

Interactions in Combined Blue Mussel and Sugar Kelp Cultures



Original images from P. Hoare, 2018 & J. Steen Booher, 2017

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A literature review and assessment of available studies on the combination of seaweeds and shellfish in integrated multitrophic aquaculture

Jort Rootlieb
Aquaculture research group
HZ University of Applied Sciences

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Supervisors:

Bastienne Vriesendorp | Scientist Bioinformatics at Corbion and founder of Aquatic Ape
Jouke Heringa | Lecturer / researcher at HZ University of Applied Sciences

Graduation lecturer:

Bram Verkruijsse | Lecturer / researcher at HZ University of Applied Sciences



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Preface

In order for me to obtain my bachelor of “Aquatic Ecotechnology” at HZ University of Applied Sciences, I am obligated to perform and write a research on a relevant topic. In the last year of this education program, I was able to attend and complete a course on aquaculture, mainly provided by researchers of the internal research group “Aquaculture”. As my prior final thesis project was cancelled due to the COVID-19 situation, I was granted an opportunity to graduate with this literature review and analysis by Bram Verkruyisse and ultimately, Jouke Heringa, a lecturer and researcher at the Aquaculture research group. Soon thereafter, I was introduced to Bastienne Vriesendorp, a scientist in bioinformatics at Corbion and seaweeds enthusiast. Throughout working on my final thesis, Bastienne and Jouke supported me with the setup and approach of my research through regular meetings. I would therefore like to thank Bram for his efforts in finding me a new topic and providing general support but especially Bastienne and Jouke for guiding me in my attempt to produce a graduation worthy literature review and analysis. Moreover, I would like to thank Loes de Jong who was also involved in discussing different aspects in my final thesis.

Summary

This study originates from an increasing demand for seaweed cultivation. HZ University of Applied Sciences, together with farmers, under the umbrella “Living Lab Schouwen Duiveland” will conduct a field study to assess the possibilities for introducing brown seaweed cultivation at or near existing mussel line culture sites and to find which processes may positively or negatively impact production as a result from this combination. Therefore, the current scientific states of shellfish and seaweed integrated multitrophic aquaculture (IMTA) are explored in the present study.

With this literature review and analysis, elaborations on biological, chemical, and physical processes derived from a multitrophic aquaculture setup with macroalgae- and shellfish populations are provided, integrated and applied. How they may affect each other’s environment and thus productivity draws an idea of the positive and negative aspects of combining macroalgae and shellfish. With elaboration on different feedback loops and their subcategories, the severity of advantages and disadvantages in a multitrophic aquaculture system are determined through a collection of different studies. The collected references and the obtained insights from this literature review may yield a general framework for the proposed field studies on multitrophic aquaculture with *Mytilus edulis* (Linnaeus, 1758) and *Saccharina latissima* ((Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders, 2006) hanging cultures in Lake Veere, the Eastern Scheldt and the North Sea later this year.

Overall, the interactions between *S.latissima* and *M.edulis* prove beneficial for both production and the environmental quality near the poly culture sites. Key parameters found include oxygen cycling, nutrient cycling, carbon cycling, biofouling, light availability, and water movement. In short, it was found that the direct effects of seaweeds cultivation on shellfish cultures include output of oxygen, both decreasing benthic nutrient fluxes and providing temporary refuge from ocean acidification. On the other hand, seaweeds decrease current velocity, causing shifts in supply of seston and settlement potential of epiphytes but also competing with algae for inorganic nutrients. Looking at the positive direct impact of

shellfish cultures on seaweed cultivation, it was found that they enhance light availability through algae consumption and inorganic nutrient availability through waste output but also consume epiphytes before settlement on seaweed blades. The additional inorganic nutrients from shellfish waste however may induce algae blooms, diminishing light availability and in periods of nutrient shortage, they indirectly compete for inorganic nutrients with seaweed through algae consumption.

For an *S.latissima* and *M.edulis* poly-cultivation setup, it is important that specific impacts are allowed to influence both cultures. This can be ensured by integrating *S.latissima* in close proximity to *M.edulis* long-lines, oriented on main current direction for optimal transport of nutrient and seston fluxes. Yet, there should be some room between the separate longlines as direct contact of *S.latissima* blades and *M.edulis* cultivation lines can induce mechanical stress on both species.

Through the discussion section, it seems recommendable to measure and/or monitor several parameters during the pilot study. Important parameters include oxygen concentrations, nutrient concentrations, current velocity, current direction, *S.latissima* blade quality/ nutrient contents, *M.edulis* meat quality/ nutrient contents and shell quality, *M.edulis* ammonia output, carbon concentration/ calcite saturation/ pH, irradiance (water transparency), chlorophyll-a and *M.edulis* filtration rates. It may also be interesting to monitor and identify epiphyte settlement on the cultivation system and cultured species.

1 Introduction

In the South Western Delta of the Netherlands, seaweed farmers are gaining momentum but struggle to find appropriate sites for production as most of the delta is owned by different parties. Blue mussel (*Mytilus edulis*) longline farmers own permits for culture practices and generally leave 30% of their culture area free of production to obtain a better-quality product. This possibly brings a solution for the production site shortage where seaweeds are grown in close proximity of mussel cultures.

HZ together with farmers, under the umbrella “Living Lab Schouwen Duiveland” will conduct a field study to assess the possibilities for introducing brown seaweed cultivation at or near existing mussel line culture sites and to find which processes may positively or negatively impact production as a result from this combination. In autumn, at three locations throughout the South Western Delta, studies will be executed to evaluate the positive and negative interactions between both *M.edulis* and integrated *S.latissima* grown on longlines. Around the world, the interactions between seaweeds and shellfish are (currently) explored and their findings may possibly yield valuable insights for the future integrated multitrophic aquaculture of *M.edulis* and *S.latissima* in the South Western Delta of the Netherlands.

1.1 Research questions

The following research questions will help with gaining an understanding of the processes and interactions within integrated multitrophic aquaculture of *M.edulis* and *S.latissima* on long lines.

Main question:

How can biological, chemical and, physical processes in integrated multitrophic aquaculture systems, consequential to culturing macroalgae and shellfish on long lines, be expected to affect local productivity (quantity and quality the of cultured species)?

Sub questions:

- 1. What are the key biological, chemical, and physical parameters that dictate shellfish and macroalgae production?*
- 2. What main biological, chemical, and/or physical processes derived from and within an integrated multitrophic aquaculture system containing both macroalgae and shellfish are expected to affect production?*
- 3. To what extend are these biological, chemical, and/or physical processes interlinked and beneficial or detrimental for successful production within integrated multitrophic aquaculture system containing both macroalgae and shellfish?*

2 Theoretical background

2.1 Seaweed longline culture

Initial seaweed stocks commonly come from hatcheries where zoospores are collected from adult algae (FAO, 2004a). Suspended in saltwater troughs, these zoospores are allowed to form sporophytes. The sporophytes are attached to strings and after one month they reach a length of >1 mm and obtain a relatively sturdy fixation (Forbord, 2012). At that point, the juvenile sporophytes can be transplanted on to longlines in the coastal or marine environment to grow up to three meters in length, depending on local environmental factors but primarily light intensity and the supply of nutrients (nitrate, ammonia and phosphorus) (Wald, 2010; Deltares, 2017). One method for the grow out in commercial kelp cultivation is practiced with longline systems in coastal areas. These system designs commonly consist of multiple horizontal, continuous or dropped culture (polypropylene) ropes, suspended at a certain depth by buoys, and held in place with anchors (Wald, 2010; Peteiro et al., 2016) (figure 1). Different designs are used like dropped lines, continuous long-lines or horizontal long-lines, depending on environmental factors like current velocity and tidal difference.

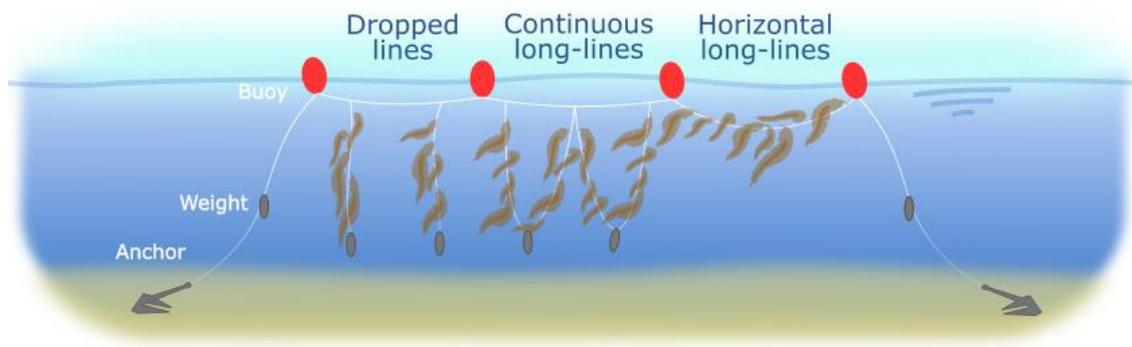


Figure 1: Conceptual drawing of common long line systems in seaweed cultivation.

In the Netherlands, considering local environmental parameters, the growing season for the latter of “consumable” brown seaweeds or winter kelps spans from October to April/ May with fastest growth in spring (Kints, 2019; Concept projectvoorstel combinatie wierenteelt hangcultuurkweek, 2020). Yields of brown seaweeds, grown on longlines was 80-400 tonnes/ha/yr throughout cultivation sites worldwide (Bruton et al., 2009). Production of sugar kelp or *S.latissima* has been reported to be most efficient at lower temperatures (5-20 °C); in a salinity of 24 to 35 ppt; with low to mild currents(0,1 to 1,52 m/s); a light intensity of 16,2 to 104,3 $\mu\text{mol m}^2 \cdot \text{s}$; a pH between 8,1 and 8,5; a total nitrogen concentration of 10 $\mu\text{mol/l}$; a carbon concentration of 90 $\mu\text{mol/l}$; and a phosphorus concentration of 0,4 to 0,63 $\mu\text{mol/l}$ (table 1).

Table1: Physical and chemical requirements for optimal production of *S.latissima*.

Parameter	Optimal range	Species	Setup	Reference	citations
temperature	10 - 15 °C	<i>S. latissima</i>	Flow trough system	Fortes & Lüning, 1980	238
	5 - 17 °C	<i>S. latissima</i>	NA	Machalek et al., 1996	96
	5 - 20 °C	<i>S. latissima</i>	Batch culture	Davison & Davison, 1987	78
	5 - 10 °C	<i>S. latissima</i>	Wierderij	Groenendijk et al., 2016	6
salinity	24 - 32 ppt	<i>S. latissima</i>	culture tanks	Gerard et al., 1987	38
	25- 35 ppt	<i>S. latissima</i>	Sea	Kerrison et al., 2015	101
	32 ppt	<i>S. latissima</i>	Wierderij	Groenendijk et al., 2016	6
current velocity	< 1,52 m/s	<i>S. latissima</i>	North Sea	Buck & Buchholz, 2005	90
	0,1 - 0,25 m/s	<i>S. latissima</i>	Sea	Kerrison et al., 2015	55
	< 1 m/s	<i>S. latissima</i>	model (MARIN)	Groenendijk et al., 2016	6
	< 0,4 m/s	<i>S. latissima</i>	NA	Berndsen et al., 2018	NA
	0,11- 0,55 m/s	<i>S. latissima</i>	Arctic sea	Matsson et al., 2019	8
light intensity	70 - 250 $\mu\text{mol m}^{-2}\text{s}^{-1}$	<i>S. latissima</i> and others	Flow trough system	Fortes & Lüning, 1980	238
	16,2 - 104,3 $\mu\text{mol m}^{-2}\text{s}^{-1}$	<i>S. latissima</i>	Bay (natural light)	Peteiro & Freire, 2012	82
	20 - 30 $\mu\text{mol m}^{-2}\text{s}^{-1}$	<i>S. latissima</i>	Lab (continuous light)	Han & Kain, 1996	41
acidity	pH 8,1 - 8,5	<i>S. latissima</i>	NA	Kerrison ET AL., 2015	101
total nitrogen	10 $\mu\text{mol/l}$	<i>S. latissima</i>	NA	Chapman et al., 1978	165
	0,014 mg N/l	<i>S. latissima</i>	NA	Kain, 1991	43
carbon	90 $\mu\text{mol/l}$	<i>S. latissima</i>	C:N ratio = +/- 9	Van Duren et al., 2019	9
phosphorus	0,63 $\mu\text{mol/l}$	<i>S. latissima</i>	N:P ratio = +/- 16	Redfield, 1934	1533
	0,4 $\mu\text{mol/l}$	<i>Laminaria digitata</i>	Mesocosms	Pedersen et al., 2010	70

2.2 Shellfish longline culture

In the Netherlands, most blue mussel or *M.edulis* stocks' seed supply for aquaculture are collected from the wild using spat collectors during summer and autumn (spatfall) in the Eastern Scheldt and the Wadden Sea. This is potentially supplemented by shellfish nurseries more often in the future as shellfish breeding methods are optimized (Stichting ProSea, 2020; FAO, 2004b).

