Aus dem Institut für Tierzucht und Tierhaltung
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

Integrated Multi - Trophic Aquaculture of Mussels (*Mytilus edulis*) and Seaweed (*Saccharina latissima*) in the Western Baltic Sea

Dissertation
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"In der lebendigen Natur geschieht nichts,

was nicht in einer Verbindung mit dem Ganzen stehe."

Johann Wolfgang von Goethe

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Beiträge zur Optik und Anfänge der Farbenlehre

Contents

General Introduction

Intensive aquaculture and the possible associated impact on the environment fostered the development of environmentally friendly culturing systems. The combination of different trophic levels in Integrated Multi-Trophic Aquaculture (IMTA) is currently one of the most promising approaches in sustainable aquaculture. The integration of fed and extractive farm components aims to transform the waste of one organism into a value for another (Buschmann et al. 1996, Chopin et al. 2001, Neori et al. 2004). The biofiltration of particulate and dissolved matter from fish cages is the main purpose for extractive farm components. Seaweeds, that retain dissolved nutrients, and filtrating organisms, that deplete the water from particulate matter, aim to create an environmentally friendly system with almost no nutrient discharge. Many IMTA studies investigate the combination of different trophic levels including finfish, shellfish and seaweed. However, only few studies exist about the combination of solely extractive organisms like algae and bivalves (Qian et al. 1996, Evans and Langdon 2000, Langdon et al. 2004, Mao et al. 2009). Even without the additional benefit of fish farming, the mutual interaction of cultured shellfish and algae is considered positive. On one hand, the filtration of mussels and their ammonia excretion enhances the growth of algae, while, on the other side, seaweed species improve water quality through depletion of dissolved nutrients and generation of oxygen.

IMTA assumes a high potential for a great diversity of species combinations within one farm site. However, most prominent extractive organisms for IMTA in temperate regions are blue mussels (*Mytilus edulis*) and kelp (*Saccharina latissima*).

Besides their worldwide ubiquitous distribution, blue mussels are key species in benthic habitats. This filtrating organism represents one of the most important bivalve species in Europe and, in particular in the Baltic Sea. *M. edulis* occurs from marine salinities to brackish water of > 5 PSU (Remane and Schlieper 1971). It can withstand a wide temperature range and can even survive freezing (Seed 1992). Due to their high salinity tolerance, they represent the dominant bivalve species in the Baltic Sea, where they occur in high abundances. Mussels inhabit hard bottom as well as soft bottom substrate (Newell 1989). Although the brackish conditions offer a refuge for adult mussels from marine predators (e.g. *Asterias rubens*), mussel population remains vulnerable to non-aquatic predators like Eider ducks (*Somateria mollisima*) (Meixner 1980, Guillemette 1998).

Despite the increasing attention to Baltic Sea mussel farming in recent years, shellfish aquaculture also had a long tradition in Germany and Denmark (Möbius 1870, Henking 1929). In the 19th century, mussel farming was practised using trees or piles of wood that were installed in the sediment along the coast (Prange 1925). A well described example is the mussel culture technique of the fishermen from Ellerbek, near Kiel. Meyer and Möbius (1872) described this culturing system in full detail and Möbius (1886) left no doubt about the quality of the mussels. With increasing traffic in the Kiel Fjord, the associated pollution of the water, and the occupation of the coastline by harbours, mussel culture was shut down shortly after the turn of the century (Hoffmann 1949). In the second half of the 20th century, some investigations were carried out on mussel and oyster farming in the Flensburg Fjord in Germany (Meixner 1969, Meixner 1972, Meixner 1990). Also at the Mecklenburg coast, successful trials on blue mussel cultivation were performed (Böttcher 1990, Böttcher and Mohr 1992). However, none of these investigations had led to a commercial mussel culture in the Baltic Sea until 2009.

Blue mussels show high growth rates in the Baltic Sea. Due to their filtration activity they significantly reduce the particulate load of the water (Vahl 1972, Bayne and Widdows 1978, Møhlenberg and Riisgård 1978), thereby enhancing water transparency and quality. Hence, mussel cultivation provides a valuable environmental service (Lindahl et al. 2005). *M. edulis* therefore represents a potential candidate for nutrient restoration of Baltic coastal waters, as well as for incorporation in IMTA.

The tissue composition of mussels of 10 % fat and 64 % protein of dry matter of meat (Berge and Austreng 1989) exhibits their importance as high value products for human nutrition (shell length > 50 mm) (Duge 1916, Noelle 1981), as well as for animal feed stuff (Brühl 1918, Grave 1974, Berge and Austreng 1989, Jönsson et al. 2011, Nagel et al. 2013). Besides food and feed, mussels are also in the focus of pharmaceutical use (Badiu et al. 2008, Hagenau and Scheibel 2010).

The brown seaweed, *S. latissima,* is endemic in the western Baltic Sea. Due to its low salinity tolerance, it is restricted to higher saline conditions as in the Kattegat and the Belt Sea (Nielsen 1995, Karsten 2007). Besides salinity, water temperature significantly determines distribution and growth performance of this cold water adapted algae (Druehl 1981, Davison and Davison 1987).

Despite the traditional cultivation of its relative *S. japonica* (Kombu) in Asia, Baltic cultivation of *S. latissima* was not practised until 1994, when it became used as raw material for cosmetics (oceanBASIS GmbH 2013).

