal increases in nutrient concentrations are associated with higher growth rates (Kain 1989). Since kelp size is determined by the ratio of erosion to the production of biomass, the increases in temperature, nutrients, and light allow kelps to grow to their maximum size in late spring/early summer. In late summer nutrients are limited and there would be more erosion of the blades leading to a reduction in size in autumn. In Autumn light once again can become limiting for growth (Gagné et al. 1982). These conclusions are supported by the nutrient levels and daylight hours at this site being suboptimal for these species in this location Figure 3-2, (Kerrison et al. 2015)).

The kelps *L. digitata* and *L. hyperborea* are known as “season anticipators”, meaning they change their growth and reproductive strategies in an annual rhythm based on an environmental trigger (most likely light), instead of on the environmental condition in the moment (Kain 1989; Lüning and tom Dieck 1989). The timing of reproduction could be linked to increases in erosion. For instance in *Ecklonia radiata*, a kelp species endemic to Australia, tissues were weakened as part of their spore release strategy (de Bettignies et al. 2013). In *L. digitata* and *L. hyperborea* the months with maximum erosion followed one or two months after the months where most reproductive tissue was found on the blades (Figure 3-2A). This could indicate that tissue loss is a method of spore release in nature and provides supportive evidence for the hypothesis from de Bettignies et al. (2013) that erosion rate will peak with spore release.

The hydrodynamics of the coast determine both maximum size, and shape of kelps (Spurkland and Iken 2012; Millar et al. 2020; Zhu et al. 2021). Larger blade sizes may make kelps more prone to breakage, wounding, and dislodgement (Roberson and Coyer 2004; de Bettignies et al. 2013). In this study I found that kelp blades were generally smaller in winter, and bigger in summer. This is consistent with findings from other studies where kelps were smaller in winter (de Bettignies et al. 2013), and will adapt their morphology to the hydrodynamic environment (e.g. (Millar et al. 2020)). Kelp structural flexibility allows them to reconfigure and reorient under conditions where they are impacted by high water speeds and achieve sizes that would not normally be expected in high energy conditions (Denny 2006). Higher water flow may be the reason why *S. latissima* and *L. digitata* blades were smaller in winter when waves were higher and more impactful. As there are more waves in winter the kelps only survive if they reduced their size. Kelps can change their morphology to higher currents but would take a longer time to change back to their earlier morphology. In *Eclonia radiata* individuals transplanted to an exposed site changed their morphology in under three months, but those transplanted to sheltered sites kept their original traits until 6 months later (Fowler-Walker et al. 2006) when presumably the tissue had been replaced. Smaller, thicker blade tissue may be an adaptation to reduce erosion, fragmentation, and dislodgement. However thickened tissues are also less effective at photo synthesis (Agusti et al. 1994; Sakanishi et al. 2017), meaning the tissues would need to be replaced when conditions improve after a period where kelps were subjected to high water impacts. The change in morphology is most likely due to the tensile forces from hydrodynamic drag. Blade morphology typical of an exposed site can be artificially induced by loading blades with weights (Gerard 1987; Koehl et al. 2008).

Many of the ecosystem services that kelps provide are dependent on their structural and morphological characteristics (see Table 3-1). Kelps provide habitat, food, and refuge for a wide variety of organisms (Smale et al. 2013). Kelp blades erode annually, providing particulate matter for suspension- and deposit feeders (Vanderklift and Wernberg 2008; Krumhansl and Scheibling 2011, 2012), and marine meiofauna and microbes (Gilson et al. 2021). The habitus of kelps, in this study in particular *L. hyperborea*, directly provide distinct habitats on their blade, stipe and holdfast for invertebrates (Christie et al. 2003; Hereward et al. 2018). More extensive kelp forest habitat is also associated with a higher abundance of fishery resources, presumably due to an increase in prey items and protection of targeted species in the kelp canopy (Bertocci et al. 2015).

There is an interest to research if coastal habitats, including kelp forests, can provide coastal protection (Duarte et al. 2013; Morris et al. 2020). The morphological traits of kelps determine if a kelp forest would be effective as coastal protection, in particular the traits that affect their drag, like flexibility (Morris et al. 2020) and blade shape (Hondolero and Edwards 2017). Seasonal changes in kelp size and morphology would impact the potential of a kelp forest to attenuate currents.

Aquaculture development of seaweed farms is currently gaining increasing attention and support from policy makers for the ecosystem services that seaweeds provide, while also being a valuable form of revenue (Kim et al. 2017; Hasselström et al. 2018). Among these services are the provision of habitats for marine organisms (Smale et al. 2013), removal of nutrients (Wang et al. 2013; Jiang et al. 2020), carbon assimilation (Pessarrodona et al. 2019; Bayley et al. 2021), and cultural services (Hasselström et al. 2018; Gundersen et al. 2021). The ecosystem services provided by a kelp farm are not necessarily the same as those of a kelp forest, but similar to forests, kelp farm services can tie into their morphology, for instance the blades acting as shelter for marine organisms (Christie et al. 2003; Deza and Anderson 2010). If the kelps would be deployed and harvested in the same year, as is practice now on most seaweed farms (e.g.

(Edwards and Watson 2011)), they would provide these functions for only a small part of the year. On top of that, some of the epiphytic organisms present in and on kelps would be unwanted in an aquaculture setting (Forbord et al. 2020a). This also ties into the age of the kelp on a farm. After removal of kelp biomass from a natural kelp forest it takes several years for the habitat to be restored (Teagle et al. 2017). This is an indication that the ecosystem function provided by younger kelps do not fulfil the same requirements that older kelps do. And this in turn would severely impact the ecosystem effects of a kelp farm. Any study on the ecosystem services provided by kelp farms should take these factors into account when discussing the benefits of kelp farms on their environment, and equating the service provided by natural kelp forests with kelp farms should be done with utmost care.

Kelp morphology is also of importance for kelp farming. For instance, in high-energy environments, such as offshore locations, where increased wave action may lead to the development of smaller blades. Additionally, the morphology of kelp blades can affect the quality of the product (Peteiro and Freire 2013). By understanding the impact of kelp morphology on yield, resilience, and product quality, farmers can select suitable kelp species and optimize growth conditions for successful aquaculture.

Climate change influences both the number and intensity of storm events, as well as temperatures (IPCC. Intergovernmental Panel on Climate Change 2014; Allan et al. 2020). The consequences of changes in water temperature and hydrodynamics could influence the timing of life events (e.g. reproduction, scenescence), as well as the morphology of kelps. This would have a knock-on effect on the ecosystem services that kelp provide since a lot of kelp ecosystem services are tied into their morphological traits. In terrestrial ecosystems the phenomenon of ecological mismatching – where the timing of seasonal life history events between different species fail to match up because of climate change- has been widely studied (e.g. (Saino et al. 2011)). However, the potential for ecosystem mismatching in kelp has not been studied. Further research on kelp ecosystem services, the effects of environmental changes on kelp morphology, and potential knock-on effects into the wider ecosystem is needed.

Conclusion:

The ecosystem services provided by kelps are considered increasingly important in a world that is facing unprecedented losses in biodiversity. *S. latissima, L. digitata* and *L. hyperborea* are foundational biogenic habitat formers in western-European coastal ecosystems. Seasonal changes in environment affect species differently and are most likely caused by the combination of factors influencing erosion and production of biomass, such as exposure to hydrodynamic forces increasing erosion, and production being influenced by nutrients and light conditions. Climate change is predicted to increase temperature, affecting the timing of reproduction, as well as increase the intensity of storm events (IPCC. Intergovernmental Panel on Climate Change 2014; Allan et al. 2020). Both factors have an impact on kelp morphology and will most likely influence the ecosystem services that these species would provide. More research is needed into how environmental changes would influence kelp morphology and their ecosystem services.

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Contains information provided by Cefas and funded by Environment Agency, licensed under the Cefas WaveNet Non-Commercial Licence v1.0. I further acknowledge the E.U. Copernicus Marine Service Information (marine.copernicus.eu). for the data they’ve made available on their website, and which has helped with the creation of this work.

Chapter 4 - Preservation and cryopreservation of the spores of *Laminaria digitata* and *Laminaria hyperborea*

Abstract:

Kelps are an essential group in seaweed cultivation, but to grow kelps commercially their meiospores are required. While protocols exist for obtaining kelp meiospores and for storing kelp materials long term, these protocols have not been optimised. This study tests the effects of sori storage time on meiospore release and germination, as well as the effects of dimethyl sulfoxide (DMSO) concentration in meiospore cryopreservation. Sori of *Laminaria digitata* and *Laminaria hyperborea* were stored for up to 4 days before meiospores were released into culture medium and checked for spore release, settlement, germination, and germ tube growth. Meiospore release was maximal when sori of *L. hyperborea* had been stored for 48 hours, but for *L. digitata* the highest number released was after 24 hours. There was no effect of sori storage time on meiospore germination rate. In a separate experiment the meiospores of these kelps were also exposed to the cryoprotectant DMSO (0,5,10,15,20,25%) before being frozen to -40 °C and plunged in liquid nitrogen. No meiospores of *L. digitata* survived the liquid nitrogen treatment, and only 5% of *L. hyperborea* spores survived in the 5% DMSO treatment. The results of this study indicate that for an optimal release of spores the storage time of sori for these species should be between 24 and 48 hours, but there could be seasonal effects on spore release and spore quality that need to be studied further. Cryopreserving kelp spores is possible, but survival rates vary between species and are generally low. These results indicate that alternative methods of storing kelp materials should be explored.

Introduction:

The cultivation of seaweeds is currently being developed in Europe to lead to a more sustainable way of producing food (Grebe et al. 2019). Kelps (*Laminariales*) are considered one of the most commercially important group of species for cultivation, contributing to over 40% of all seaweed harvests in 2018 by weight (Chopin and Tacon 2020). Most of this production is still taking place in Asia, but there are several initiatives aiming to expand kelp aquaculture in Europe (e.g. (Peteiro et al. 2016; Van Der Molen et al. 2018; Barbier et al. 2019; Thomas et al. 2019; Huntington and Cappell 2020; Vincent et al. 2020)). For the industry to get started spores are needed. Currently two methods are used to obtain kelp spores: they can be obtained from reproductive tissues (sori) of adult kelp, or from cultures where kelp spores are grown into gametophytes in a suspension (Edwards and Watson 2011; Alsuwaiyan et al. 2019). Two limitations of these methods are firstly that there has been little research on the optimisation of spore release (Alsuwaiyan et al. 2019), and secondly that maintaining kelp gametophytes in suspension is not a suitable method to store kelp microscopic stages long term (e.g. over 1 year) (Hoffmann and Santelices 1991).

Spore release in kelp is generally done in three steps: first sori is excised and cleaned, then it is desiccated for a period of time, and lastly the sori is immersed in a seawater medium to release zoospores (Alsuwaiyan et al. 2019). Desiccation is thought to apply an osmotic shock which would induce the expulsion of zoospores. Desiccation time is not consistent across studies, neither within nor between species (Alsuwaiyan et al. 2019). While an extended desiccation time can reduce spore release (Fonck et al. 1998) there have been no studies on the effect of desiccation period on spore viability and gametophyte performance.

The long term preservation of kelp spore and gametophyte cultures is needed to preserve heirloom strains, facilitates in the creation of cultivars, ecosystem restoration, and diverse research applications (Wade et al. 2020; Goecke et al. 2020). Preservation of kelp materials can be done at temperatures above 0 °C, but the viability of samples preserved at these temperatures reduces over time. For instance over 75% of *Macrosystis pyrifera* gametophytes were non-viable after three years of storage at 10 °C in low light conditions (Barrento et al. 2016). An alternative method is by using cryopreservation. Preservation in liquid nitrogen is reliable for long term storage, does not require a lot of labour or space and involves less risk of contamination than non-frozen storage. The most widely studied technique is ‘two-step’-cooling (Taylor and Fletcher 1999a; Yang et al. 2021). In two-step cooling the materials are cooled to a temperature between 0 and -40 °C in a step that is referred to as pre-freezing. Temperatures lower than -40 °C are less effective as the membrane becomes impermeable below this temperature (Karlsson et al. 1994). The pre-freezing step allows the material enough time to reduce its water content so that less intracellular ice crystals will be formed (Wolkers and Oldenhof 2015; Elliott et al. 2017). The pre-freezing step is followed by a second rapid cooling step where the material is immerged into liquid nitrogen. Generally the viability of algal materials stored in this manner would be low (Kuwano et al. 1996) unless a cryoprotectant is added (van der Meer and Simpson 1984; Renard, P., Arbault, S., Kaas, R., Perez 1992; Taylor and Fletcher 1999b). Cryoprotectants are solutes that protect living cells from freezing damage by limiting ice formation. The absorption of cryoprotectants by cells prevents the formation of intracellular ice crystals, but at the same time exposes the cell to toxic cryoprotectants. The toxicity of a cryoprotectant can be dependent on the species or strain it is used on (Taylor and Fletcher 1999a; Heesch et al. 2012), species life stage (Kuwano et al. 1992), the type of cryoprotectant (Zhuang et al. 2015), concentration (Choi and Nam 2012), and exposure time (Cañavate and Lubian 1994). This has led to optimal cryoprotectant substance and concentration being determined empirically (Taylor and Fletcher 1999a). Popular cryoprotectants are dimethyl sulfoxide (DMSO), glycerol and methanol and optimal concentrations are often between 5-20% (Taylor and Fletcher 1999a).

There have been several studies on the (cryo-)preservation of commercially valuable kelp species (e.g. *Undaria pinnatifida* in (Nanba et al. 2009; Wang et al. 2011), or *Saccharina japonica* (Zhang et al. 2007a, b)), but most of these studies focussed on preserving gametophytes. Considering that in smaller cells the intracellular water can be extracted more rapidly during freezing than larger cells (resulting in less intracellular ice) (Dumont et al. 2004), small cells such as spores might be easier to preserve.

This chapter describes a study on the preservation of the meiospores of two species of kelp: *Laminaria digitata* and *Laminaria hyperborea*. The objectives were: 1) to test how prolonged periods of sori desiccation impact spore release, attachment, germination, and germ tube growth, and 2) to test the effects of two-step cryopreservation with different concentrations of the cryoprotectant DMSO to find an optimal concentration for preserving spore viability. Developing a method for the long-term storage of kelp spores would allow for a seedbank to be established, making it possible to preserve genetic diversity and trade in seaweed spores.

Method:

Experiment 1: Effect of sori storage time on spore release, attachment, germination, and early growth

Sample collection

Five fertile *Laminaria digitata* individuals were collected on the 16th of July 2018, and 3 fertile *Laminaria hyperborea* on the 12th of March 2020, from the shore at Langland Bay, Swansea, Wales (N 51' 33'52.9" W 4' 00'45.5"). Reproductive kelp samples were placed into plastic bags and brought to the laboratory. Methodology for sori preparation and spore release was based on the procedure by Edwards and Watson (2011). Sori (95g for *L. digitata* and 86g for *L. hyperborea*) was removed from the individuals with a scalpel and were cleaned by scrubbing them with paper towels with autoclaved filtered seawater (Whatman^{TM} Grade 1 qualitative filter paper). The blades were wiped dry with clean paper towels and scrubbed again for 3 times in total to remove as much mucilage and epiphytes as possible. Cleaned sori was cut into pieces of approximately 4 cm^2. All sori pieces were mixed and divided into 12 stacks. Each stack was weighed and wrapped in dry paper towels. Ten stacks were stored at 2 °C, the remaining 2 were immediately used to measure spore release. This time was taken as t=0h though it was 7 hours after the harvest of the individuals from the field. The following steps were repeated with the remaining sori stacks after 12, 24, 48, 72 and 96 hours of refrigerated storage.