One method to grow out the collected spat to a marketable size is on longlines, similar to those discussed in seaweed longline culture except for the horizontal long line (figure 2). "Floats are connected together by horizontal lines that support a large number of vertical ropes where mussels are grown" (FAO, 2004b). Again, different layouts (dropped lines and continuous long-lines) are applied depending on environmental factors like current velocity and tidal differences.

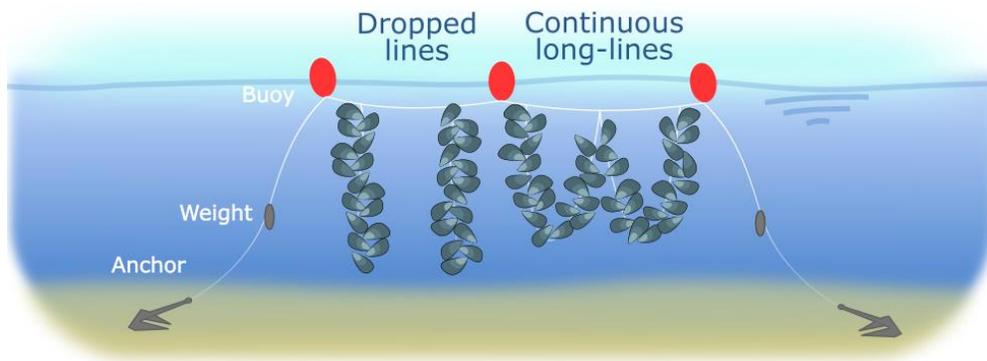


Figure 2: Conceptual drawing of common long line systems in shellfish cultivation.

In less than two to three years, the mussels reach a marketable size of at least 4 cm with the main period of growth from March to October (Widdows et al., 1979) and they are harvested for consumption. When local environmental conditions are beneficial to *M.edulis* (table 2), a yield of 18-20 tonnes/ha/yr can be achieved (FAO, 2004b). To add a note on oxygen supply requirements, Zwaan & Eertman, 1996 mention that bivalves are able to respire in anoxic conditions but also during dryfall for quite some time.

Table2: Physical and chemical requirements for optimal production of *M. edulis*.

Parameter	optimal range	Species	setup	Reference	citations
temperature	10 - 20 °C	<i>M.edulis</i>	Wild and cultured in loch	Stirling, 1995	139
salinity	18 - 32 ppt	<i>M.edulis</i>	Wild and cultured	Gouletquer et al., 2009	NA
current velocity	0,1 - 1 m/s	<i>M.edulis</i>	model	Kapetsky et al., 2013	83
	0,2- 0,8 m/s	<i>M.edulis</i>	NA	Animal+1., 2019	NA
acidity	pH 8,1 - 8,5		Wild		
oxygen supply	5 mg/l	<i>M.edulis</i>	post-harvest storing	Barrento et al., 2013	11
	> 3,0 mg/l	<i>M.edulis</i>	Volkerak – Zoommeer	Smaal & Wijsman, 2014	NA
chlorophyll- a	> 0,5 µg/l	<i>M.edulis</i>	Offshore	Kapetsky et al., 2013	83

2.3 Carrying capacity

As all organisms in an aquatic environment require and occupy an ecological niche (e.g. physical habitat, consume food, respire, and excrete waste), there is a limit to population sizes and species composition. This is therefore determined by limiting factors, balancing the demand of all present populations and the supply of all requirements supported by the ecosystem (Smaal et al., 2013). For bivalves in particular, as Dame and Prins, 1998 defined, “The carrying capacity relies on water residence time, primary production time and bivalve clearance time”. In terms of aquaculture, production carrying capacity is applied in cultivation potential of crops, taking into consideration system proprietary limiting factors (e.g direct and indirect nutrient availability). In marine aquaculture, the ecological carrying capacity is also taken into consideration as this dictates the maximum stocking and farming density of aquaculture species, without compromising wild populations in the same aquatic system on the long run (Smaal & Wijsman, 2014). Yet, significant ecological changes as a result from cultivation practices (for the better, but more likely for the worst) are surely realistic, despite the best efforts of the farmers. Therefore, societal acceptance or social carrying capacity is leading in marine aquaculture (Smaal et al., 2019).

2.4 Seaweed and shellfish IMTA

With integrated multitrophic aquaculture (IMTA), two or more species from different trophic levels are cultured in proximity. Often, one specie’s waste products (excess nutrients) are reused by another species while both organisms depend or benefit from each other in some form of synergistic relationship (Chopin et al., 2012). With the combination of cultivated species in different trophic levels, farmers aim to mimic natural ecological processes to obtain productivity with a higher nutrient removal efficiency, better quality of the products due to synergisms, and better economic security through product diversification (Chopin et al., 2012).

In Integrated multitrophic aquaculture, seaweed and shellfish are often referred to as extractive species, taking care of waste products efflux and feed spillage from the target species which are usually finfish. They (shellfish and seaweed) are deployed as bio mitigation measures for uptake of excess nutrients and after harvest, as feed for the target species or an additional aquaculture product (e.g. Zhang et al., 2019). The sole combination of shellfish and seaweed cultivation seems less represented in current day integrated multitrophic aquaculture and research. However, several synergistic but also some antagonistic relationships in this polyculture are expected within the local production system (Concept projectvoorstel combinatie wierenteelt hangcultuurkweek., 2020) (figure 3).

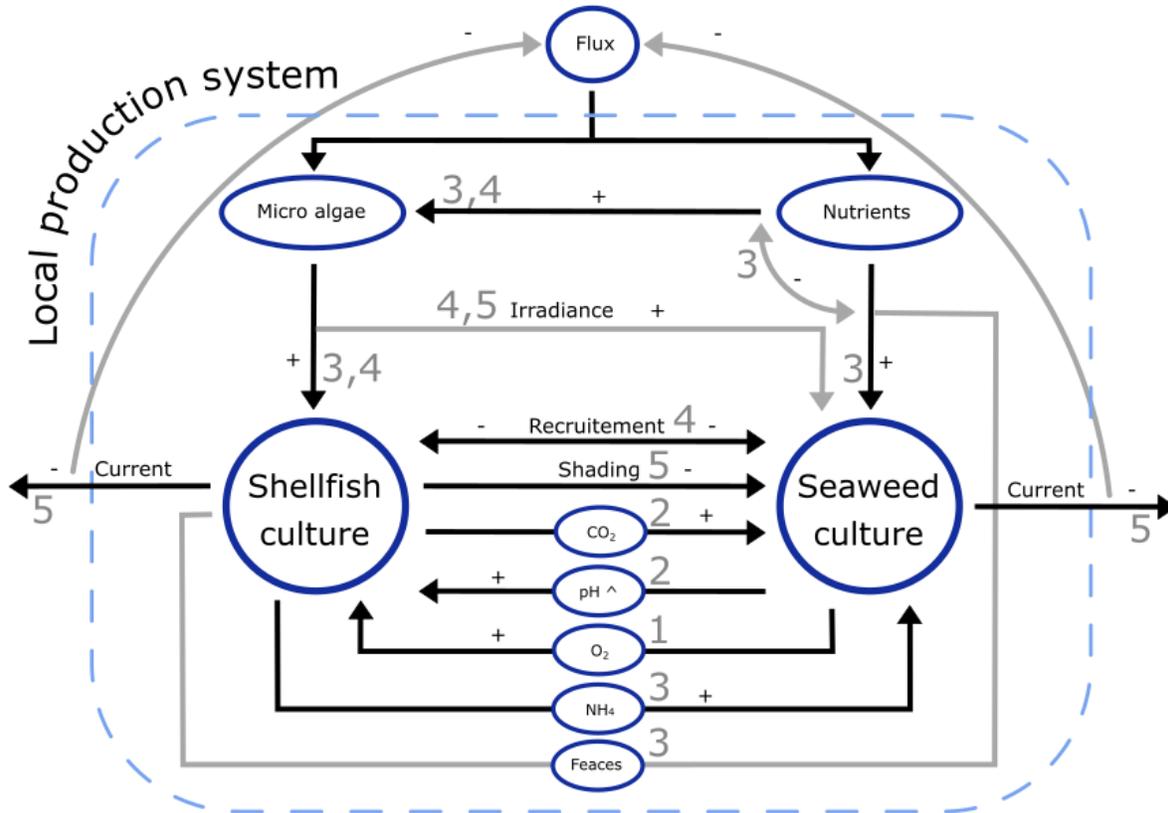


Figure 3. A feedback loop impression of processes and effects in multitrophic aquaculture with mussels and seaweeds, related to feedback loop themes (1 = oxygen cycling; 2 = carbon cycling; 3 = nutrient cycling; 4 = ecological habitat; 5 = physical habitat).

2.5 Environmental conditions of pilot sites

Starting from September 2020, three pilot sites will be prepared for polyculture studies where Sugar kelp (*S. latissima*) and potentially some other seaweeds will be implemented near existing blue mussel (*M. edulis*) longline aquaculture sites in the North Sea, Eastern Scheldt and Lake Veere (figure 4). No measurements of hydrodynamics are available at the pilot sites or nearby measurement stations. Yet it can be expected that tidal difference induces irregular current velocities. Data on light intensity are not available from monitoring stations of Rijkswaterstaat. and Water temperature will fluctuate throughout the seasons, reaching down to -2 and up to 24 °C in the North Sea and Eastern Scheldt while temperature in Lake Veere ranges from 6 to 18 °C. Salinity in the North Sea and Eastern Scheldt is roughly 32 ppt and from 17 (winter) to 28 (summer) ppt in Lake Veere (Vries et al., 2015). Chlorophyll-a, total nitrogen, total phosphorus and oxygen concentrations measured at nearby monitoring stations of Rijkswaterstaat are transformed to average values per season throughout several years, showing seasonal variety of the availability for parameters required for growth in *M.edulis* and *S.latissima* (table 3).



Figure 4: Overview of future pilot sites in the Eastern Scheldt, North Sea and Lake Veere.

Table 3: Chlorophyll-a, total nitrogen, total phosphorus and oxygen seasonal averages per monitoring station, indirectly derived from Rijkswaterstaat through <https://wateringo.rws.nl>

Mattenhaven - Wissenkerke		Average value per season			
Parameter:	Period:	Spring:	Summer:	Autumn:	Winter:
Chlorophyll-a (µg/l)	2005-2018	8,48	4,18	2,16	0,91
Total nitrogen (mg/l)	2005-2017	0,58	0,38	0,45	0,77
Total phosphorus (mg/l)	2005-2017	0,03	0,04	0,06	0,06
Oxygen (mg/l)	2005-2017	9,88	6,23	7,84	9,99
Buitenhaven - Walcheren 2 km uit de kust		Average value per season			
Parameter:	Period:	Spring:	Summer:	Autumn:	Winter:
Chlorophyll-a (µg/l)	2005-2018	17,05	8,05	3,68	1,84
Total nitrogen (mg/l)	2005-2017	0,64	0,39	0,57	0,86
Total phosphorus (mg/l)	2005-2017	0,05	0,05	0,11	0,11
Oxygen (mg/l)	2005-2017	10,25	8,07	8,18	9,82
Geersdijk - Soelekerkepolder oost		Average value per season			
Parameter:	Period:	Spring:	Summer:	Autumn:	Winter:
Chlorophyll-a (µg/l)	2005-2018	5,79	7,32	4,89	1,03
Total nitrogen (mg/l)	2005-2017	0,92	0,51	0,69	1,61
Total phosphorus (mg/l)	2005-2017	0,14	0,18	0,21	0,17
Oxygen (mg/l)	2005-2017	10,22	8,18	8,22	10,40

In table 3, it can be seen that the phytoplankton concentrations (feed for mussels and other shellfish) differ throughout the sample locations and seasons. The highest average values are found in the North Sea and overall, a higher concentration is measured during spring, and summer (the main growing period of *M.edulis*) (Widdows et al., 1979). In winter, chlorophyll-a is relatively low at all locations but still sufficient for optimal growth of *M.edulis* (>0,5 µg/l) (Kapetsky et al., 2013). Oxygen concentrations are higher during winter and compared with locations, it is lowest in the Eastern Scheldt. Yet, average values of oxygen concentrations do not

subseed optimal growth conditions for *M.edulis* (> 3,0 mg/l) (Smaal & Wijsman, 2014). In terms of inorganic nutrient availability, *S.latissima* requirements are met throughout the seasons and pilot sites.