The perennial macroalgae *S. latissima* exhibits high growth rates and thus, high biomass production in the western Baltic Sea. Due to the uptake of dissolved nutrients like ammonia, nitrate, and phosphorus, and thereby enhancing water quality, seaweed cultivation provides a substantial environmental service. *S. latissima* therefore represents a useful candidate for nutrient restoration of Baltic coastal waters, as well as for incorporation in IMTA. The biochemical composition of this seaweed exhibits a high potential for diverse use options like in pharmaceutics and cosmetics (Choi et al. 2013, Ruxton and Jenkins 2013, Goecke et al. 2012), human food (MacArtain et al. 2007, Holdt and Kraan 2011) or animal nutrition (Leonard et al. 2010).

Certainly, *M. edulis* and *S. latissima* play important roles in the natural ecosystem. As key species, mussels link the benthic with the pelagic system (Kaspar et al. 1985, Dame et al. 1991, Smaal 1991). *S. latissima* is an important perennial primary producer (Kain 1979, Bartsch et al. 2008). Both species offer shelter and a habitat for countless invertebrates and other biota. Although mussels and seaweeds

inhabit similar habitats, *M. edulis* and *S. latissima* do not exist in close combination in nature. This might be either due to a low settlement success of mussel larvae near *S. latissima* (Dobretsov and Wahl 2001) because of excretion of repellent agents from the seaweed. Another possibility is the reduced reproduction success of seaweed close to mussels as reported for the sexual production of the two brown seaweeds *Cystoseira compressa* (Benedetti-Cecchi et al. 1996) and *Fucus vesiculosus* (Albrecht 1998). The physical abrasion of algae by sharp mussel shells might also contribute to a low abundance of seaweed in mussel beds. Considering a vulnerable, fragile ecosystem like the Baltic Sea, mussels and seaweeds face special environmental benefits but also limitations. Thus, their specific biological requirements need to be considered for a successful incorporation in integrated aquaculture.
The health of the ecosystem crucially determines the suitability and the success of open aquaculture systems.
The study area of this thesis was located in the Baltic Sea and more in precisely in the Kiel Bight. The Baltic Sea is a diverse ecosystem, which is characterised by a strong eastward salinity gradient. Due to its almost closed topography, various threads like eutrophication, development of oxygen minimum zones, and pollution affect the environment and the associated biota (Remane and Schlieper 1971, HELCOM 2003, Nausch et al. 2011). Anthropogenic exploitation of coastal ecosystems is suspected to have major effects on the marine habitat and its resources. Highly occupied areas like the Kiel Fjord, are most susceptible for pollution.

Main sources of pollution in the Kiel Fjord:

- shipping industry (private, trading or military) including oceanic transport, shipyards and ports with the associated discharge of hazardous substances like paints, lacquers, metal dust, solid or fluid sewage from ships (cooling waters, ballast waters) and airborne pollution from exhaust emission;
- the world's busiest water way, the Kiel Canal (95 ships daily, in 2012); its discharge of nutrients and pollution is highly varying and not sufficiently known;
- the river Schwentine; with a mean yearly discharge of 490 t nitrogen, 24 t phosphorus (Nausch et al. 2011);
- sewage treatment plant in Bülk, near Kiel; discharges yearly 140 t nitrogen and 4.5 t phosphorus (Nausch et al. 2011);
- Kiel power plant; releases seawater with elevated temperature and discharges chemicals via antifouling treatment (*Dipolique 154*);
- rain water; drainage inflow is installed every 50 m in the City of Kiel;
- beach tourism.

Despite the relatively high risk of pollution, fish aquaculture (*Oncorhynchus mykiss*) and seaweed farming (*Saccharina latissima*) have been successfully practised in the Kiel Fjord since 1980s and

1990s, respectively. Starting in 2010, a three years joint project ('Extractive Baltic Aquaculture of Mussels and Algae', funded by the Deutsche Bundesstiftung Umwelt (DBU)) aimed to install an IMTA farm system of mussels (*M. edulis*) and seaweed (*S. latissima*) in the Kiel Fjord. According to the main project aim, this Ph.D. thesis was restricted to the western Baltic Sea and in particular the Kiel Fjord.

Generally, IMTA farm components are installed apart from each other to avoid a direct, physical contact and moreover, to prevent fouling and clogging of substrates. Nonetheless, a near installation of farm components is also imaginable, e.g. in highly occupied waters like the Kiel Fjord, where limitation for space requires the highest spacial exploitation. Despite the environmentally friendly target, an artificial combination of different species as in IMTA might also exhibit ecological limitations.

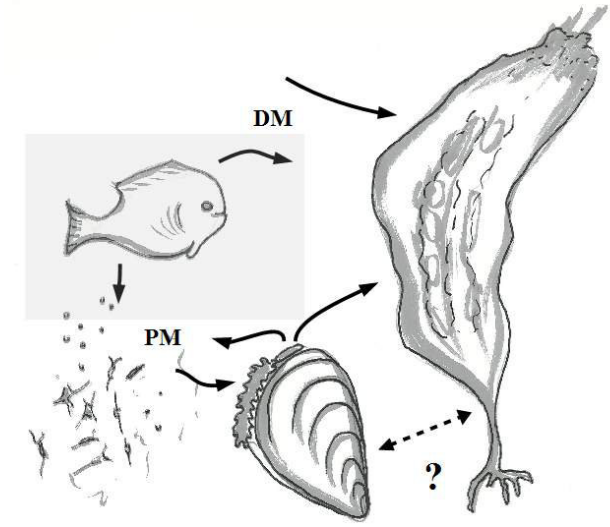

Fig. 1: IMTA system. Continuous lines show fluxes of particulate (PM) and dissolved matter (DM) within the organisms, dashed line represent the uncertain interspecific interaction between shellfish and seaweed. Due to the focus of the present study, fed aquaculture of finfish is excluded from considerations (grey area).