Spore release

Each sori stack was placed in a ringer flask with 200mL of filtered autoclaved seawater enriched with F/2 media (Lin 2005). The flask was covered with aluminium foil and stirred with a clean metal spoon at least every 5 minutes. An aliquot was taken with a sterile plastic transfer pipette after 5 minutes, and again every 10 minutes later until the sori had been in the seawater for 65 minutes. A spore release time of 60 minutes is most commonly used (Alsuwaiyan et al. 2019), but was in this case extended by 5 minutes to take an additional sample. The concentration of spores in the solution was determined using a hemocytometer. With a light microscope (at x200 magnification) the number of spores in the four corner grids and the central square in the hemocytometer were counted. The following equation was used with the hemocytometer counts to calculate spore concentration:

$$\text{Average number of spores in } 0.5\ \mathrm{mm}^3 * \frac{1}{0.5\ \mathrm{mm}^3} * \frac{1000\ \mathrm{mm}^3}{1\ \mathrm{mL}} = \frac{\mathrm{Spores}}{\mathrm{mL}} \qquad (4\text{-}1)$$

The sori were removed by filtering the solution through a 42 µm mesh. The spore density was measured four times and averaged to obtain the final spore count.

Settlement success

For each treatment two clean glass slips were placed at the bottom of a plastic petri dish with a diameter of 5cm. Each petri dish was filled with a mixture of spore suspension and culture media to attain an estimated spore density of 50,000 spores per mL for the *L. digitata* experiment, and 100,000 spores per mL for the *L. hyperborea* experiment. Total volume was always 10mL. For some treatments of the *Laminaria digitata* the sori did not release enough spores to obtain the targeted density, a lower spore density was used to measure settlement success (Table S0-4).

The petri dishes were stored in an incubator at 7.5 °C for 24 hours (12L:12D). The glass slips were carefully removed from the petri dish and gently dipped into filtered autoclaved seawater to remove any unattached spores. One slip was placed in a new petri dish with 10mL of culture medium and placed back in the incubator to use later for the determination of germination rate and germ tube growth. The other glass slip was used to quantify settlement success. The glass slips were placed under a light microscope. Ten views per slip were haphazardly selected at x400 magnification of which an image was taken with an Olympus UC30 digital camera (0.094 mm^2 per view). These images were later used to count the number of attached spores per area.

Six days later (7 days post-spore release) the glass slips were taken from the remaining petri dishes for the corresponding treatment. Ten haphazardly selected views were taken again. The number of gametophytes and spores were counted and used to determine the percentage of germination success. Ten additional images of gametophytes were taken as well. The germ tube length of these gametophytes was measured using the programme ImageJ (version 1.51k).

Since spore density was not equal between treatment groups for the measurements of attachment success. These values were corrected to account for differences in starting spore density using the following formula:

$$corrected\ attachment = number\ of\ spores\ attached * \left(\frac{target\ number\ of\ spores}{starting\ number\ of\ spores}\right) \tag{4-2}$$

with the number of spores attached being a count of the number spores per field of view for the microscope at the used setting, the target number of spores being the aimed total amount of spores in the medium used (1.000.000 in 10 mL), and the starting number of spores being the actual measured amount of spores in the medium.

Statistical tests

All statistical tests were performed in R Studio Version 1.4.1106 (RStudio Team 2021). Sample size for spore release was too small to be statistically tested. For spore attachment, spore germination, and germ tube length the data were tested for normality with a Shapiro-Wilk Normality Test and homogeneity of variance by using Levene’s test. Where these assumptions were met a one-way ANOVA was performed. If not, a Kruskal-Wallis one-way ANOVA was performed as a non-parametric alternative, combined with a Pairwise Wilcoxon Rank Sum Test adjusted with Benjamini & Hochberg procedure to compare levels between groups.

Experiment 2: Effects of different concentrations of the cryoprotectant DMSO on germination and early growth of spores

Plant material

Reproductive *Laminaria digitata* were collected by hand from the shore at Langland Bay, Swansea, Wales on the 2nd of September 2019. L*aminaria hyperborea* were collected on March 12th 2020 from the same location (N 51’ 33’52.9” W 4’ 00’45.5”). The kelps were taken to the laboratory where they were cleaned with seawater in the same way as described. Sori of 3 individuals were excised and stored at 2 °C in the dark for 36 hours to dessicate the sori. This time was chosen based on experiment 1. The sori were cut into pieces of approximately 4 cm^2. Sori were placed into 0.5L of autoclaved seawater enriched with F/2 culture medium in a DURAN bottle. The bottle was covered with aluminium foil and stirred with a clean metal spoon at least every 5 minutes. After 1 hour the sori were removed by filtering the spore solution through a 42µm mesh. Spore density of the final solution was measured with a haemocytometer.

Experimental treatments

After filtering out the sori, 0.8 ml of the spore solutions were added to cryogenic ampoules and mixed with DMSO to obtain solutions with 5 different concentrations of cryoprotectant (0,5,10,15,20% v/v). There were three experimental conditions which the resulting mixes of spore solution and cryoprotectant were subjected to in this study. These conditions correspond with the steps in the two-step cooling method where first a material is exposed to a cryoprotectant, followed by a step where the solution is slowly cooled to sub-zero temperatures called the “pre-freezing step”, and lastly a rapid cooling step where the solution is plunged into liquid nitrogen. Each experimental condition was tested in a multifactorial experiment with the five concentrations of cryoprotectant for a total of 15 experimental treatments. Each treatment was tested in quintuplicate. An overview of the methodology is presented in Figure 4-1.

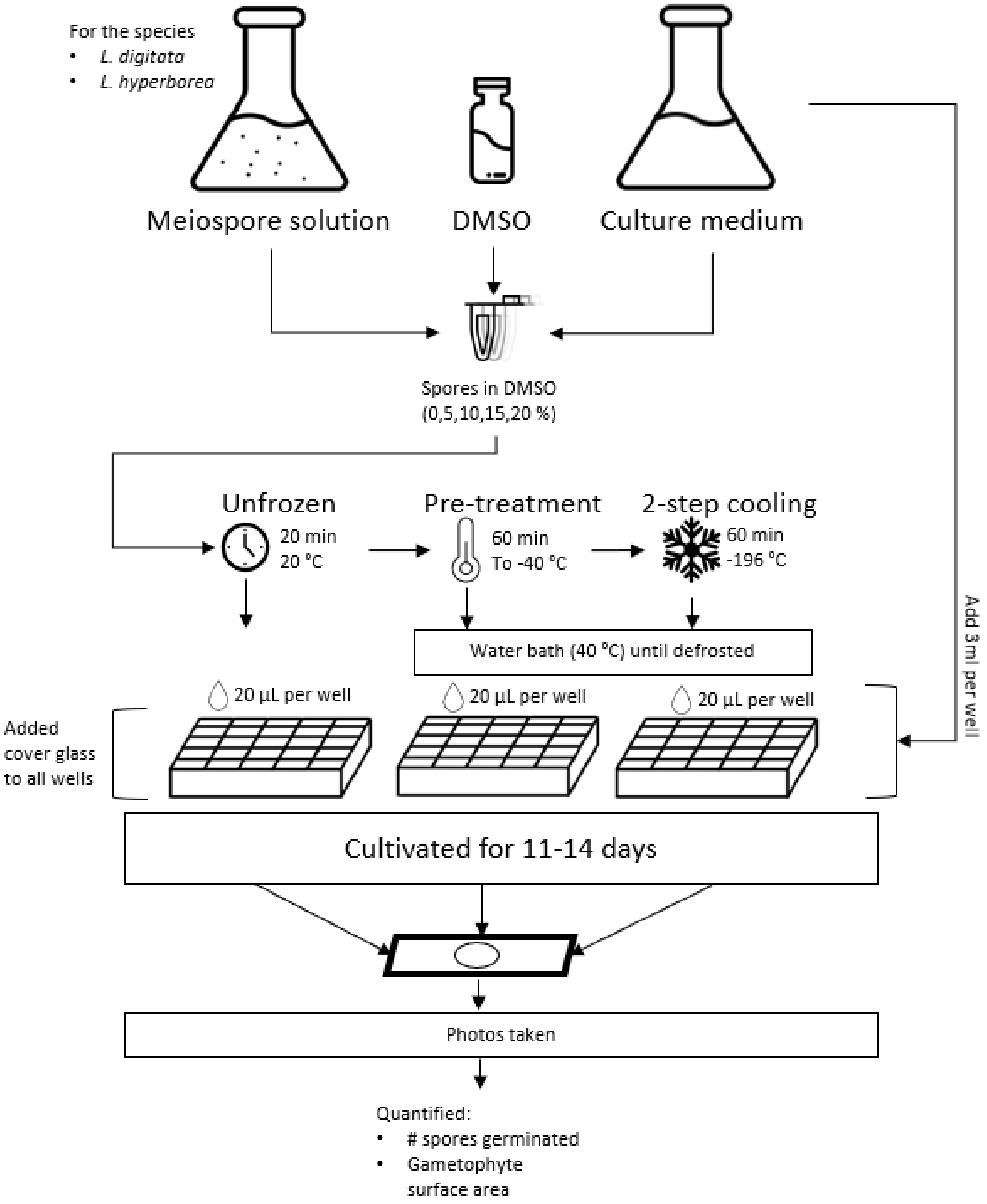


Figure 4-1: Schematic overview of the methodology to test the effect of DMSO (dimethylsulfoxide) concentration on the viability of spores of *L. digitata* and *L. hyperborea*.

All ampoules with the mixes were left at room temperature (20 °C) for 20 minutes to allow the cryoprotectant to diffuse into the spore cells. Samples after exposure to only the cryoprotectant were taken from the ampoules and checked for viability according to the method described in the section “viability test” below.

Freezing and thawing treatment

The ampoules for the pre-freezing treatment, and the 2-step cooling treatment were placed in Nalgene Mr. Frosty™ Cryo 1 °C Freezing containers (Nalgene Ninc International, Rochester, NY, USA) filled with isopropyl alcohol. These freezing containers are designed to reduce the temperature at -1 °C/min. The containers were placed in a -80 °C freezer for 60 minutes to freeze the samples to -40 °C. Final temperature was verified by measuring the isopropyl alcohol with a low temperature thermometer.

Only the ampoules for the two-step cooling treatment were immersed into liquid nitrogen. This happened immediately after the ampoules had cooled to -40 °C in the pre-freezing step. Ampoules were stored in liquid nitrogen for 1 hour. Afterwards all frozen samples were thawed quickly by placing them in a 40 °C water bath until defrosted (1 to 2 minutes) and transferred to a crushed ice bath. This temperature was chosen to dethaw the samples as this temperature yielded optimal results in earlier studies on seaweed cryopreservation (Zhou et al. 2007; Lalrinsanga et al. 2009). The effects of long-term frozen storage of samples was not tested in this experiment as there is little or no detectable decline of cell viability after years of storage at -196 °C (see (Taylor and Fletcher 1999a; Yang et al. 2021) and sources therein).

Viability test

0.2 mL of spore solution was taken from each of the ampoules and added to a pre-prepared repli-dish (10 x 10 cm, 25 compartments). Each well contained a 15 mm diameter borosilicate glass slip and 3mL of autoclaved enriched seawater. Spore solutions were randomly assigned to wells in the repli-dishes. The repli-dishes were placed in an incubator for 11-14 days (due to time limitations it was not possible to measure all spores on the same day) at 10 °C, 50 µmol m^{-2} s^{-1} photon flux density, with a 12L:12D photoperiod. Illumination was provided by white fluorescent lights.

After the culture period, spore germination was quantified by examining the glass slips under a light microscope. Nine haphazardly selected views of the coverslip were examined, and images were taken at x400 magnification with an Olympus UC30 camera. If no spores were found while taking images, extra spores and gametophytes were searched for manually to use for size determination. The surface area of photographed gametophytes was measured with ImageJ to quantify growth.

Statistical analysis

A Kruskal-Wallis test at $p<0.05$ was used to determine the difference in size of gametophyte surface area at the end of the culture period for the germinated spores. A pairwise Wilcoxon test with P-values adjusted according to the Benjamini & Hochberg procedure was used to determine the differences in gametophyte surface area between the different combinations of concentrations of DMSO and steps taken in the two-step cooling method.

Results:

Experiment 1: effect of sori storage time on spore release, attachment, germination, and early growth

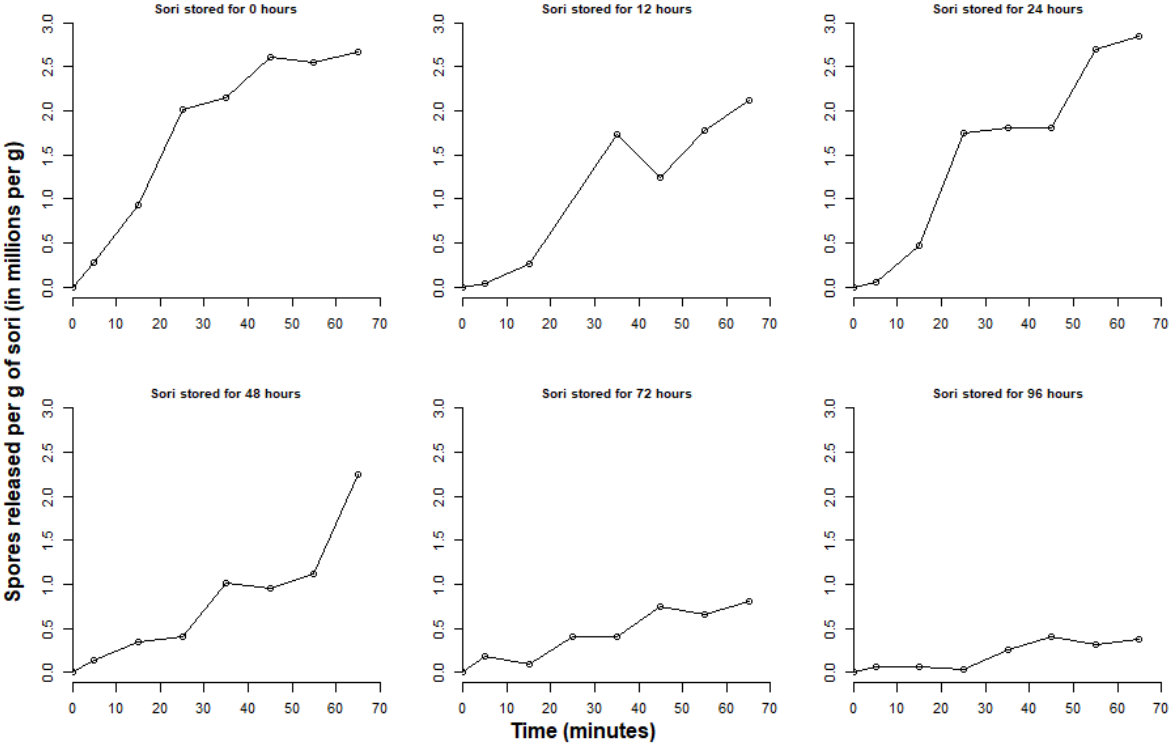


Figure 4-2: Spore release of *Laminaria digitata* sori stored at 2 °C for different times (number of experimental units per treatment(n)=2).