To provide an estimate for determining the limiting factor in nutrients availability near the pilot locations, the total nitrogen and total phosphorus concentrations from table 3 are transformed to a molair unit (M) and the redfield ratio is applied for reference to the ideal nutrient ratio (N:P = 16:1) enhancing optimal algal growth in table 4a (Redfield, 1934). The Redfield ratio value is then interpreted to identify the limiting factor if present in table 4b. Because different species of algae require different nutrient ratios for optimal growth conditions, a range of 10 – 20 is used instead of 16.

Table 4a and b show that total phosphorus near the Mattenhaven pilot location stands as a limiting factor for algal growth in spring, summer, and winter, and is especially deficient in spring (within the main growing period for *S.latissima* (Kints, 2019)).

Near Mattenhaven, the nutrient ratio is within the optimal ranges during autumn. Regarding the Buitenhaven pilot location, Phosphorus is limiting at spring and winter as well, but sufficient during summer in addition to autumn. Data from Geersdijk shows a somewhat different result from the other two pilot locations: Total phosphorus is deficient only in winter, but total nitrogen is deficient in summer and autumn, suggestively induced by high phosphorus loads from agricultural runoff into Lake Veere (Nolte et. al., 2006). Yet, the nutrient ratio near Geersedijk is balanced during spring. Overall, phosphorus is the limiting nutrient near all pilot locations during winter, and the only location where nitrogen limitation turns up is near Geersedijk. These parameters and their limitations are important for both the production of *S. latissima* and the food source (phytoplankton) for *M. edulis*. However, average concentrations of total nitrogen are higher than the minimal requirements for optimal growth of *S. latissima* (0,014 mg/l) (Kain, 1991) in every season at all locations. Total phosphorus requirements for *S. latissima* similar to *Laminaria digitata* (>0,4 µmol/L= 0,0124 mg/l) (Pedersen et al., 2010) are also met in all cases.

Table 4a: Redfield ratio values of total Phosphorus (P) and total nitrogen (N). Values are result of P/M in Molair, thus if n >20, P is limiting, if n<10, N is limiting.

Location near:	Spring:	Summer:	Autunm:	Winter:
Mattenhaven	42.75	21.01	16.59	28.38
Buitenhaven	28.31	17.25	11.46	17.29
Geersdijk	14.53	6.27	7.27	20.94

Table 4b: Overview of limiting factor per season for each pilot location. P = total Phosphorus, N = total Nitrogen and – means none of the two considered nutrients are a limiting factor.

Location near:	Spring:	Summer:	Autunm:	Winter:
Mattenhaven	P	P	-	P
Buitenhaven	P	-	-	P
Geersdijk	-	N	N	P

3 Methodology

To answer the research questions provided in the introduction section, a primarily qualitative literature review has been executed over the course of three months, consulting several scientific databases through google scholar, accessible with an HZ student licences.

3.1 Phase 1, quick scan, and literature collection

This research includes a systematic literature study. The search approach consists of collecting relevant key terms for the combination of each feedback loop and respective theme (appendix. 1). Additional relevant references found in the collected literature are also explored applying the snowballing concept. The amount of studies reviewed per feedback loop theme ((1) oxygen availability, (2) carbon cycling, (3) nutrient cycling, (4) ecological habitat, (5) physical habitat) are according to what is available but limited roughly to 10 relevant documents. During this phase, most of the literature was collected, where the objective, location, experimental setup and studied organisms are noted in the catalogue.

3.2 Phase 2, Reviewing results

The relevance of the literature collected in phase 1 was determined by assigning scores to the studied organisms, the environment where the experiment took place and the local climate (table 5). The assigned scores are based on the situation at the future pilot study, therefore the combination of blue mussel or *M.edulis* and sugar kelp or *S.latissima*, in a marine/ estuarine temperate environment are valued most. If findings are based on multiple species combinations, environments or climates, an intermediate score is taken for the relevance assessment. In turn, an average score is assigned (<5,9= not relevant; 6-7,9= relevant, >8= very relevant). If one of the assessment sections is missing in the reviewed study, an average will be determined, discarding that particular detail. Together with an elaboration on the score, this approach aids in selecting the most important studies to analyse in more detail and it indicates the importance of what they found. The relevant and very relevant sources and their findings per feedback loop theme, conclusions and gaps in knowledge are added to the catalogue allowing for reviewing project details systematically and thus keep track of relevant information. The snowball method was used to find other relevant literature by using key documents per feedback loop theme that form the starting point, and other studies mentioned in the

Table 5: Literature relevance framework for assessment

Species combination	Score:
Finfish, seaweed and shellfish	4
Seaweed and shellfish	6
Blue mussel and seaweed	8
Sugar kelp and shellfish	8
Blue mussel and sugar kelp	10
Environment	
Lab	4
Mesocosm	6
Pond	8
Marine/ estuary	10
Location	
Arctic	6
Tropic	6
Temperate	10

bibliography are explored subsequently. If other relevant sources turned up within the literature collection from phase 1, these may be added to the catalogue. The relevant statements (results) found in the search are divided in themes: (1) oxygen availability; (2) carbon cycling; (3) nutrient cycling; (4) ecological habitat; (5) physical habitat and an elaboration is provided in the result section.

3.3 Phase 3, analysis

With the catalogue, expectations can be drawn on which environmental processes within multitrophic aquaculture systems growing both macroalgae and shellfish play an important role for production. The level of relevance determined in phase 2 will also be considered in drawing a conclusion upon which parameters are deemed most important based on the recurrence of topics. From the results, the processes related to shellfish/ seaweed interactions are collected and it is determined whether it concerns positive or negative effects. From this “summary”, the main topic per key process is determined and classified in either oxygen cycling, nutrient cycling, carbon cycling (and ocean acidification), biofouling, light availability, or water movement. Moreover, the effects of cultivation on environment and ecology are also taken into consideration and potential interspecific (seaweed/ shellfish) impacts are assumed. In turn, the different key processes are interlinked when either interference or enhancement of the concerning main topic seems plausible. From this data, feedback loops are composed per main topic and elaborations are provided, primarily using the “summaries” of the key processes and information from the theoretical background. Finally, in “application” the components of the feedback loops are elaborated in sense of the expectation on severity of impact within the multitrophic aquaculture system containing both macroalgae and shellfish, related to the environmental situation at the future pilot sites.

4 Results

4.1 General findings

Based on the yield of relevant findings from the reviewed studies per feedback loop theme within the catalogue, the assumption was obtained that certain environmental conditions, processes and interactions are more important than others. The studies that were found were either in Dutch or English, and not all predetermined search terms were used or resulted in finding literature where the search term concerned the main topic of the study.

It seems like the availability of experiments primarily focussing on the interactions between seaweed and shellfish culture are limited as there is quite some overlap with the studied topics and researchers/authors. However, local conditions, study approach and species combinations differ strongly throughout the collected literature. Despite these differences, all selected literature was valued relevant or very relevant.

Several studies were found, involving shellfish, seaweeds, finfish, and combinations between these groups. The most relevant experimental field studies have been conducted, involving different species of kelp, bivalves or finfish, which were monitored and/or cultured in coastal and marine environments (Figure 5).

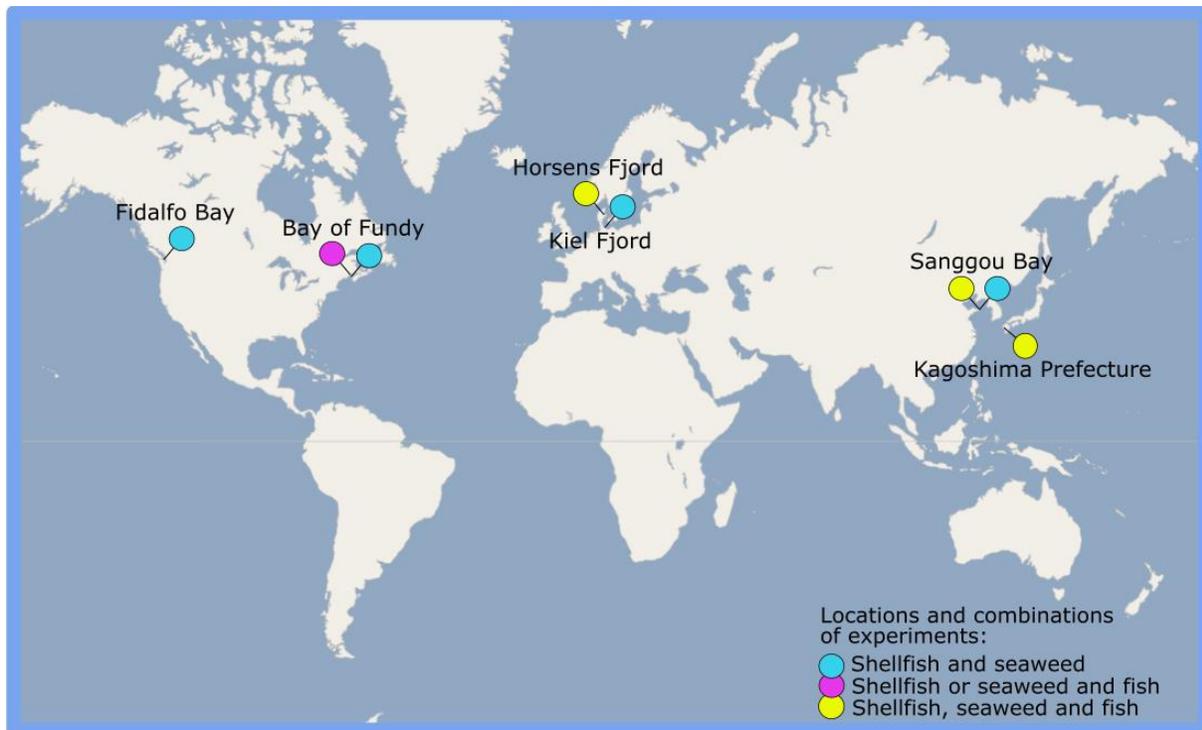


Figure 5: locations where relevant studies have been conducted, providing insight in dispersion of IMTA experimental and/ or cultivation activities involving seaweed, shellfish and/or finfish in coastal and marine environments.

4.2 (1) Oxygen cycling

It seems that changes in oxygen availability, induced by integration of shellfish and seaweed does not interfere nor enhance to a point of detrimental effects in the marine cultivation environment, suggestively to be explained by the anaerobic respiration of *M. edulis* (Barrento et al., 2013) and the photosynthetic respiration of seaweeds.

In Sanggou bay, Ning et al., 2016 found that levels of dissolved oxygen (DO) were near saturation at a polyculture area where kelp and oysters are grown in proximity, in contrast with the oyster monoculture area elsewhere in the bay, where DO levels were lower. Near the bottom at the oyster monoculture area, DO levels were found to be below the saturation, indicating a positive effect on the DO availability when kelp culture (providing DO) is within proximity to, in this case, oyster culture. These environmental properties (nearly saturated DO and growing kelp) also helps with efflux of nitrogen oxide (NO_x^-), and the decreased influx of both dissolved organic nitrogen (DON) and phosphate (PO_4^{3-}), due to assimilation by kelp (Vonk et al. 2008).

Moreover, Zhang et al., 2009 found that benthic life (normally avoiding localized oxygen depletion level due to deposition of prior suspended material as a result from oyster respiration (Christensen et al., 2003)) were present at polyculture sites of shellfish and seaweed, due to the photosynthetic activity in Sanggou Bay.

In terms of oxygen availability, the polyculture of seaweeds and shellfish as practiced in Sanggou bay is considered sustainable despite the increased deposition rate of suspended organic matter due to shellfish respiration.

4.3 (2) Carbon cycling

Shellfish consume particulate organic carbon (POC) to be embedded in their shells as calcium carbonate (CaCO_3) but also excrete CO_2 for this calcification process together with aerobic respiration (Tang et al., 2011). These processes cause a decrease of oxygen saturation levels and shifts in the carbonate equilibrium (Copin Monte'gut and Copin Montegut 1999). However, dissolved inorganic carbon (DIC) is transformed into organic carbon through photosynthesis by seaweeds (e.g. Tang et al., 2011).