Regardless of distance, the different culture species are connected in IMTA within the aquatic environment and are able to interact with each other. Seawater is the most crucial vector for species interaction. It carries chemical cues, e.g. microbiota and soluble substances that originate from wild habitats as well as from farmed species (Wieczorek and Todd 1998, Hadfield and Paul 2001). Ecological interactions, whether directly (physically) or indirectly (chemically), can be supporting, but can also be limiting. In particular, during periods of elevated vulnerability, like early life cycle stages, species interactions are of special concern. However, although it is a determinant factor for a successful production, there is weak knowledge on species interactions in integrated aquaculture.

The particular objective of this thesis was to investigate the potential and the associated species interaction of integrated mussel and seaweed cultivation in the western Baltic Sea. Therefore, different research questions were answered:

I – Is mussel production for human food possible in the Kiel Fjord, nowadays?

Mussel production was practised 100 years ago, but vanished due to polluted waters and occupied coasts. Although natural habitats have recovered, the Kiel Fjord exhibits a relatively high risk of pollution. Therefore, mussel production for human food consumption was evaluated on a small scale pilot farm in the urban environment in the Kiel Fjord. Mussel larvae abundance, mussel growth, and analyses of food safety measurements (algae toxins, microbiology, chemical contaminants) were observed and criteria for organic certification were investigated.

(*Introducing Chapter I: Mussel production potential in an urban environment in the Western Baltic Sea. The revival of an almost forgotten tradition*).

II – Is IMTA of M. edulis and S. latissima possible in the Western Baltic Sea?

Mussel production was suspected to be less suitable for the Baltic Sea because of low growth rates, thin shells and less meat of *M. edulis*. Seaweed (*S. latissima*) is close to the edge of its distribution and suffers from diverse stress like low salinity and eutrophication. Therefore, the potential for extractive marine aquaculture of *Mytilus edulis* and *Saccharina latissima* was evaluated at four different locations in the Kiel Bight. Production yields (growth and condition) of mussels and seaweed were determined monthly during major season of combined farming. Species carbon and nitrogen allocation was analysed and related to physiological metabolisms as well as habitat conditions (nutrient availability).

(*Chapter II: Extractive Aquaculture of mussels (Mytilus edulis) and seaweed (Saccharina latissima) in the Baltic Sea).*

III – Is mussel settlement influenced (inhibited or supported) by seaweed?

Mussel settlement is crucial factor in mussel cultivation. Excretions of macroalgae are known to inhibit mussel larvae and thus, are suspected to negatively influence mussel settlement in IMTA. Therefore, larvae occurrence and settlement of *Mytilus edulis* was observed within the water column at two locations in the Kiel Fjord as a function of seaweed abundance. Mussel substrates were exposed to different seaweed treatments (juvenile and adult sporophytes, seaweed crude extract). Mussel settlement was microscopically determined on commercially applied mussel seed collectors.

(*Chapter III: Foul play in IMTA – how seaweed can counteract settlement of mussel juveniles*)

IV – Can mussels support seaweed development during early life stages?

During early life stages, seaweed development is an important factor for later biomass production and harvest yield. Close to the edge of its distribution in the Baltic Sea, especially seaweed juveniles are suspected to suffer diverse stress. Mussel ammonia excretion provides additional nutrients that are required for seaweed growth and biomass development. Consequently, the development of *Saccharina latissima* was investigated during alternation of generations in the lab and in a subsequently following field study as a function of bivalve abundance. Specific development stages of juvenile seaweed sporophytes were defined and their abundance was observed microscopically in the lab. The expected growth supporting effect of mussel abundance during the seaweeds early development in the lab was followed in a field study. The biomass production and the biochemical composition (tissue carbon and nitrogen contents) of young seaweed sporophytes were determined and related to their prior exposition to mussels.

(Chapter IV: Increasing production of seaweed crop by fertilisation at an early nursery stage).

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- Chapter I -

Mussel Production Potential In An Urban Environment In The Western Baltic Sea

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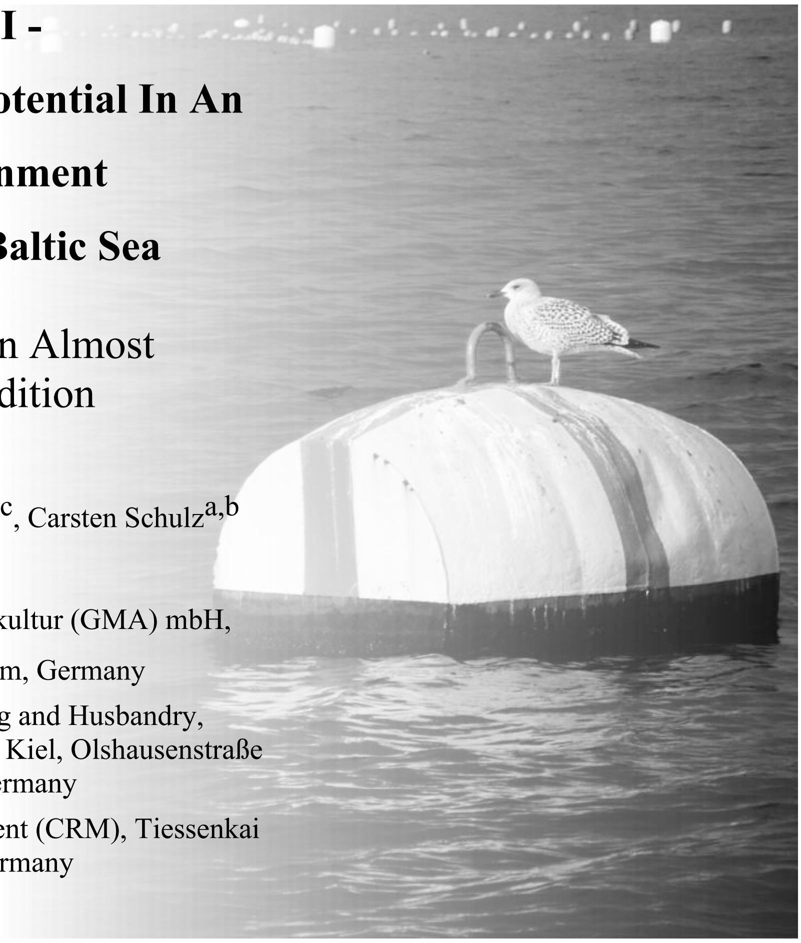
The Revival Of An Almost Forgotten Tradition



