# Sustainable Kelp Aquaculture in Sweden

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# ABSTRACT

Seaweed aquaculture is gaining more interest worldwide, including Europe. However, despite its long coast seaweed farming is still very minor in Sweden. The overarching aim of this thesis is to develop sustainable cultivation methods for Sugar kelp (*Saccharina latissima*) in Sweden, with more specific focus on (1) the effect of seaweed farming on ecosystem services and its environmental impact, (2) spatial location of farm sites, and (3) the development of new techniques and knowledge for selective breeding programs.

An ecosystem services assessment combined with the analysis of various environmental parameters in the field were used to study environmental impact associated with a two-hectare kelp farm. The ecosystem services assessment indicated positive or no effect on provisioning (e.g. food, biomaterials), supporting (e.g. habitat, biodiversity), and regulating (e.g. mitigating eutrophication) ecosystem services. However, some cultural ecosystem services such as recreation and aesthetic values, were likely negatively affected. The results from the environmental impact study showed that the seaweed farm has very limited negative environmental effects, but can rather have a positive effect on some environmental parameters.

The selection of suitable cultivation sites in coastal waters is essential for the sustainable establishment and further development of seaweed aquaculture in previously unexplored regions. In two field studies I investigated spatial growth patterns of *S. latissima* for optimising its nutrient mitigation capacity, crop yield, and crop quality (i.e. biofouling). The results indicate that there is relatively large spatial variation in growth and nutrient mitigation capacity of farmed seaweed biomass. Biofouling and growth decreased with increasing exposure levels, indicating that wave exposure is an important factor for site selection.

Furthermore, the capacity to conserve genetic diversity through cryo-preservation of gametophytic cells (i.e. haploid life stage) was found to be an attractive preservation method. The results show that after thawing the preserved gametophytes may serve as seed stock for onward cultivation or in selective breeding programs. A successful domestication commonly starts with a base knowledge of the genetic population structure and diversity of the species of interest. Therefore, this was assessed for *S. latissima* along the Swedish west coast, using genomic sequencing (ddRAD). The results suggest a well-connected meta-population along the Swedish west coast, but with clear signals of adaptive divergence between sub-populations, most likely driven by environmental

selection. This indicates strong natural selection in the heterogeneous marine coastal environment, leading to local adaptations despite high gene flow and connectivity.
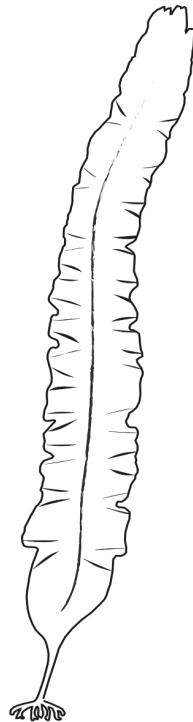
# POPULÄRVETENSKAPLIG SAMMANFATTNING

I Europa och över hela världen finns ett växande intresse för vattenbruk och odling av makroalger. I Sverige är algodling än så länge en mycket liten verksamhet, trots goda förutsättningar och en lång kustlinje. Det övergripande syftet med denna avhandling är att utveckla hållbara odlingsmetoder för sockertare (*Saccharina latissima*) i svenska vatten. Mer specifikt har följande aspekter undersökts: (1) algodlingens miljöpåverkan och effekter på ekosystemtjänster, (2) algodlingens placering, och (3) kunskap och tekniker för växtförädling och avel av sockertare.

Algodlingens miljöpåverkan undersöktes i en två hektar stor testodling i Kosterhavet. En analys av odlingens effekter på ekosystemtjänster visade på positiva eller inga effekter på *försörjande* (t.ex. livsmedel, biomaterial), *stödjande* (t.ex. livsmiljöer, biologisk mångfald) och *reglerande* ekosystemtjänster (t.ex. att mildra övergödning). För vissa *kulturella* ekosystemtjänster kopplade till friluftsliv och estetiska värden fanns troligen en negativ effekt. En miljökonsekvensstudie visade på mycket begränsade negativa miljöeffekter, snarare kan en makroalgodling vara fördelaktig ur miljösynpunkt med positiva effekter på bottenfaunan.

Att kunna välja lämpliga odlingsplatser är avgörande för att etablera och utveckla en väl fungerande makroalgodling. I två fältstudier undersökte jag hur odlingsplatsens läge påverkade sockertarens upptag av näringsämnen och därmed förmåga att bidra till minskade övergödningseffekter, och platsens påverkan på skördens storlek och kvalité (mängden påväxtorganismer/biofouling). Resultaten tyder på att det finns relativt stora variationer i näringsupptag och tillväxt beroende på växtplats. I mer exponerade odlingslägen minskade mängden påväxtorganismer men också algernas tillväxttakt, vilket visar att vindar och vågor är viktiga faktorer vid valet av odlingsplats.

Till grund för en framgångsrik växtförädling/avelsprogram behövs baskunskap om populationsstruktur och genetisk biodiversitet hos arten i fråga. Därför undersökte jag detta hos sockertare med hjälp av dna-sekvensering (ddRAD). Resultaten visar att längs svenska västkusten finns lokala delpopulationer av sockertare som tillsammans bildar en väl sammanhängande meta-population. Den heterogena kustmiljön ger ett variabelt selektionstryck, vilket leder till lokala anpassningar trots att det finns ett genflöde mellan delpopulationerna. För att lagra och bevara genetisk variation hos sockertare kan djupfrysning (kryokonservering) av gametofyter (den haploida livsfasen) vara en

lämplig metod. Upptinade gametofyter kan sedan användas som utsäde i odling eller i ett växtförädlingssprogram.
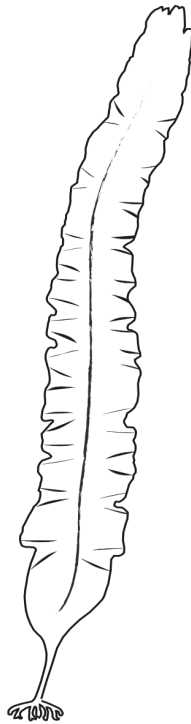
# LIST OF PAPERS

This thesis is based on the following papers, which is referred to in the text by their roman numerals:

**PAPER I:** Hasselström, L., **Visch, W.**, Gröndahl, F., Nylund, G. M., & Pavia, H. (2018). The impact of seaweed cultivation on ecosystem services-a case study from the west coast of Sweden. *Marine Pollution Bulletin*, 133, 53-64.

**PAPER II:** **Visch W.**, G.M. Nylund, M. Kononets, P. Hall, H. Pavia. Environmental impact of kelp (*Saccharina latissima*) aquaculture. *Submitted*

**PAPER III:** **Visch W.**, P. Bergström, G.M. Nylund, M. Peterson, H. Pavia, M. Lindegarth. Spatial differences in growth rate and nutrient mitigation of two co-cultivated, extractive species: the Blue Mussel (*Mytilus edulis*) and the Kelp (*Saccharina latissima*). *Submitted*

**PAPER IV:** **Visch W.**, G.M. Nylund, H. Pavia. Growth and biofouling in kelp aquaculture (*Saccharina latissima*); the effect of location and wave exposure. *Submitted*

**PAPER V:** **Visch W.**, C.R. Menendez, G.M. Nylund, H. Pavia, M. Ryan, J. Day. (2019) Underpinning the development of seaweed biotechnology: Cryopreservation of brown algae (*Saccharina latissima*) gametophytes. *Biopreservation and Biobanking*, 17(5), 1-9

**PAPER VI:** Thomson A., **W. Visch**, P.R. Jonsson, G.M. Nylund, H. Pavia, M. Stanley. Drivers of local adaptation and connectivity along an environmental transition zone in the sugar kelp, *Saccharina latissima*. *Manuscript*

# INDEX

# ABBREVIATIONS

| | |
|---|---|
| BACI | Before-After Control-Impact |
| BACIP | Before-After Control-Impact Paired |
| COI | Cytochrome c Oxidase I |
| ddRAD | double-digested Restriction site Associated DNA |
| DMSO | Dimethyl sulfoxide |
| FAO | Food and Agriculture Organization of the United Nations |
| GEA | Genotype Environment Association |
| ha | hectare |
| IMTA | Integrated Multi-Trophic Aquaculture |
| MBACI(P) | Multiple Before-After Control-Impact (Paired) |
| mtDNA | mitochondrial DNA |
| Mtons | Million tonnes |
| n | haploid or sample size |
| N | population size |
| PES | Provasoli's Enriched Seawater |
| psu | practical salinity unit |
| *S. japonica* | *Saccharina japonica* |
| *S. latissima* | *Saccharina latissima* |
| *S. longissima* | *Saccharina longissima* |
| SNP | Single Nucleotide Polymorphism |
| SSR | Simple Sequence Repeats |
| v/v | volume per volume |
| WFD | Water Framework Directive |
| y/yrs | year / years |
| 2n | diploid |

# BACKGROUND & AIMS

Seaweed aquaculture is gaining more interest worldwide, including Europe. However, despite its long coast it is still very minor in Sweden. As seaweed cultivation does not use fertilisers, pesticides, fresh water irrigation, or arable land, it is an attractive complement to crop cultivation on land. To establish a sustainable seaweed aquaculture industry, a better understanding of the environmental effects and factors that determine site selection is crucial. In addition, the development of high performing strains may allow for higher crop yields of the produced product.

On this basis, the main goal of the present thesis is to understand the prerequisites for the development of a sustainable seaweed aquaculture industry. The thesis starts with a qualitative assessment of the ecosystem service provided by a kelp farm, followed by a quantitative analysis of a wide array of environmental parameters possibly affected by seaweed farming in the area. Particular attention is given to potential implications for a future seaweed aquaculture industry, especially in the context of sustainability. This is followed by an investigation into site selection for farm locations, both in terms of yield and quality of the seaweed biomass. Finally, the long-term stable preservation of male and female gametophytes using cryopreservation and the diversity, connectivity and population structure in *Saccharina latissima* along the Swedish west coast is assessed. This may offer a knowledge base that can be useful for conservation and management policy of future seaweed cultivation practices in the area, as well as for selective breeding programs.

More specifically, the aim of each paper was:

**PAPER I:** Affected ecosystem services: To qualitatively assess the impact of seaweed cultivation on ecosystem services, more specifically whether or not current status of the ecosystem services along the Swedish west coast would be positively or negatively affected by the cultivation of kelp. The assessment includes regulating and supporting services, along with provisioning and cultural services in a holistic ecosystem services assessment.

**PAPER II:** Environmental impact: To investigate the effect of a seaweed farm on its environment a quantitative assessment was made analysing a multitude of environmental parameters using an asymmetrical before and after impact (BACI) design. This would

provide necessary empirical evidence for future management policies and legislation regarding licencing of farm sites along the Swedish west coast.

**PAPER III:** Spatial variation in growth and nutrient mitigation: The purpose was to investigate spatial differences in growth rate as well as nutrient mitigation of two extractive species that are often used in integrated multi-trophic aquaculture (IMTA) systems: the blue mussel (*Mytilus edulis*) and the kelp (*S. latissima*). This would provide aquaculture industry with criteria for the selection of suitable cultivation sites and thereby optimize nutrient mitigation efforts in eutrophied coastal areas or in IMTA systems.

**PAPER IV:** Exposure related biofouling and growth: The main objective was to assess the effect of wave exposure and spatial variation on the biofouling and crop yield of cultivated kelp (*S. latissima*). This transplantation experiment was conducted along the Swedish west coast, with three different wave exposures along various geographic scales (km to m-scale). Differences in fouling and growth as a result of wave exposure or geographic location could be helpful in selecting cultivation sites in new farm locations.

**PAPER V:** Cryopreservation of gametophytes: The purpose was to develop a method for the long-term preservation of living material of *S. latissima* through cryopreservation. This would facilitate the development of a future biobank capable of conserving commercially interesting strains, acting as a resource for future breeding or other experimental purposes, as well as for the genetic resource management of wild populations.

**PAPER VI:** Population structure: The objective was to investigate the diversity, connectivity and population structure in *S. latissima* along the Swedish west coast. This would offer baseline knowledge for future domestication, but will also provide information for conservation and management policies in the area.

# INTRODUCTION

## Seaweed aquaculture

The current world aquaculture production, approximately 131.4 million tonnes (Mtons), continues to grow and must double by 2050 in order to satisfy global demand for aquatic protein (FAO 2018b). With over 30 Mtons in 2015, the global seaweed aquaculture industry contributes substantially to the total biomass production. As a result of an increased interest, there has been a rapid annual growth in seaweed cultivation of almost 8% during the last decade. The production is primarily dominated by two Asian countries, namely China (47.9 %) and Indonesia (38.7 %) (FAO 2018b). Approximately one third of global seaweed production in 2014 was from the two kelp species *Laminaria japonica* and *Undaria pinnatifida*, with China and Korea as the main kelp producing countries. The vast majority of the globally harvested seaweed biomass is farmed, with less than 2% of the total from wild harvested seaweed (FAO 2018a).