4.3.1 Ocean acidification

As a result, from increasing atmospheric carbon dioxide (CO_2) uptake through the ocean's surfaces and local waste products from respiration, the carbon chemistry in the water is altered. This leads to ocean acidification (OA) where increased levels of carbon dioxide (CO_2), hydrogen (H^+) (lowering pH) and bicarbonate (HCO_3^-) decrease the availability of carbonate (CO_3^{2-}), aragonite (Ω_{Ar}) and calcite (Ω_{Ca}) which are essential for shell development (Feely et al., 2004). Therefore, the decrease of pH potentially threatens growth and survival of calcifying organisms, including *M.edulis* (Clark & Gobler, 2016). This process is most prevalent in the open ocean while in near shore environments, the carbon chemistry, and

thus alkalinity or acidity (pH) are more often subjected to fluctuation due to biological activity (Duarte et al., 2013; Waldbusser & Salisbury, 2014). Kelp can decrease the effects of ocean acidification on shellfish locally (within several meters depending on kelp to shellfish ratio and hydrodynamics) and temporally (during photosynthetic activity), causing strong diurnal fluctuations in oxygen (O₂) and calcite (Ω_{Ca}) saturation in seawater (Chopin, 2015).

In the Skokomish Delta, United States of America, the effects of natural seaweed and seagrass bed's bioremediation potential on clams (*Ruditapes philippinarum*) has been examined by means of a field study. It was found that clam survival and recruitment were not impacted, and no significant differences were found in pH throughout the different treatments. Yet, the size of clams near macrophyte and macroalgae beds was compromised, in comparison with those grown in locations where macroalgae and seagrass were absent, contradicting their hypothesis and suggesting that growth was influenced due to unidentified factors associated with aquatic vegetation (Greiner et al., 2018). With another field study, the effects of bladder wrack (*Fucus vesiculosus* Linnaeus, 1753) were observed to facilitate temporal calcification of *M. edulis* in acidified aquaria and marine cultivation sites near natural kelp beds in Kiel Fjord (Wahl et al., 2018). Chopin, 2015 came to the same conclusion while studying integration of seagrasses and macroalgae to finfish and shellfish monocultures, indicating that this adaptation helps reducing the effects of OA through absorption and assimilation of dissolved inorganic CO₂ locally. Han et al., 2013 observed a positive effect on calcification potential through the presence of kelp (*Gracilaria lemaneiformis*) opposed to monocultures of shellfish (*Chlamys Farreri*) in experimental field microcosms in Sanggou bay. Another study, reviewing the impacts of OA and exploring mitigative measures for bivalve farming proposed the integration of macroalgae to other shellfish cultures as an adaptive measure to overcome future OA problematics in shellfish aquaculture (Tan & Zheng, 2020). The review of Buck & Langan, 2017 on possibilities for open-ocean aquaculture mentioned that the sequestration of CO₂ and increasing pH induced by seaweeds could reduce OA in coastal environments (Clements and Chopin, 2017). Fernández et al., 2019 also reviewed positively on the carbon assimilation potential of both seaweed and shellfish cultivation, proposing seaweed and shellfish IMTA for the mussel aquaculture industry of Chile.

4.3.2 Carbon fluxes

In West Bengal, India, an experiment on the performance of IMTA with different combinations including fed species; *Mugil cephalus*, *Liza tade* (Mulletts), *Peneaus monodon* (tiger shrimp) and extractive species; *Ipomoea aquatica* (water spinach) and *Crassostrea cuttackensis* (oyster) in brackish water ponds displayed a decreasing trend on the levels of dissolved organic carbon (DOC), relative to the amount of different species cultured together. Additionally, particulate organic matter (POM) increased in all ponds throughout the experiment but the final measurements were parallel to DOC contents measured at the end of the study (Biswas et al., 2020). Looking at benthic carbon fluxes, it was found that the correlation between dissolved oxygen (DO) influx and temperature dictate carbon fluxes rather than total organic carbon (TOC) (waste product from shellfish and finfish cultivation) in the benthic environment of Sanggou Bay (Ning et al., 2016). Accordingly, compared to shellfish and finfish monoculture sites, significantly lower carbon fluxes were observed at IMTA sites with seaweeds besides shellfish and or finfish as photosynthetic activity increased dissolved oxygen (DO) influx locally while also consuming CO₂. Condition incides (health)

of *M.edulis* cultivated both in monoculture and in close proximity to *S.latissima* have been monitored throughout one year, showing no significant differences in carbon content of the meat nor shell growth related to interaction with *S.latissima* between the different treatments (Rößner, 2013). For *S.latissima* during the grow out period, carbon accumulation through photosynthesis may be enhanced by luxury uptake of nitrogen in early life stages, induced by fertilization of *M.edulis* during nursery nursery (Chapman et al., 1978; Rees 2007). This positive interaction was tested successfully by the differences biomass development in *S.latissima* observed in the mesocosm and field experiment in Kiel Fjord (Rößner, 2013).

4.4 (3) Nutrient cycling

Although nutrients are fundamental to aquaculture ecosystems, information on accurate internal nutrient cycles in IMTA systems is still lacking in literature (Sequeira et al., 2008; Troell et al., 2009; Tang & Fang, 2012; Chopin, 2013; Ning et al., 2016).

Accurate account of nutrient fluxes between the benthic environment and the water column in IMTA systems is essential for aquaculture management. According to Buck & Langan, 2017, in terms of competition, there is little interaction between seaweeds and shellfish as shellfish feed on organic nutrients and seaweeds access inorganic nutrients. Looking at nutrient fluxes, shellfish even enhance the availability of some inorganic nutrients (Jansen, 2012). However, an excess of inorganic nutrients in the form of aquaculture wastes (e.g. nitrogen and phosphorus) from fed aquaculture feed spillage and faeces may cause eutrophication, potentially causing “harmful” algal blooms. Nutrient absorbers (e.g. seaweed) can mitigate the effects of eutrophication by competing for resources with phytoplankton and in turn removing dissolved nutrients from the system through harvest. Moreover, suspension feeders (e.g. shellfish) feed on algae and can disperse other particulate organic wastes as Zhang et al., 2019 described in his literature review and field study on bio mitigation from IMTA at Kagoshima Prefecture, Japan.

In addition to impacts related to algae blooms, account of benthic fluxes is also important. High bio deposition (faeces and pseudo faeces) by organisms actively grown can cause a substantial increase in organic content of the sediment (Hatcher et al., 1994; Christensen et al., 2000; Ferrón et al., 2009; Lee et al., 2011). In the sediment, nutrients can then be resuspended in the water column via sedimentary mineralization, resulting in degradation of the water quality (Fanning et al., 1982; Chau 2002; Qin et al., 2004). A case study done in Sanggou Bay - China investigated the impacts of aquaculture on benthic nutrient fluxes in the IMTA system, and sedimentary mineralization processes (Ning et al., 2016). The case study implicated that benthic nutrient fluxes were significantly lower in IMTA systems compared to monoculture systems due to efficient nutrient recirculation. Furthermore, it was suggested that benthic nutrient fluxes in IMTA systems were caused by dissolved oxygen levels (DO) at different culture areas rather than by sedimentary total organic carbon (TOC) generated from cultured organisms. Ning et al., 2016 suggested that seasonal variations were controlled by nutrient concentrations and temperature related to aquaculture. Similarly, a Danish field experiment in the Horsens Fjord by Marinho et al., 2015 concluded that the peak in January and annual minimum in May-July of nitrogen content in close proximity

to an IMTA system (blue mussel (*M.edulis*) and rainbow trout (*Oncorhynchus mykiss*)) matched the seasonal trend of ambient concentrations.

Skriptsova & Miroshnikova, 2011 performed a laboratory analysis where the effluent of shellfish waste products (phosphates and nitrogen- primarily in form of ammonium) were strongly reduced by seaweeds in both the flow-through and static treatments. Marinho et al., 2015 illustrated that there is potential for assimilation of nutrients, particularly nitrogen, by *S.latissima*. Seaweeds cultivated nearby fish farms generally hold a higher nitrogen content, and utilisation of waste nutrients from fish farms may be depended on factors as nutrient loading, current speed and direction, and distance to nutrient source. It was further noted that cultivated mussels contribute to local dissolved nitrogen concentration of which seaweed in an IMTA system takes advantage if cultured in close proximity. The combination of oysters and different seaweeds as extractive components of IMTA in land based low-saline ponds proved to provide efficient accumulation of nitrogen from nitrogen dioxide (NO₂), nitrite (NO₃⁻) and total ammonia nitrogen (TAN), but also phosphorus from phosphate (PO₄³⁻) (Biswas et al., 2020). Troell et al., 2009 identified and analysed the challenges in offshore IMTA. He found that seaweed and shellfish as an extractive component besides finfish cultivation benefit in periods with limited availability of organic and inorganic nutrients as growth rates were 50% higher as a result from nutrient uptake and particulate waste filtration, than those cultivated without cultured fish in proximity. In Bocabec Bay, Canada, the cultivation of *S.latissima* near salmon cages proved beneficial as production of seaweed was 46% greater compared with the reference site (*S.latissima* monoculture site) due to enriched levels of nutrients from salmon faeces and feed spillage (Chopin et al., 2004).

With a field and lab study in Kiel Fjord, the growth enhancing effect of *M.edulis* on *S.latissima* in early life stage has been studied. Here, Rößner, 2014 found that a higher biomass of *S.latissima* was obtained compared with the reference site, likely due to the local ammonia excretion of *M.edulis* cultivation during hatchery providing nitrogen reserves during grow-out (Rößner, 2014; Chapman & Craigie, 1977). However, the nitrogen contents in *S.latissima* and in the surrounding waters were not significantly different between the field sites in Kiel Fjord, and nutrients are also taken up indirectly in *M.edulis* tissue (Ferreira et al., 2011). Yet, for *S.latissima* farming in the Baltic, she recommends the combination of this crop with *M.edulis* cultivation to temporarily compensate for nutrient limitation through faeces and ammonia excretion. For a high efficiency of ammonia and phosphorus removal and optimal growth of seaweed Mao et al., 2009 and Ajjabi et al., 2018 advises a shellfish: seaweed ratio of 3:1. Large scale implementation of seaweeds to shellfish cultivation sites in Chili may therefore create an ocean acidification and eutrophication buffering environment by producing an additional aquaculture product (Fernández et al., 2019).

Assessing the IMTA options for abalone and seaweed in land based ponds, Nobre et al., 2010 noticed a deficiency of nutrients as waste production of the abalones and the supplied seawater did not meet the requirements for intensive seaweeds cultivation in a semi closed system. Chopin et al., 2015 even indicated the bio mitigation potential of seaweeds, proposing to introduce nutrient trading credits (NTC) for an increased economic feasibility of integration or cultivation of seaweeds. As these organisms are effective nutrient scrubbers (Chopin et al., 2001), this could add an additional 23% of profit to present seaweed cultivation practices throughout the world (Chopin et al., 2012).

4.5 (4) Ecological habitat

Through the literature collection, ecological habitat was mainly studied to explore biofouling, either as a function of habitat provision from IMTA structures and their species or to find ways of dealing with the negative effects from these “marine pests”, decreasing crop quality as they attach to and compete with or damage the cultured organisms.

4.5.1 Biofouling

Seaweed cultivation is prone to epiphytes as opportunistic benthic algae attach to the aquaculture structures and seaweeds themselves, competing for nutrients, space, and light (Buschmann and Gómez, 1993). For *S.latissima* cultivation, this problem becomes ever more present seasonally as accumulation of epiphytes increases during the warmer months (spring and summer) (Marinho et al., 2015; Førde et al., 2016). Additionally, biofouling accumulation on aquaculture structures and seaweeds is observed to be less intense in exposed environments. Biofouling on shellfish aquaculture can increase weight, possibly causing detachment (Fitridge et al., 2012); cause physical damage through their method of attachment or predation (Carroll et al., 2015); reduce shellfish fitness via competition of resources or compromising bivalve functioning (Woods et al., 2012).