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Increasing demand for high value seafood products in a situation of stagnating traditional fishery resulted in high growth rates for the aquaculture sector. But the regional potential is individually depending on various factors. In the Kiel Fjord, mussel aquaculture has been performed in history, but decreased with industrialization and the associated habitat degradation. Nowadays, as aquatic habits has recovered, the production of high value food products like mussels seem again to be feasible with regard to nutritional, ecological, legal and also economical aspects.

Until 1906 Ellerbek, a small village of fisher- and ferrymen, existed at the east coast of the Kiel Fjord (Prange 1925). Their most valuable income in summer was the famous smoked sprat „Kieler Sprotten“. Additionally, farmed mussels, known as „Kieler Pfahlmuschel“, served as another income during winter months, when fishing was almost impossible. Mussels were cultivated on five meter long oak, alder or beech trees. The stems were manually pushed into the sediment in a water depth of approx. 4-5 m. Up to 2000 - 4000 trees were installed this way per mussel field, from which five existed in the Fjord. After mussel spat settled in early summer, mussels grew on the „musseltrees“ for 3 – 4 years until final harvest. They were sold on regional markets and also transported to markets in Hamburg, Prague and Budapest (Möbius 1870).

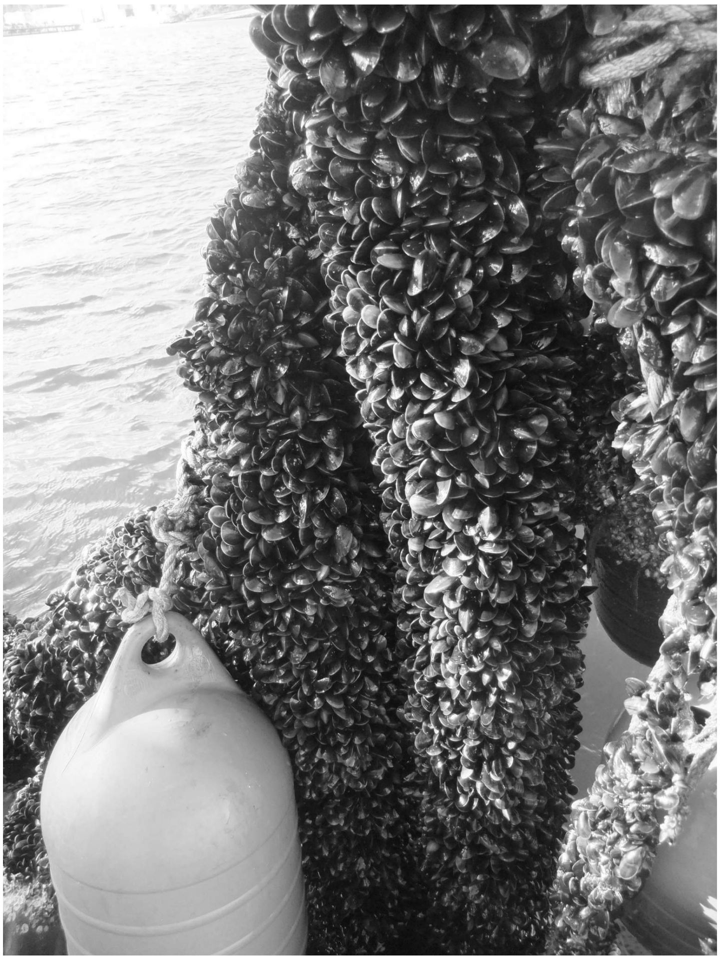

Fig. 1: mussel culture rope

In the end of the 18th century Kiel became an important naval port. Due to the increasing traffic on the water and the associated incremental water pollution the mussel cultivation was shut down.

Today, more than 100 years later, Kiel has grown to a state capital city with approximately 200 000 inhabitants. The Fjord is still an important cruise harbour and forms the Baltic entry of the Kiel channel. However, the water quality has recovered and the Kiel Fjord and blue mussels (*Mytilus edulis*) are abundant. The salinity of ~1,5 % and a constant current speed of 1 – 3 cm/s provide good hydrographical conditions for mussel aquaculture and therefore led to the decision to revive the tradition of mussel farming.

In 2010 the Deutsche Bundesstiftung Umwelt (DBU) funded a three years joint project between Coastal Research & Management (CRM) and the Gesellschaft für Marine Aquakultur in Büsum (GMA) to develop a longline musselfarm in the Kiel Fjord.

Fig. 2: longline with buoys (11 l)

The musselfarm was rather designed as an upgrade of an already existing algae farm (*Saccharina latissima)* of CRM. Mussels and algae are grown combined in an integrated system. The production field is located in a military restricted area close to the Kiel Channel. It has an average water depth of 10 meters and size ranges over 100 x 60 meters. According to the main current direction, that passes parallel to the long side of the area, the used longlines extend to a length of 100 m. Both ends of the longlines are permanently fixed by screw-in-anchors. The length of the production substrates of approx. 3 m provide sufficient space between seafloor and cultured organisms.