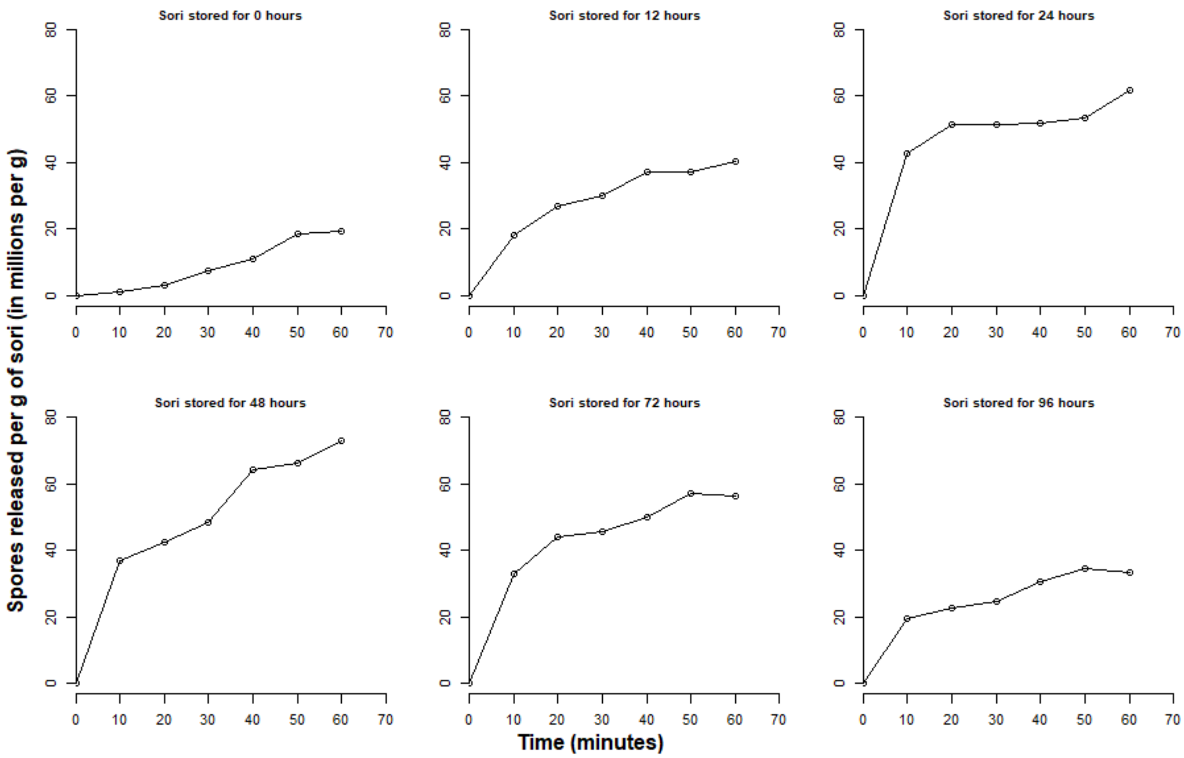


Figure 4-3: Spore release of *Laminaria hyperborea* sori stored at 2 °C for different times (number of experimental units per treatment(n)=2).

Laminaria digitata sori released more spores per gram of sori when it was stored at 2 °C for less than 72 hours. At that storage time spore release was faster and final spore concentrations were higher than when sori was stored for 72 or 96 hours. When sori was stored for 96 hours the release of spores was reduced by 87% compared to the number of spores released after 24 hours (Figure 4-2 and Figure 4-4A). Spore release was highest in sori stored for 0 and 24 hours. *Laminaria hyperborea* sori released more than 10 times as many spores per g sori than *L. digitata* (Figure 4-3). *L. hyperborea* spore release was fastest immediately after putting the sori into seawater, with 69% of spores being released within the first 10 minutes for sori that had been stored for 24h. The highest number of spores were released when *L. hyperborea* sori was stored for 48h (Figure 4-5A). The lowest number of spores were released when the sori had not been stored (0h treatment, 73% less spores released than after 48h storage).

Spore attachment success for *L. digitata* increased when sori were stored for 24 hours or more (Figure 4-4B). Spore attachment was highest when the spores were released after a 72h sori storage time.

Germination rates of *L. digitata* spores were highly variable regardless of sori storage time and no significant differences were found (Figure 4-4C). Seven days after spore release the average *L. digitata* germ tube length was between 25 and 30 µm. Only when sori was stored for 96 hours did mean germ tube length fail to reach that size within seven days (Figure 4-4D).

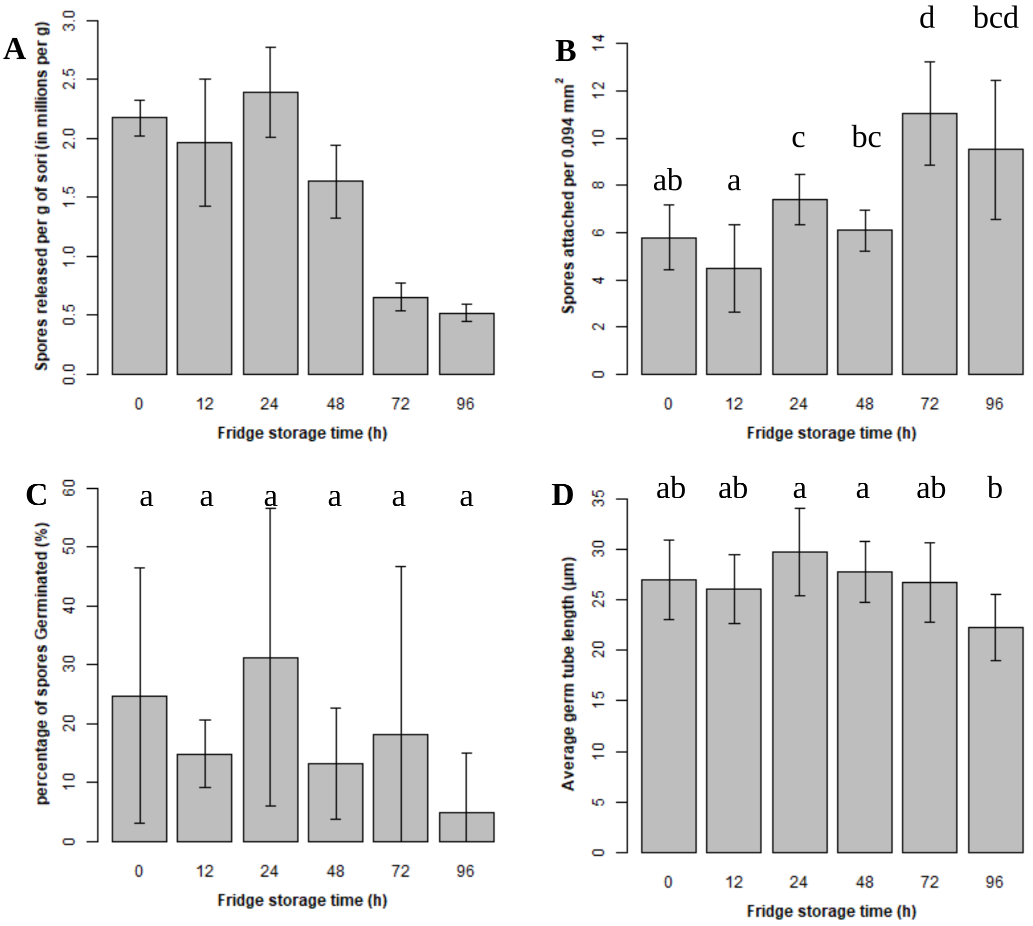


Figure 4-4: Effects of *Laminaria digitata* sori storage time (0, 12, 24, 72 and 96 hours) on spore release (A), attachment (B), germination percentage (C) and early growth (D). Spore release is presented in number per gram of sori +- 2std. error (n=2 + 6 pseudoreplicates). Spore attachment is presented in the number of spores observed to have attached, corrected for differences in starting density +- 2SE (n=4). Spore germination is presented in percentage of spores germinated out of total +- 2SE (n=4). Average germ tube length was measured 7 days after spore release +- 2SE. n= 34 to 45 depending on treatment. Different letter groups indicate statistically significant differences among

treatment groups within species (determined using a paired Wilcoxon rank sum tests). Treatments with the same letter are not significantly different from each other. A p-value of < 0.05 was considered statistically significant for all tests.

The spore attachment success of *L. hyperborea* was higher when their sori was stored for 24 hours or less (Figure 4-5B). When sori was stored for longer the mean attachment success was reduced (28% reduction between 12 and 96 hours). *L. hyperborea* germination rates were high for all treatment with an average of 83% of spores successfully germinating (Figure 4-5C). No statistically significant differences in germination rates were found based on sori storage time. Germ tube length of *L. hyperborea* was affected by sori storage time. Germ tube length was highest when spores were released after 48 hours (22.0 ± 0.29 µm) and lowest when spores were released after 0 or 12 hours (18.4 ± 0.27 µm and 18.4 ± 0.23 µm respectively) (Figure 4-5D).

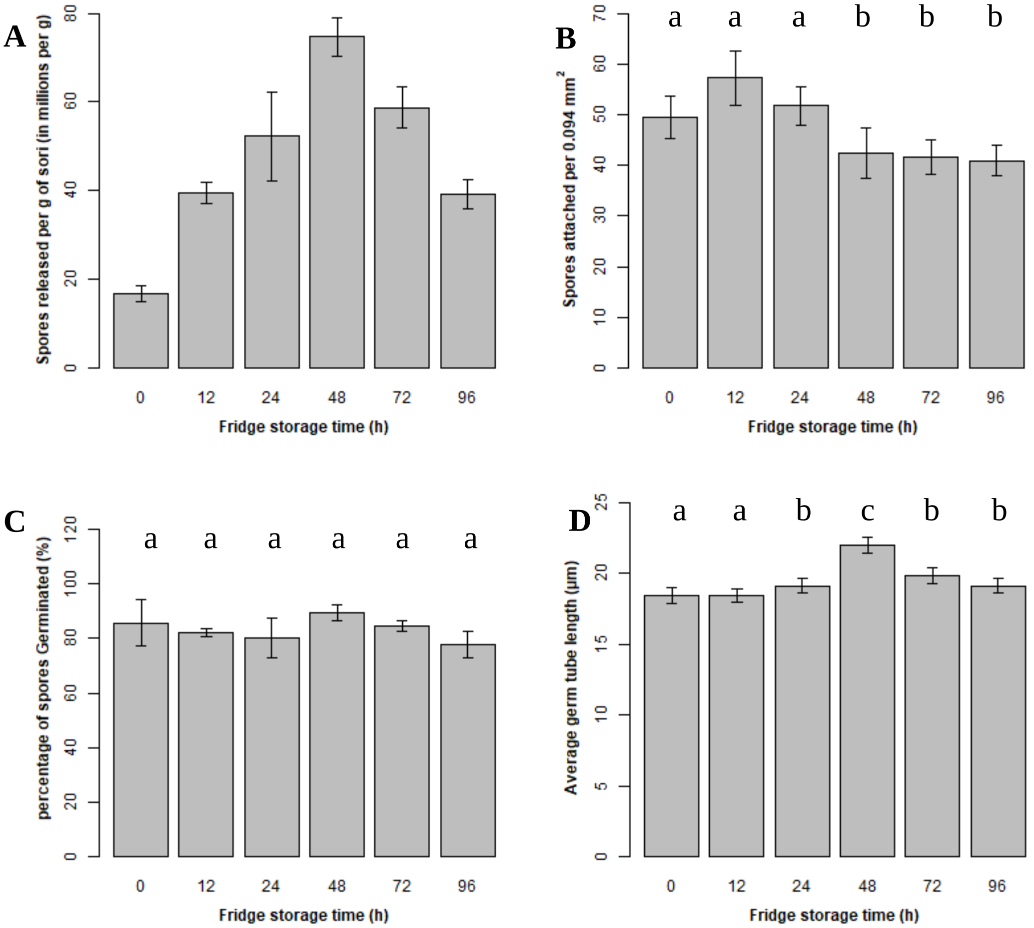


Figure 4-5: Effects of *Laminaria hyperborea* sori storage time (0, 12, 24, 72 and 96 hours) on spore release (A), attachment (B), germination percentage (C) and early growth (D). Spore release is presented in number per gram of sori +- 2std. error (n=2 + 6 pseudoreplicates). Spore attachment is presented in percentage of spores attached out of total +- 2SE (n=4). Spore germination is presented in percentage of spores germinated out of total +- 2SE (n=4). Average germ tube length was measured 7 days after spore release +- 2SE. n=384 to 556 depending on treatment. Differences in spore attachment and germ tube length were tested with a Kruskal-wallis one-way ANOVA. Different letter groups indicate statistically significant differences among treatment groups within species (determined using using paired Wilcoxon rank sum tests). Treatments with the same letter are not significantly different from each other. A p-value of < 0.05 was considered statistically significant for all tests.

Experiment 2: Effects of different concentrations of the cryoprotectant DMSO on germination and early growth of spores

After 11 to 14 days the germination rate of meiospores (i.e. with visible germ tubes or developing gametophytes) was found to have been severely affected by both cryoprotectant concentration and cryopreservation steps (Figure 4-6). There were no *L. digitata* spores found to have germinated after having been plunged into liquid nitrogen, regardless of DMSO concentration. Only a single germinated spore was seen after spores were frozen to -40 °C. No meiospores germinated when they had been exposed to a concentration of DMSO higher than 15%, even when they had not been frozen (Figure 4-6).

When tallying all the germinated spores a similar pattern is seen. Only 4 germinated spores were found after being subjected to the -40 °C pre-freezing treatment (Figure 4-7). The gametophyte growth was only different between the 0% and 5% DMSO treatments (Kruskal-Wallis ranked sum test, χ^2=16.456, p<0.01) but seemed to otherwise be unaffected by the freezing treatments or DMSO concentration.