Bannister et al., 2019 studied biofouling in marine aquaculture (finfish, shellfish and seaweeds) through a review of recent research and developments. They proposed the strategies to cultivate seaweeds in exposed environments and/ or to harvest the crop before water temperature increases (also mentioned in Troell et al., 2009). Additionally, he noted that encouraging grazing by herbivores may also help in countering biofouling as these organisms consume either suspended epiphytes before settlement or after, depending on the species. Rößner, 2013 also observed this positive effect in mesocosms where the filtration by *M.edulis* (consuming epiphytes and other microalgae, reducing biofouling and increasing light availability), combined with a sufficient supply of nutrients, supported early life stage development and faster growth of *S.latissima* during grow-out in the fjord. Spatfall however may induce losses in seaweed biomass as observed in Chile where a heavy load of mussels settled on the *Gracilaria* culture (Halling et al., 2004). At the *S.latissima* cultivation sites of Norway, fouling of *M.edulis*, amongst other epiphytes has also been reported during summer months (Stévant et al., 2017). Another study on the influence of *S.latissima* on *M.edulis* also observed the heavy loading of *M.edulis* spat on seaweed blades during summer months, but in periods with lower water temperature, healthy blades were seldom affected (Rößner, 2013).

4.5.2 Habitat provision

The introduction of structure and cultured organisms attracts a multitude of species in succession, some of them colonizing the aquaculture structures or crops while others prey on these “fouling” species (Chopin et al., 2012). However, the waste production of aquaculture practices may compromise oxygen availability, depending on the cultivated species and in turn decreasing biodiversity in and around the cultivation area.

In Gullmar Fjord, Sweden, it has been suggested that natural benthic community’s species diversity and biomass below *M.edulis* longline monoculture sites may be reduced through accumulation of organic material and sedimentation of particulate organic nitrogen (PON) (Lindahl et al., 2005). Opposed to *M.edulis* cultivation, with the right aquaculture system design, seaweed cultures can effectively act as a restoration measure by providing habitat, adding structures, and attracting aquatic organisms while also inserting a nett supply of oxygen in and around the culture sites (Buck et al., 2017). In context to ocean acidification, Wahl et al., 2018 mentioned that plankton blooms may occur (from aquaculture waste products), resulting in hypoxic conditions and decreasing bio mitigation efficiency of seaweeds by shading as observed in Kiel Fjord. Yet, the algae bloom can act as an ample food source for calcifying filter feeders, which in turn strengthens their ability to cope with ocean acidification (Ramajo et al., 2016).

4.6 (5) Physical habitat

The physical habitat was also studied often as e.g. currents are highly important for transport of food and waste but also the structural integrity of marine aquaculture structures.

4.6.1 Irradiance

For this parameter, only the effects on seaweed or more specifically *S. latissima* apply as shellfish do not directly depend on light intensity. A study in Horsens Fjord, Denmark found that photosynthetic saturation of *Saccharina Latissima* was not fully achieved in winter months (December- February) where light intensity reaches down to $48 \mu\text{mol m}^2 * \text{s}$ (Marinho et al., 2015). However, according to other studies it was found that a range of 16,2- 250 $\mu\text{mol m}^2 * \text{s}$ can maintain growth, yet possibly supplemented by luxury uptake when plenty light and nutrients ware available (Fortes & Lüning, 1980; Peteiro & Freire, 2012; Han & Kain, 1996). Marinho et al., 2015 also indicated that the thermal conditions during the main growing period of *S. latissima* were not optimal as water temperature reached down to 0 °C in winter (below the threshold of 5 °C (Machalek et al., 1996; Davison & Davison, 1987; Groenendijk et al., 2016)), likely explaining the relatively low yield. Another study, executed in Kiel Fjord, Denmark found that despite the enhanced water transparency induced by algae bloom filtration by *M. edulis*, the cultivation system (longlines) casts a shadow on the first few meters from the surface in its close vicinity. In this study however, the first few meters of *S. latissima* seeded lines have been destroyed primarily due to

mechanical stress but nevertheless, the growth of *S. latissima* below the *M. edulis* lines was considered good (Rößner, 2013). Light limitation or UV protection was also observed in Kiel Bight- Baltic Sea, which was part of the previous study where the constructions for trout cultivation (including piers, cages, and pontoons) cast a shadow upon *S. latissima* culture lines. In contrast, a much lower shading effect on *S. latissima* culture lines was observed with the longlines and buoys of *M. edulis* cultures. However, the light limitation was deemed to be mainly due to the limited overall hours of sunlight in winter months (Rößner, 2013).

4.6.2 Water movement

Water movement affects both seaweeds and shellfish production through possible dislodgement, interception of seston supply, nutrients and waste fluxes. Single bladed seaweeds found as present in *S. latissima* are found to generate turbulence and swirl, decreasing mainstream velocity (Eckman et al., 1998; Eckman & Duggins, 1991; Hurd, 2000). Alteration of the hydrodynamic environment was also observed as a result from aquaculture facilities in Sanggou Bay, causing water current reduction while reducing nitrogen and particulate matter fluxes (Zhang et al., 2009; Shi et al., 2011). As a result from a decreased current velocity, in the period of algae blooms and a lowered filtration rate by *M. edulis*, seston accumulated near the culture ropes in Kiel Fjord. It took some time before this surplus of particulate organic matter was decreased significantly by the cultured *M. edulis* (Rößner, 2013). Hydraulic stress induced by the *S. latissima* cultivation is not suspected to have a significant impact on *M. edulis* attachment in low current environments (Koehl et al., 2003; Rößner, 2013). Strong currents, however, will pose a threat for the attachment of both shellfish (byssal thread) and seaweeds (holdfast), potentially leading to biomass losses in IMTA due to dislodgement from culture ropes (Halling et al., 2005). The parallel positioning of the culture lines on dominant current direction at an integrated kelp and scallop co-culture system was found to be a problem as seaweed blades wrapped around the scallop nets, cutting of the supply of particulate organic matter (POM), normally brought in by the currents (Zhang et al., 2019).

Geiner et al., 2018 assessed the natural remediation by macroalgae and seagrass on ocean acidification and found that current induced flapping and waving of macroalgae reduced water movement and influenced settlement of particles (Ginsburg & Lowenstam, 1958). This effect both causes a decrease (Carroll & Peterson, 2013) and increase (Peterson et al., 1984) in the availability of algae for bivalves. Generally, with a very high current velocity, the removal efficiency by macroalgae is often decreased as retention time at the thalli (blade surface) is shorter (Skriptsova & Miroshnikova, 2011). However, a moderate current or low velocity and thus nutrient flux causes an increase in nutrient uptake rates as more nutrients are constantly available (Buschmann et al., 2001). Moreover, the current induced flapping and waving of the blades of *S. latissima* in a lab experiment have been found to decrease the diffusive boundary layers on the blade's surfaces significantly, allowing for an enhanced renewal thus nutrient flux at the blade's surfaces (Huang et al., 2011). This statement is supported by another lab experiment where macroalgae had a higher nutrient uptake rate (2-3 times) in a continuous flow system opposed to the uptake rates found in a static system (Skriptsova & Miroshnikova, 2011). Given this information, the combination of cultures may either decrease a high current velocity, positively impacting culture circumstances, or decrease nutrient fluxes in a decreased slow current environment, both depending on the scale of the culturing system.

5 Discussion

The latter of findings on oxygen cycling originates from field studies in Sanggou bay, including different combinations of shellfish, seaweeds, and finfish in mono- and integrated cultures. Nutrient cycling was represented in a multitude of studies from literature review to full scale field experiments. The information on carbon cycling comprises of experimental results from both lab-, mesocosms- and, field studies. Findings on biofouling are collected from several studies in the field or lab environment and reviews on the impact of biofouling in shellfish and seaweed monocultures. What was found on light availability was primarily based on field studies in Danish Fjords and supplemented with findings from some other studies. Water movement was represented by a multitude of lab and field experiments.

5.1 Method

Throughout the literature collection phase, not all search terms were used nor yielded relevant studies. Most of the relevant studies were found searching on “shellfish and seaweed IMTA”, supplemented by nutrient, biofouling, oxygen and carbon dioxide in combination with the “shellfish and seaweed IMTA. Yet, plenty results were found throughout the collected literature and It seems like the important topics were represented as multiple findings relevant for the different “feedback loop themes” were regularly found within one paper. In addition to using search terms, the principle of snowballing also yielded a few relevant studies.

The latter of collected material originates from discussion sections, also including several other references that were not included in the catalogue. Some of those references were not open access and the goal of collecting roughly 10 findings per relevant “feedback loop theme” was surpassed in most cases. With the implementation of the relevant literature studies and their findings, the measured parameter section was neglected as for some studies, there were simple too many- or with literature reviews, no measurements. Likewise, the gap in knowledge section was seldomly used as this was not provided in most studies. The literature relevance analysis potentially was too mild as no collected references were not relevant according to the scores it received on project location, scale, and species combination.

A total of 28 studies were reviewed and yielded 2 relevant findings for oxygen cycling, 10 for carbon cycling, 15 for nutrient cycling, 11 for ecological habitat, and 8 for physical habitat. More studies were available, but it seemed like the most important and relevant researches were collected. Within most separate findings, different factors and processes were included in relation to the main topic of the subtract from the discussion sections, leading to an overall abundance of feedback loop related findings. As the environmental properties and setup of the studies differ, the collected findings were mostly qualitative, rather than quantitative.

With the analysis of the collected results, relations between processes and factors from different studies were interpreted and integrated in feedback loops per assigned topic. Yet, not all findings on indirect interactions fitted in the separate feedback loops and were therefore elaborated in text, separately but in relation to the factors and processes from feedback loops.

In the application section of the discussion, the separate feedback loop and elaborations from integration are further interpreted, linked to species requirements and the pilot site conditions in order to obtain an idea of the importance of certain factors and processes.

5.2 Integration

In the following chapter, the main interactions and associated factors as stated in literature are identified and elaborated per main topic, discussed in the following paragraphs. The main findings yielded from the literature review are summarised in Appendix 2. From here, feedback loops are created, containing direct relations in processes relevant to the main topic. In the produced feedback loops, the arguments of processes are indicated with a code, e.g. oxygen cycling 1 (OC1), originating from appendix 2. Further interpretation of potentially relevant factors that may influence discussed interactions are elaborated using indirect arguments and additional findings from the results section.

5.2.1 Oxygen cycling

An overview of interactions in direct relation to oxygen cycling between shellfish and seaweed in IMTA systems in the form of a feedback loop is given in figure 6. Relevant factors include: Oxygen concentrations, nutrient cycling and flux, presence or likelihood of algae growth/ blooms, and carbon (CO₂) concentrations.

The results show that seaweed culture inserts a nett supply of dissolved oxygen in and around culture sites, increasing biodiversity and availability of oxygen for uptake by other cultured species (OC2, NC7 and OC1). Oxygen produced by photosynthesis increases local nitrogen oxide (NO_x-) efflux and decreases benthic carbon and other nutrient fluxes (OC4, NC7 and CC3). Dissolved inorganic nutrients are taken up by seaweeds for growth, but together with the addition of nutrient from shellfish waste excretion, the limited maximum uptake rate of seaweed species near cultivation sites may cause occurrence of algae blooms. Algae blooms may decrease oxygen concentrations or potentially induce hypoxic conditions (OC3, LA2).

Results also show that heavy currents, increase nutrient supply (WM4 and WM5), but reduce nutrient availability for uptake by seaweeds (WM5). If heavy currents do not limit nutrient uptake by algae competing for the available nutrients (NC9), low current velocity may enhance the negative oxygen concentration feedback loop, ultimately limiting production yield, whereas a steady slower flow allows for higher nutrient removal efficiency in seaweeds (NC10). In addition, heavy currents limit settlement of epiphytes on seaweed, allowing for more efficient photosynthesis and thus oxygen production.

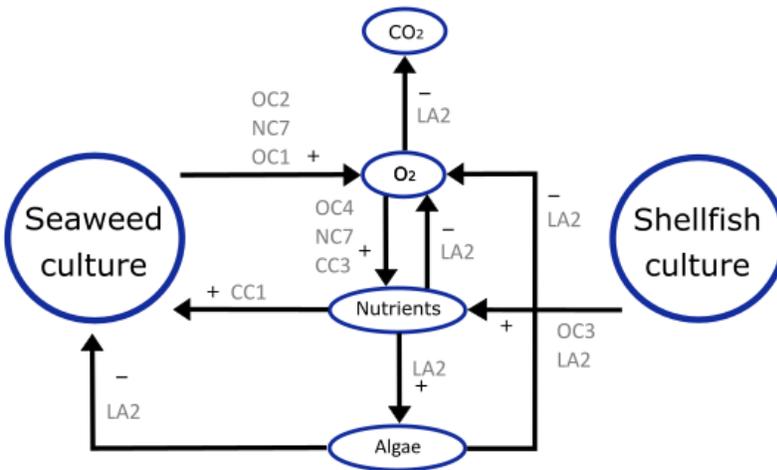


Figure 6: Feedback loop interactions in direct relation to oxygen cycling between shellfish and seaweed in IMTA systems. Main factors and operators of relevance to oxygen cycling identified from the results are shown in black rectangles. The dotted blue line illustrates the boundaries of the IMTA system. Arrows indicate a direct interaction or relationship, for which it is indicated whether the operator enhances (+) or limits (-) the degree, extent, likelihood or presence of the next component.