In late spring, when water temperatures exceed 12 °C, the natural mussel population provides a regular spatfall with yearly constant high mussel larvae abundances. Since 2009 the amount of mussel larvae ranged between 16 000 (2011) and 90 000 (2009) larvae / m³ at peak. The young mussels (shell length ~0,5 mm) settle initially on mussel spat collectors. After three months at the latest, the mussel spat is transferred into mussel socks (polypropylene and cotton, different mesh sizes). Mussels remain in these substrates until they are harvested in the following winter. Substantial shell growth occurs at water temperatures above 13 °C. Mussels reach market size of minimum 55 mm within 18 months.

The high production potential of mussels is reflected by comparatively high shell growth rates as well as by high meat contents and short recovery time from poor condition. Meat content of mussels were high in November 2010, December 2010, May 2011 (> 50%), and low in March 2011 and April 2011 (39 and 44 %). Similar pattern is reflected by the mussel condition index.

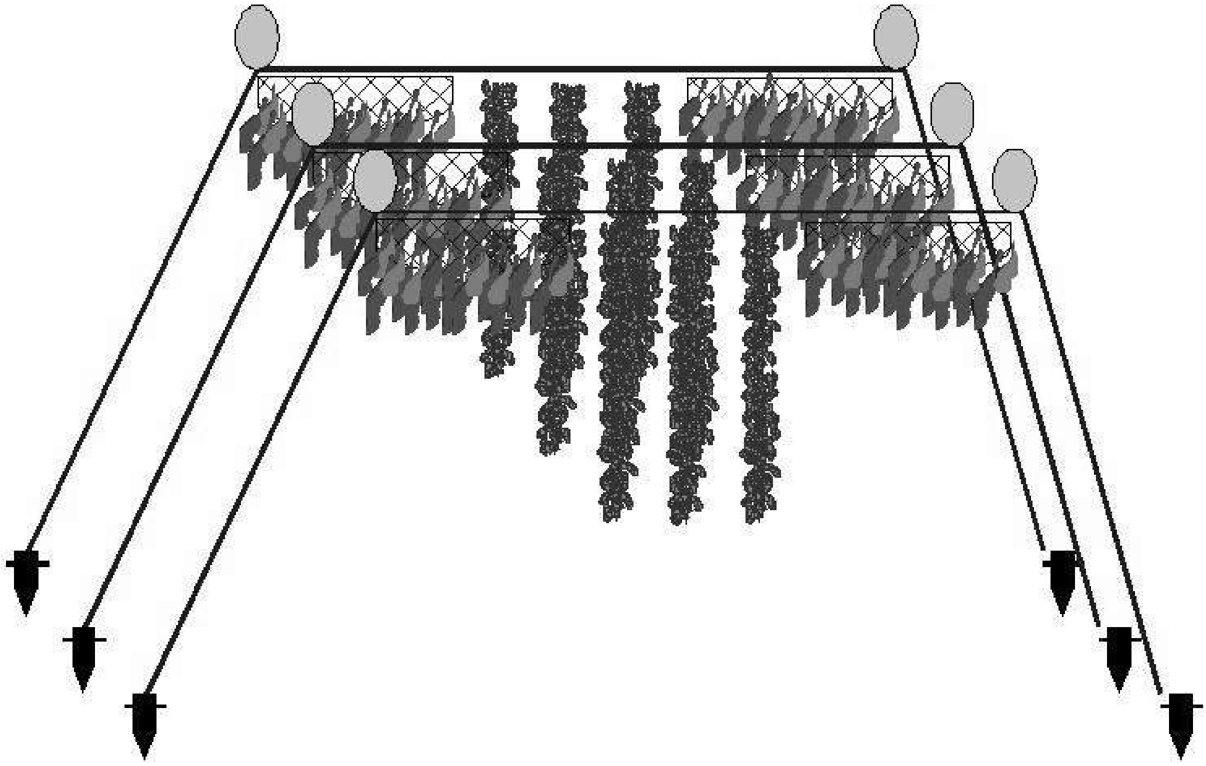

Fig. 3: Sketch of the integrated longline system for algae and mussel cultivation

The clear drop in the mussel quality parameters in March and April 2011 is probably caused by low food availability during the long and cold winter 2010. On the other hand it could also be explained by the inappropriate sampling material of the mussels in this time. Due to an invasion of Eider Ducks in January 2010, almost all mussels that grew outside the socks and had a good condition, were eaten up by the birds. Unfortunately, the risk of predation by birds was underestimated at that time. But until now the invasion was a singular event and mainly due to the harsh winter conditions. Nevertheless there will be arrangements (flutter tape, noises) to prevent future invasions.

In addition to biological parameters which show a great potential for food mussel aquaculture, food safety measurements have to be taken into account. According to the EU regulations 852, 853 and 854 /2004, specific rules are defined to organise official controls on products of animal origin intended for human consumption (European Commission 2004a, 2004b, 2004c). Therefore the status of algaetoxins, microbiological quality and chemical contaminants are essential parameters for food mussel production in the EU.

During the monitoring from 2010 – 2012 in Kiel, no algaetoxins occurred in analysed mussels (monthly measurements in 2010 and during mussel season in 2011 and 2012). The bacterial load (*E.coli*) was analysed at the same time and appeared to be closely related to water temperature. The microbiological quality of the shellfish water "Kiel Fjord" was proved „A" (< 230 cfu / $g_{mussel\ meat}$) except during summer months where it was proved „B" (230 – 4600 cfu / $g_{mussel\ meat}$). According to these findings, mussels can be sold fresh during winter harvest. All further analyses of chemical contaminants like heavy metals, organic pollutants or other harmful residues were uncritical.