Within a European context, seaweed aquaculture has gained renewed and strong interest during the last 15 to 20 years. Countries all along the North Atlantic and North Sea have shown interest in the cultivation of varies kelp species, such as *Saccharina latissima* that is the main species of interest within the present thesis (Kerrison et al. 2015; Peteiro et al. 2016). At present, relatively small-scale pilot studies have been set-up to develop cultivation techniques for native kelp species and promote the seaweed aquaculture industry in the area (Marinho et al. 2015; Sanderson et al. 2012; Stévant et al. 2017; Rolin et al. 2017). As a result, commercial enterprises have emerged in order to supply the increasing demand for traceable, locally produced, high quality seaweed products, such as Hortimare[1] (NL), Seaweed Energy Solutions[2] (NO), Ocean rainforest[3] (Faroe Islands), and KosterAlg[4] (SE) amongst others.

Kelp farms are typically situated in nearshore coastal environments where there is easy access in semi-exposed sites with sufficient currents that provide nutrient rich seawater for good biomass growth without damaging the crop or infrastructure. Although large-scale offshore production of seaweed biomass has been proposed as a possibility (Buck and Buchholz 2004; Buck et al. 2018), at present offshore seaweed farming only involves investigations at small pilot scales (Buck et al. 2017). A farm typically consists of horizontal long-lines (Fig. 1A), positioned at approximately 2 m depth by anchored ropes on both ends. Alternatively, farmers use suspended horizontal header ropes with dropper ropes that hang vertically down to approximately 3-5 m (Fig. 1B), or in between horizonal long-lines, a method commonly used in Chinese kelp farms (Fig. 1C).
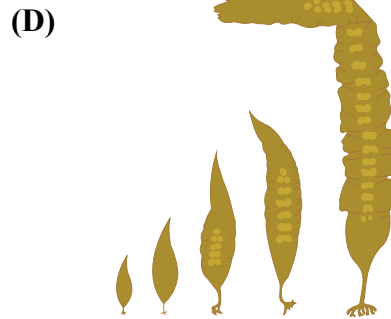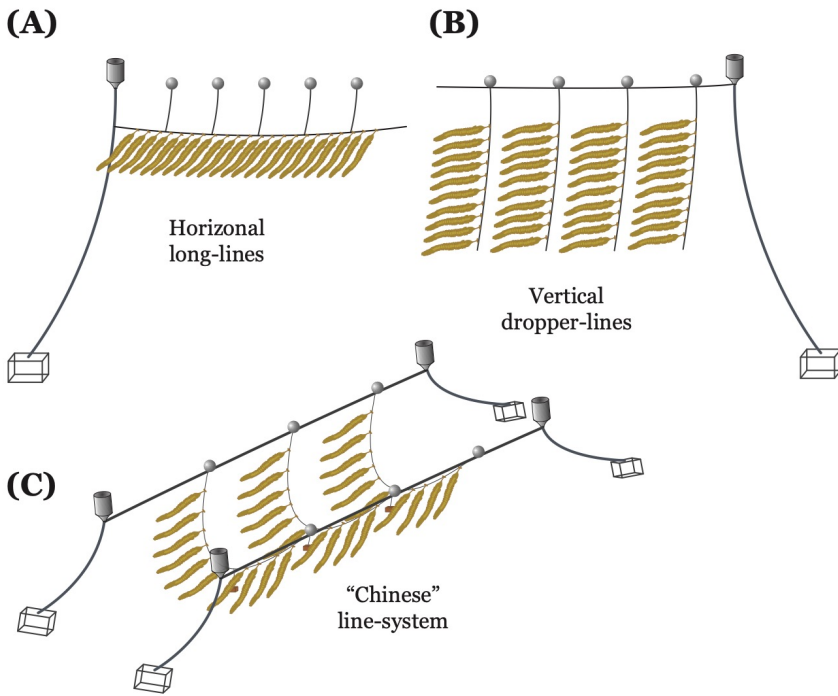
**Fig. 1.** (A) farm set-up with horizontal seeded long lines as used in this thesis; (B) vertical seeded dropper lines; (C) horizontal long-lines that position the seeded lines in between them. (D) The cultivation period of *Saccharina latissima* in relationship to the seawater temperature and day length in coastal waters at the farm site in the Kosterhavet National Park, Sweden. Points indicate monthly means and error bars show standard deviation (n=30).
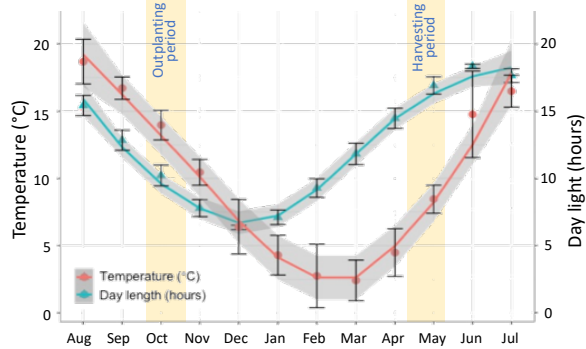
The cultivation period of kelp is primarily dictated by seasonal changes in seawater temperatures and biofouling (Førde et al. 2015; Rolin et al. 2017). In most temperate coastal regions in the northern hemisphere, the cultivation season generally starts when seawater temperature gets below 15°C in October-November, and lasts until rapid increase in biofouling that coincides with an increase in seawater temperature (Saunders and Metaxas 2007; Scheibling and Gagnon 2009; Park and Hwang 2012; Freitas et al. 2016) dictates the harvest in April-June (Fig. 1D). In European temperate regions, the most productive periods, i.e. with highest daily growth rates, are in early Autumn and Spring, when daylight is abundant (Broch et al. 2019). Accordingly, early deployment in Autumn and delayed harvest in Spring can increase the yield substantially (Broch et al. 2019); personal observation). As later harvest is commonly restricted by settlement and growth of other organisms on the blades, biofouling is therefore considered one of the major challenges and constraints in the development and growth of the seaweed aquaculture sector (Stévant et al. 2017; Lüning and Mortensen 2015; Getachew et al. 2015).

## Ecosystem services and environmental impact of seaweed aquaculture

As seaweed cultivation is gaining increased interest world-wide, it will expand further beyond Asia into other regions with high production potential, such as the temperate regions of the North Atlantic. However, environmental concerns related to impacts of aquaculture, primarily fed and shellfish farming, have long been recognised (Naylor et al. 2000; Wu 1995; FAO 2018b). Seaweed aquaculture is often considered as the least environmentally damaging form of aquaculture (Folke et al. 1998; Roberts and Upham 2012). Supplementary to the produced biomass, seaweed farms can also provide additional ecosystem services. Ecosystem services can be broadly defined as the ecosystem's direct and indirect contributions to human well-being (TEEB 2010), and there are often divided into final and intermediate services (Costanza et al. 2017). A commonly used framework assessing ecosystem services is based on supporting, regulating, provisioning and cultural services (MEA 2005; Costanza et al. 2017). The current status of these ecosystem services has been assessed by Bryhn et al. (2015) for Swedish coastal waters including the Skagarrak (Fig 2). This report provides a useful starting point from which to assess the impact of seaweed cultivation on ecosystem services in this area (**paper I**).

Environmental effects related to seaweed aquaculture have thus far primarily been explained as likely changes in the ecosystem based on prior knowledge of seaweed ecophysiology and impacts from other types of aquaculture (Campbell et al. 2019; Roberts and Upham 2012; Wood et al. 2017; Titlyanov and Titlyanova
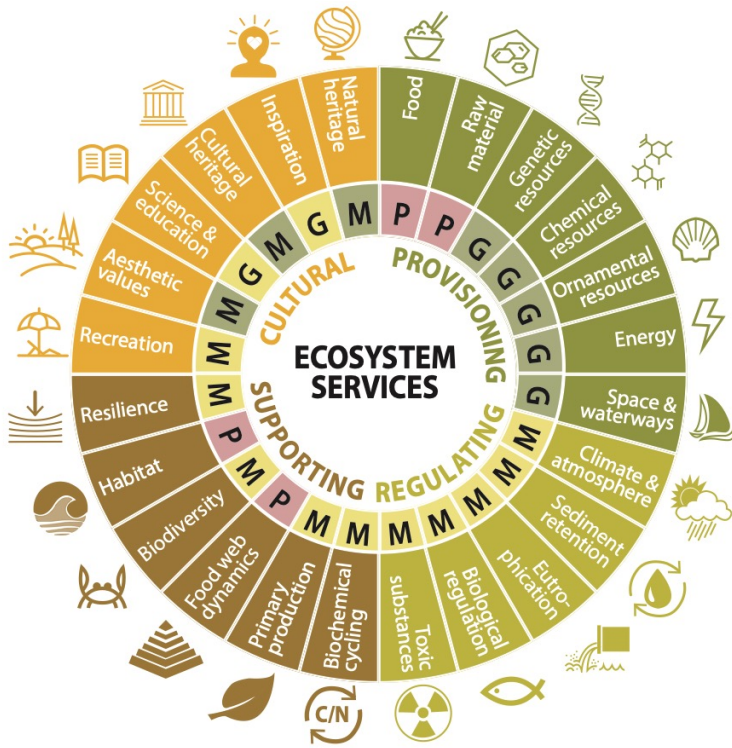
**Fig. 2**. Ecosystem services assessed for Swedish seas by Bryhn et al. (2015). With the status of the service; good (G), moderate (M), and poor (P).

2010). In addition, there is a limited body of work providing primary data associating such changes with seaweed farming (Zhang et al. 2009; Walls et al. 2017a; Walls et al. 2016; Buschmann et al. 2014). Inferences have been made about potential drivers of environmental change due to seaweed aquaculture. A review by Campbell et al. (2019) suggests that the main drivers are, in order from high-to-low risk:

1. Release of reproductive material;
2. Facilitation of diseases, parasites and non-native species;
3. Absorption of kinetic energy;
4. Addition of cultivation systems (mortality megafauna);
5. Absorption of nutrients;
6. Artificial habitat creation;
7. Absorption of light;
8. Release of dissolved and particulate matter;
9. Addition of noise;
10. Addition of cultivation systems (impact via infrastructure);
11. Absorption of carbon.

The main goal of an impact assessment is to evaluate whether a particular stressor has changed the environment, which components are negatively affects, and to estimate the magnitude of the effect (Smith 2014). Discriminating impacts from natural changes in the system can be challenging and has led to the development of various monitoring designs.

A common framework used in environmental impact assessments is Before-After Control-Impact (BACI) design (Green 1979). The basis of the BACI framework is that data is collected from the putative impacted site and a control site before and after the activity. There are four basic types of BACI designs, each using analytical models that address different questions (Table 1). In the classic BACI there is no replication in time and space (Fig. 3A). A more detailed sampling strategy incorporates Before and After sampling at the Control and Impact sites with Paired samples in time (BACIP) (Fig. 3B). As the putative impact may modify variances rather than means, an asymmetrical sampling design that incorporates multiple randomly selected control localities can be compared to one impact location all sampled several times Before and After the start of the putative impact. Examples of such asymmetrical designs are MBACI(P) and Beyond-BACI sampling designs, see Fig. 3C and Fig. 3D respectively (Underwood 1991, 1994, 1993, 1992). In addition, if data collection of the impacted site Before impact is not possible, a range of randomly chosen, undisturbed, control sites sampled randomly in time may serve as Before data (Underwood 1994).
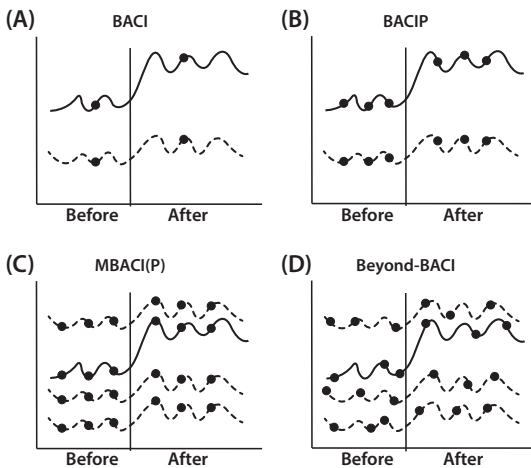


**Fig. 3.** Timing of sampling (black dots) to detect environmental impacts (indicated by the horizontal bar) at control sites (- -) and impact location (—). (A) BACI design; with a single time of sampling before and after impact in one control and one impact site. (B) BACIP design; one impacted site and one control location, paired sampling three times before and three after impact. (C) MBACI(P) design; asymmetrical sampling with three control locations and one impacted site, sampled three times before and after impact. (D) Beyond BACI design; random sampling times from periods.