5.2.2 Nutrient cycling

An overview of interactions in direct relation to nutrient cycling between shellfish and seaweed in IMTA systems in the form of a feedback loop is given in figure 7. Relevant factors include: Oxygen concentrations, nutrient concentrations, carbon dioxide concentrations, biodiversity, currents and the presence or likelihood of algae growth (or blooms).

Results showed that the influx of nutrients and seston is dictated by the current velocity and direction, and that heavy currents increase nutrient supply but reduce nutrients available for uptake by seaweeds (WM4, WM5). Shellfish consume particulate organic matter, indirectly removing inorganic nutrients from the water column (NC6, NC18). However, it was found that direct interspecific competition between seaweed (inorganic nutrient uptake) and shellfish (organic nutrient uptake) seems to be limited (NC15, NC18), and cultured mussels contribute to local dissolved nitrogen concentrations, enhancing nutrient availability for seaweeds (NC1, NC3). *M. edulis* faeces and ammonia excretion even allow luxury uptake in *S. latissima* during nursery (NC2). Furthermore, luxury uptake of nitrogen due to fertilization by shellfish enhances carbon uptake of nearby seaweeds during photosynthesis (CC1), further demonstrating the benefits of shellfish cultivation in proximity to seaweeds. During ambient nutrient limitation, seaweed, but also shellfish may benefit from nearby finfish cultivation (NC12).

Although inorganic nutrients are made available for seaweeds by shellfish, an excess of inorganic nutrients from settled waste by shellfish cultivation can compromise local biodiversity by causing hypoxic conditions, and enable occurrence of algae blooms that may cause hypoxic conditions and a decrease of light availability as well (OC3, LA2). In addition to this effect, particulate organic matter is assimilated less efficiently by lower biodiversity in pond cultures (NC19), which would further affect growth and production of cultured species. However, seaweeds can mitigate eutrophication by competing with algae through consumption of dissolved inorganic nutrients if environmental conditions allow a high photosynthesis efficiency (NC9).

Although nutrient supply is increased by heavy currents, it reduces nutrient uptake by seaweeds (WM5), where a steady flow allows for a higher nutrient removal efficiency (NC10). As also discussed in paragraph 5.2.1 (oxygen cycling), this may indicate that tidal currents, velocity/ retention time are important factors regarding effective competition for nutrients of seaweeds with algae and may be an important factor limiting occurrence of algae blooms. As flapping and waving of seaweeds decreases main current velocity and efflux of waste (WM3), current velocity and steadiness as a factor in regard to competition for available nutrients between algae and seaweeds may prove a delicate relationship and require further investigation.

Photosynthesis by seaweed (or algae) plays a key role in nutrient cycling, as the nett release of oxygen by seaweeds increases nitrogen oxide (NOx-) efflux locally, also benefiting biodiversity (NC7). In addition, photosynthesis induced accumulation of nutrients in seaweeds leads to a reduced influx of dissolved organic nitrogen (DON) and phosphate (PO43-) (NC8). Benthic nutrient fluxes on the other hand, are reduced by dissolved oxygen content (DO), rather dan total organic carbon (TOC) in IMTA systems (OC4).

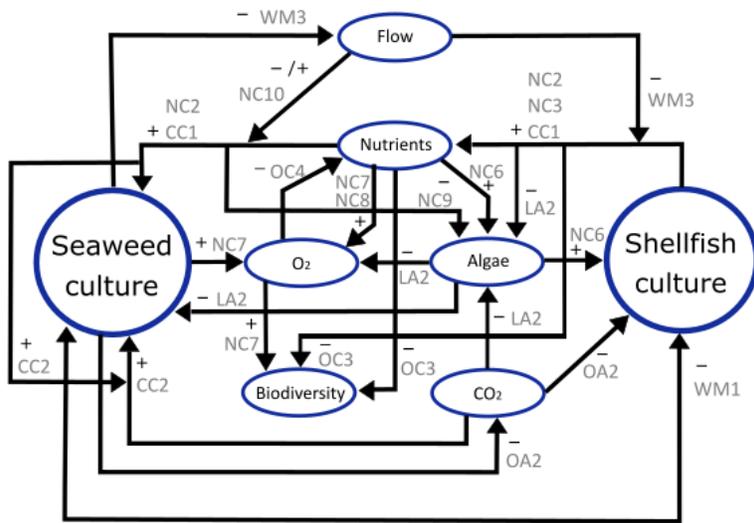


Figure 7: Feedback loop interactions in direct relation to nutrient cycling between shellfish and seaweed in IMTA systems. Main factors and operators of relevance to nutrient cycling identified from the results are shown in black rectangles. The dotted blue line illustrates the boundaries of the IMTA system. Arrows indicate a direct interaction or relationship, for which it is indicated whether the operator enhances (+) or limits (-) the degree, extent, likelihood or presence of the next component.

5.2.3 Carbon cycling

In Seaweed and shellfish IMTA, carbon cycling is altered through photosynthesis and respiration of cultivated species. The direct interactions and factors from the result section are summarized in figure 8. Relevant factors include nutrient availability, carbon availability, algae, oxygen availability, biodiversity and water movement.

Cultivation systems and standing stocks of shellfish and seaweed decrease flow velocity, altering transport of seston, ambient- and shellfish excreted organic and inorganic nutrients (WM3, WM5, NC10). In a low current environment, a high level of dissolved carbon may accumulate in suspension and enhance ocean acidification, compromising shell development in shellfish (QA1, OA2). However, due to a higher

biodiversity, initiated by seaweed net input of oxygen (OC1) decreases dissolved organic carbon (CC2). As a result from shellfish excretion, inorganic nutrient levels increase locally (NC1, CC1), potentially initiating algae blooms (LA2) and reducing oxygen levels in the local environment (OA1, OA2) but also enhancing seaweed carbon uptake efficiency which might counter hypoxia through photosynthesis (OC3). Local algae blooms can however be diminished by shellfish filtration (LA1, NC6) and competition with seaweeds for inorganic nutrients (NC9). Carbon uptake efficiency through seaweed photosynthesis enhanced by fertilization of inorganic nutrients from shellfish waste (NC1, OA2) and nutrient supply from the environment (NC10), lowers acidity and thus increases calcite saturation for shellfish shell development (OA1).

Furthermore, the algae and suspended epiphyte filtration by shellfish increases light availability, enhancing seaweed photosynthesis potential (LA1) and thus its ocean acidification mitigation efficiency.

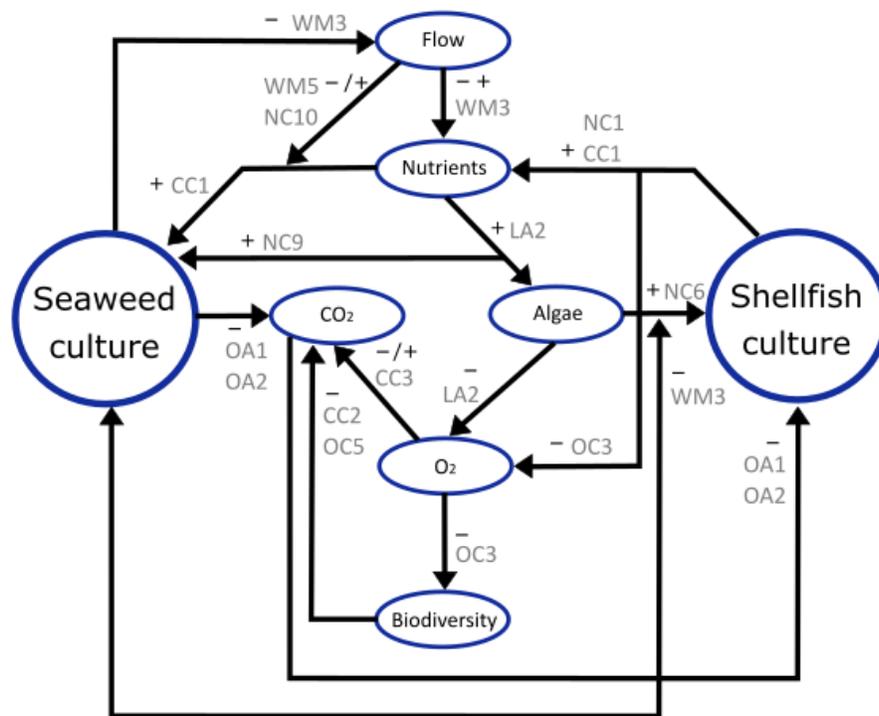


Figure 8: Feedback loop interactions in direct relation to carbon cycling between shellfish and seaweed in IMTA systems. Main factors and operators of relevance to carbon cycling identified from the results are shown in black rectangles. The dotted blue line illustrates the boundaries of the IMTA system. Arrows indicate a direct interaction or relationship, for which it is indicated whether the operator enhances (+) or limits (-) the degree, extent, likelihood or presence of the next component.

5.2.4 Biofouling

In IMTA, biofouling can compromise the growth efficiency of seaweeds and shellfish through direct damage from attachment but also through competition for light and inorganic nutrients or seston. Therefore, in figure 9, the processes and factors from the direct interactions have been put into context of biofouling. Important factors in this feedback loop are oxygen availability, biodiversity, epiphytes, water movement (flow) and water temperature.

Shellfish filtration of suspended particles decreases the number of epiphytes before settlement but during spatfall, the young shellfish can heavily load on seaweed blades and culture structures (BF1, BF3).

Settlement of epiphytes on seaweeds and culture structures is enhanced when water temperature is relatively high (BF3) but in exposed environments, settlement is diminished due to high current velocities (BF3, BF2). Moreover, the nett release of oxygen from photosynthesis allows a higher biodiversity at the culture site, possibly causing a decrease in epiphytes through predation, grazing and filtration by mobile benthic and pelagic species (OC1, BF1, NC19).

Settlement of epiphytes on seaweeds in colder periods was significantly less and healthy blades were often unaffected, therefore, suggesting that the enhanced supply of nutrients by shellfish waste excretion weaponizes seaweed resilience in terms of biofouling (NC1, NC2). Likewise, the current induced influx of inorganic nutrients is determined by current velocity and direction and with a steady flow, seaweeds are able to subtract more nutrients from the water column, increasing their resilience against biofouling (NC10, WM4, WM5). A higher biodiversity may decrease the availability of organic carbon which are transformed into inorganic carbon through respiration, available for seaweed growth thus again strengthening the blades resilience to biofouling (OC5, CC2).

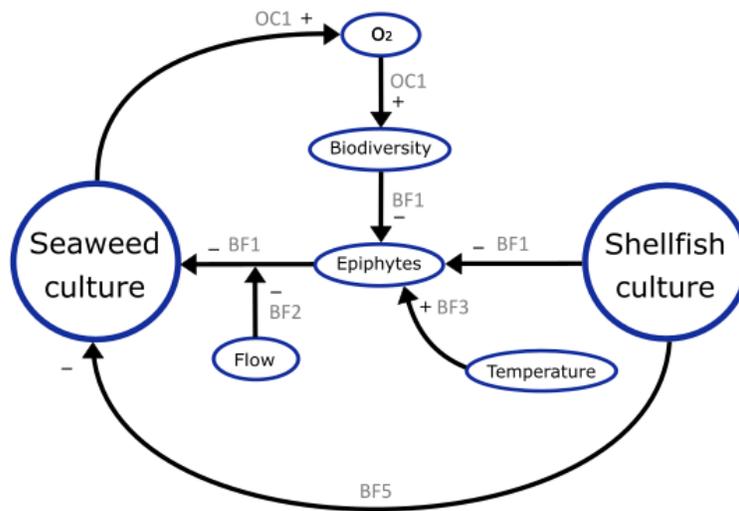


Figure 9: Feedback loop interactions in direct relation to biofouling between shellfish and seaweed in IMTA systems. Main factors and operators of relevance to biofouling identified from the results are shown in black rectangles. The dotted blue line illustrates the boundaries of the IMTA system. Arrows indicate a direct interaction or relationship, for which it is indicated whether the operator enhances (+) or limits (-) the degree, extent, likelihood or presence of the next component.