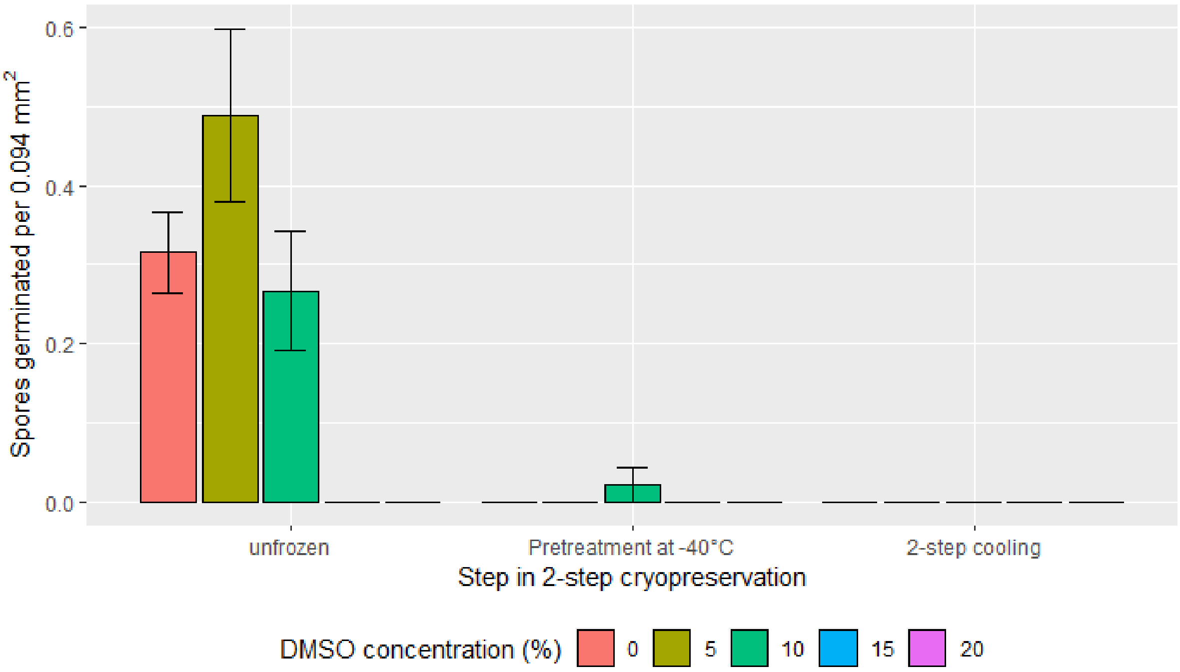


Figure 4-6: The number of *Laminaria digitata* spores germinated per 0.094 mm^2 area after exposure to different concentrations of the cryoprotectant DMSO. Spores were suspended in 0 to 20% DMSO in seawater, cooled to -40 °C at -1 °C/min in a pre-treatment and then plunged in liquid nitrogen with 2-step cooling. Values are in mean ± S.E (n=5)

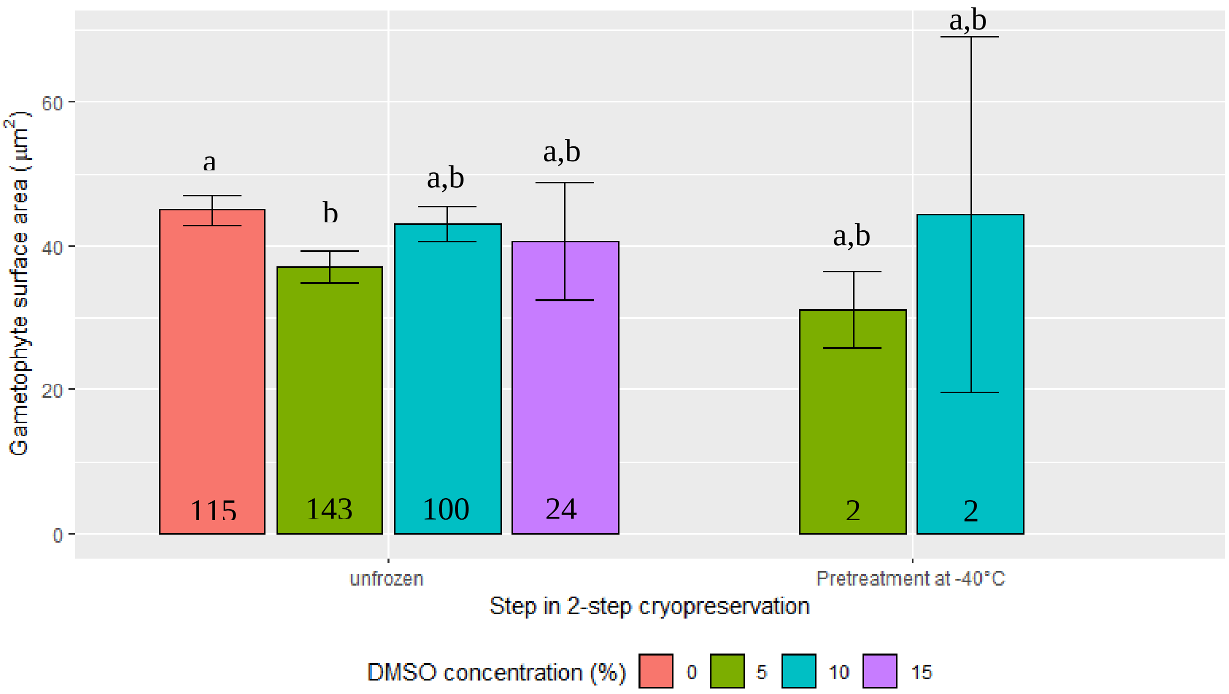


Figure 4-7: Gametophyte surface area of *Laminaria digitata* after exposure to different concentrations of the cryoprotectant DMSO. Spores were suspended in 0 to 20% DMSO in seawater, cooled to -40 °C at -1 °C/min in a pre-treatment. Values are in mean ± S.E. Number of measurement shown at the bottom of the bar. All spores that were immersed in LN after the pre-treatment did not germinate and are thus ommitted from the figure. Different letter groups indicate statistically significant differences among treatment groups within species (determined using using paired Wilcoxon rank sum tests). Treatments with the same letter are not significantly different from each other. A p-value of < 0.05 was considered statistically significant for all tests.

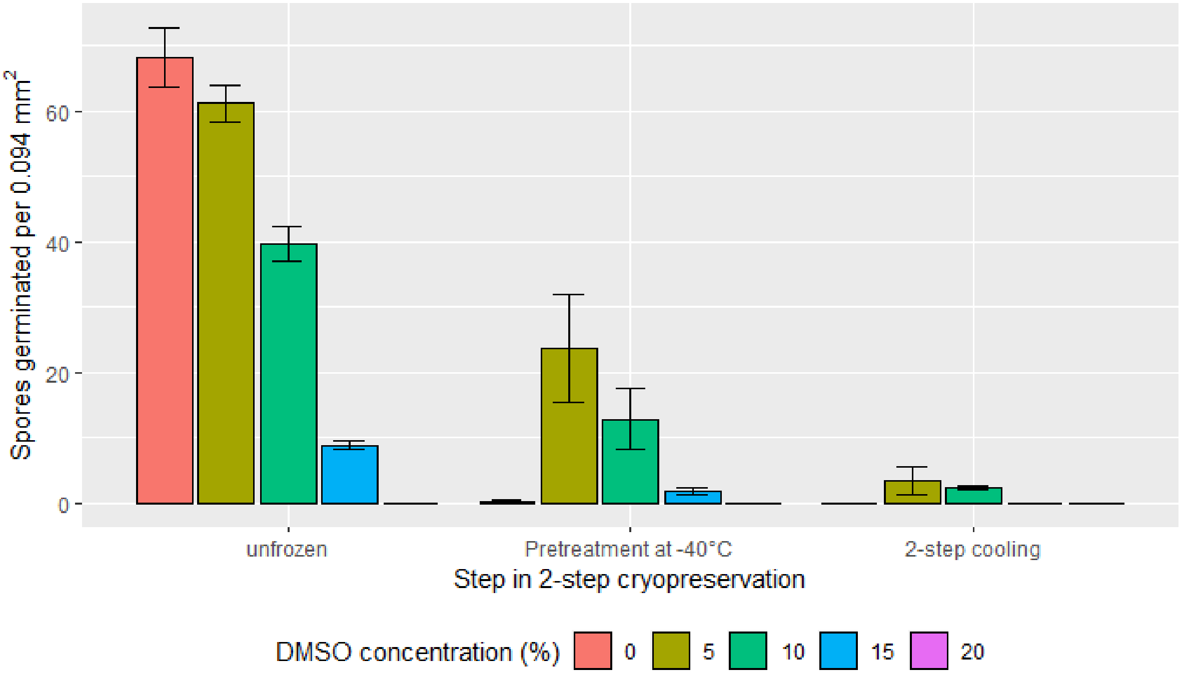


Figure 4-8: *Laminaria hyperborea* spores germinated per 0.094 mm^2 area after exposure to different concentrations of the cryoprotectant DMSO. Spores were suspended in 0 to 20% DMSO in seawater, cooled to -40 °C at -1 °C /min in a pre-treatment and then plunged in liquid nitrogen with 2-step cooling. Values are presented as mean ± S.E (n=5)

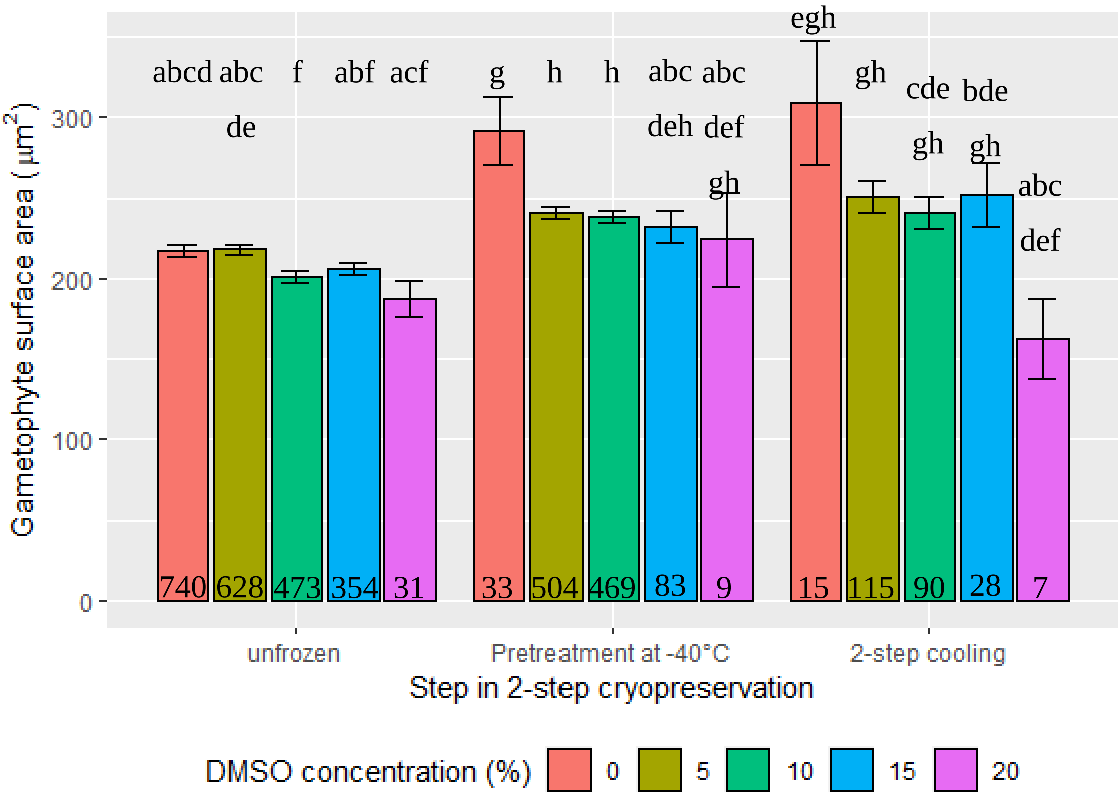


Figure 4-9: Gametophyte surface area of *Laminaria hyperborea* after exposure to different concentrations of the cryoprotectant DMSO. Spores were suspended in 0 to 20% DMSO in seawater, cooled to -40 °C at -1 °C/min in a pre-treatment and then plunged in liquid nitrogen. Values are in mean ± S.E. Number of measurement shown at the bottom of the bar. Different letter groups indicate statistically significant differences among treatment groups within species (determined using using paired Wilcoxon rank sum tests). Treatments with the same letter are not significantly different from each other. A p-value of < 0.05 was considered statistically significant for all tests.

L. hyperborea spore germination was highest under the control treatment (0% DMSO and not frozen) at an average of 68 germinated spores being visible per field of view of the microscope (Figure 4-8). Putting the spores through the -40°C pre-treatment or the full 2-step cooling process led to a severe reduction in germination rates, with a 65% and 95% reduction (compared to control) for the most successful treatments (both at 5% DMSO treatment). Higher DMSO concentrations reduced germination in the unfrozen samples, but their absence in the freezing treatments led to almost no spores surviving.

Size of the *L. hyperborea* gametophytes was higher when meiospores had not been exposed to DMSO (Figure 4-9). Mean gametophyte size was higher in the spores that had been frozen than the spores exposed to the same DMSO concentrations but hadn't been frozen.

Discussion:

Sori storage effects on release and quality of kelp meiospores

I found that meiospore quality was affected by sori desiccation period, and that this effect was not the same for *L. digitata* and *L. hyperborea*. The number of spores released, spore attachment, and germ tube growth of spores of *L. hyperborea* was much higher than that of *L. digitata*. In *L. digitata* spore attachment was higher when when sori was stored for 72h, but the number of spores released was highest at 24 hours. The increased attachment at 72h did not offset the lowered number of spores released, leading to the conclusion that for *L. digitata* the optimal desiccation period is 24 hours. In *L. hyperborea* spore release and germ tube growth was highest when sori was desiccated for 48 hours, but spore attachment was higher when desiccation time was 24 hours or less. For *L. hyperborea* the recommended desiccation period between 24 and 48 hours. This study provides one of the first results into the optimisation of zoospore release of *Laminaria digitata* and *Laminaria hyperborea* (see review by (Alsuwaiyan et al. 2019)).

Current protocols recommend desiccation periods of 18 to 24h for *L. digitata* (Edwards and Watson 2011) and 14 to 24h for another member of the family Laminariaceae, *S. latissima* (Flavin et al. 2013). No manual for spore release of *L. hyperborea* was found. These desiccation periods are roughly similar to the optimum found in this study.

One of the few studies done on the optimisation of sorus spore release took place on *Lessonia nigrescens* and *L. trabeculata* (Fonck et al. 1998). In *L. nigrescens* desiccation did not affect spore release until 24h, when it was reduced by over 50%. But in *L. trabeculate*, spore release was at its highest after 12h and was reduced by over 90% when desiccated for 0 or 24 hours. However, the germination rates of these spores started to become heavily reduced with longer sorus desiccation times. This study has similarly found large differences in spore release between species of the same genus, as well as an effect of sorus desiccation time on spore release. But contrary to the findings by Fonck et al. (1998) spore germination rate in this study was unaffected by desiccation time. These results indicate that sorus desiccation time can have differing effects on spore release and spore quality parameters (e.g. germination rate, attachment success, and germ tube growth) depending on species.

The quality of the spores in this study could be influenced by seasonal or environmental effects that were not considered. Spore release after sori storage for 0h was similar to spore release from sori stored for 24h in *L. digitata*. While the reproductive period of *L. digitata* is between April and December, with an optimum in late summer (Bartsch et al. 2013), the temperature on the shore on the sampling day for *L. digitata* was 20 °C. This is high enough to damage the cellular structure of the blade (Simonson et al. 2015) meaning the sori could have experienced stress before the start of the experiment which could have negatively affected spore quality as well as induced spore release earlier after desiccation. The meiospore germination rate in this study of 30% was lower than was found in other studies (Olischläger and Wiencke 2013; Zacher et al. 2016). The number of spores released per fertile area is known to vary throughout the year (e.g. (Joska and Bolton 1987)). The germination rates of spores of *L. digitata* (Olischläger and Wiencke 2013), growth rates of *Saccharina latissima* (Nielsen et al. 2016a) and *Ecklonia radiata* (Mohring et al. 2013) also vary based on time of year. Current protocols to obtain spores from wild kelps often mention a reproductive period, but do not mention that spore quality can depend upon time in the reproductive period as well (Edwards and Watson 2011; Flavin et al. 2013; Redmond et al. 2014). It is unknown if the seasonal and environmental effects would have influenced the spore quality parameters of *L. digitata* between different treatments, but it likely influenced overall spore quality and could have obscured the effect of desiccation time on number of spores released for *L. digitata*.

A major limitation in this study is that only two replicates were used for the calculation of spores released from the sori. The reason for this was a practical limitations in manpower and equipment available in the lab. It takes approximately 5 minutes to count the number of spores in a sample using the method from this study. In this study the effects of sori storage time on spore release was one of the main research goals, I therefore prioritised the amount of detail I could collect on spore release over time, rather than the amount of replicates (see Figure 4-2 and Figure 4-3). This study provides preliminary evidence that most *L. hyperborea* spores are released soon after the sori is put back into the water after the desiccation period, while this is not the case for *L. digitata* (Figure 4-2 and Figure 4-3). These results would need to be corroborated by future studies to see if these conclusions hold and that this is not because of seasonality or low number of replicates.