**Table 1.** A selection of the basic designs commonly used to evaluate putative impact. Adopted from (Downes et al. 2002).

| | BACI | BACIP | MBACI(P) | Beyond-BACI |
|---|---|---|---|---|
| **Control and impact** | 1 each | 1 each | Many | Many, hierarchical |
| **Sampling times** | 1 | Many | Many | Many, hierarchical |
| **Treatment of space** | Fixed | Fixed | Random | Random |
| **Treatment of time** | Fixed | Random | Fixed | Random |
| **Replication** | Only subsamples within each location | Times within each period. Subsamples at each location | Locations. Times used to characterise Before and After. Subsamples at each location | Locations at each spatial scale. Times at each spatial scale |
| **Underlying logic / question** | Was the Impact location the same, relative to the Control on the two times they were sampled? | Did a change occur at Impact location relative to Control location, in a way that was unexpected, given background of temporal change at the two locations? | Did the impact location(s) change relative to the group of Control locations, in a way that was un-expected given the background pattern of changes among these control locations? | At a given scale of space or time, did the Impact location(s) change relative to the group of Control locations, in a way that was unexpected, given the background pattern of changes among these control locations? |
| **Value / utility** | May be used in subsequent meta-analysis to provide information for future management. | Appropriate choice when only one control is possible. Most useful when Control vs. Impact differences in well-behaved (i.e. two locations track similarly in the absence of the human activity). | Simply the best when the spatial scale of impact can be defined, either by accurate predictions of the kind of impact or management decision about kinds of change to be regulated. | Provides lots of information at multiple (discrete, possibly arbitrary) scales; most use as guide to better distributing effort for estimation of unknown effects. |

The aforementioned approaches rely on a significant interaction between treatment (i.e. Impact vs. Control) and period (i.e. Before vs. After) to demonstrate a putative effect. Regardless if the statistical hypothesis testing is significant, the direction of the effect (i.e. effect size) can be determined using BACI contrasts by comparing differences in mean of the depend variable between treatments and periods:

$$\text{BACI} = (\text{Impact}_{\text{AFTER}} - \text{Impact}_{\text{BEFORE}}) - (\text{Control}_{\text{AFTER}} - \text{Control}_{\text{BEFORE}})$$

It is generally considered that a positive value indicates a larger increase (or reduced decrease) between periods at the impacted location relative to controls. Conversely, a negative value indicates a larger decrease (or reduced increase) between periods at the impact sites relative to the control sites (Chevalier et al. 2019).

An evidence-based estimate of the environmental impact of seaweed cultivation is done in **paper II**, which assessed a wide array of environmental parameters (i.e. benthic infauna and benthic mobile macrofauna, benthic oxygen flux, associated mobile and sessile organisms, dissolved nutrient composition and concentrations, and shading) during a two-year cultivation period using an asymmetrical Before-After Control-Impact (BACI) design.

## Site selection for seaweed aquaculture

As seaweed farming is expanding in Europe and North America, site selection becomes increasingly important. For cultured kelp, the selected farm site must provide a suitable environment during the growth period. Environmental factors such as temperature, salinity, light, water motion, and nutrient availability are essential for productivity and quality of the crop (Kerrison et al. 2015). Despite the phenotypic plasticity of kelps (Fowler-Walker et al. 2006; Bartsch et al. 2008), optimal growth is usually found within a relatively narrow range (see Table 2). However, it can vary between kelp species and strains/ecotype (Bartsch et al. 2008).

One of the major factors affecting primary productivity in coastal areas is nutrient availability (Mallin et al. 1993). However, excess nutrient loading leads to eutrophication of marine coastal waters, resulting in a multitude of impacts on coastal ecosystems (Diaz and Rosenberg 2008; Cloern 2001; Rabalais et al. 2010). In response to growing problems associated with eutrophication, a number of national and international policies have been implemented in order to prevent further degradation and mitigate problems where they occur. These actions have traditionally focussed on controlling and reducing external nutrient loads, but increasing urgency and perceptions of lacking progress has recently led to an increased interest in active measures (Duarte and Krause-Jensen 2018; Gren et al. 2009). One suggested solution is to use extractive bivalve or seaweed aquaculture as they utilise the inorganic and organic excess nutrient for their growth (Bricker et al. 2017; Ferreira and Bricker 2016; Seghetta et al. 2016; Kellogg et al. 2014; Rose et al. 2014; Petersen et al. 2016; Lindahl 2011).

Cultivation of extractive species can occur as a separate measure or in combination with fed species. The latter approach is referred to as integrated multi-trophic aquaculture (IMTA), and is often proposed as an environmentally friendly aquaculture practices (Chopin et al. 2001; Troell et al. 2009). The basic concept of using extractive aquaculture to mitigate the effects of eutrophication

**Table 2.** Summary of growth response of *Saccharina latissima* to different environmental parameters. Adapted from Kerrison et al. (2015),

| | | |
|---|---|---|
| **Temperature (°C)** | Optimal | 5 – 15 |
| | Reduced | > 17 |
| **Salinity (psu)** | Optimal | 24 – 35 |
| | Reduced | < 21 |
| **Water motion** | Optimal | Moderate to high currents (0.1 – 0.25 m s$^{-1}$) |
| | | Can growth well in strong currents (>0.25 m s$^{-1}$) |
| **Nutrients** | Optimal | between 5 - 20 µM NO$_3^-$ (Roleda and Hurd 2019) |
| | Optimal | ≥ 0.3 µM PO$_4^{3-}$ |
| **pH** | Optimal | 8 – 8.5 |
| | Reduced | Large deviation from optimal limits carbon uptake |
| **Depth** | Optimal | 1 – 2 m |
| | Reduced | max. 4 – 5 m (depending on light penetration) |
| **Density** | | Unknown |

in coastal waters is to consider excess amounts of nutrients as a resource to be recycled, rather than a waste product. However, as the relationship between environmental factors and growth of seaweed and/or bivalves is complex and often interactive (Bergström et al. 2015; Bartsch et al. 2008), site-selection for extractive aquaculture is challenging. Therefore, spatial growth differences and nutrient mitigation capacity of two extractive marine organisms, the blue mussel *Mytilus edulis* and sugar kelp *Saccharina latissima*, was investigated in **paper III**.

European temperate coastal regions have shown to be excellent environments for the cultivation of sugar kelp, *Saccharina latissima*, which is ubiquitously found at the rocky shores of the North Atlantic (Stévant et al. 2017; Marinho et al. 2015; Sanderson et al. 2012; Peteiro et al. 2016). Environmental conditions can vary extensively in near shore waters that are often used as cultivation sites, such as archipelagos and fjord systems, thereby affecting seaweed growth and quality of farmed crop (Kerrison et al. 2015; Bruhn et al. 2016; Kim et al. 2015). Seasonal changes in seawater temperatures have previously shown to dictate the grow-out period of kelp, as it coincides with biofouling (Fig. 1D; Førde et al. 2015; Rolin et al. 2017). In addition, biofouling can vary profoundly within a relatively small geographic range, unrelated to seasonal changes (Matsson et al. 2019). Hydrodynamic forces (strong currents and wave action) have been suggested to cause this variation, as previous studies have reported different biofouling cover on seaweeds in sheltered, semi-exposed and exposed localities (Peteiro and Freire 2013b; Mols-Mortensen et al. 2017; Bruhn et al. 2016; Matsson et al. 2019). This indicates that wave exposure and/or water current might be important to consider for site selection in order to reduce biofouling and increase crop yields. Selecting

sites with limited biofouling may enhance the overall yield as it extends the grow-out period of the crop during the spring/early summer when light availability increases. Hence, the effect of wave exposure and location on biofouling and growth of farmed kelp was studied in **paper IV**.

## Seaweed domestication

Selective breeding has previously shown to benefit plant and animal production systems as it generally leads to an improved quality, consistency and traceability of the cultured crop or animal (Evenson and Gollin 2003). Genetic improvements could successfully be implemented in kelp because of the relatively high genetic diversity, the possibility of sexual propagation (Bartsch 2018), relative short generation time, and the wish from the industry for genetic improvement (Robinson et al. 2013). There are generally three options for genetic improvement of seaweed as listed by Robinson et al. (2013):

1) Continuous selective breeding program;
2) Line breeding and production of hybrid lines; and
3) Genetic transformation or modification.

A continuous selective breeding program is essentially natural selection in a controlled way. It is a stepwise process that builds on the improvements of previous generation. As crossing with existing wild genotypes or back-crossing is possible, loss of fitness due to inbreeding can be limited. Line breeding and production of hybrid lines applies inbreeding with trait selection in order to create homozygote "inbred" lines, typically using a finite closed population. These lines are then crossed (i.e. hybridized) to produce phenotypic superior individuals to the original selected parents. Higher performance can be achieved if the selection intensity was strong enough when creating the inbred lines, or from heterosis, also called hybrid vigour. In kelp, inbred lines are relatively easily produced, as kelp species self-fertilise (Schiel and Foster 2006) and clonal gametophyte cultures can serve as seed stock for onward cultivation (Barrento et al. 2016).

Currently, most of the selective breeding programs for kelp are in eastern Asian countries where line breeding is applied in combination with inter- or intra-specific hybridization to develop high performing "cultivars" with superior phenotypes. Historically, breeding of kelp started in the early 1960s with selective breeding. The use of mutagens (e.g. radiation) combined with the successful cloning of male and female gametophytes in the 1970s allowed for inter-specific hybridization in the 1980s. Further targeted selection, continuous self-crossing, and intra-specific hybridization in the 1990s led to 18 certified kelp cultivars

**Table 3.** An exclusive list of the *Saccharina* cultivars mentioned in the literature (n=32) and their respective mode of domestication. One originates from Korea, the remainder is farmed in China. *S. jap = Saccharina japonica*; *S. lon = S. longissima*; *S. lat = S. latissima*.

| Type of breeding | Parental generation (female x male) | Name of cultivar | Note | Reference |
|---|---|---|---|---|
| **Selection and inbreeding** | *S. jap* | Haiqing No. 1 & 2 | First breeding line | Fang et al. 1962 |
| | | No. 860 & 1170 | Inbred line + x-ray treatment | IOOMF and Oceanology 1976 |
| | | Zaohoucheng No. 1; Line 7; Lianza No. 1; | Inbred lines | Tian and Yuan 1989; Li et al. 2007; Liu et al. 2012; Li et al. 2015; Li et al. 2008 |
| | | Fujian | Late maturing, no systematic selection | Zhang et al. 2011 |
| | | Da Ban; Ben Niu | | Zhao et al. 2016; Liu et al. 2012 |
| | | Dongfang No. 7* | Derived from intra-specific hybrid | Hwang et al. 2019; Li et al. 2016 |
| | | Huangguan No. 1*; Sanhai*; 205*; Jeongwan No. 1* | | Liu et al. 2014; Zhang et al. 2016b; Hwang et al. 2019 |
| **Hybridization** | *S. jap* | Danhai No. 1 | Inter-specific using parthenogenesis | Fang et al. 1983 |
| | | Dongfang No. 6* | Korean ecotype x Lianza no. 1 | Hwang et al. 2019; Li et al. 2015 |
| | | B013 | Crossing distantly related individuals | Zhao et al. 2016 |
| | *S. jap* x *S. lon* | Danza No. 10. | Chinese x Japanese | Fang et al. 1985 |
| | | Pingbancai | | Zhang et al. 2016b; Zhang et al. 2016a |
| | | Line LZZ | Combined with continuous self-crossing | Li et al. 2008; Li et al. 2007 |
| | | Dongfang No. 2* | | Hwang et al. 2019; Li et al. 2007; Li et al. 1999 |
| | | Dongfang No. 3* | Line 7 x Line LZZ | Hwang et al. 2019; Li et al. 2008 |
| | *S. lon* x *S. jap* | 90-1* | male Zaohoucheng No. 1. | Zhang et al. 2007a |
| | *S. jap* x *S. lon* | Yuanza No. 10 | high yield variety | Zhang et al. 2011 |
| | | Xinbenniu | | Zhang et al. 2019 |
| | *S. jap* x *S. jap/S. lat* | Rongfu* | Fujian x Yuanza No 10 | Hwang et al. 2019; Zhang et al. 2011 |
| | *S. jap/S. lat* x *S. jap* | Ailunwan* | Yuanza No.10 x Fujian | Zhang et al. 2016b |

*\* certified cultivars in China and Korea (Hwang et al. 2019)*

currently farmed in Korea and China alone (see Table 3; Hwang et al. 2019). In Europe and North-America ethical considerations prohibit the use of inter-specific hybrids as they are seen as foreign species and the use of local strains is encouraged (Barbier et al. 2019).