5.2.5 Light availability

Light availability is necessary for photosynthesis and therefore an essential parameter in seaweed cultivation. In this paragraph, the processes and relations related to light availability are provided in figure 10 and elaborated based on the result section, in the following paragraphs. Relevant factors include algae, epiphytes, nutrients, water movement and water temperature.

The deposition of particulate organic matter from shellfish culture may leach and increase inorganic nutrient availability, potentially inducing algae blooms which reduce light availability (CC1, LA2). However, in a seaweed and shellfish IMTA, algae concentrations are reduced by shellfish through filtration (LA1, BF1). Moreover, shellfish waste products also enhance the availability of inorganic nutrients, allowing for luxury uptake in the blades and more efficient carbon uptake by the cultured seaweeds (CC1),

strengthening seaweed coping mechanism for periods with shortage of irradiance. Biofouling on seaweed blades and aquaculture structures may also limit the availability of light and potentially competition on inorganic nutrients but accumulation of epiphytes may be reduced through shellfish filtration and these organisms settlement ability is compromised in exposed and cooler environments (BF1, BF4, BF2, BF3). On the other hand, Shellfish spatfall can load heavily on the seaweed blades.

When looking at indirect processes, seaweeds could prevent poor light conditions from eutrophication as they may outcompete algae through uptake of inorganic nutrients (NC9). A temporal lack of light availability for photosynthetic processes could potentially be countered with the excessive amounts of assimilated nutrients as a result from luxury uptake through shellfish waste excretion obtained in early life stages (CC1).

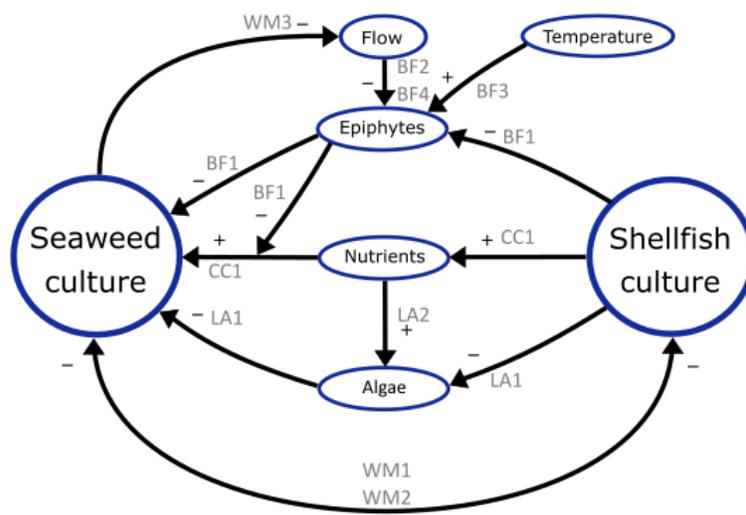


Figure 10: Feedback loop interactions in direct relation to light availability between shellfish and seaweed in IMTA systems. Main factors and operators of relevance to light availability identified from the results are shown in black rectangles. The dotted blue line illustrates the boundaries of the IMTA system. Arrows indicate a direct interaction or relationship, for which it is indicated whether the operator enhances (+) or limits (-) the degree, extent, likelihood or presence of the next component.

5.2.6 Water movement

Water movement is an essential physical parameter in aquaculture. Relevant processes include the in and efflux of organic-, inorganic nutrients, seston and settlement of epiphytes which may be strongly affected by current direction and speed and have potential impacts on seaweed and shellfish cultivation. In this subparagraph, the feedback loop (figure 11) with the direct interactions in water movement are provided and elaborated, including relevant indirect processes and their relations to the direct interactions from the result section.

Results show that seaweed blades alter the hydrodynamic environment through slowing down main current velocity (WM3). With a decreased current velocity in exposed environments, the supply or influx of nutrients and seston are lower but retention and thus availability and nutrient removal efficiency in seaweeds and shellfish increases, depending on the direction of flow. (NC10, WM4, WM5). A downside in current reduction comes with settlement potential of epiphytes, excessively accumulating on seaweed

blades and aquaculture structures in periods where the water is calm and warm, and a reduced waste efflux (BF2, BF4, WM3). When seaweeds and shellfish are cultured in very close proximity, current induces flapping and waving of seaweeds can cause the blades to wrap around the shellfish lines, cutting off the supply of organic- and inorganic nutrients (WM1, WM2). Moreover, a high current environment might cause dislodgement of both shellfish and seaweeds.

Looking at indirect impacts related to current velocity, it was found that with decreased nutrient effluxes (waste products) can be assimilated through direct uptake by seaweeds and indirectly reduced through chemical reactions resulting from a higher net supply of oxygen from seaweed photosynthesis locally (NC7, NC8, OA1, OC1, NC17). Moreover, a reduced nutrient efflux can initiate algae blooms, competing with seaweeds for inorganic nutrients and potentially causing hypoxic conditions but on the other hand also increasing the supply of seston to be consumed by shellfish (LA1, LA2, NC6). With a very low current velocity, the inorganic nutrients influx from shellfish respiration (if sufficient algae are available) only will not meet the growth requirements in large seaweed cultivation (NC5).

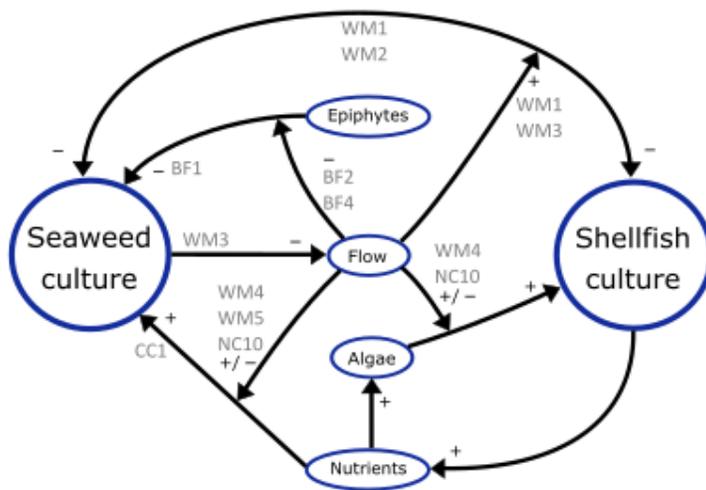


Figure 11: Feedback loop interactions in direct relation to water movement between shellfish and seaweed in IMTA systems. Main factors and operators of relevance to water movement identified from the results are shown in black rectangles. The dotted blue line illustrates the boundaries of the IMTA system. Arrows indicate a direct interaction or relationship, for which it is indicated whether the operator enhances (+) or limits(-) the degree, extent, likelihood or presence of the next component.

5.3 Application

In this chapter, an elaboration on key factors for IMTA production found in the integration section are provided. Therefore, integrated factors and operators are put in contrast with the theoretical framework sections (shellfish and seaweed requirements and the environmental conditions at pilot sites), defining plausibility of occurrence and potential severity of impact. Through this approach, relevance of parameters to be monitored can be suggested for the recommendation section, in addition to an indication on field study setup.

5.3.1 Parameters in relation to species interactions

Overall, the production of *S.latissima* and *M.edulis* in a polyculture seems to be more beneficial than monoculture for both the farmer and the environment. Below, the most important processes are put in to contrast with *M.edulis* and *S.latissima* poly culture.

The importance of photosynthetically enriched oxygen levels solely for meat growth in *M.edulis* seems not to be very high as this organism is able to depend on anaerobic respiration. Yet, due to global and local increased input of carbon, calcification (shell growth) may be compromised through a lack of calcite saturation as a result from ocean acidification if not for carbon assimilation and the nett output of oxygen by seaweeds. Moreover, the waste excreted in *M.edulis* cultivation could cause hypoxia and eutrophic conditions but in seaweed and shellfish IMTA, oxygen saturation is enhanced through photosynthetic output locally which may lessen the likelihood of hypoxia and occurrence of eutrophic conditions. Therefore, enriched oxygen levels may enhance nutrient limitation but to increase oxygen levels effectively, ample nutrients are required for photosynthesis in seaweeds in the first place.

Through photosynthesis by *S.latissima* and filtration by *M.edulis*, ambient nutrient concentrations can effectively be accumulated. Where *S.latissima* requires inorganic nutrients for growth, *M.edulis* gain mass through feeding on algae which are also phototrophic. These processes provide a nett input of oxygen and through *M.edulis* respiration, an increased load of nutrients locally. Oxygen in turn is involved in chemical processes, breaking down particulate faeces and pseudo faeces, leaching inorganic nutrients to become available for *S.latissima* production. The additional availability of inorganic nutrients allows for a higher carbon uptake in *S.latissima*, in turn increasing calcite availability for shell production. Yet, these inorganic nutrient availability can also induce algae blooms, leading to eutrophic conditions which will diminishes the ecological quality of the cultivation site while inhibiting photosynthesis as light is intercepted to reach the *S.latissima* blades. Current velocity and direction dictate the supply and availability of nutrients and seston as mentioned in water movement.

Ocean acidification induced by the increased levels of carbon in the water column cause pH to drop and calcite saturation to decline. Looking at shellfish and seaweed IMTA, this has a negative effect on shell development, whilst shellfish also excrete carbon and other inorganic nutrients. Seaweeds however benefit from these additional inorganic nutrients, depending on flow velocity and can effectively utilize carbon through accumulation, furthermore increasing oxygen availability locally which counters the lack of calcite saturation for calcification processes in shellfish during photosynthetic activity. The nett supply

of oxygen further allows for a higher biodiversity which increases fluctuation in pH. Another result from enhanced nutrient levels is the potential for algae blooms to occur. These events might decrease the availability of nutrients and light for seaweeds, but in an IMTA system, competition from algae is compromised by shellfish filtration.

Biofouling is not a requirement for seaweed and shellfish growth, on the contrary, it inhibits their ability to access nutrients and physically damage as a result from epiphyte attachment and activities are observed. Seaweeds are able to counter settlement of epiphytes as an increase in biodiversity through enhanced oxygen levels initiates a higher predation and grazing pressure on these “marine pests”. Likewise, shellfish consume epiphytes in suspension before settlement but during spat fall, offspring has been reported to settle on seaweed blades heavily. Moreover, the reduction of currents as a result from aquaculture structures and grown species allows for efficient settlement of epiphytes and this is further enhanced when temperatures are relatively high. Yet, when temperatures are low, settlement of epiphytes takes place on healthy seaweed blades sporadically.

Shellfish excrete additional inorganic nutrient in the local environment. These inorganic nutrients are partially assimilated by seaweeds and may potentially initiate algae blooms. A high algae concentration diminishes light penetration and thus decreases photosynthesis potential and seaweed growth. Moreover, epiphytes on seaweed blades and cultivation systems may further intercept sunlight. Shellfish however feed on suspended epiphytes and algae, and enhance seaweed health through supplemented inorganic nutrients lowering the impact of potential eutrophication and the accompanying irradiance deficiency. Additionally, mitigation of poor light conditions might be obtained through seaweeds outcompeting algae for inorganic nutrients.

Current velocity determines the availability of supplied inorganic nutrients for production of *S.latissima* and seston for *M.edulis* cultivation. Heavy currents increase supply but may diminish availability for uptake where low currents enhance water residence time and accessibility to inorganic nutrients and seston for production. Seaweed blades flap and wave on cultivation lines due to water movement, potentially wrapping around shellfish lines if cultivation is too cramped. In shellfish and seaweed IMTA, the aquaculture system and standing stocks decrease current velocity. Waste excretion from shellfish potentially accumulates within the system if current velocity is very low but seaweeds are able to absorb these excessive nutrients, especially when ambient nutrient input is limited.

5.3.2 Parameters in relation to environmental conditions

Based on the environmental conditions available for the pilot sites in the Scheldt delta, present oxygen levels pose no issue for shellfish cultivation. However, pilot sites do experience limitations in phosphorus and in Geersdijk, nitrogen is also a limiting factor for seaweed growth in summer and autumn. This period however is outside the main growing season for *S.latissima* and phosphorus and nitrogen compounds are readily available throughout the pilot locations in every season. Eutrophication however might pose a problem for the environmental quality in the absence of *S.latissima* but *M.edulis* cultivation will remain throughout the years, taking benefit from algae blooms as a result from eutrophication locally in the summer months.

During the *S.latissima* main growth period, phosphorus compounds are the limiting factor in Mattenhaven and Buitenhaven, while in Geersdijk, nitrogen is limiting in autumn and phosphorus in winter. Yet the minimum requirements for successful production of *S.latissima* are met thus the additional supply of nutrients from *M.edulis* cultivation may be considered excessive. Seston or chlorophyll-a is also readily available in all locations throughout the year but during winter, photosynthesis inhibition in *S.latissima* through algae blooms seem unlikely.