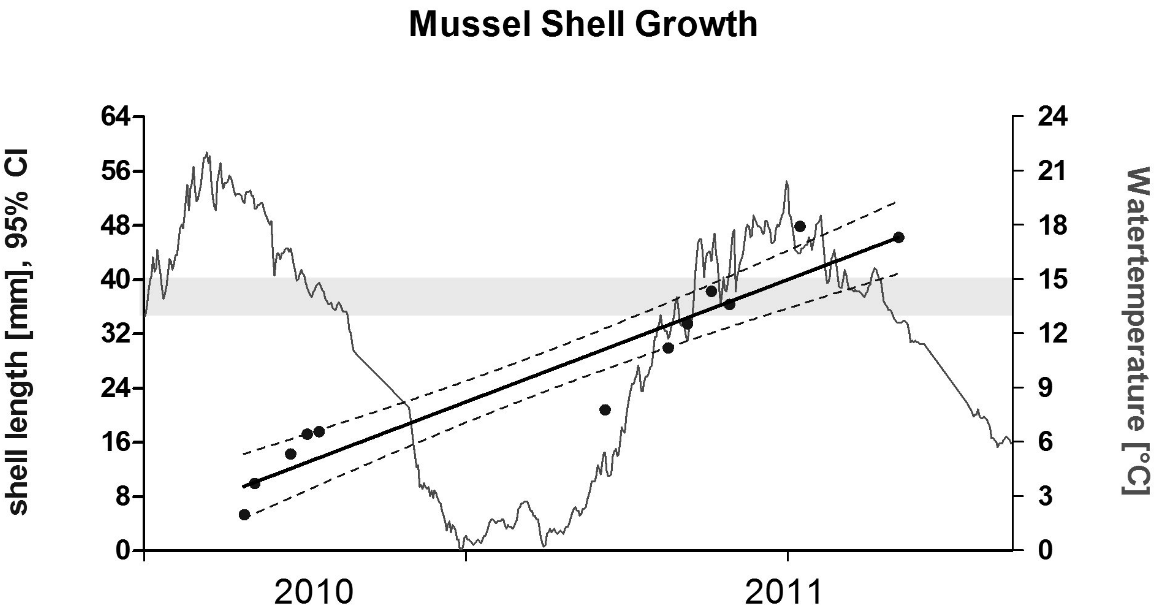

Fig. 4: mussel shell growth in the Kiel Fjord pilot farm

Compared to mussel fishery by dredging, mussel farming on longlines has a low environmental impact. Therefore the mussels of the Kiel Fjord are certified organic according to the (European Commission 2009) since 2011.

Moderate hydrographic conditions, high growth rates and clean waters suggest that mussel aquaculture in the Kiel Fjord is profitable from the biological, legal and also economical point of view. The mussels represent high value products of both: quality and price.

References

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European Commission (2004b) Council regulation No. 853/2004 laying down specific hygiene rules for on the hygiene of foodstuffs

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European Commission (2009) Council Regulation (EC) No 710/2009 amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production

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- Chapter II -
Extractive Aquaculture of mussels (*Mytilus edulis)* and seaweed (*Saccharina latissima*) in the Baltic Sea

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Abstract

Seaweed and mussel cultivation have been in focus of the Baltic Sea region in recent years. Environmental limitations like brackish water and low current velocity due to missing tidal currents, as well as societal barriers such as limited financial capacity and social acceptance are still challenging factors for its rapid development. This investigation evaluated key criteria for the integrated aquacultural potential of *Mytilus edulis* and *Saccharina latissima* in the western Baltic Sea.

Algae and blue mussel growth rates and conditions were determined monthly in a monitoring study at four sites along the Kiel Bight during the season of a potential combined cultivation, from October 2010 until May 2011.

Seaweed growth was significantly dependent on locations, ranging from almost no growth (0.08 ± 0.06 cm day^{-1}) at all locations except Kiel West in November 2010 to a maximum growth rate of 0.8 ± 0.1 cm day^{-1} in Kiel East in April 2011. In Kiel West, seaweed grew constantly 0.5 ± 0.1 cm day^{-1}. Seaweed biomass exhibited highest values 24.10 ± 2.35 [% DW] in Kiel West and lowest values of 12.11 ± 1.18 [% DW] in Eckernförde. Seaweed C/N ratio of 55.26 ± 10.36 indicated a nitrogen limitation at Kiel West, whereas no such limitation was observed at the other locations (C/N ratio 26.64 ± 3.31).

Mussel shell length increased substantially from initially 17.5 ± 6.6 mm to 28.2 ± 1.5 mm from October 2010 to January 2011 and was reduced from February until May 2011 with almost no growth. Whereas mussels grew similarly in size at all study locations, their condition varied seasonally and regionally. During Winter 2010, the condition indices [gDW_{meat} $gDW_{shell}^{-1}100$] ranged from 22.5 at the most eastern site to 26.2 at the most western site. During March 2011, low condition indices of 9.3 ± 0.7 were observed at all locations. A reduced mussel condition with a concurrently elevated C/N ratio of 5.2 ± 0.2 in Kiel West and 4.9 ± 0.1 in Kiel East in May 2011 indicated the beginning of spawning in mussels. The constant C/N ratio of mussel tissue of 4.1 ± 0.1 in Eckernförde and Marina Wendtorf in spring 2011 indicated no spawning.

According to the high meat contents (46.9 to 54.2 %) of mussels (> 43 mm) and the good seaweed growth rates, the western Baltic Sea exhibits suitable conditions for mussel and seaweed cultivation. Both organisms considerably contribute to the nutrient retention in eutrophic waters of the Baltic Sea, and as potential extractive components in the vicinity of fed aquaculture.