Genetic transformation and modification, such as gene editing, has not been used for the production of varieties in the seaweed aquaculture industry to date (Hwang et al. 2019). Changes in ploidy, mutagenesis, manipulation to add genes or control their expression have previously been explored in microalgae (Brodie et al. 2017), but few studies report findings on seaweed (Qin et al. 2012). This is primarily due to that there are plentiful and complete genomic information for microalgae allowing for gene editing (Nymark et al. 2016), whereas genomic knowledge is limited for seaweed (Mikami 2014). This is however changing, as recent research efforts have enabled whole genome-sequences of several commercially farmed seaweed species, such as *Saccharina japonica* (Ye et al. 2015), *Chondrus crispus* (Collén et al. 2013), and *Pyropia yezoensis* (Nakamura et al. 2013). These studies shed light on essential information for genetic engineering, such as physiology, evolution, and reproduction. However, the diverse genetic backgrounds and variance of life histories among seaweed strains may prove to be an obstacle for genetic engineering (Lin and Qin 2014).

There is currently a limited foundational knowledge base from which to develop a selective breeding program in Europe. Therefore, **paper V** addresses issues related to the long-term stable storage of seaweed cells that can be used in future selective breeding programs and/or for onwards cultivation. This will allow for hybridization experiments as well as backcrossing. **Paper VI** provides a baseline knowledge of genetic diversity and population structure, important for future conservation and management policy in Sweden. But also help domestication, where it can be informative for future selective breeding programs. This was done using a seascape genomic approach, applying a combination of high throughput genomic-wide sequencing (ddRADseq) with connectivity and migration analysis across an environmentally constrained distribution in the North Sea-Baltic Sea transition zone. This study presents the first of such investigation for *S. latissima*.

# METHODS

In this section I summarise the methods used in this thesis. A more detailed description can be found in the specific papers.

## Farming kelp - the hatchery and grow-out phase

During the hatchery phase, seeded cultivation lines are produced in the laboratory, where the reproduction cycle and development can be controlled. A detailed description of the production of standardized juveniles in the laboratory can be found in Forbord et al. (2018), and the protocol used in **paper II**, **III**, and **IV** contain only minor variations.

As the main focus of this thesis is the cultivation of *Saccharina latissima*, a description of its life-cycle is included (see Fig. 4). The heteromorphic life-cycle of *S. latissima* contains a diploid (2n) macroscopic sporophytic phase and a haploid (n) microscopic phase. An adult sporophyte forms sporogenous tissue (sorus) on the mid to distal central part of the blade in which spores are produced. In natural communities sori are primarily formed in reproductive adult individuals from October to January (Bartsch et al. 2008). The spores are released into the water column and settle on hard substrates. The settled spores germinate and develop into gametophytes. The female gametophyte forms oogonia that develop eggs and the male gametophyte forms antheridia that produces sperm.
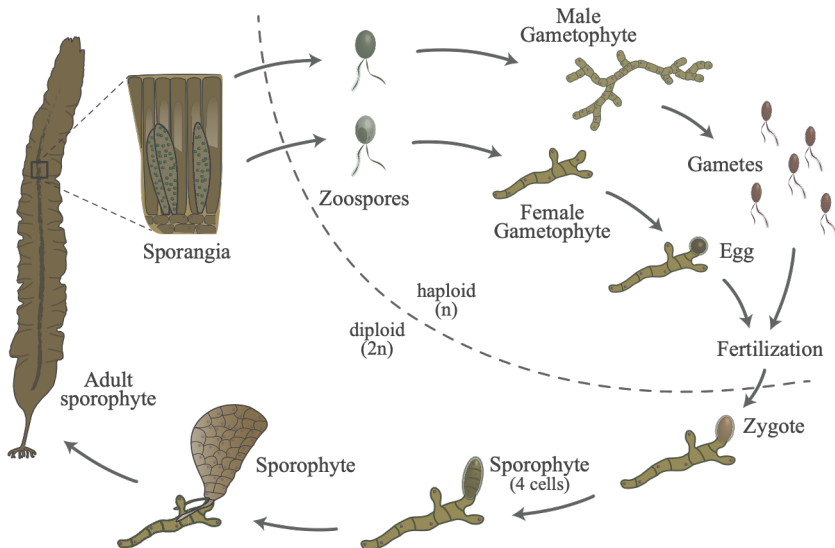


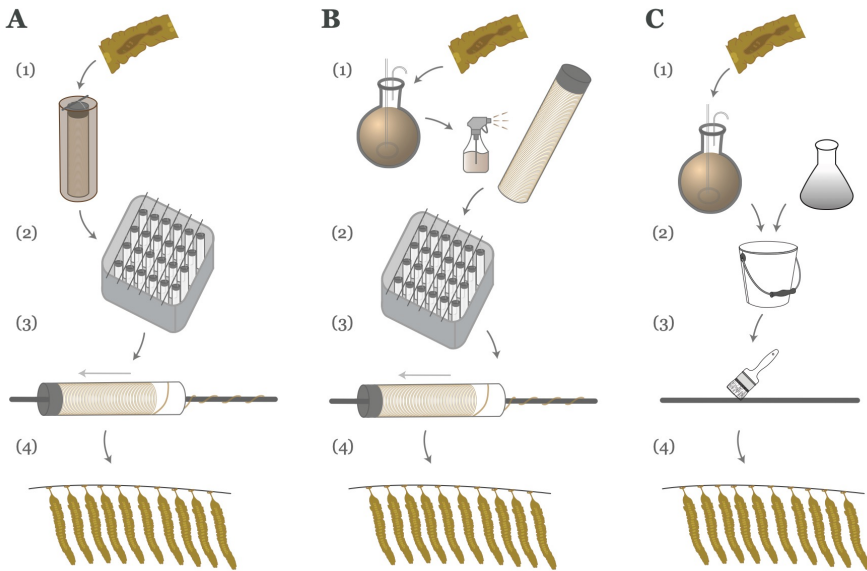**Fig. 4.** Life cycle of kelp (e.g. *Saccharina latissima*).

**Fig. 5.** The three production methods for seeded long lines. (A) allows motile spores to settle onto the collector; (B) first cultures gametophytes in flask and sprays them onto the collector; (C) combines gametophytes and a "binder" into a solution that is used to seed the cultivation line directly.

Mature eggs release pheromones that acts as an antheridium releaser and attracts the newly released sperm to ensure fertilization (Müller et al. 1985). After fertilization the zygote develops into a new sporophyte. Year-round artificial production of sori and spores in adult sporophytes is possible by a combination of short-day treatment and removal of the blade meristem (Pang and Lüning 2004; Forbord et al. 2012). This method has been successfully applied throughout this thesis (**paper II, III,** and **IV**).

There are roughly three methods for the production of seeded lines that are deployed out at sea. The first two methods use cultivation lines that are coiled around fabric long lines, the third seeds the crop directly onto long lines (Fig. 5).

The first method allows motile spores to settle onto collectors with cultivation lines and develop into sporophytes under laboratory conditions (see Fig. 5A; **paper II** and **III**). An advantage is that the motile spores attach themselves, thereby ensuring attachment to the cultivation line. A disadvantage is that the indoor hatchery phase is relatively long (> 1 month), increasing the likelihood of contamination of e.g. diatoms, bacteria, or other epiphytes.

In the second method the spore solution is kept in aerated glass flasks (Fig. 5B). The spores are developed into gametophytes that are subsequently sprayed onto collectors (**paper IV**). Further development and attachment of seedlings takes place under laboratory conditions in tanks until they are ready for deployment out at sea. The main advantage is an improved control over the gametophyte growth phase, less risk of contamination, and flasks are more space efficient than tank systems. A disadvantage is that it still requires indoor tanks, albeit during a shorter time period thereby reducing risk of contamination.

The third and last method eliminates the use of collectors and tanks during the laboratory phase. Gametophytes and/or small sporophytes are cultured in flasks before they are seeded with a binder solution that assist with the attachment to the cultivation line (Fig. 5C; Kerrison et al. 2018). Seeding can be done at sea right before deployment, therefore this method is referred to as "direct seeding". The main advantage is that it requires very little laboratory space and resources during the hatchery phase. A disadvantage is that there is less control over the attachment of the crop that might detach during the first days after deployment before it is properly adhered, especially in high energy environments (Mols-Mortensen et al. 2017).

**Clonal gametophyte cultures**

The development of a clonal stock culture can be achieved by isolating male and female gametophytes and is a prerequisite in selective breeding programs. The cultivation of inbred lines and/or inter- and intra-specific crossings using clonal gametophyte isolates involves several steps: (1) formation of sorus and spore release, (2) spore settlement and development, (3) isolation and propagation, and (4) hybridization. Any of these steps can be controlled by adjusting the temperature, light, and nutrients. A detailed protocol description of the practical derivation of clonal stock cultures can be found in Bartsch (2018), and the protocol used in **paper V** contains only minor variations.

The first step involves the collection of fertile sorus tissue, as described earlier in the section "Farming kelp - the hatchery and grow-out phase". Once the sorus tissue is prepared, the second step is spore release. Each laboratory has its own methods to release spores, but it is generally a three step process; pre-treatment, desiccation, and post-desiccation (Alsuwaiyan et al. 2019). Throughout this thesis, pre-treatment involved thorough cleaning of the tissue with autoclaved seawater without the use of chemical disinfection and wiped dry. In the desiccation step the sorus tissue is placed overnight in a cool, dark, and humid place, e.g. refrigerated and wrapped in damp/wet paper towel. The post-desiccation step involves spore

release. This step differs from the protocol for mass release of spores (Fig. 6A) (Forbord et al. 2018), as only very few spores are needed for clonal gametophyte cultures.
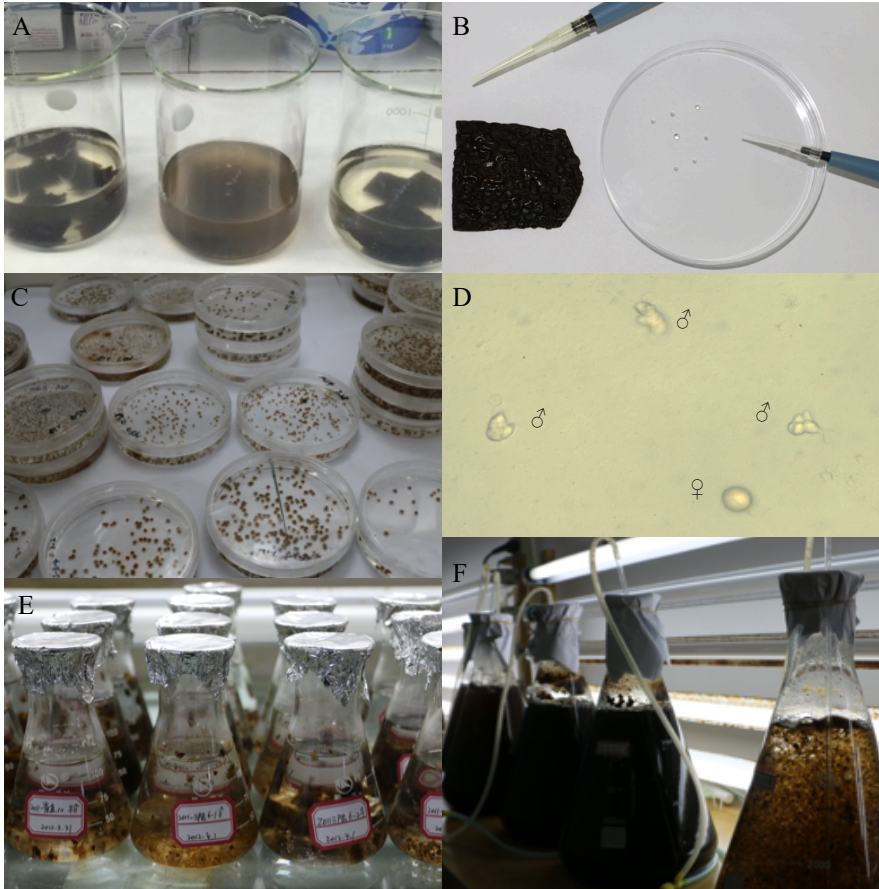


**Fig. 6.** Spore release, gametophyte isolation and clonal propagation of gametophytic biomass. (A) The mass release of spores from pre-treated sorus tissue, with the dark/turbid water indicating spore release; (B) petri dish with small droplets with spores released by the sorus tissue; (C) petri dish with mixed male and female gametophytes; (D) settled male (♂) and female (♀) gametophytes; (E) isolated gametophytes vegetatively propagated; and (F) 5 L flask with cloned gametophytes.