Some of the differences in the number of spores released per unit of fertile area between *L. digitata* and *L. hyperborea* can be explained due to differences in spore production of these two species. The annual spore production of a *L. digitata* individual is estimated to be less than 4% of a *L. hyperborea* (Chapman 1984; Joska and Bolton 1987). The reason for this difference has not yet been studied. Both germination rate and number of spores released was several times higher for *L. hyperborea*, but *L. digitata* showed a higher germ tube growth. This could indicate that *L. digitata* is better at establishing a spot on the coast, but more spores of *L. hyperborea* would find a place on the shore due to their higher numbers. Both species have different optima for light, temperature, salinity, and other factors that would lead to differences in habitat suitability (Westmeijer et al. 2019).

The release of zoospores in current experimental protocols is likely to be more reflective of trial and error method in a lab setting, than a biological basis (Alsuwaiyan et al. 2019). Currently desiccation is thought to apply an osmotic shock to induce meiospore expulsion (Alsuwaiyan et al. 2019), but the mechanisms behind spore release are not fully understood. In nature, sporogenesis could weaken the tissue, making tissue erosion part of the strategy for spore release (de Bettignies et al. 2013). To better understand how spore release is triggered in different species we need to quantify the zoospore density over time and relate this to changes in environment. Some success has been found in spore quantification by using quantitative Polymerase Chain Reactions (qPCR)(Nagasato et al. 2020). This research so far has only been done on species endemic to Japan, and more research is needed on how zoospore release depends on seasonality, environmental factors, or other variables in nature.

This study provides one of the first examples of an attempt at optimisation of the release of zoospores, and tests on how spore quality is influenced by sori desiccation time.

Besides desiccation time, a lot of other variables, including temperature, light conditions, cleaning method, spore release medium, and species effects, have been studied in a limited capacity or not at all (Alsuwaiyan et al. 2019). As there are over 400 studies utilising zoospore release protocols, the release and quality of spores is essential to the understanding of some of the ecological effects of environmental factors. In industry application a maximal spore release can also help in the creation of seaweed farms. For the species tested here, the recommended desiccation time is between 24 and 48 hours.

Cryopreservation of kelp spores using DMSO

This study found that the concentration of the cryoprotectant can help in the survival of *L. hyperborea* spores when frozen in liquid nitrogen, but that most spores will not survive the process. This treatment was ineffective in preserving *L. digitata* spores since no viable spores were found after cryopreservation. For *L. hyperborea* the optimal concentration of DMSO to act as cryoprotectant is 5% of culture medium. Gametophyte size of both *L. digitata* and *L. hyperborea* gametophytes showed a high level of variation within treatments. There seemed to be a small negative effect of increased DMSO concentration though this was not always statistically significant. This is one of the first studies attempting to preserve kelp meiospores through cryopreservation. This study provides one of the first experiments on the ability to preserve phaeophyte spores (see review by (Yang et al. 2021)), as well as the first study where any materials of *L. hyperborea* were cryopreserved.

In cryopreservation, a variety of different approaches are available. These include choice of life stage (sporophyte, meiospore, gametophyte), cryoprotectant, and cryoprotectant concentration. So far most of the studies on brown algae have focussed on using gametophytes (Yang et al. 2021) since studies on using sporophyte materials is generally less successful as extracellular ice crystals formation can disrupt the tissue (Wolkers and Oldenhof 2015). This study is one of the few that used spores. Small cells such as spores are thought to be easier to preserve, for instance *Ulva rigida* gametes could be preserved longer at sub-zero temperatures than thalli or germlings of the same species (Gao et al. 2017). However, in this study most spores did not survive the freezing process. While there have not been studies on the spores of either *L. hyperborea* or *L. digitata*, there has been a cryopreservation experiment on the meiospores of *Saccharina japonica*. Here the highest viability after cryopreservation was 50% (Zhang et al. 2007b). Yet the viability for gametophytes was even higher at 84% (Zhang et al. 2007c). While these studies had slightly different approaches, it could be that the composition of kelp spores make them less suitable for cryopreservation. Though there is still only limited evidence.

The optimal concentration of the cryoprotectant DMSO of 5% for *L. hyperborea* was lower than optimal concentrations found in other studies, and higher concentrations were more lethal in this experiment. Half of all *S. japonica* spores remained viable when pre-treated with 10% DMSO for 15 minutes (Zhang et al. 2007b) and the same concentration was optimal for the cryopreservation of gametophytes of *S. latissima* (Visch et al. 2019). The toxicity of DMSO alone (without any freezing) for *S. japonica* spores reduced viability by only 3% with a 5% (v/v) DMSO pre-treatment of 15 minutes (Zhang et al. 2007b). Choice in cryoprotectant type and concentration can be essential in affecting survival of the germplasm. The effectiveness of a cryoprotectant is dependent on three properties: firstly, the compound must be soluble in water and remain soluble at low temperatures. Secondly, it must have a low toxicity so that it can be used at high enough concentrations to have an effect. And thirdly, it must be able to penetrate into the cells through the cell membrane, so generally small polar but uncharged substances or hydrophobic molecules work best (Wolkers and Oldenhof 2015).

A limitation in this study was the fact that DMSO could not easily be removed from the spore solutions after cryopreservation. When thawing the cryopreserved materials any cryoprotective additives will need to be removed. Usually this is done by diluting the samples and washing of the algal materials with culture medium (e.g. (Lalrinsanga et al. 2009)) or by centrifugation and removal of supernatant (e.g. (Zhou et al. 2007; Piel et al. 2015)). Here the spore solution was diluted, but complete removal of the cryoprotectant was not possible, with around 1.25% (v/v) of DMSO still present in the final growth medium for the treatment with 20% DMSO. This could have contributed to mortality after cryopreservation. Though the cryoprotectant was not completely removed, the 16-fold dilution was better than those used in similar studies where 6-fold (Kuwano et al.

2004; Nanba et al. 2009) or 7-fold (Lalrinsanga et al. 2009) dilutions were used with similar concentrations of cryoprotectants.

Besides cryopreservation, other methods exist where kelp materials were stored for longer periods of time. These have explored the cold storage of *Macrocystis pyrifera* and *Fucus edentatus* at non-cryogenic temperatures with mixed effectiveness (Bird and McLachlan 1974; Barrento et al. 2016). In addition, the spores of *Undaria pinnatifida* can also remain viable for over 14 days when kept at 18 °C (Forrest et al. 2000) and the "over-summering" of gametophytes and microscopic sporophytes (Lee and Brinkhuis 1988) indicate that macroalgal gametophytes can survive for long periods of time (Schoenrock et al. 2021). This potential can be further explored, potentially in combination with the induction of freezing tolerance (Bird and McLachlan 1974; Ben-Amotz and Gilboa 1980).

Conclusion:

This study has investigated the protocols surrounding spore release, and spore cryopreservation.

Meiospore release was maximal when *L. hyperborea* sori was stored for 48 hours, or 24 hours for *L. digitata*. The germination rate of the meiospores released by sori of both species was unaffected by storage time. For maximal spore release the sori of these species should be stored between 24 and 48 hours. But future protocols should also mention that the quality of spores obtained can differ throughout the reproductive period.

Considering the 100 and 95% mortality in this method of meiospore cryopreservation of the two macroalgae species tested in this study, a vast number of spores would need to be stored to have enough to start a seaweed farm, or for the preservation of samples for biodiversity. This indicates that this method would need to be further refined before being successfully applied. Options for additional studies could be to use different life stages or cryoprotectants, or to further develop completely new techniques not based on cryopreservation that are used in the storage of other materials.

Chapter 5 - General discussion

5.1 Introduction

Seaweed farming has the potential to provide a food and biomaterial resource that is currently not fully utilised in the western world. Since they do not require land, fertilisers, or pesticides they could help us meet the increasing food demand for a growing world population, while at the same time improving water quality parameters locally by reducing eutrophication and acidification. Many questions still surround the seaweed aquaculture industry in the UK and Europe. The broad aim of this thesis was to increase the knowledge base surrounding the cultivation and ecosystem services of kelp species endemic to western Europe. The results of the studies performed have addressed several of the key questions about the successful cultivation of kelps, including questions on site selection, morphology, effects of water composition parameters, and obtaining spores for further cultivation.

To help seaweed aquaculture in the UK and Europe to become a success we need to have a deeper understanding of how to best farm kelps. The main objectives of this thesis were:

1. The development of a model to predict what sites would be suitable for a *Saccharina latissima* seaweed farm, particularly in an estuary.
2. To assess the effects of salinity and nitrate concentrations on the settlement, germination, germ tube growth, and gametophyte growth of *Saccharina latissima*, *Laminaria digitata*, and *L. hyperborea*.
3. To describe the changes in morphology of *Saccharina latissima*, *Laminaria digitata*, and *L. hyperborea* over the seasons, and describe how these morphological traits correlate.
4. To assess two options for obtaining kelp spores: one focussing on storing kelp materials long term as a seedbank, in which the effectivity of the cryoprotectant DMSO was tested in the cryopreservation of kelp spores. And one focussing on how to obtain the maximal amount of spores from reproductive materials through measuring the effects of sori storage time on the germination rate and early growth of kelp spores.

These objectives were explored through a variety of methods including: the application of models, collation of public data, literature review, observational studies, and experiments. Here I present a summary of the results of this thesis (ch 5.2), followed by a discussion on how these findings match current literature (ch 5.3), and in conclusion give recommendations on how to advance with this knowledge, as well as speculate on how the future of seaweed aquaculture and seaweed ecology will change in the coming years (ch 5.4 and 5.5).

5.2 Summary of thesis results

In **Chapter 1** it was found that by modelling the seaweed growth of *Saccharina latissima* a reasonable expectation could be made into how well individuals of this species could grow in specific environmental conditions. Some of the limitations of this method are that the model required a large amount of environmental data measurements which would not be known at many potential sites. The model is currently also only available for one species. Though it could be relatively simple to simulate a different kelp species by changing some of the variables in the model, this new model would then need to be verified again.

The effects of the water quality parameters nitrogen content and salinity on the settlement, germination and growth of kelp spores was the topic of **Chapter 2**. The effects of salinity and nitrate were similar between species but differed between life stages. Reductions in salinity had a negative effect on germination rate, while reductions in nitrate concentration impacted growth. Though using gametophytes as the basis for growing kelps is still being studied (e.g. (Forbord et al. 2020b)), the results nevertheless indicate that a large section of an estuary, where salinities are reduced and nitrate concentrations are increased, could provide the environmental conditions suitable for setting up a seaweed farm. It should be noted that there are other environmental, technical and social factors that would determine a site’s true suitability for a farm. A question remaining from this study is whether it is the reduction in salinity that is an issue in seaweed growth, or if it is the fluctuation in salinity. Consistently having to adapt intracellular osmolarity would be a source of stress on the individual that could negatively impact both survival and growth of kelps and this requires further study.

The morphology of kelps contributes to both the ecosystem services they provide, as well as their potential as a food (ingredient). I investigated the changes in morphology of kelps on the shore over a two-year period to see what the seasonal differences were in **Chapter 3**. The findings line up with some of the research that has been done on erosion and biomass of the kelp species investigated. While the population of *L. hyperborea* had a sizable blade and a long stipe throughout the year, this was less the case for *L. digitata* and *S. latissima*. This means that *L. hyperborea* can provide many of its ecosystem functions throughout the year where other species could not.

The acquisition of kelp spores was the topic of **Chapter 4**. Here I studied the potential of storing kelp spores of *L. hyperborea* and *L. digitata* by using the two-step cooling method with DMSO as a cryoprotectant, as well as the effect of sori storage time on spore release, settlement, and growth. While the cryopreservation method in this study has had some success in the past, all *L. digitata* spores and the vast majority of *L. hyperborea* spores were no longer viable after cryopreservation regardless of DMSO concentration. This indicates that this method is unlikely to be cost effective for spore storage and for the successful preservation of kelp reproductive materials other techniques need to be investigated. The storage time of sori had an impact on the number, as well as the quality, of the kelp spores they released. For both *L. digitata* and *L. hyperborea* spore release was optimal with a storage time of 24 to 48 hours. These results are similar to current methodologies advised in kelp farming manuals even though there is still a lack of published work corroborating the method from these manuals.

5.3 Overview of findings in the context of current literature

Site selection and environment

This thesis expands upon an existing model that can be further developed to help potential farmers, governments, or other stakeholders select a good site for a seaweed farm. Earlier research exists where methods for site selection for aquaculture were developed and/or applied. These vary wildly in species used, spatial resolution of results, target audience, and whether they include legal and/or social factors. Most of the current literature focusses on site selection by applying a multiple-criterion selection method based on social, economic, and environmental factors (e.g. (De Sousa et al. 2012; Liu et al. 2013; Thomas et al. 2019). The issue lies in how environmental factors are incorporated into the decision-making process. In this method a site parameter, such as nutrient concentration, is given a single numerical score (for instance from 1 to 8, like in (Liu et al. 2013)). However, parameters such as nitrogen concentration, temperature, and turbidity often change throughout time, meaning that a single value for a location could potentially not accurately represent monthly, or even hourly, changes in the system. Other site selection studies have solved this issue by adopting ranges for environmental variables (from unsuitable to suboptimal to optimal) and looked at climatologies of environmental variables, ensuring that extreme values remain within a species their tolerance limits (Marine Management Organisation 2019; Kershaw et al. 2021). In this thesis I opted for a different approach, using a seaweed growth model instead of applying a multi-criteria evaluation. There have been other studies that applied seaweed growth models to multiple sites (Broch et al. 2013, 2019; Van Der Molen et al. 2018; Fossberg et al. 2018; Aldridge et al. 2021) but these have mostly focussed on bioremediation (Broch et al. 2013; Fossberg et al. 2018), or were dependent upon 3-D hydrodynamic/biogeochemistry models that are not widely available everywhere (Van Der Molen et al. 2018; Broch et al. 2019). This thesis provided a suggested methodology for site selection that is possible for sites if there is data available on water quality parameters.

While a variety of seaweed models exist (e.g. (Lehre Seip 1980; Gagné and Mann 1987; Friedlander et al. 1990; Lee and Ang 1991)) most of these models do not factor in enough environmental factors that prediction of a good site is possible. Currently only the models of *Enteromorpha* sp. (Martins and Marques 2002), *Ulva lactuca* (Lamprianidou et al. 2015; Lavaud et al. 2020), *Undaria pinnatifida* (Murphy et al. 2016), *Saccharina latissima* (Broch and Slagstad 2012; Venolia et al. 2020), and *Saccharina japonica* (Zhang et al. 2016) take environmental factors into account enough that they can be meaningfully used for site selection. Further models would need to be developed for this method to be applied in the site selection of different seaweed species.

As a part of this thesis the effects of salinity and nitrate on spore settlement and gametophyte growth were also studied. These were studied from the point of view of applicability for site selection, but these factors also tie into the distribution of seaweeds. The first steps a kelp spore must overcome is to establish itself on the coast, after which it turns into a gametophyte and needs to grow and produce sperm or an ovum. Only a very small percentage of kelp spores end up contributing to the establishment of a full-grown sporophyte (an estimated two in a million for *L. digitata* (Chapman 1984)). With kelp forests under threat by environmental impacts (Mineur et al. 2015; Smale et al. 2019) we need a good understanding of their establishment so we can protect them, and in some cases re-establish them. There are a variety of projects working on kelp forest restauration (Layton et al. 2020; Fredriksen et al. 2020).