Key words:

Baltic Sea, nutrient retention, *Mytilus edulis*, *Saccharina latissima*

Introduction

Despite the fact that interest in Baltic Sea aquaculture has increased in recent years, development of sea farming is still in its infancy (SUBMARINER 2013, Lindahl and Zaiko 2012).

The brackish condition, eutrophication as well as water pollution in the Baltic Sea, were suspected to be key environmental challenges for Baltic aquaculture (Matczak et al. 2012, Lindahl and Zaiko 2012). Baltic aquacultural potential is also influenced by low, wind driven current velocity, missing tidal currents, eutrophication of coastal waters (Gerlach 1990, HELCOM 2003), as well as the occurrence of oxygen depleted zones triggered by water stratification in summer (Böttcher and Mohr 1992). These environmental conditions in combination with an underdeveloped market for regional products and the strict regulations (e.g. HELCOM 2007), might explain the limited interest of private investors for the aquacultural business. In contrast, sustainable aquaculture is supported by the European Union through special funding, e.g. EFF (European Commission 2006). The demand for seafood is increasing rapidly (FAO 2012) and according to the CFG (Common Fishery Policy of the EU), aquaculture is seen as one of the most important sectors to fulfil this demand (European Commission 2013). However, environmental concerns like discharges of nutrients or pollutants contribute to the overall weak social acceptance of aquaculture. An environmentally friendly approach is Integrated Multi-Trophic Aquaculture (IMTA), which incorporates extractive organisms, like mussels and seaweed, within traditional fish farming (Chopin et al. 2001, Barrington et al. 2009, Abreu et al. 2011). According to the EU regulation 710/2009, extractive aquaculture of mussels and seaweed on suspended longlines is considered environmentally harmless and can be certified as organic in the EU (European Commission 2009).

However, as far as we know, there is no European IMTA facility yet that operates in a commercially profitable way and without financial support from research pilot projects or governments.

At present, European seaweed farming is used for nutrient reduction in aquaculture sites (Birkeland 2009, Holdt 2009). It is also aimed to use seaweed for generation of bioenergy (biofuel) (Bruton et al. 2009, Fry et al. 2012, Hughes et al. 2012), as well as for use in the cosmetic (oceanBASIS GmbH 2013) and pharmaceutical industry (LFA 2012). The mussel farms in the more saline region of western Baltic Sea (> 12 PSU) presently focus on production for human nutrition; while the farms located in the regions with low salinity may be more appropriate for production of animal feed (Lindahl 2011).

Seaweed growth and biochemical composition is generally influenced by nutrient concentration, light, salinity and temperature (Lüning et al. 1990). Similarly mussel condition and growth depends on water quality, food availability and temperature (Bayne and Widdows 1978). These specific environmental requirements affect the biological productivity of the seaweeds and mussels, and hence, not every site suits both organisms. Common site selection criteria have been developed for the marine environment (Laing and Spencer 2006, Radiarta et al. 2011), but not yet for brackish systems like the Baltic Sea.

The objective of this study was to evaluate the potential of integrated Baltic Sea aquaculture systems of mussel and seaweed production by determining the key biological criteria like species growth, condition, and nutrient allocation of carbon and nitrogen. As such, this study is the first to evaluate the suitability of the Baltic Sea for seaweed and mussel farming.

Material and Methods

In this study, the potential for the cultivation of *Mytilus edulis* and *Saccharina latissima* was evaluated at four locations in the western Baltic Sea. Both species are endemic in the Baltic Sea and thus, are adapted to brackish conditions (Remane and Schlieper 1971, Nielsen 1995). Monthly sampling was carried out on monitoring modules from October 2010 until May 2011. Biotic site selection criteria like species growth and condition were determined for both organisms and were compared with hydrographical data.

Hydrography at monitoring sites

All study sites were located in the Kiel Bight in the western Baltic Sea (Fig. 1) with an average water depth of less than 12 m. The most western sites were located on the north and south coast of Eckernförde Bay. As both sites mirrored one another hydrographically and biologically, data sets were pooled (ECK, 54°27' N, 9°51' E). Another two sites were located in the Kiel Fjord. Kiel West (KW, 54°22' N, 10° 9' E) was located at an algae/mussel farm on the west coast of the Fjord. Kiel East (KE) was located at the east coast of the fjord nearby a trout farm, close to the Kiel power plant (54°20' N, 10°10' E). Although both Kiel sites were geographically as close as the Eckernförde locations, they differed substantially in hydrography and thus were regarded as different sites. The fourth site (MW) was located in a small harbour in Marina Wendtorf (54°25' N, 10°16' E) on the east coast at the edge of the Kiel Fjord.

The current velocity was wind driven and ranged from 0.0 to 10.8 cm s^{-1} with a mean velocity of 2.5 ± 1.1 cm s^{-1} (measured at the algae / mussel farm in November 2009 – August 2010, with current meter SD6000, sensordata).

Water temperatures [°C] and light intensity [LUX] were hourly measured with HOBO ® pendant temperature / light data loggers at 1 m below surface. With respect to the spectral sensitivity of the plants, the illuminance [LUX] was converted into quantum irradiance [µmol $m^{-2}s^{-1}$] by multiplying with 0.02 (Lüning 1981).