After desiccation the sorus tissue is cleaned with dry/damp paper towel so to remove unviable spores that where released premature. Small droplets of growth media (e.g. half strength PES with $GeO_2$) are pipetted onto the sorus tissue (Fig. 6B). After 5-10 min the droplets can be pipetted from the sorus tissue onto a Petri dish and spores are checked for mobility and density under an inverted microscope. Ideally, a droplet should contain approximately 30-50 mobile spores

that is subsequently diluted in a new Petri dish filled with culture media. Higher spore densities will complicate isolation in a later stage. In order to prevent unicellular gametophytes (Fig. 6D) from becoming fertile and develop into sporophytes, the culture conditions have to be adapted. Red light or very low-light intensities, combined with culture medium free of trace-metals (especially iron) and relatively high temperatures have shown to inhibit gametogenesis (Lüning and Dring 1975; Lüning 1980). A monthly media change is sufficient, but more frequent changes generally lead to higher vegetative growth rates. After one or two months the gametophytes are visual and can be isolated (Fig. 6C). When the initial spore density in the Petri dish was low enough and well-separated, it was found to be more convenient to delay isolation with a few weeks or months. At that stage the gametophytes were more easily isolated with forceps and the sex could be distinguished (personal observation). Further propagation of isolated gametophytes is done by fragmentation using a sterile pipet tip for small samples (<50 ml concentrated gametophyte solution; Fig. 6E) or a blender for larger quantities (>50 ml concentrated gametophyte solution; Fig. 6F). Once a sufficient biomass is established, hybridization crosses can be made by selecting the strains of interest, mix male and female gametophytic cells (1:1), and culture conditions are altered to induce gametogenesis (i.e. increase light intensity, include trace-metals in the culture media and lower the temperature).

## Measuring environmental impact of seaweed aquaculture

There are various sampling strategies to distinguish natural temporal and spatial variability from anthropogenic environmental impact (see above). Possible environmental impacts from seaweed aquaculture have been suggested previously, e.g. Campbell et al. (2019). However, the actual detection of an effect may still prove to be difficult, and "case-based" decisions need to be made about which factors to include in the assessment that are likely affected.

In **paper II**, a 2 ha seaweed farm (i.e. impact site) was located in the Koster archipelago on the Swedish west coast within the Skagerrak region of the North Sea. This archipelago is part of the Kosterhavet national park, which is the most species rich marine area in Sweden (Morf 2010). The cultivation period started in September/October and the biomass was harvested in April/May. The productivity was up to 15 kg wet weight m$^{-1}$ seeded long line, which estimates to a total harvest of 78 tonnes per year.

An asymmetrical experimental design was used, combining multiple paired sampling strategy Before-After Control-Impact (MBACIP) with a Beyond-BACI approach, to estimate the putative impact of a seaweed farm compared to four control locations. The specific sampling design was adjusted according to each

environmental variable. For example, effects on benthic oxygen flux, benthic infauna, and benthic mobile macrofauna was compared between years (`Before´ is 2016 and `After´ is 2017), because the putative impact on these parameters was expected to be fixed between years. Changes in dissolved inorganic nutrients, however, were compared between sampling periods within years (`Before´ is February and `After´ is May for both 2016 and 2017), as dissolved inorganic nutrients were not expected to be affected by the seaweed farm between years.

As a predictor of benthic response to organic enrichment caused by the seaweed farm, the benthic oxygen flux (mmol m$^{-2}$ day$^{-1}$) was measured *in situ* using two benthic chamber landers (e.g. (Tengberg et al. 2003; Ståhl et al. 2004; Almroth et al. 2009). In addition, changes in benthic infauna species composition was analysed by sediment samples (van Veen grab; 0.1 m$^2$) randomly taken at the seaweed farm and the four control locations. To compare species diversity between sediment samples three different variables/indexes were calculated: the rarefaction, the effective number of species, and the benthic quality index, in accordance with the EU Water Framework Directive classification of the sediment of coastal regions across Sweden.

The effect of a seaweed farm on epibenthic mobile macrofauna was assessed using baited cages (Carapax cod cages). Four cages were randomly deployed on the sea floor at each sampling location (i.e. seaweed farm and four control locations). After 3-6 days the caught organisms were identified, counted, and the cages were re-baited for a second sampling. The accumulated catch per cages was used for comparing the species composition between the seaweed farm and the control locations. In addition to epibenthic mobile macrofauna, associated organisms attracted to the cultivated kelps were analysed by sampling five 50 cm long-line with cultivated seaweeds (incl. blade, stipe, and holdfast) randomly within the farm into a net-bag with a 200 μm mesh size.

As seaweed assimilate inorganic dissolved nutrients for their growth from the environment, they may have an effect on nutrient levels in the surrounding seawater. This was assessed by analysing the concentration of dissolved inorganic nutrients (i.e. $NO_3^- + NO_2^-$, $NH_4^+$, $PO_4^{3-}$, and $SiO_2$) in sampled seawater (10 ml) at 2 and 5 m depth from within the seaweed farm and the control locations in February and May of 2016 and 2017. In addition, to measure light attenuation (i.e. shading effects) caused by the seaweed farm three light loggers were randomly deployed within the farm and at the control locations at 5 m depth (the seaweed lines were placed approx. 2 m deep).

## Cryopreservation

One of the aims of this thesis was to develop a low-cost protocol for the stable and long-term storage of gametophytic cells, as future selective breeding depends partly on the capacity to preserve genetic recourses. For this purpose, a two-step controlled-rate cooling method was applied, using four different cooling methods with cryoprotectants from various classes. The general workflow of the cryopreservation procedure and the viability assay used in this study is shown in Fig. 7.

Male and female gametophytes, derived from a *S. latissima* sporophyte, were isolated and allowed to develop clonally by fragmentation. The *S. latissima* gametophyte colonies were transferred into the cryogenic vials containing 1 ml of chilled (10°C) cryoprotectant solution, in triplicate. After 15-30 min incubation under ambient light conditions, the samples were subjected to the different controlled rate cooling methods. Additionally, viability was evaluated after direct plunging the samples, with and without the different cryoprotectants, into liquid nitrogen.

The following cryoprotectants were used:
- dimethyl sulfoxide (DMSO) (5% v/v);
- D-sorbitol (9% v/v) together with DMSO (10% v/v);
- polyethylene glycol (10% v/v);
- methanol (10% v/v);
- polyethylene glycol (5% v/v) together with methanol (5% v/v)

The following controlled and passive freezing protocols were used:
- controlled-rate cooler;
- Stirling cycle freezer (nitrogen-free);
- Mr. Frosty® freezing container;
- CoolCell® freezing container

After storage in liquid nitrogen the vials were rapidly thawed, washed, and transferred into a 6-well plate with PES medium for the recovery phase. Viability was assessed at day 10, 24, 35, and 52 post-thawing, using five levels of culture viability (no viability; 0-20% viability; 20-50% viability; 50-80% viability and >80% viability). Culture viability was visually estimated as the proportion of brown coloured cells (i.e. viable cells) of the total number of gametophytic cells within a sample. Sporophyte development was assessed at day 35 or 52 days post-thawing by crossings with non-cryopreserved male or female gametophytes as appropriate.
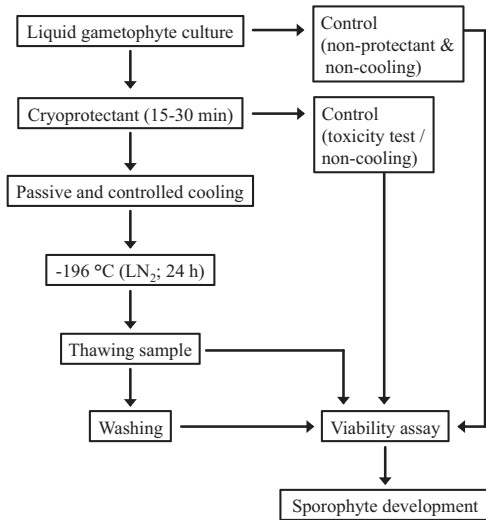
**Fig. 7**. Procedure for the cryo-preservation of *S. latissima* gameto-phytes and the viability assay.

## Population structure and connectivity

The focus of **paper VI** was to assess the diversity, connectivity and population structure of *Saccharina latissima* along the Swedish west coast using ddRAD-sequencing. Thus far, studies that have investigated these questions have primarily used non-coding simple sequence repeats (SSRs) or mitochondrial DNA (mtDNA) markers, such as Cytochrome c Oxidase I (COI) (Table 4; Fig. 8). The SNP-based population analysis used here (ddRADseq) allows the investigation of functional coding regions of the genome and offers a means of analysing signals of adaptation in natural populations, over large geographic areas.

Sampling was conducted at 9 stations, where 20 individuals (N=180) were collected at each station either by snorkelling (depth <2 m) or by trawling (depth >10 m: Ven and Mölle) (Fig 14.). From each individual, genomic DNA was extracted for sequencing from a 1 cm$^2$ meristematic tissue sample using the NucleoMag Plant kit (Machery Nagel) (Fort et al. 2018) with an additional gel-purification step. The purification step was necessary as brown algae typically contain a high amount of polysaccharides that have to be removed to enable the extraction of high-quality DNA (Panova et al. 2016). The double-digest RADseq library was prepared using a modified version of Peterson et al. (2012). A more detailed description of the library preparation, bioinformatic data processing, migration analysis, and particle dispersal modelling and connectivity analysis can be found in the "Material & Methods" section of **paper VI**.

**Table 4.** List of studies focusing on *Saccharina latissima* genetic diversity and structure using microsatellite based markers or mtDNA (i.e. COI)

| Reference | Type and number of markers | Number of sites and location |
|---|---|---|
| Paulino et al. 2016 | Microsat. 12 | 3 European |
| Guzinski et al. 2016 | Microsat. 32 | 6 NE Atlantic (European) |
| Nielsen et al. 2016 | Idem. Paulino et al. 2016 | 8 NE Atlantic (Kattegat) |
| Luttikhuizen et al. 2018 | Microsat. 10 + mtDNA | 8 NE Atlantic (European) |
| Mooney et al. 2018 | Microsat. 7 | 14 Ireland-UK west coast |
| Neiva et al. 2018 | Idem. Paulino et al. 2016. + mtDNA | 7 NE Atlantic (North-American) 15 NW Atlantic 2 NE Pacific |
| Breton et al. 2018 | Idem. Paulino et al. 2016 | 5 NW Atlantic (Maine, USA) |
| Evankow et al. 2019 | Idem. Guzinski et al. 2016 | 8 Norway |
| Næss 2019 | Idem. Paulino et al. 2016 | Norway (2 fjord systems) |

Local adaptation – analysing putative loci under selection – was investigated using three approaches; (i) Fst-based island model and (ii) Fst-based hierarchical model, and (iii) a Bayesian approach. Genotype environment associations (GEA) were analysed for various environmental parameters such as, temperature, salinity, and chlorophyll *a* concentration (for list of all variables see Table x in **paper VI**), using and Fst-based Bayesian differentiation method (*Bayescenv;* De Villemereuil and Gaggiotti 2015). Loci identified by the GEA-analysis we subsequently evaluated (BLASTn and Blast2Go) for their functional properties. Connectivity between populations along the Swedish west coast was assessed for its direction (i.e. north-south or south-north) using SNPs as well as applying a particle dispersal model simulating 5 day spore dispersal in the area over 1 generation and stepping-stone 4 and 32 generations.
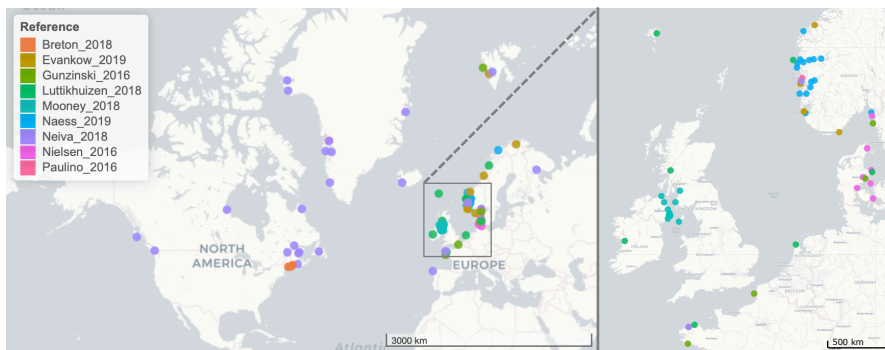


**Fig. 8.** Overview of the sampling sites across Europe and North America that have applied SSR or mtDNA markers in their genetic population analysis of *S. latissima*. The coloured dots represent the sampling site of a specific study, with the North Sea region highlighted (right panel).

# MAIN RESULTS AND DISCUSSION

## Overview of key findings

The main results of the present thesis in relation to the specific aims outlined above were:

**PAPER I:** The results suggest that provisioning (e.g. food, biomaterials), supporting (e.g. habitat, biodiversity), and regulating (e.g. mitigating eutrophication) ecosystem services are mainly positively affected or unaffected, while some cultural ecosystem services (e.g. recreation, aesthetic values) are likely negatively affected.

**PAPER II:** The results show that seaweed aquaculture has limited negative environmental effects, especially compared to other forms of aquaculture such as fish and bivalve farming, but can rather have a positive effect on some environmental variables.