A potential alternative to using multi-criteria selection, or modelling growth for site selection could be by applying species distribution modelling. Species distribution models combine occurrence or abundance with environmental data to predict where a species could be established (Elith and Leathwick 2009). Effectively this is very similar to the identifying the suitable environmental parameters for seaweed cultivation. Of course, there would be differences in how these models would need to be applied, for instance depth is often used in seaweed distribution models (Yesson et al. 2015a; Marcelino and Verbruggen 2015; Jayathilake and Costello 2020), which would be less of an issue when using seaweed farms (potential farming sites can be pre-selected relatively easily to be deep enough to ensure that blades do not touch the seafloor, yet not so deep that anchoring costs become too expensive). Species occurrence data (global data available through Global Biodiversity Information Facility) can be combined with a species distribution modelling program like MaxEnt and environmental data sets (Assis et al. 2018). Through this relatively low-effort method sites suitable for seaweed cultivation could be found.

An additional benefit to using this method is that it would be very easy to make additional predictions as to how site suitability would change because of climate change. Species distribution modelling compares presence data with environmental factors at that time. By using predictions for temperatures and nutrients for future climate change scenarios the suitability of a site in the future could be predicted.

Morphology

Ecosystem services provided by kelps are tied together with their morphological traits. Understanding how morphology changes through time and environmental effects is important to understand how ecosystem services will be impacted. The seasonal differences in blade size between the kelps *L. digitata, L. hyperborea* and *S.latissima* are not the same (this study). Besides environmental factors, factors such as age and location on the shore also influence the morphology of kelps (Kain and Jones 1963; Teagle et al. 2017).

Algal morphology does not only impact their ecosystems functions, but also their marketability. For instance through the substantiality value, a simple index for *Laminaria* species cultivated in Asia in order to assess the blade quality for human consumption (Peteiro and Freire 2009). Plus, logically a larger seaweed would sell for more. Wild harvesting is currently still a source of seaweeds globally (0.9 million tonnes in 2018)(Chopin and Tacon 2020). Understanding how environmental impacts affect kelp forests, total biomass of the standing stock, individuals, and their traits helps in making decisions how much of the kelps can be harvested without impacting their ecosystem services excessively. If seaweeds are harvested from the wild, their ecosystem services must be balanced with their value as a product to ensure that the seaweed is not overharvested and the value from collecting the seaweeds does not outstrip the value of the services the seaweeds provide on the shore. Ecosystem services are often not factored into the valuation of seaweeds on the shore.

Spore preservation

In Norway on the island of Svalbard stands the Global Seed Vault, with the aim to store backups of seed samples of the world's crop collection (Westengen et al. 2020). Currently there are over one million samples in the vault, but seaweeds are still missing from their collection because there is not currently a way to store seaweeds effectively in a simple way for long term. The fact that we cannot store seaweeds long term has several consequences: we cannot set up a seed bank, there are extra challenges with cultivation of cultivar variants, and selling and trading of kelp material becomes more difficult. In Asia they have already implemented a variety of cultivars of seaweeds (Hwang et al. 2019). Domestication of a crop is associated with genetic bottlenecks and reducing diversity (Loureiro et al. 2015; Valero et al. 2017). Conserving the crop wild relatives- the wild plant taxa related to a crop- will help in maintaining a wide variety of genes. In the future, depending on how seaweed farms are managed and how widespread they will be, genes from cultivars could get into the wild population reducing the genetic diversity of the species altogether. The risk of cross-breeding wild and farmed seaweeds has been highlighted as one of the potential environmental impacts of seaweed farming (Campbell et al. 2019) and is one of the reasons why fertile material to start farms in the UK is normally collected in close proximity to the farm.

Currently cryopreservation seems to be the most promising option to store kelps long term. There have been a wide variety of studies on all sorts of materials and life stages of seaweeds (Yang et al. 2021). In multicellular tissues any extracellular water results in the formation of ice crystals, disrupting the tissue (Wolkers and Oldenhof 2015). This is especially an issue with storing thick thallus. In species with thin thallus, the extracellular water can be more readily removed, especially in species which have evolved to tolerate a higher amount of desiccation such as the genus *Ulva* and *Porphyra/Pyropria*. For instance *Porphyra umbilicalis* can be stored at -80 and -20 ^{o}C for 12 months with 100% survival (Green and Neefus 2014). For the species with thick thallus other life stages need to be stored. Considering that in smaller cells the intracellular water can be extracted more rapidly during freezing than larger cells (resulting in less intracellular ice) (Dumont et al. 2004), small cells such as spores were thought to be easier to preserve. The findings of this study contradict this hypothesis. Survival for both species tested was generally much lower than for the storage of gametophytes in similar protocols (Yang et al. 2021).

This thesis also discussed the optimisation of spore release in kelps. Further optimisation would allow seaweed farmers to get larger quantities of spores more easily. Currently the number of spores available is not a concern. The number of spores released from a single individual can, under the right circumstances, provide enough spores to set up a whole farm (Chapman 1984; Edwards and Watson 2011). It is possible that in the future when certain cultivars are used, these spores might be more valuable. But even in that situation the number of spores from a single individual would still be a lot. Because of this, there probably has not yet been a need in the seaweed industry to develop optimal methods for obtaining large amounts of spores.

5.4 Identifying challenges ahead and recommendations for future research

Economy

Seaweed farming has the potential to provide an additional food source and help us meet our goals for sustainable growth in the marine and maritime sectors (Duarte et al. 2022). Kelp farming in the UK and in Europe in general is still in its early stages, and there are technical, legal, and economical barriers to overcome for seaweed cultivation to become a successful industry (Huntington and Cappell 2020; Vincent et al. 2020).

One of the things holding back a further growth of the seaweed aquaculture industry in Europe is its profitability. Reports often find that the feasibility of a seaweed industry is tied into the market value of the seaweeds (Van Den Burg et al. 2016; Camus et al. 2019). To improve the profitability of seaweed production seaweed farmers, researchers and other stakeholders can focus on two broad tactics: reduce costs of production or increase the value of the product.

Increasing the value of seaweed products

The value of seaweed varies widely depending on their application, with their value as pharmaceuticals, cosmeceuticals, and nutraceuticals being the highest per kilogram (Chopin and Tacon 2020). Followed by that is their value as an ingredient or food for human consumption and this is how most of the biomass of farmed seaweed in the Chinese market is used (Hwang et al. 2019). Seaweeds are generally not part of the average European diet, but in Asia they are regularly eaten as part of the diet (Nisizawa et al. 1987; Hwang et al. 2019; Tanaka et al. 2020). While the aquaculture of seaweeds in Europe is still in its early stages, aquaculture in Asia has had a longer history going back to the 1940s. In particular, Korea, Japan and China are already applying advanced techniques to breed cultivars of their most farmed seaweeds (Hwang et al. 2019). In the meantime, seaweed cultivation in Europe and the Americas has remained focussed on how to grow seaweeds in a way that is profitable (Van Den Burg et al. 2016; Camus et al. 2019; Hasselström et al. 2020). Another challenge for economic feasibility of seaweed aquaculture in western countries has to do with the high costs of the installation and material for the farm, as well as high hourly wages, increasing production costs compared to China and Indonesia (Van Den Burg et al. 2016). In Japan wages are higher than in other countries in Asia, and more equipment in required. Due to higher production costs the cultivation of *Porphyra* is only possible because it has a hight market price and is appreciated as an integral part of Japanese cuisine (Edwards and Watson 2011). All in all, currently aquaculture of seaweeds in Asia has a bigger market, a bigger output, and a larger knowledge base than in most western countries. In my opinion, these factors are underestimated in much of what is being reported in the news about the European seaweed industry (for examples see (Chang 2007; Azania Jarvis 2015; Quinn 2018; Marshall 2020)). But so far seaweeds have not had as explosive a growth as these newspapers predicted, nor have they really stepped into the marketplace. This could have to do with the level of familiarity the public has with seaweeds. It is still often seen as a "weed" or "slimy", and even for those interested in cooking with seaweeds there are limited options available for purchasing European seaweed species(personal observation).

There are opportunities for increasing the perception of seaweeds and seaweed farming though. In Sweden, when asked about their opinions on aquaculture most respondents chose positive or neutral responses. Groups with a higher awareness of aquaculture tended to be more positive than those with a low awareness. More respondents were supportive of aquaculture on the Swedish west coast (over 80% positive for seaweeds compared to 60% positive for aquaculture in general) (Thomas et al. 2018). There could be potential benefits in increasing awareness and understanding of seaweeds, as well as promoting new or traditional dishes featuring local species (Mouritsen 2017; Rioux et al. 2017; Rees 2019).

Seaweeds have distinct olfactory properties that need to be taken into account when incorporating them into other foods (Vilar et al. 2021). There is potential in seaweeds being used as a novel ingredient in forms of western cuisine as a source of umami taste where it could be used in dishes like ice cream, fresh cheese, or bread (Mouritsen et al. 2012). Much of the negative perceptions of seaweed could stem from an unfamiliarity with it as a food product and could be improved upon by further education and marketing. While there has been a rise in popularity of foods with seaweeds, this has mostly been focussed on Asian dishes (e.g. sushi, ramen) containing seaweed species endemic to Asia (Winberg 2011). In Western-Europe we have species that are similar in morphology and ecosystem function to those widely cultivated for food in Asia. A variety of seaweeds grow on the shores of the UK that could be analogous to some of the popular seaweeds in Asian cuisine (Table 5-1). Yet the popularity of seaweeds is still much less than in Asia and seaweed products tend to only be available to consumers in the UK from specialist shops (Bouga and Combet 2015). Growing this market segment could increase the profitability of farming seaweeds. Developing new products, based on European seaweeds, could contribute to this (Mouritsen 2017).

Besides food applications, seaweeds also contain compounds which are used as pharmaceuticals, nutraceuticals, and cosmeceuticals. These components are the most valuable seaweed based products with the highest price per kilogram (Chopin and Tacon 2020). The potential of seaweed-based products as pharmaceuticals is significant, and further research is needed to fully explore their potential. This could be achieved by either further developing the methods to process seaweeds to extract valuable components, or improving the level of evidence for effectiveness of seaweed pharma- and nutraceuticals (Hafting et al. 2015). A high level of evidence is required by regulatory agencies before health claims can be made to aid marketing and without these claims the highest return on investment may not be achieved by investors(Hafting et al. 2015).

Table 5-1: Overview of seaweeds produced for food/feed by species in 2018 and potential European homologue (FAO 2020; Chopin and Tacon 2020) Omitted from this table are the genera *Eucheuma*, *Gracilaria*, *Kappaphycus* since these are mostly used to produce agars and carrageenans. These three genera provided a combined 44.6% of the total global seaweed aquaculture production in 2018.

% of global seaweed production (by weight)	Species (common name)	Potential European alternative (common name(s))
35.3	*Saccharina japonica* (sweet kelp, ma-konbu)	*Saccharina latissima* (Sugar kelp, Royal kombu)
7.2	*Undaria pinnatifida* (wakame)	*Alaria escuelenta* (Atlantic Wakame, dabberlocks, winged kelp)
8.9	*Porphyra/Pyropia* spp (nori)	*Porphyra/Pyropia* spp. (purple laver)

An alternative option is to include the ecosystem service valuation -placing monetary value on the services provided by the natural environment- as part of the product they provide. While not all values surrounding ecosystem services can be associated with a monetary value, doing so could give legislators and business stakeholders clearer incentives into investment in, and legislation concerning the environment. The value of a kelp forest can fluctuate depending on the species, density, size, services provided, and other factors (Williams and Davies 2019; Bayley et al. 2021). For instance, in the Falkland islands the value of a kelp forest is estimated at £3.24 million per km per year (Bayley et al. 2021), while in West Sussex it was estimated at £12.6 thousand per km per year (Williams and Davies 2019). Since cultivated kelps grow from suspended ropes instead of being attached to benthic substrates the services provided by kelp farms could differ substantially from those of kelp forests (Walls et al. 2016). For kelp farms there is limited information available on what ecosystem services they provide, what monetary value these would have, and if other parties – like government or fisheries - could or would provide additional funds to the kelp farm owner for these services.

One ecosystem service that is already being sold is carbon sequestration through carbon accreditation schemes (Godfrey 2022). Since kelps grow relatively quickly and store carbon they can aid in the local sequestration of CO_2 and help buffer eutrophic or acidic waters (Froehlich et al. 2019). Care needs to be taken that any carbon captured remains captured though, and currently there are not many options for the long-term sequestration of carbon. Compared to woody biomass on land the faster turnover rate of seaweed biomass limits its potential to act a long-term store for carbon (Troell et al. 2022).

Decreasing production costs with new techniques

In current research new ideas are proposed that could reduce production costs of seaweeds or have other benefits. The costs, possibility, and benefits of these techniques can be very location dependent. While what is presented below is not an exhaustive list, it does give an idea of current research ideas that are being developed.

The integration of seaweed farms with other industries, particularly other forms of aquaculture, can have synergistic effects. Integrated Multi-Trophic Aquaculture (IMTA) has shown to reduce the pressure on the environment since seaweeds or filter feeders would take up nutrients from the fed species (Roleda and Hurd 2019). It should be noted that IMTA does not always require a fed species though. Alternatively, seaweed farms could be integrated with offshore wind farms since this would provide a multifunctional use of a limited space (Buck et al. 2004).

There are proposals to develop currently unused areas as sites for seaweed cultivation. Currently most of the production of seaweeds takes place in coastal areas and shallow oceans. But there have been proposals to expand to areas further offshore (Buck et al. 2004; Lehahn et al. 2016; Fernand et al. 2017) and there has been some early success in offshore kelp cultivation (Bak et al. 2020). In areas where nutrients are limiting, local nutrient conditions can be increased with artificial upwelling which can increase yield of seaweeds and turn sites with low levels of nutrients into areas that are suitable for seaweed aquaculture. This technique has been tested in Asia (Fan et al. 2019), but not yet in Europe. There are also schemes suggesting land-based aquaculture where seaweed is grown in tanks or pools on land, either to grow transgenic or invasive species without risk of escapes (Qin et al. 2005), for local benefits to the environment (Iersel and Flammini 2010; Friedlander and van Rijn 2018), or to utilise low local land and labour costs (Iersel and Flammini 2010). Some seaweeds, such as *Ulva* or *Palmaria palmata*, may even grow better in tanks.

In Eastern-Asia the development of seaweed cultivars has already contributed to both quality of food as well as the total biomass produced (Hwang et al. 2019). At least 47 certified seaweeds have been used in commercial cultivation in Asia (Hwang et al. 2019), but in the seaweed industry outside of Asia studies into the creation of cultivars have been limited to only a few studies (Kraan et al. 2000; Umanzor et al. 2021). New cultivars can be selected to improve commercially valuable traits, environmental adaptability, and disease resistance (Hwang et al. 2019; Campbell et al. 2019).

Besides cultivation techniques, also new harvesting techniques are being considered. For wild seaweeds the technique and intensity of harvesting efforts influence the recovery of cut seaweed beds and their communities (Mac Monagail et al. 2017). Through harvesting the distal end of a kelp and leaving the meristematic tissue intact, the canopy can regrow more quickly, and the stipe and holdfast can keep providing their ecological functions (Thompson et al. 2010; Campos et al. 2021). For large wild seaweeds mechanical harvesting can be done through trawling, mechanised cutting, hand cutting/raking, or collection of beach-cast materials (Lotze et al. 2019). Trawling and dredging have negative impacts on the local environment and requires a fallow period to allow recovery of the kelp bed (Christie et al. 1998; Lotze et al. 2019). The impacts of applying mechanical harvesting techniques can depend on location, algal regenerative ability, and harvesting pressure (Wilding et al. 2021). Maximum sustainable levels of harvests can be variable too based on local environmental conditions (Mac Monagail et al. 2017).