The pilot sites are in coastal environments, meaning that pH fluctuates, and a calcite shortage might not be much of an issue. Yet, during summer and autumn when *S.latissima* is absent, the nett supply of oxygen and accumulation of carbon is not at place. From the environmental data of Rijkswaterstaat, no recent measurements of carbon concentration have been collected but assuming that pH is fluctuating, it can be noted that calcite is temporarily available for calcification (shell development) of *M.edulis*. At Geersdijk, it is expected that a higher carbon loads exists, originating from agriculture and accumulated in suspension and soil due to the potentially lower current environment.

Significant impacts of biofouling on *S.latissima* are not expected as settlement of epiphytes is reduced during the main growing period and before temperature increases, the seaweeds are harvested. In the Eastern Scheldt however, loading of seaweed sporophytes on shellfish has been reported but not from cultivated *S.latissima* as they are harvested before they release their spores.

Algae blooms are less likely to occur in the main growing period of *S.latissima*. Though, in winter, daylight is more scarce but due to luxury uptake from *M.edulis* excretion, *S.latissima* seems to remain productive despite compromised light availability. No data was found on light availability in or near the pilot sites but considering the findings from Danish field studies, light availability might not pose a serious threat for *S.latissima* cultivation.

No data is available on the hydrodynamic environment at the three pilot sites. However, Buitenhaven and Mattenhaven are sheltered in harbours but subjected to tidal movement and as of now, Geersdijk is situated in a semi closed system. Yet, it is unknown weather current velocity is sufficient for *S.latissima* (0,1 to 1,52 m/s) and *M.edulis* (0,1 to 1 m/s) cultivation.

6 Conclusion

The first two research questions; “*What are the key biological, chemical, and physical parameters that dictate shellfish and macroalgae production?*”; “*What main biological, chemical, and/or physical processes derived from and within an integrated multitrophic aquaculture system containing both macroalgae and shellfish are expected to affect production?*” are answered as follows.

Throughout the gathered literature, oxygen was found to be involved in multiple processes. It plays an important part in chemical processes of nutrient and waste cycling but also ocean acidification mitigation. Moreover, ample oxygen availability allows for an increase of biodiversity in and around the IMTA cultivation site. Nutrients, primarily nitrogen and phosphorus compounds are directly and indirectly essential for growth of both *S.latissima* and *M.edulis*. The in- and output of nutrients is related to factors like oxygen concentrations, carbon dioxide concentrations, and biodiversity. Moreover, algae blooms can have a strong impact on supply and availability of nutrients. It seems that ocean acidification due to higher levels of ambient carbon become ever more present in the natural environment and through aquaculture waste output. Relevant factors regarding the carbon cycle include nutrients, algae, oxygen, flow velocity and biodiversity and these are enhanced or inhibited through different processes. Shellfish and seaweed quality and ability to grow is also determined by potential interference of biofouling. For settlement of epiphytes, biodiversity, oxygen concentration, flow velocity and temperature dictate the level of impact. Light availability is a direct factor that determines productivity of seaweeds as this relies on photosynthesis. Algae, epiphytes, nutrients, and water flow have been found to take part in processes that affect light availability. Water movement seems to be an important parameter. The results show that flow velocity and direction may directly enhance or inhibit processes in nutrient cycling and biofouling. In addition to the direct impacts, water movement is indirectly involved in a multitude of key processes and factors in seaweed and shellfish production.

The third research question; “*To what extent are these biological, chemical, and/or physical processes interlinked and beneficial or detrimental for successful production within integrated multitrophic aquaculture system containing both macroalgae and shellfish?*” does not have a straightforward answer as the level of impact depends on a multitude of variables in interrelated factors and processes. Therefore, it proved rather complicated to include quantifications thus, the results were based on qualitative results. Researchers and farmers recognize environmental remediative properties and carried out promising trials with seaweed and shellfish polycultures. However, several aspects of the factors and processes taking place in seaweed and shellfish IMTA are not fully understood. Yet, combining seaweed and shellfish is often recommended in the collected studies as their findings indicate an overall beneficial interspecific relationship.

7 Recommendations

7.1 Monitoring and measurement suggestion

To gain insight in quantification on seaweed or shellfish derived impacts on processes and factors determining *M.edulis* and *S.latissima* production in the South Western Delta, the following parameters should be measured and/or monitored at the pilot study sites. Oxygen concentrations in and around the cultivation sites should be recorded regularly, in relative positions to the dominant current directions and thus nutrient fluxes. Moreover, the *S.latissima*'s photosynthesis efficiency or blade quality should be monitored as "unhealthy" seaweed has a lower uptake of carbon and a lower output of oxygen. As ambient inorganic nutrient availability is already sufficient at the pilot sites, it may be wise to also monitor the excessive input of ammonia from *M.edulis*. Together with measuring nutrient content in both *S.latissima* blades and *M.edulis* meat, this may bring insight in the local nutrient balances and efficiency of direct and indirect nutrient uptake. As essential information of carbon concentrations at the pilot sites is missing, it seems wise to measure carbon and acidity prior to and throughout the production process. Moreover, carbon accumulation in *S.latissima* can provide insight in the efficiency of carbon uptake and thus, ocean acidification mitigation potential. Furthermore, as carbon concentration dictate pH and thus calcite saturation it might also be interesting to keep track of shell development in *M.edulis*. Monitoring of epiphytes does not seem very important, yet it might be interesting to gain insight in which species settle on *S.latissima* and *M.edulis* in the different pilot sites as it may suggest the habitat provision potential of marine aquaculture. To track light availability, it is essential to gain insight in levels of irradiance near the seaweed cultivation lines. Additionally, water transparency could be monitored in and around the cultivation site to estimate the positive effects of IMTA considering light availability. Moreover, chlorophyll-a could be measured alongside shellfish filtration rates. As there is no data available on current velocities and directions while this parameter is very influential in IMTA productivity success, it seems obvious to include monitoring of water movement in and around the cultivation sites.

7.2 IMTA setup suggestion

The optimal *M.edulis* and *S.latissima* IMTA design is highly dependent on the hydrodynamic environment but to enhance interspecific interactions, separate cultivation lines should be in relatively close proximity. Positioning on the current should facilitate transport of *M.edulis* waste products to *S.latissima* longlines, and the nett output of oxygen and enhanced calcite saturation towards *M.edulis* longlines. To ensure the occurrence of beneficial interspecific relations derived from enhanced oxygen input and nutrient/ carbon cycling the *M.edulis* and *S.latissima* cultivation systems should be in relatively close proximity. For a balanced and effective IMTA system in terms of nutrient cycling and ocean acidification mitigation, a ratio of 3 shellfish to 1 seaweed in terms of biomass was advised. In terms of epiphyte settlement, it seems important to allow ample flow through the cultivation sites as these organisms can barely settle in exposed environments.

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9 Appendixes

Appendix 1. Search terms per feedback loop theme

General search terms in combination with “shellfish and seaweed IMTA” and terms used to search the separate literature on relative factors or processes in text:

- Oxygen availability
 - o Oxygen supply/ demand
 - o Respiration/ photosynthesis
 - o Hypoxia
- Carbon cycling
 - o Ocean acidification
 - o Bio mitigation/ bioremediation
 - o Calcification/ calcite
 - o Respiration/ photosynthesis
- Nutrient cycling
 - o Faeces/ leaching
 - o Decomposition
 - o Nitrification
 - o Food availability/ algae / phytoplankton (chlorophyll-a)
- Ecological habitat
 - o Biofouling/ epiphyte/ bio deposition
 - o Habitat/ nursery (fish)
 - o Carrying capacity
- Physical habitat
 - o Dislodgement
 - o Current/ retention/ refreshment
 - o Light penetration/ irradiance/ algae bloom

Appendix 2. Direct and indirect interactions

Table 6: Direct and indirect interactions in form of arguments, subtracted from the result sections and applied in integration.

Direct interactions of shellfish on seaweeds			
Positive interactions	Code	Negative interactions	Code
Algae are consumed by shellfish, increasing light availability for seaweeds enhancing growth.	LA1	Seaweed blades can tangle around shellfish, cutting off inorganic nutrient supply.	WM1
Filtration by shellfish decreases the number of epiphytes in suspension before settlement on seaweed.	BF1	Algae blooms may occur (due to high nutrient levels near shellfish cultivation sites), potentially causing hypoxia and a decrease of light availability for seaweeds.	LA2
Luxury uptake of nitrogen due to fertilization by shellfish enhances carbon uptake of seaweeds during photosynthesis.	CC1	In land based IMTA ponds, supply of nutrients from the inlet and abalones waste effluent did not meet the requirements for intensive seaweed cultivation.	NC5
Shellfish enhance availability of inorganic nutrients for seaweeds.	NC1	Shellfish consume particulate organic matter, indirectly removing nutrients from the water column.	NC6
M.edulis faeces and ammonia excretion allow luxury uptake in S.latissima during nursery.	NC2	Heavy loading of shellfish spat on seaweed blades has been observed	BF5
Cultured mussels contribute to dissolved nitrogen concentration locally.	NC3		
Direct Interactions of seaweeds on shellfish			
Positive interactions	Code	Negative interactions	Code
Seaweed culture can act as a restoration measure by providing habitat and inserting a nett supply of oxygen in and around the culture sites.	OC1	Seaweed blades can wrap around shellfish, cutting of organic nutrient supply.	WM2
Due to the photosynthesis induced nett release of oxygen, nitrogen oxide (NOx-) efflux and biodiversity increases locally.	NC7	Flapping and waving of seaweeds decreases main current velocity and efflux of waste.	WM3
Photosynthesis induced accumulation of nutrients in seaweeds leads to a reduced influx of dissolved organic nitrogen (DON) and phosphate (PO ₄ ³⁻).	NC8	Clams grown in vicinity to wild seaweed and seagrass beds were compromised in growth due to unknown reasons.	NA1
Large scale implementation of seaweed at shellfish cultivation sites may buffer local ocean acidification through carbon uptake.	OA1	Seaweeds can mitigate eutrophication by competing with algae trough consumption of dissolved nutrients.	NC9
In polyculture sites, dissolved oxygen levels are enhanced by seaweeds compared to shellfish monoculture sites.	OC2		
Ocean acidification can be mitigated by seaweeds through absorption and assimilation of dissolved inorganic CO2 locally providing calcite saturation.	OA2		
Non interactive/ indirect effects			
Arguments	Code	Arguments	Code
A steady flow allows for higher nutrient removal efficiency in seaweeds.	NC10	Near salmon cultivation sites, the growth conditions (nutrient levels) for S.latissima are enhanced through faeces and feed spillage.	NC11
Current velocity and direction dictate influx of nutrients and seston.	WM4	During ambient nutrient limitation, extractive seaweed and shellfish benefit from nearby finfish cultivation.	NC12
Heavy currents increase nutrient supply but reduce nutrient availability for uptake by seaweeds.	WM5	Benthic nutrient fluxes in IMTA are reduced by dissolved oxygen content (DO) rather than total organic carbon (TOC).	OC4
Exposed environments (high currents) decrease settlement of epiphytes.	BF2	Decreasing trends of dissolved organic carbon were observed by introducing a higher biodiversity in pond IMTA.	OC5
Accumulation of epiphytes on seaweeds increases during the warmer months.	BF3	Ambient nitrogen contents were parallel to local nitrogen content near a shellfish and finfish polyculture site seasonally.	OC6
With high current velocity, accumulation of epiphytes on seaweeds decreases.	BF4	No significant differences in shell meat carbon content and shell growth have been found as a result from seaweed/ shellfish interactions.	OC7
Biodiversity may be compromised locally due to accumulation of settled waste from shellfish culture as this potentially causes hypoxic conditions.	OC3	Direct interspecific competition between seaweed (inorganic nutrient uptake) and shellfish (organic nutrient uptake) is limited.	NC15
In coastal environments, pH fluctuates due to relatively high biological activity.	OA3	Enriched levels of nutrients from salmon faeces and feed spillage can locally increase S.latissima production by 50%.	NC17
High biodiversity causes lower levels of dissolved organic carbon (DOC).	CC 2	Oysters and seaweeds as extractive components in pond IMTA efficiently assimilate nitrogen- and phosphorus compounds (NO ₂ , NO ₃ ⁻ , TAN).	NC18
Benthic carbon fluxes are dictated by dissolved oxygen (DO) and temperature.	CC3		