**PAPER III:** The primary results indicate that there is a spatial mis-match between optimal conditions for growth in the blue mussel (*Mytilus edulis*) and the seaweed (*Saccharina latissima*). The nutrient mitigation capacity of nitrogen and carbon was estimated for *M. edulis* to approximately 700 kg N and 6,600 kg C ha$^{-1}$yr$^{-1}$ of particulate nutrients. The nutrient mitigation capacity of *S. latissima* was estimated to about 100 kg N and 1000 kg C ha$^{-1}$yr$^{-1}$ of the dissolved nutrients.

**PAPER IV:** The results show that biofouling decreased with increase wave exposure, but growth generally increased with decreased wave exposure. Relatively large spatial variation, from m-scale to km-scale, was found for both biofouling and growth. Additionally, wave exposure affected tissue composition, with higher carbon but lower nitrogen and water content at exposed sites compared to moderate and sheltered sites.

**PAPER V:** Cryopreservation was shown to be a useful option for the long-term preservation of *S. latissima* gametophytes, with viable cells in all treatment combinations. The highest viabilities for both cryopreserved male and female gametophytes were found using controlled-rate cooling methods (i.e. controlled-rate cooler and

Stirling cycle freezer) combined with DMSO 10% (v/v) or DMSO (10%) + D-Sorbitol (9%) as cryoprotectants. Higher viabilities were noted of male compared to female gametophytes.

**PAPER VI:** The results revealed relatively low differentiation between *S. latissima* populations along the Swedish west coast. Nevertheless, a degree of hierarchical structure was observed, with southern populations clustering distinctly apart, and northern and central populations also diverging. Despite the observed well mix populations the results suggest a strong role for divergent local selection and adaptation within populations across the region.

## Ecosystem services and environmental impact of seaweed aquaculture

As seaweed aquaculture practice is expected to increase and expand into new areas, a better understanding of the impact on the environment and ecosystem services is important. The results of **paper I** and **paper II** provide an overview of these impacts in a qualitative ecosystem services assessment and a quantitative environmental impact assessment. The ecosystem services assessment indicated positive or no effect on provisioning (e.g. food, biomaterials), supporting (e.g. habitat, biodiversity), and regulating ecosystem services (e.g. mitigating eutrophication). However, some cultural ecosystem services (e.g. recreation, aesthetic values) were likely negatively affected (see Table 2, **paper I**).

Supporting services contribute indirectly to human wellbeing and include biogeochemical cycling, primary production, food web dynamics, biodiversity, habitat, and resilience. A cascade representing a causal chain from the farm's habitat-generation to the effects on human well-being through ecosystem services is shown in Fig. 9. The habitat provided by the farmed kelp may support
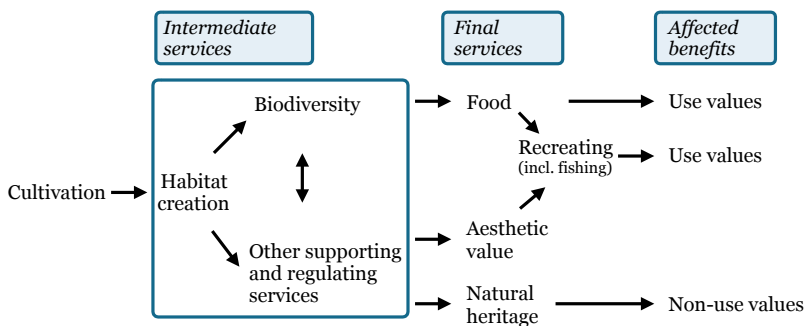


**Fig. 9**. Cascade representing a causal chain from the cultivation's provisioning of habitat to ecosystem services and benefits.

biodiversity (see **Paper II** for a discussion about the potential effect on biodiversity) as well as other supporting and regulating services, which also interact. While these interactions are not fully known, these intermediate services can potentially generate increased food provision, which can benefit commercial and/or recreational fishing. Further, although the effects may be very small, aesthetic values may be positively affected by habitat creation, which in turn may imply positive impacts on recreation in nearby areas (e.g. use values for divers) or non-use values related to the natural heritage.

Regulating services are both direct and indirect services, and include climate and atmospheric regulation, sediment retention, regulation of eutrophication, biological regulation, and regulation of toxic substances. Seaweed cultivation is thought to offer bioremediation services through the uptake of dissolved nutrients, thereby mitigating coastal eutrophication upon harvest (Lüning and Pang 2003; Fei 2004). The complexity of the possible benefits associated with a less eutrophicated state makes it difficult to link ecosystem functions – i.e. intermediate services – with final provisioning and/or cultural services such as food, recreation aesthetic values, or natural heritage. The uptake of dissolved nutrients results in benefits through a cascade that includes ecosystem services (Fig. 10).

Provisioning services presented here are mainly final services and include food, raw material, genetic -, chemical - and ornamental resources, energy, and space and waterways. An evident direct service provided by a seaweed farm is the crop that is produced which has a multitude of potential uses, from food and feed, to health products and biochemicals (Holdt and Kraan 2011). The socio-economic effects of seaweed farming vary greatly, as there are large differences in market values between these products.
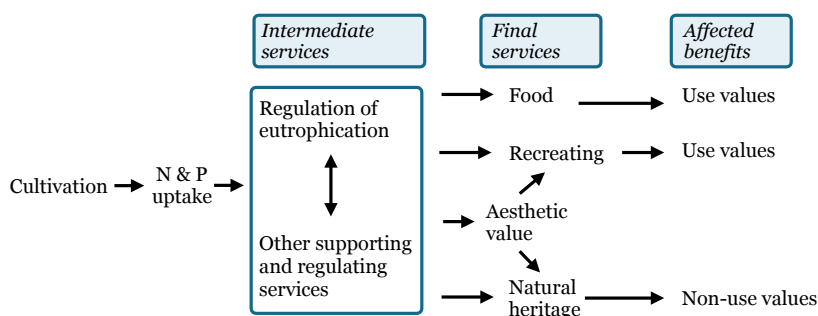


**Fig. 10.** Cascade representing a causal chain from the cultivation's uptake of N & P to ecosystem services to benefits.

Cultural services are both direct and indirect services that include recreation opportunities, aesthetic values, science and education, cultural heritage, inspiration, and natural heritage. Some of these services could be indirectly affected by a seaweed farm. For example, the present seaweed farm located in the Kosterhavet National Park was part of an exhibition in 2017 with visitors from the public, schools and policy makers. The cultivation also stimulates many new research questions and potentially industry innovations (Lansstyrelsen 2017). Concerning the cultural heritage, economic activity in Swedish coastal villages has since many decades been in decline due to a change in the structure of the fishing industry from small- to large scale (Söderqvist and Hasselström 2008). New activities and industries in coastal villages is likely to partly counteract this trend. Recreational values may, in contrast, be affected negatively by seaweed cultivation (Cabral et al. 2016), either by the physical presence of the farm, impact on aesthetic values, or through other farm related activities in the area. These impacts could be ameliorated by for example reducing visibility during cultivation period, selecting "non-recreational" sites, and the complete removal of the visual infrastructure whilst the seaweed biomass is absent (e.g. summer season).

The results of **paper II** indicate that the installation of a seaweed farm may have a positive effect on benthic infauna, with an improvement of the benthic ecological status – as formulated by the EU water framework directive – at the farm site. This contrasts with other aquaculture practices such as fish or shellfish aquaculture (Edwards 2015; Smaal et al. 2018), where significant changes in benthic infauna have often been attributed to a strong organic enrichment (Pearson and Rosenberg 1978). So far, few studies have investigated benthic effects of kelp cultivation, but they all report minimal negative impacts on benthic infaunal communities (Walls et al. 2017b; Zhang et al. 2009; Buschmann et al. 2014; Zhou 2012).

The installation of the seaweed farm was found to attract a multitude of organisms that use the cultured seaweed biomass either as habitat or as shelter. However, baited cage fishing on the sea floor indicated that the benthic mobile macrofauna was not affected by the seaweed farm. Previous studies show that bivalve aquaculture attracts benthic mobile fauna due to increased food supply and the creation of new artificial habitat on the sea floor (Morrisey et al. 2006; D'Amours et al. 2008). Seaweed aquaculture can be expected to be similar in benthic habitat creation (e.g. anchors), but is likely to differ from mussel cultivation in terms of food supply for benthic macrofauna. A light attenuation of approximately 40 % at 5 m depth was noted at the peak of the seaweed biomass, just before the harvest. As the farm site of the present study was chosen such that macrophytes where absent at the sea floor, no effect on autotrophic communities

were measured. It is recommended to avoid well vegetated areas and habitats that are protected under the EU-WFD, such as seagrass meadows or maerl beds (Campbell et al. 2019). No changes were observed in benthic oxygen flux and dissolved nutrient concentrations between farm and control sites. Combined, these results show that seaweed aquaculture has limited negative environmental effects, especially compared to other forms of aquaculture such as fish and bivalve farming, but can rather have a positive effect on some environmental variables.

## Site selection

The selection of farm sites with suitable characteristics is essential for the successful establishment of seaweed aquaculture. Therefore, **paper III** explores the spatial growth differences and nutrient mitigation capacity of kelp and bivalves in an open-ended fjord system. The effect of wave exposure and location on the growth and biofouling of farmed kelp was investigated in **paper IV**.

The results from **paper III** show that the optimal location for growth, and thus the capacity for removing nutrients, differs between the blue mussel *Mytilus edulis* and the kelp *Saccharina latissima* (Fig. 11). Mussels grew at a higher rate in sheltered sites located in inshore areas, whereas the growth of seaweeds increased in more exposed coastal areas. For mussels, the dry soft tissue growth in sheltered areas was up to double that in exposed sites, while the growth for seaweeds showed a fivefold increase in wet weight at exposed sites compared to sheltered inshore sites. The results on mussel growth is consistent with previous studies (Bergström et al. 2013, 2015), showing a high variability in growth of *M. edulis* at larger spatial scales (tens of km) compared to smaller scales (m to a few km) and more specifically that growth-rates are higher in sheltered inshore areas compared to the more exposed coastal sites. The spatial variation of *S. latissima* growth is similar to the results found in a Danish estuary/fjord-system where higher growth were observed in more saline areas compared to inshore areas (Bruhn et al. 2016). This suggests that processes at the larger km-scale, such as the recipient bay or water body in which a farm is located, contribute substantially more to variation in seaweed growth than variation within a bay or water body. Furthermore, the nutrient composition of the seaweed demonstrated high carbon and lower nitrogen content at exposed marine sites compared to relatively lower carbon and higher nitrogen content at sheltered inland sites. Previously, variation in nutrient composition of *M. edulis* was found to be primarily caused by age and size of the mussel, but that there was very little spatial variation (Bergström, unpublished results). This is corroborated by the relatively low variability of total nitrogen content in the soft tissue, as well as the low content and variation of nitrogen in the shells compared to the total amount nitrogen observed in previous studies (see Table 3, **paper III**). Chemical analysis for *S. latissima* indicated
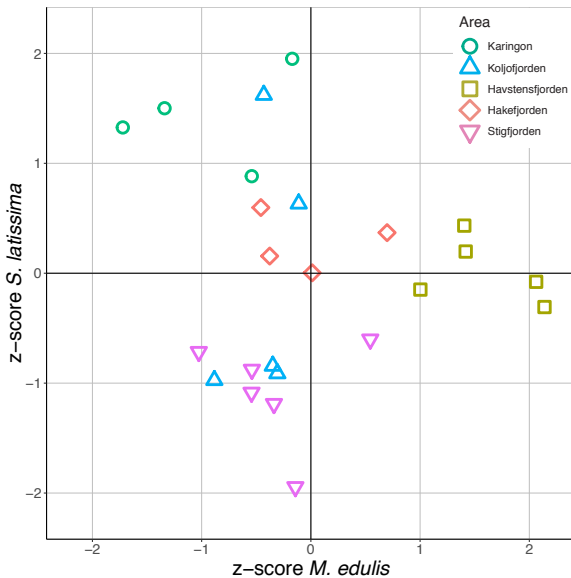
**Fig. 11.** Graphical comparison of Z-scores (for calculations see **paper III**) of *S. latissima* wet weight growth and *M. edulis* dry soft tissue growth per site within area. Käringön (green circles) is the more exposed coastal area, whereas Havstensfjorden is the most inshore coastal area.

nitrogen and carbon levels (N: 2.7- 3.6 % and C: 25.8 and 27.7 % dry weight) comparable to levels found in other fjord environments (Bruhn et al. 2016; Kim et al. 2015; Broch et al. 2013; Broch and Slagstad 2012).