Climate change and additional challenges

Climate change is going to change how seaweeds are grown (Fernández 2011; Harley et al. 2012; Chung et al. 2017), their species distribution (Assis et al. 2016; Küpper and Kamenos 2018) and ecosystem functioning (Pessarrodona et al. 2019). These changes are going to affect both wild kelp forests as well as aspects concerning commercial kelp cultivation.

Kelp forests are affected by climate change in a wide variety of ways including abundance (Yesson et al. 2015b), species composition. The stressors originating from climate changes can be interactive, making precise predictions of consequences of climate change difficult to accurately predict (Harley et al. 2012). Acidification and increases in temperature both affect the reproduction and early life-history of seaweeds

(Bartsch et al. 2013; Gaitán-Espitia et al. 2014; Xu et al. 2015). At a species level there are four responses possible to these changes in the environment: persistence without adaptation, persistence with adaptation, migration, or extinction (Harley et al. 2012). Future distributions of kelp forests are likely to change. Biodiversity and the ecosystems provided by kelp forests are under threat by ocean warming (Smale 2020).

How climate change will impact seaweed production is still unclear, much of the distribution of kelps is influenced by temperature maxima (Bartsch et al. 2013; Rogers-Bennett and Catton 2019; Arafeh-Dalmau et al. 2020). Seawater temperature is usually at its highest in late summer around August or September. With kelp farming the standard practice is to usually have harvested the seaweeds before that time (Edwards and Watson 2011; Flavin et al. 2013; Redmond et al. 2014; Campbell et al. 2019). There is little research available on how the changes in environment from climate changes – including increases in ocean temperatures, climate variability, acidity- will affect commercial kelp cultivation. Considering that climate change is expected to lead to threats to food security (Diouf 2009) and that we need to increase our production by 25 to 70% by 2050 (Hunter et al. 2017) the effects of climate change on seaweed cultivation need to be better understood.

Somewhat ironically, seaweeds are touted as both a victim of climate change (Babcock et al. 2019), and as a possible solution to it (Chung et al. 2011; Froehlich et al. 2019). Since climate change is expected to lead to higher temperatures, greater rainfall variability, and more frequent extreme weather events some of our current food production systems will be under threat (Diouf 2009). Some of these pressures can be alleviated by alternative food sources that would not be affected by these pressures to the same degree. The large production or seaweeds currently seen in particular in China and Indonesia (FAO 2020) take up large amounts of nitrogen and phosphorous that could have otherwise eutrophied sections of the nearby ocean (Jiang et al. 2020). While seaweed cultivation is not expected to offset the carbon emissions from global aquaculture (Froehlich et al. 2019), the CO_2 absorbed does help in the sequestration of carbon for a short term. In the long term this carbon would be released into the atmosphere again unless moved into long term storage, for instance the deep-sea

(Krause-Jensen and Duarte 2016), or by turning it into biochar (Zhang and Thomsen 2019). It should be noted that the potential for seaweeds to permanently sequester carbon at a meaningful geological timescale is still uncertain (Troell et al. 2022). While forms of carbon offsetting can be part of an individual or company strategy to help the environment, the main focus needs to remain on reducing emissions (Hooper et al. 2008) and the role seaweeds can play in reducing the amount of carbon released from food systems and other activities (Troell et al. 2022).

Future research

In much of the text written above, there were suggestions on specific research. In this section three more general avenues of research are suggested where I believe there is potential for further development that could improve both the seaweed industry and our knowledge on seaweeds in general: 1) kelp forest ecology and climate change effects, 2) seaweed chemical composition and applications, and 3) seaweed industry and marketability. What can be noted is that these research field encompass the concepts people, planet, profit. They also require an interdisciplinary approach. Collaborations between multiple areas of knowledge are essential in an area where there are many stakeholders and many people affected. Many of the issues that are currently facing the planet require more than just knowledge on nature, but also knowledge on marketing, economy, social aspects, chemistry, and more.

Kelp forest ecosystem services and climate change effects

Kelp forests provide a wealth of ecosystem services (Smale et al. 2013; Bayley et al. 2021), and some of these are provided by kelps farms as well (Kim et al. 2017; Hasselström et al. 2018). The exact nature, and variable surrounding these services are not always well understood. For example, in this thesis the seasonal morphology of kelps was studied. But it is unclear which ecosystem services would be affected by morphology. Can certain species provide different functions based on their morphological differences? And since climate change is expected to shift the distribution of many kelp species (Assis et al. 2016), would the new species provide the same services?

The ecosystem services provided by kelp forests could also be looked at from a more financial point of view: what monetary benefits are they providing? The capture of carbon, bioremediation, shelter provided to fish, aesthetic value, and other services provide benefits that are possible to quantify (Vásquez et al. 2014; Bayley et al. 2021; Hynes et al. 2021). Better understanding of the services provided could provide incentives for governments or businesses to invest in seaweed production. Since much of the local benefits of kelp farming include both biophysical as well as socio-economic effects, such as job provision, coastal protection, habitat provision, bioremediation, and carbon fixation. We need to better understand what these benefits are, in what situation they would arise, and who would be benefiting. This information could help businesses and governments to see the benefits of seaweed aquaculture and would make them more inclined to invest in seaweed farm projects.

One of the other ecosystem services attributed to kelps that is currently becoming increasingly more financially valuable is their potential to capture carbon. Multiple companies are already selling or advertising carbon offset certificates (e.g. The Seaweed Company, Carbon Kapture, Running Tide, Pull To Refresh, and Kelp Blue). But the long term storage of carbon in kelps is dependent on the end goal of the specific product (Hasselström et al. 2018). Some companies sell the seaweed they grew, which means the company no longer has any control on whether this carbon will remain sequestered, while other companies plan on sinking the seaweeds to the deep sea, where it is unclear what effects they will have on the local ecosystem, biochemistry, or whether they will remain in the deep sea. The sequestration schemes should be grounded in a solid scientific basis, which is currently not the case.

The distribution of kelp species is affected by climate change induced temperature increases (Simonson et al. 2015; Smale et al. 2019). Since kelp forests provide valuable services, we need to have a good understanding where they are and have tools to monitor changes to them. Currently kelp forest distributions can be observed through high resolution satellite imagery (Huovinen et al. 2020). At a smaller scale there is the potential to observe and quantify kelp forests in more detail with drones (Kellaris et al.

2019). Drones can be further explored as a potential method to monitor local kelp forests relatively cheap.

The carbon sequestration potential of kelp forests is currently not yet fully understood. Naturally growing seaweeds donate organic carbon to nearby other blue carbon habitats (seagrasses, mangroves, tidal marshes) where material can be buried in the sediment, or can be transported to deep ocean (Krause-Jensen and Duarte 2016; Jones et al. 2022). Much of the donation of organic carbon happens through the degradation of seaweeds into detritus (Gilson et al. 2021; Jones et al. 2022), but the factors influencing degradation needs further research. In addition, the sequestration of carbon from kelp forests into the deep sea is thought to be location dependent, but the factors influencing this sequestration are still not fully understood.

Seaweed chemical composition and applications

Seaweed chemical composition is influenced by environmental variables which can change depending on location and season (Gevaert et al. 2001; Schiener et al. 2014; Zhang and Thomsen 2019). The composition of a seaweed also affects its further processing (Milledge et al. 2014), nutritional quality (Wells et al. 2017), and palatability (Rioux et al. 2017). Seaweed composition has allowed it to be used as an ingredient in foods (Rioux et al. 2017; Okhotnikov et al. 2020), biofuel(Milledge et al. 2014), as hydrocolloids (Porse and Rudolph 2017), animal feed (Makkar et al. 2016), and a wide variety of other applications including cosmetics and pharmaceuticals.

Seaweed can provide a potential source for biofuel production, however the efficiency of this process is dependent upon its chemical composition (Fernand et al. 2017; Tabassum et al. 2017). Understanding how environmental factors affect kelp composition can help understand in creating the conditions to grow kelps for specific purposes. We also must face engineering challenges in the optimisation of design of kelp bioreactors for this application to become economically viable (Milledge et al. 2014).

Another application that is currently being explored is the use of seaweeds as a feed for livestock to improve production, reduce methane emission (when fed to cattle), and improve meat quality (Costa et al. 2021). Since a large section of land is being used for the production of animal feeds (Ritchie and Roser 2013) switching to marine based feed production would open up more land for other uses. In addition, feeding ruminants seaweed has been linked to reductions in methane emissions (Min et al. 2021). Since the release of 1 kg of methane is considered to have the same global-warming potential as that of 25 kg of CO_2, the emissions of methane should be reduced where possible (IPCC. Intergovernmental Panel on Climate Change 2014). Future research should focus on how the incorporation of different seaweeds into the diets of livestock animal will affect their growth, health, flavour, and carbon impact.

The potential for genetic improvement of seaweeds is great. Some countries in Asia have already successfully developed cultivars of seaweeds (Hwang et al. 2019). Crops like tomatoes, maize, other, have had a long history of crop improvement, but seaweeds are still in the early stages of this process. Different cultivars could be made that would suit different end users, for instance with increased hydrocolloid concentration or specific morphological traits (e.g. stipe length, blade size).

Seaweed industry and marketability

There is a rising interest in expanding sustainable marine production in Europe as part of the European Blue Growth strategy (European Commission 2021), but currently the legal and economic feasibility of seaweed farming is still unclear for potential future seaweed producers making it difficult for them to get financial backing. This is slowly changing. Phyconomy, a project to track the emerging economy of seaweed in the western world, found that investments into seaweed doubled from 2020 to 2021 (17 to 34), with the amount invested growing by 36% (Phyconomy 2022). Stakeholders should encourage further collaboration between producers and established players in the food sector. There are indeed already initiatives and organisations in the UK aiming to create such networks between industry, academia and governments (e.g. The Scottish Seaweed Industry Association, the Seaweed Alliance, Algae Innovation Platform, Alga-UK). Direct engagement between producers and the food sector could encourage closer collaboration and bring in financial backing and a broader client base (Van Den Burg et al. 2021).

Seaweeds in Asia are a common food ingredient (Nisizawa et al. 1987; Hu et al. 2021), but in western Europe they are still not common. Sushi has become a widely available dish featuring seaweed, and ingredients like wakame and kombu are featured in some recipes available on British recipe websites (personal observation). Slowly more UK seaweeds are being incorporated into British cooking culture with some famous chefs featuring UK seaweeds in their cooking (Blanc 2021; The Hairy Bikers 2022). A notable exception that has traditionally been a part of British food culture is laverbread, a traditional Welsh food made by boiling *Porphyra* for several hours. By analysing cookbooks, recipe websites, and the availability of seaweeds in shops the presence and popularity of seaweeds in a country's food culture can be monitored.

In addition to the perception of seaweed food products the perception of seaweed farms so far seems to be neutral or positive (Thomas et al. 2018). However, this research has only been done in Sweden and might not be representative of acceptance in other countries.

5.5 Closing statement

We -as inhabitants of this planet- have our work cut out for us. Agriculture needs to provide for a higher amount of people with more high-quality food, that is both sustainable and does not require a high amount of land use. Seaweed aquaculture can contribute to achieving those goals.

Food and drink is currently contributing 17% to the greenhouse gas emissions in the EU and 28% of material resource use (European Commission 2011). In January 2020 a paper was published with over 11,000 scientists signatories from 153 countries highlighting the urgency of climate change, as well as the achievability of six proposed steps to lessen its worst effects (Ripple et al. 2020).

The problems facing our current generation are clear, but there are solutions. To reduce our impact on the planet we can reduce our consumption of animal products and restore ecosystems both on land and in sea (Aleksandrowicz et al. 2016; Li et al. 2021; Lim et al. 2022). Turning farmland back into their original ecosystem would be a part of that. Half of all habitable land is currently used for agriculture. If we pursue an agricultural model where seaweed aquaculture would replace part of terrestrial agriculture, we will be able to produce food, feed, and biomaterials without having to increase our use of freshwater, fertilisers, and land.

This thesis tackled some of the current questions and challenges in kelp aquaculture in the UK and Europe. From this work it has become apparent that while there is still more research to be done, there is also enough knowledge that from a biological point of view kelp farms can become successful. Were governments, businesses, and other stakeholders to invest in seaweed aquaculture the future will not merely look bright, it will look green, red, and brown.

Appendix:

Appendices for Chapter 1

Table S0-1: datasets collected with quantitative data about the Milford Haven Area

Database	Variables	Locations	Description	Timeframe	Source
Bathing Water Quality	Salinity	Group1, group3	Measurements of water quality parameters from in and around the Milford Haven Waterway as part of the Bathing Water Quality Data collected under the Bathing Water Directive by the NRW/EA (?). Weekly data with large gaps between years.	1993 - 2000	MHPA, Acquired with the help of Sue Burton
Surface Water Temperature Archive	Temperature	group1, group2, group3, group4, group5, group6, group7	Time series of surface water temperatures can provide indicators of climate change and associated ecological responses. An archive was created in 2007 as part of a research project and is a unique collation of Natural Resource Wales and The Environment Agencies water temperature data from more than 30,000 sites across England & Wales. The archive contains water temperature data (up to 2007) and site metadata. Monthly measurements.	1977 - 2008	http://lle.gov.wales/catalogue/item/SurfaceWaterTemperatureArchiveUpTo2007/?lang=en
Environmental Water Quality	Nitrate Salinity Temperature	group1, group2, group3, group4, group5, group6, group7	Data collected by/for The Milford Haven Waterway Environmental Monitoring Steering Group. Composed of Data from Urban Waste Waters Treatment Directive monitoring, Data from Dangerous Substances Directive monitoring, Data from Shellfish Waters Directive monitoring, and data from water quality monitoring route (MHWEMSG). Irregular measuring frequency with often gaps of months between measurements for a location.	1989 - 1999	MHPA, Acquired with the help of Sue Burton
Water Quality Assessment Milford Haven Estuary	Nitrate Salinity Temperature	group1, group3, group5, group6	Data on water parameters from the 2014 report “Water Quality Assessment: Milford Haven estuary” written as part of the Project “D58 Mussel carrying capacity in Milford Haven” by Chris Lowe, Christian Lønborg and Christine Gray of SEACAMS, Swansea University. Monthly or every other month.	2013 - 2014	SEACAMS, Acquired with the help of Christine Gray
Milford Haven Water Quality data 1998-2006	Nitrate Salinity Temperature	group1, group2, group3, group4, group5, group6, group7	Data collected by/for The Milford Haven Waterway Environmental Monitoring Steering Group. Composed of Data from Urban Waste Waters Treatment Directive monitoring, Data from Dangerous Substances Directive monitoring, Data from Shellfish Waters Directive monitoring, and data from water quality monitoring route (MHWEMSG) Monthly measurements.	1998 - 2006	MHPA, Acquired with the help of Sue Burton
Irradiance data	Solar readings (in W/m^2)		Solar irradiance data from a remote monitoring station based at Milford docks which measured every minute.	2015-2016	MHPA, acquired with the help of Richard Noyce

Table S0-2: overview of growth responses of *Saccharina latissima* to different environmental parameters. Based on (Kerrison et al. 2015)

Variable	Optimal	Reduced growth	Severe stress	Death
Temperature (^oC)	5-15	17-21		20-24
Salinity	24-35	15-21	5-11 (for 3-4d)	
Water motion	Low-moderate (can grow well in strong current)			
Nitrogen (µM NO_3)	10			
pH	8-8.5			

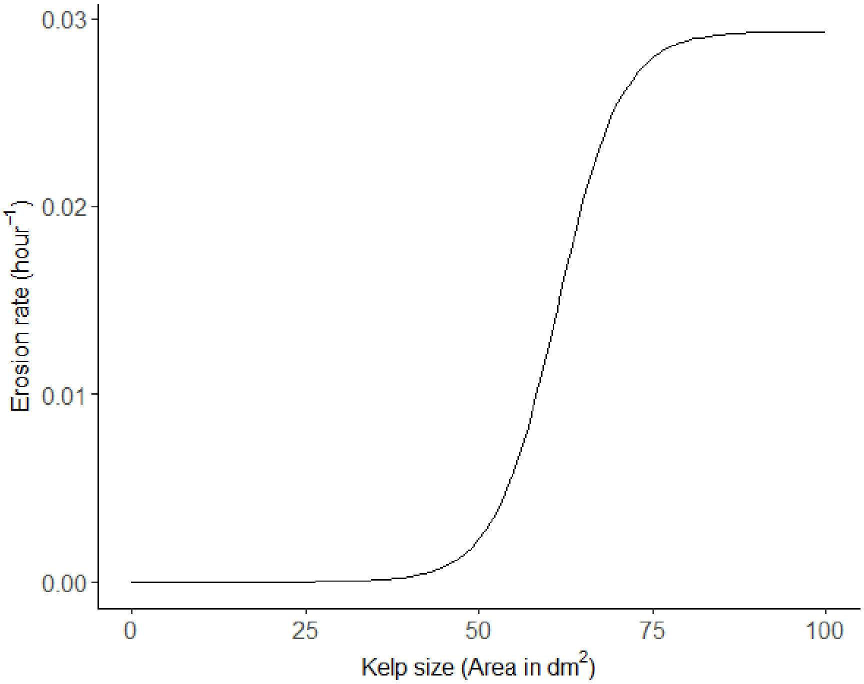


Figure S0-1: The relationship between the size of a kelp (in dm2) and the hourly erosion factor, based on (Broch and Slagstad 2012).