The estimated nutrient removal in this study imply that mussel aquaculture can be an efficient tool for mitigation of eutrophication in the coastal environment with an extraction capacity of >700 kg N ha$^{-1}$ y$^{-1}$ and >6500 kg C ha$^{-1}$ y$^{-1}$ in high-productive mussel areas. This corresponds with the findings by Petersen et al. (2014), showing that mussel cultivation has the potential to remove between 0.6-0.9 t N ha$^{-1}$ y$^{-1}$. The potential nutrient removal by *S. latissima* was estimated to approx. 100 kg N and 1000 kg C ha$^{-1}$yr$^{-1}$ for a high-productive area in the present study, which is considerably lower than a modelled scenario of 360 kg N ha$^{-1}$ y$^{-1}$ (Broch et al. 2013). This could be partially due to the relatively simple farm set-up used for the mitigation estimates in this study (seeded horizontal long-lines instead of dropper lines with more effective use of space). In relation to the needed nitrogen reduction of 4761 tonnes in the Kattegat region or the allocated nutrient reduction target of 9240 tonnes nitrogen for the Swedish part of the Baltic Sea and Kattegat combined (HELCOM 2013), extractive aquaculture could contribute substantially in order to meet these targets. For example, the cultivation of approximately 7 and 48 km$^2$ y$^{-1}$ of mussels and seaweed respectively, can reduce the total needed removal of nitrogen in the Kattegat (i.e. 4761 tonnes) by 10%. In addition, the results show that variability in growth and mitigation capacity among sites was much larger for seaweeds compared to mussels, highlighting the need

for careful site selection for seaweed aquaculture within a heterogenous coastal environment.

Biofouling is one of the major challenges and constraints in the development and growth of the seaweed aquaculture sector (Stévant et al. 2017; Lüning and Mortensen 2015; Getachew et al. 2015). The possibility to reduce biofouling by selecting farm sites based on the degree of wave-exposure and on the geographic location along the Swedish west coast was explored in **paper IV**. Biofouling occurred at all exposure levels, but the results show that it decreased with increased wave exposure, from 10 and 6 % coverage of kelp blades at sheltered and moderately exposed locations, respectively, to 3 % at exposed locations (Fig. 12). This corroborates some previous findings (Peteiro and Freire 2013a; Sogn Andersen et al. 2011). However, highest cover of fouling organisms at the site with highest exposure was found in northern Norway (Matsson et al. 2019) and in a Danish estuary where fouling was unaffected by degree of wave exposure (Bruhn et al. 2016). In addition, one of the most popular location for seaweed cultivation in Korea (Wando region) experiences significant problems with biofouling, despite relatively high water current velocities (Park and Hwang 2012). Species abundance and biofouling taxa recorded in this study were both within the same range (between 3-32 species) and species composition as previous findings from other kelp farms (Walls et al. 2017a; Matsson et al. 2019; Kim et al. 2017; Rolin et al. 2017). Fouling taxa overlapped between region and exposure, but the species richness (i.e. the number of taxa) decreased with increased exposure.

Growth, expressed as blade surface area, was found to be lower at exposed locations compared to sheltered and moderately exposed locations. Previous studies investigating yield of kelp farms in relation to exposure have focused on different exposure types (e.g. sheltered, current velocity, or wave exposure), therefore results have been ambiguous and general conclusions have been difficult to draw. For example, high exposed environments where found to generate lower yields (Sanderson et al. 2012), or higher yields at wave-exposed and sheltered sites compared to current-exposed locations (Mols-Mortensen et al. 2017). Others, however, found a positive relationship between current velocity and yield (Peteiro and Freire 2011, 2013a), or no relationship between yield and degree of exposure (Bruhn et al. 2016; Matsson et al. 2019). In natural kelp populations, exposure has shown to affect productivity, with higher growth in moderately exposed environments compared to sheltered or wave-exposed habitats (Leigh et al. 1987; Kregting et al. 2016). Besides the effect of wave exposure, the results from the present study indicate that there is also large spatial variation in both growth and
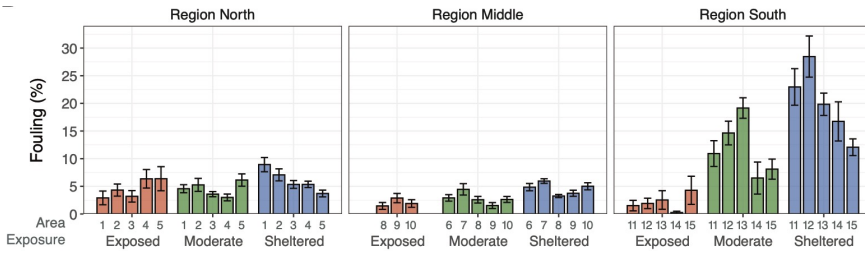
**Fig. 12.** Mean blade fouling coverage of *S. latissima* thalli. Error bars show SEM, n=20.

fouling of the seaweed biomass at the selected farm sites, with significant differences from the km-scale to the m-scale.

In addition, exposure level affected the tissue composition, with a higher carbon, but lower nitrogen and water content at exposed locations compared to moderate and sheltered sites. Morphological and biomechanical responses of seaweeds to different hydrodynamic regimes are well documented in natural communities, generally with narrow thick blades in exposed habitats and wide thin blades in sheltered habitats (Koehl et al. 2008; Bekkby et al. 2014; Hurd 2000; Hurd et al. 1996). Effects of wave-exposure on tissue carbon content was previously found to be related to an increase in carbon uptake that is allocated to structural cell wall material (Kraemer and Chapman 1991b, a). It has been hypothesised that differences in growth rate due to water motion may be related to trade-offs between blade growth rate and an increase in tissue strength (Kregting et al. 2016).

Isotope signatures (i.e. $\delta^{13}C$ and $\delta^{15}N$) also differed between exposure levels, with lower $\delta^{13}C$ concentrations at exposed locations compared to moderate and sheltered sites and higher $\delta^{15}N$ at sheltered compared to exposed and moderate locations. Based on the expected preferential uptake of $^{12}C$ in seaweeds (Keeley and Sandquist 1992), the observed difference in $\delta^{13}C$ could be explained by differences in accessibility of carbon at the different exposure levels, with less access of carbon in the sheltered localities. Although ambient dissolved nutrients where not measured in the present study, the elevated $\delta^{15}N$ in the tissue at the sheltered sites suggest a larger dominance of anthropogenic nitrogen (i.e. land-based origin) at sheltered locations compared to more moderately and exposed locations (Deutsch and Voss 2006; Raimonet et al. 2013; Viana and Bode 2015). Together, these results indicate that wave exposure is an important factor to consider in site selection for both yield as well as composition of the seaweed biomass.

**Methods underpinning seaweed biotechnology**

The need for genetic resource management and the implementation of gene banks for the safeguarding of the global seaweed aquaculture industry and genetic diversity of wild stocks has previously been recognized (Barrento et al. 2016; Cottier-Cook et al. 2016). **Paper V** explores the development of low-cost protocols for the stable and long-term storage of *Saccharina latissima* male and female gametophytes using various cryopreservation methods.

A widely applicable two-step cooling protocol for the long-term stable preservation of kelp gametophytes was successfully developed. Viability of gametophytic cells did, however, vary among the tested freezing protocols and between sexes. The highest viability levels (up to 80% for males and 20% for females) observed after cryopreservation was in samples using a controlled rate freezing method (i.e. Controlled rate cooler and Stirling cycle freezer methods) in combination with cryoprotectants DMSO (5%) or DMSO (10%) + D-Sorbitol (9%). The passive cooling methods (Mr. Forsty and CoolCell) resulted in lower viability levels (≈20%). A viability of up to 80% found here for male gametophytes is broadly comparable to previous reported survival rates using a similar two-step cooling protocol for other brown algae gametophytes (Fig. 13; Kuwano et al. 2004; Nanba et al. 2009; Zhang et al. 2007b; Piel et al. 2015; Kono et al. 1998; Sakanishi and Saga 1994). However, viability of female gametophytes was relatively low compared to previous findings (see Fig. 13). This difference may be caused by morphological differences between sexes, with slender and longer male cells compared to thicker and larger female cells (Druehl et al. 1989). This may allow the cryoprotectant to penetrate more easily into male cells whereby it loses more water than big female cells during freezing, thereby preventing intercellular ice nucleation (Pegg 2007).

Strategies for improving post-thaw viability of cryopreserved cells includes preconditioning through optimization of pre-cryopreservation culture conditions, such as temperature acclimation (Ben-Amotz and Gilboa 1980), choice of culture medium, culture age (Cañavate and Lubián 1997), salinity alterations or osmotic adjustments (Reed 2008), and low light irradiance (Nanba et al. 2009). In addition, there are other cryopreservation protocols available, but they have generally resulted in lower viability when compared to traditional two-step cooling protocols (Fig. 13). The success of different types of cryopreservation strategies for the long-term storage of brown algae gametophytes is clearly dependent upon the susceptibility of the cell type to cryoinjury as a result of ultra-low temperatures, extracellular freezing and osmotic stress. The initial controlled rate cooling phase, cryoprotectant and post-thawing washing phase had a profound influence on the gametophytes' capacity to survive these stresses, recover and
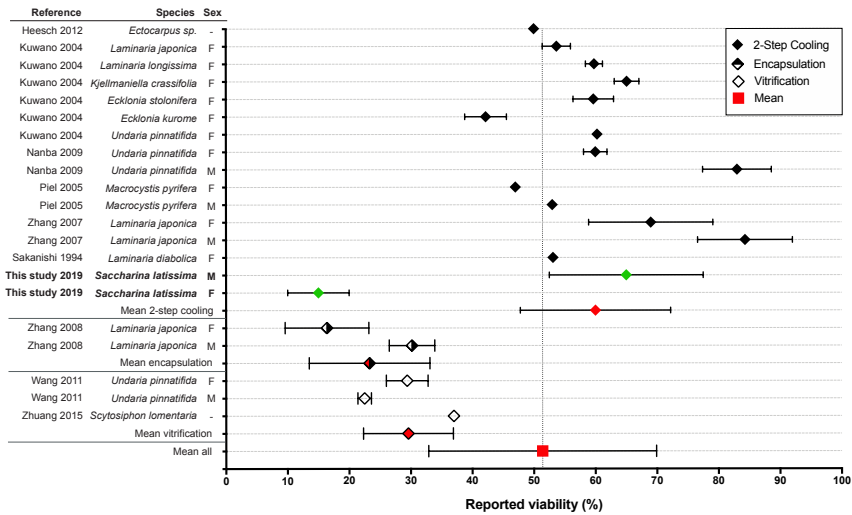
**Fig. 13.** Results from a literature review on the cryopreservation of gametophytes of various kelp species. Reported values are highest observed viability, *M* denotes male, *F* denotes female, the vertical dashed-line marks the overall mean, and error bars indicate SD.

ultimately transfer into the sporophytic life-stage. In conclusion, the controlled-rate cooling methods generated higher viability than the low-tech passive cooling methods, but both methods resulted in viable gametophytic cells with the ability to successfully complete its life-cycle.

Seaweed domestication is strongly dependent on the underlaying genetic diversity. The focus of **paper VI** was to assess the diversity, connectivity and population structure of *S. latissima* along the Swedish west coast using an integrated genomic, environmental and physical modelling approach. It was hypothesised that sharp environmental gradients (e.g. decreasing north-south salinity gradient; Fig 14A) in the region would drive strong adaptation to the local environment and increased differentiation between populations within the region. Results from the total marker set showed relatively low differentiation between populations in the region, with an average pairwise Fst of 0.039 and only a small proportion (5-7%) of variation explained at a population or group level across the region. Nevertheless, a degree of hierarchical population structure was observed (Fig. 14B), with southern populations clustering distinctly apart, suggesting some constraints to connectivity between Northern, Central, and Southern populations. In contrast, analysis of putatively adaptive loci (n=242) revealed much greater differentiation between populations (av. Fst = 0.237; 25-30% variation amongst population and groups). There was also a large number of environmentally associated loci under selection, suggesting a strong role for divergent local selection and adaptation within populations across the region. Additional physical
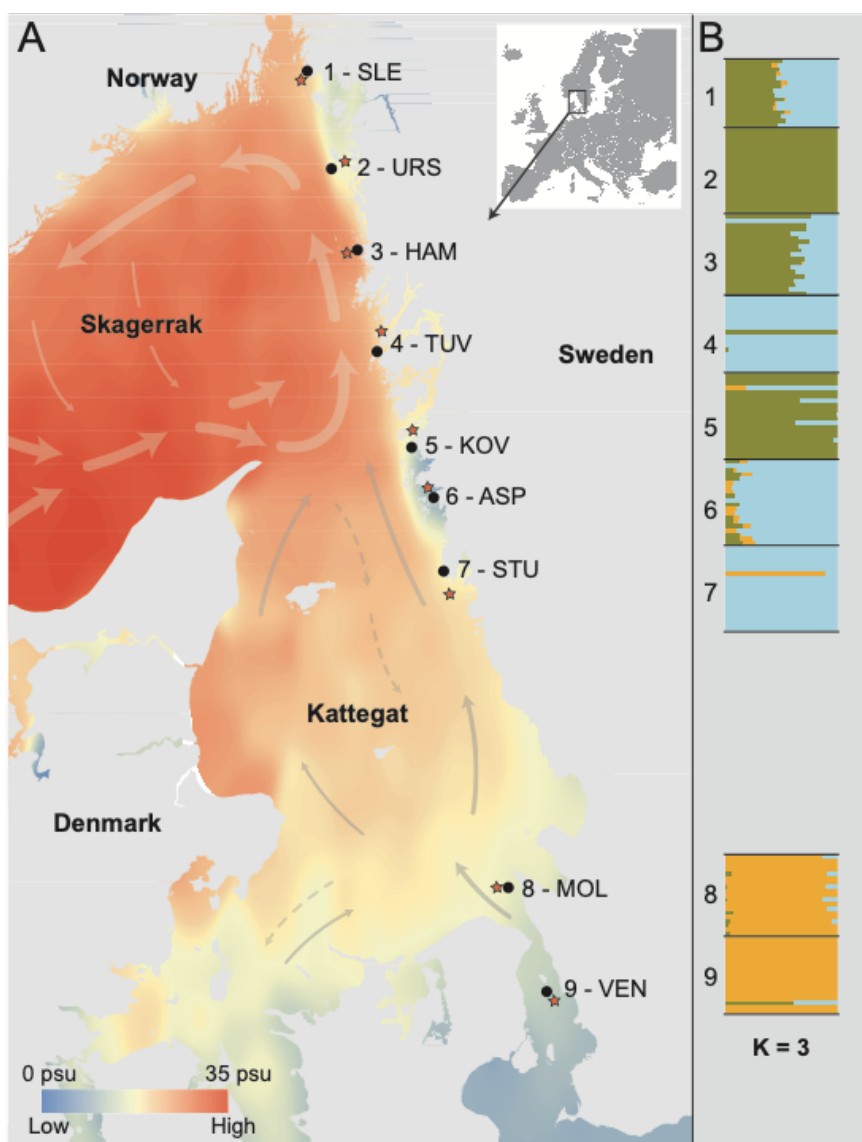
**Fig. 14.** (A) Map of sampling sites (black dots) for *Saccharina latissima* in the Skagerrak-Kattegat region of the North Sea. The background heat map shows diffusion interpolation of minimum salinity values from the ICES data-base (1980-2018). The red stars indicate the location of the closest available monitoring station with information on water chemistry and other environmental variables. The arrows indicate mean field circulation (averaged over a year), the hyphenated arrows indicate deeper "counter-currents" in the Kattegat (Svansson 1972). (B) Individual population membership plotted for the three inferred clusters ($K_{max} = 3$) based on the total marker set corresponding to the sampling locations indicated on the left panel.

modelling of dispersal across the region indicated the presence of large-scale oceanographic barriers to gene-flow between northern and central populations, but also confirmed the potential for area-wide gene-flow amongst kelp populations over multigenerational time-frames.

More specifically, the final marker set contained 9228 SNPs of which a total of 242 putative loci under selection were identified. Population summary statistics for both neutral and adaptive loci as well as the full marker set can be found in Table 5. Structure analysis of the full marker set showed a maximum likelihood at K = 3 clusters, separating the populations into a southern population (SW8, SW9), a central population (SW7, SW6, SW4) and a northerly population (SW1, SW2, SW3, SW5; see Fig 14B.). However, the pattern was not entirely linear with SW4 and SW5 associating with populations that were more distant, and relatively large proportion of admixture with stations SW1 and SW3 showing mixed assignment to northern and central clusters. When applying the structure analysis on the adaptive loci, maximum likelihood was found at K = 6 clusters, with sites SW8 and SW9 remaining closely clustered, and stations SW2 and SW5, and SW4 and SW7 also clustering together. These results are in contrast to the original hypothesis of strong differentiation across the population as well as previous findings by Nielsen et al. (2016) that indicated a decreasing diversity with lower salinity levels. It does, however, confirm previous results that revealed relatively

**Table 5**. Summary of population statistics. $n$ is number of sampled individuals, $\pi$ is the genetic diversity, *Het(o)* is the observed heterozygosity, *Het(e)* is the expected heterozygosity, and *F-is* is inbreeding co-efficient,

| Station | Acronym | Latitude | Longitude | Het(o) | Het(e) | $\pi$ | F-is | Unique Alleles |
|---------|---------|----------|-----------|--------|--------|-------|------|----------------|
| Slevik | SW1 | 59.1758 | 10.8139 | 0.253 | 0.230 | 0.238 | -0.036 | 3 |
| Ursholmen | SW2 | 58.8345 | 10.9936 | 0.236 | 0.223 | 0.229 | -0.016 | 9 |
| Hamburgö | SW3 | 58.5412 | 11.2420 | 0.239 | 0.231 | 0.237 | -0.004 | 5 |
| Tuvesvik | SW4 | 58.2155 | 11.4114 | 0.245 | 0.235 | 0.242 | -0.003 | 15 |
| Kovikshamn | SW5 | 57.8413 | 11.6798 | 0.235 | 0.226 | 0.232 | -0.005 | 2 |
| Aspholmen | SW6 | 57.6626 | 11.8365 | 0.254 | 0.242 | 0.248 | -0.011 | 4 |
| Sturöd | SW7 | 57.3938 | 11.9090 | 0.234 | 0.231 | 0.237 | 0.009 | 3 |
| Mölle | SW8 | 56.2644 | 12.4972 | 0.255 | 0.241 | 0.247 | -0.020 | 2 |
| Ven | SW9 | 55.8851 | 12.6965 | 0.241 | 0.231 | 0.238 | -0.007 | 2 |

low levels of admixture between the Skagerrak and Kattegat regions (Guzinski et al. 2016; Nielsen et al. 2016). The relatively high levels of heterozygosity observed here are consistent with high diversity and mixing between populations and can be indicative of large effective population sizes (Gagnaire et al. 2015). Such systems are considered common amongst marine broadcast spawners, where effective population sizes are often large and well connected (Selkoe et al. 2016). In addition, the Skagerrak/Kattegat region is a relatively young system (approx. 8000yrs ago; Björck 1995) and *S. latissima* may not have reached an equilibrium yet after the Last Glacial Maximum (Luttikhuizen et al. 2018).

Particle dispersal modelling suggests a relatively high connectivity between populations, with a potential dispersal of zoospores up to 130km in one generation with a 5-day drift period. The use of a stepping stone model suggested an even greater connectivity over multiple generations, with cross-regional connectivity within 4 generations from south to north. The model also indicates a general trend towards asymmetrical dispersal from south to north. Multi-generational connectivity similarly suggested a strong barrier to southerly, but not northerly dispersal, situated between Hamburgö (SW3) and Tuvesvik (SW4). The central populations, in particular Aspholmen (SW6) and Sturöd (SW7), demonstrated the greatest degree of potential connectivity. Network analysis of migration using loci under selection also highlighted SW4 as a significant sink population for adaptive loci as shown by significant asymmetric migration in to SW4 from all other stations. Combining dispersal modelling and migration approaches alongside Fst based approaches has previously been found useful in determining demographic scenarios (Jahnke et al. 2018). In line with Jahnke et al. (2018) the physical dispersal model identified a hydrodynamic barrier to southward dispersal between stations SW3 and SW4. In addition, the results identify SW4, SW6, and SW7 from the central cluster as an important location of adaptive variation, matching similar diversity hotpots for *Zostera marina* (Jahnke et al. 2018). The limited dispersal found between stations SW8 and SW9 is likely to be associated with habitat availability, as well as hydrodynamic transport. The sandy shores and relatively shallow waters combined with a low salinity in the southern part of the Kattegat reduces the quality of suitable habitat for *S. latissima*, thereby isolating populations SW8 and SW9. From SW7 northwards, the coastal zone primarily consists of rocky shores is islands, skerries and fjords, providing almost continuously high quality habitats for *S. latissima*.

Finally, genotype environmental association (GEA) identified a total of 247 associations, 71 loci as putatively in association with one or more of the 9 environmental variables (see Fig. 3 and Table 5. **paper VI**). Of these 71 loci, 61 GEA loci were also present within the initial 242 putative loci under selection.

Despite that 27 GEA loci auto-correlated with five or more environmental variables, 29 GEA loci associated with just one variable or group of variables (e.g. min. salinity and mean spring salinity). The majority of GEA loci were found to associate with mean summer temperature (n=35), mean annual chlorophyll *a* (n=35), and minimum salinity (n=33). Functional annotation analysis of all selected loci and GEA loci (n=252) indicated a relation with various functions (see Table S4 and S5 **paper VI**). Functional association of candidate loci with annotated genes can offer further insights into associations identified through GEA approaches. A number of gene functions were found for associated loci, including genes for stress response, membrane structure, and alginate and fucoidan production. This might be expected under severe temperature and salinity regimes. The frequency of stress related functions, and the high number of shared associations between environmental drivers, also suggested a degree of adaptive auto-correlation whereby stress response pathways might be selected for by a range of environmental drivers (Jones. et al. 2019).

# CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis shows that kelp farming presents a sustainable system for the production of biomass at the Swedish west coast (**paper I** and **II**). Kelp farming can have a positive impact on ecosystem services, such as providing food, forming new habitats for various organisms, and mitigating eutrophication (**paper III**). However, the development and expansion of the seaweed aquaculture industry may be hampered by negative impacts on cultural ecosystem services, such as recreational and aesthetic values. Current seaweed farms in Europe and North America are typically small to medium scale (up to 50 x 200 m lines) that, when managed and located properly, pose a low risk to the environment. However, as the industry is expanding, scale dependent impacts need to be assessed and risks need to be managed accordingly. Future targeted monitoring and further research on environmental impacts, applying appropriate sampling designs over longer temporal time scales, are therefore essential.

The cultivation of extractive species, such as seaweeds or bivalves, is a suggested solution to remove excess nutrients from coastal waters and can occur as a separate measure, or in combination with fed species in an integrated multi-trophic aquaculture (IMTA) setting. In **paper III** site selection was identified as a key factor for the capacity of two species from different trophic levels, mussel (*Mytilus edulis*) and kelp (*Saccharina latissima*), to synergistically maximise their growth and nutrient mitigation. Mussels grew at a higher rate in sheltered locations

in coastal areas, whereas seaweed growth increased in relative more exposed marine areas.

In addition to nutrient uptake, site selection was found to be influential for the mitigation of biofouling (**paper IV**). Selecting farm sites with high wave exposure was found to significantly reduce biofouling, but also to result in lower crop yields when compared to sheltered and moderately exposed farm locations. The degree of wave exposure was also found to have an effect on the composition of the farmed thalli, with an increase in carbon content, but decrease in nitrogen and water content, in exposed farm locations. The spatial variation in both biofouling and yield found in the present thesis highlight the complexity of selecting favourable farm sites in a heterogenous coastal environment. Highlighting the need for small-scaled pilot tests before full-scaled farm operations are deployed in a specific area of interest. Thus, further elucidation of the interactive and cascading effects of various environmental factors influencing growth of species from multiple trophic levels is needed.

Finally, the thesis contributes to the knowledge base concerning *S. latissima* for future selective breeding and biotechnological applications. **Paper V** showed that cryopreservation of male and female gametophytes can be a useful method for the long-term stable conservation of genetic resources, with highest viabilities in the two-step control-rate cooling methods combined with DMSO (10%) as cryoprotectant. Viability was found to be higher in males compared to females, and protocol optimisation requires primarily improving the viability of female gametophytes. Furthermore, cryopreservation has the potential to facilitate the development of a future biobank capable of conserving commercially interesting strains, thereby safeguarding the future seaweed aquaculture industry. This will act as a resource for future breeding, other experimental purposes, and the genetic management of wild populations.

In addition to efforts to preserve genetic resources, **paper VI** explored the genetic structure and diversity of *S. latissima* along the Swedish west coast using genomic markers. Results indicate well mixed populations with relatively low differentiation between populations. Nevertheless, a degree of hierarchical structure was observed, with southern populations clustering distinctly apart, and northern and central populations also diverging. Although these results may be seemingly contradictory, they suggest a strong role for divergent local selection and adaptation within populations across the region. Additionally, mitigating impacts of farmed seaweeds on their natural populations is crucial for the seaweed farming industry. The results will be important for formulating future guidelines for seaweed aquaculture in Sweden. Specifically, the results can inform about (i)

suitable distance between populations used for seed stock and cultivation sites to limit genetic pollution of natural populations by farmed strains, and (ii) the number of parental individuals used to source the seed stock to reduce negative effects of small source populations used in seaweed farms.

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