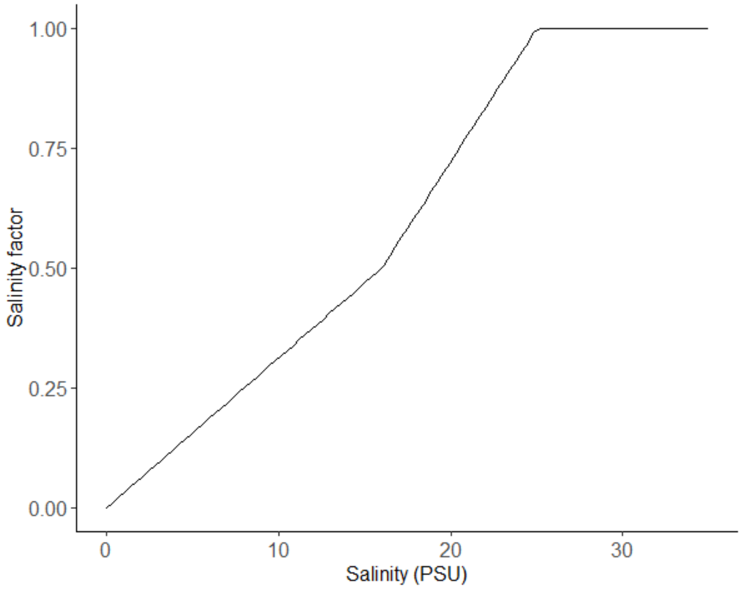


Figure S0-2: the relationship between salinity and the corrective salinity factor which was added to the Venoliamodel as part of this study,

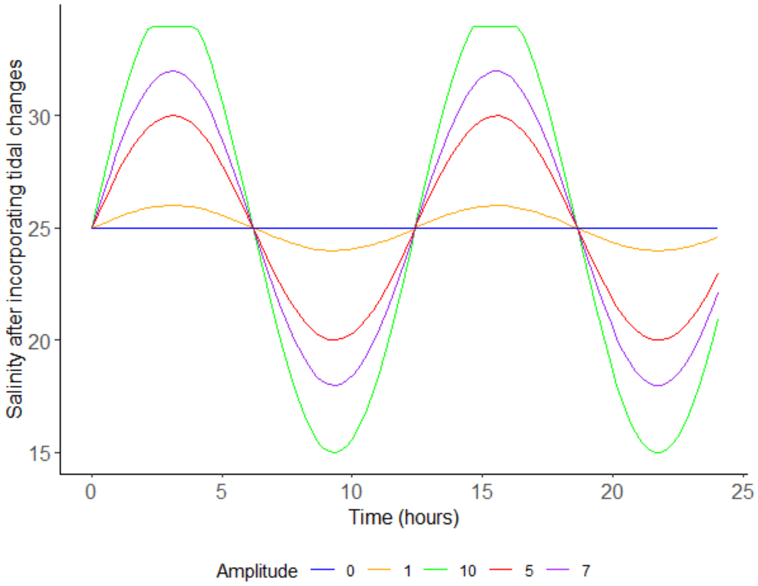


Figure S0-3: The correction for salinity which was added to the environmental data used as input for salinity. Different sites have a different amplitude of fluctuation for salinity, see Table 1-2. In this example a standard salinity of 25 was used to illustrate the hourly effects of the formula used.

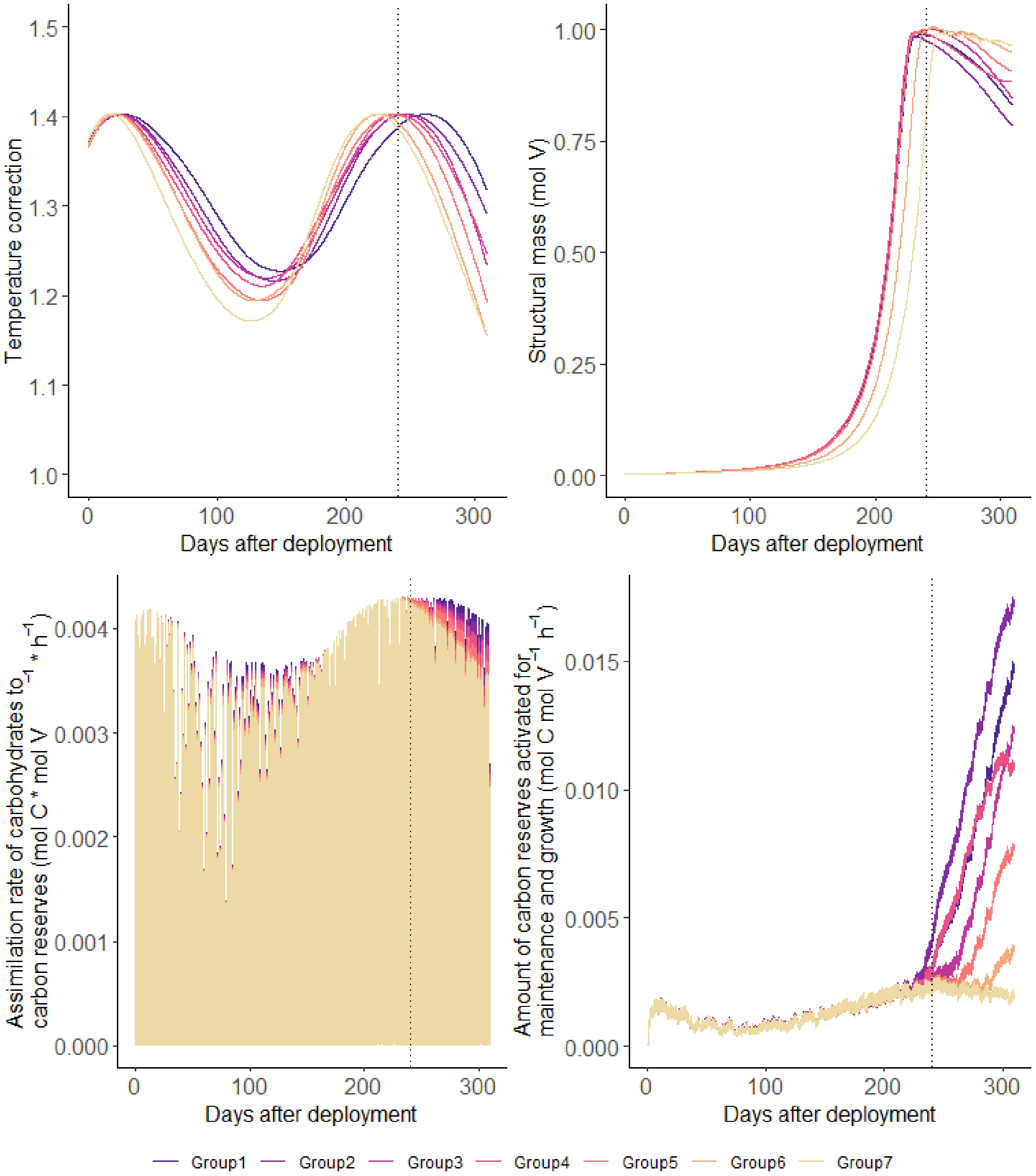
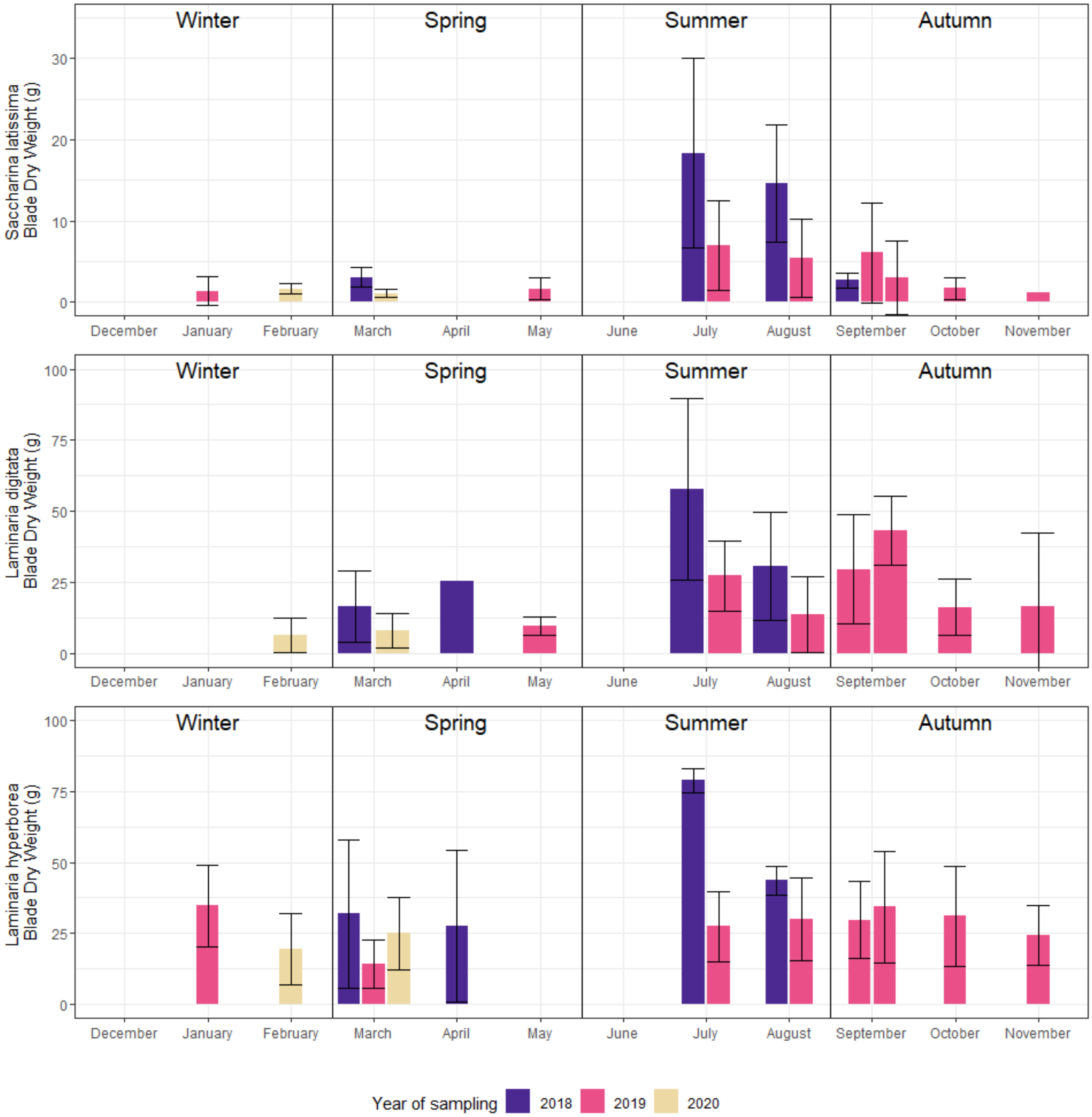


Figure S0-4: a few selected model outputs for seaweed growth in 7 sites in the Milford Haven Waterway. These include the temperature correction, the amount of structural mass in the modelled kelp (in moles V, with the V being the letter for structural weight used in this study), the assimilation rate of carbohydrates to carbon reserves, and the amount of carbon reserves activated for maintenance and growth.

Appendices for Chapter 3

Table S0-3: List of sampling dates and number of samples from this study.

day	*Saccharina latissima*	*Laminaria digitata*	*Laminaria hyperborea*
19/03/2018	3	3	2
17/04/2018	NA	1	4
16/07/2018	6	9	5
14/08/2018	5	9	7
11/09/2018	7	NA	NA
23/01/2019	8	NA	8
22/03/2019	NA	NA	6
19/05/2019	6	6	NA
04/07/2019	8	8	7
05/08/2019	6	7	6
02/09/2019	8	10	8
30/09/2019	10	6	8
29/10/2019	11	10	10
27/11/2019	1	6	8
11/02/2020	8	14	13
12/03/2020	10	16	8



Supplementary Figure 0-5: Blade dry weight for *Saccharina latissima*, *laminaria digitata*, and *Laminaria hyperborea* from Langland Bay from 2018 to 2020.

Appendices for Chapter 4

Table S0-4: Spore release and spore densities in experiment 1 on the effect of sori storage time on spore release and spore germination and growth of the kelps *Laminaria digitata* and *Laminaria hyperborea*. Spores were grown in 10 mL of medium.

Species	Sori desiccation time (h)	Spore density after spore release (spores per mL)	Spore suspension added (mL)	Culture media added (mL)	Final spore density to measure settlement success (spores per mL, in thousands)
L. digitata	0	68,500	7.3	2.7	50.0
L. digitata	0	77,000	6.49	3.51	50.0
L. digitata	12	35,500	10	0	35.5
L. digitata	12	63,500	10	0	63.5
L. digitata	24	77,000	6.49	3.51	50.0
L. digitata	24	72,333	6.91	3.09	50.0
L. digitata	48	39,500	10	0	39.5
L. digitata	48	55,500	9.01	0.99	50.0
L. digitata	72	23,000	10	0	23.0
L. digitata	72	16,500	10	0	16.5
L. digitata	96	17,500	10	0	17.5
L. digitata	96	13,000	10	0	13.0
L. hyperborea	0	696,500	1.44	8.56	100.3
L. hyperborea	0	902,000	1.11	8.89	100.1
L. hyperborea	12	2,088,000	0.48	9.52	100.2
L. hyperborea	12	2,023,500	0.5	9.5	101.2
L. hyperborea	24	1,900,000	0.53	9.47	100.7
L. hyperborea	24	3,512,500	0.29	9.71	101.9
L. hyperborea	48	3,862,500	0.26	9.74	100.4
L. hyperborea	48	4,267,500	0.24	9.76	102.4
L. hyperborea	72	2,386,700	0.42	9.58	100.2
L. hyperborea	72	3,402,500	0.3	9.7	102.1
L. hyperborea	96	1,895,000	0.53	9.47	100.4
L. hyperborea	96	1,910,000	0.53	9.47	101.2

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