Within the Chemical Baltic Sea Monitoring (COM), the State Agency for Agriculture, Environment and Rural Areas (LLUR 2011) periodically observed water parameters at 1 m below surface in a dense network of sampling stations. The COM provided salinity data, as well as nutritional parameters like total amount of nitrogen (TN) [mg l^{-1}], phosphorous (TP) [mg l^{-1}], Chlorophyll a [µg l^{-1}] and Secchi depth [m]. According to the location of the monitoring sites, adequate official observation stations of COM were chosen (Fig. 1). Hence, ECK (A), KW (B), KE (C) , and MW (D) data corresponded to COM station no. 7 (a), 59 (b), 103 (c), and 90 (d), respectively.

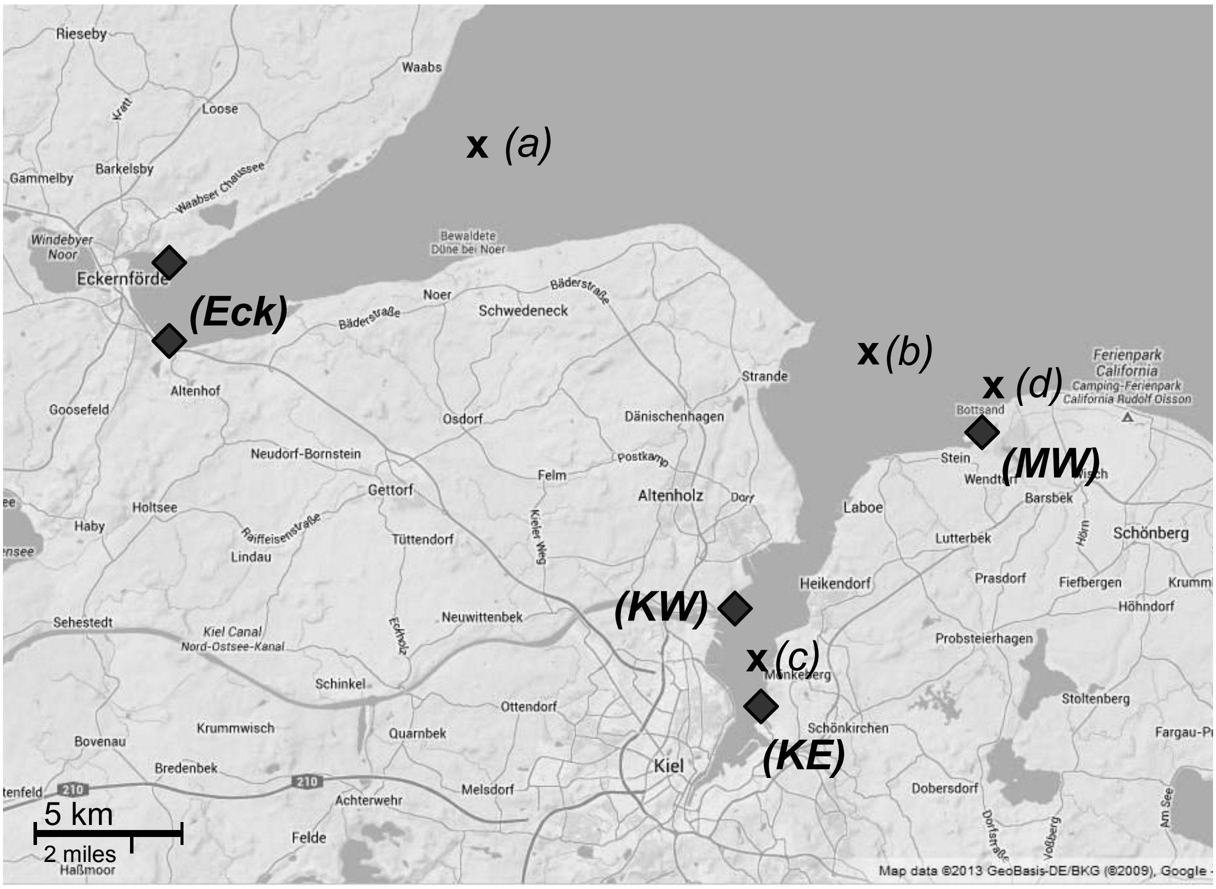

Fig. 1: Monitoring locations in the Kiel Bight: Eckernförde (ECK), Kiel West (KW), Kiel East (KE), Marina Wendtorf (MW); and COM stations: (a) No. 7: Boknis Eck, (b) No. 59: Kleverberg, (c) No. 103: Mönkeberg and (d) No. 90: Kolberger Heide. © Google Maps

Experimental mussel and seaweed culture system (EMSC)

EMSC modules were installed in three (October 2010– January 2011) and four (February – May 2011) replicates. Each module (Fig. 2) consisted of a mussel production unit, a seaweed unit, and a temperature logger (HOBO ® pendant, onset data loggers). Modules were mounted on a pier (ECK, MW) or on aquacultural infrastructure (KW, KE) at 1 m below water surface. Mussels and seaweed used in the monitoring modules were produced on suspended longlines at the algae / mussel farm in the Kiel Fjord (KW). Juvenile mussels originated from the mussel spatfall in the end of June 2010. Their mean shell length (SL) was 17.1 ± 6.6 mm (n = 109) when filled in one meter long cotton bisected socks with a mesh size of 10 mm (MSC-5L-black, 38 mm, Flying Dutchman Marine) in October 2010. After socking, mussels were able to move out and to attach themselves outside the mussel socks.

Seaweed individuals originated from sporulation in autumn 2009 and thus were 12 month old when attached to the experimental units. Adult plants were maintained in cooled tanks at 15 °C and low light conditions (31.2 ± 6.6 µmol $m^{-2}s^{-1}$) from June until October 2010. Rhizoids of three seaweeds per replicate were plait into a twisted rope, which was directly fixed at the lower part of the mussel socks. Seaweed fronds were cut 10 cm above the cauloid to enable a comparable length measurement at all sites.

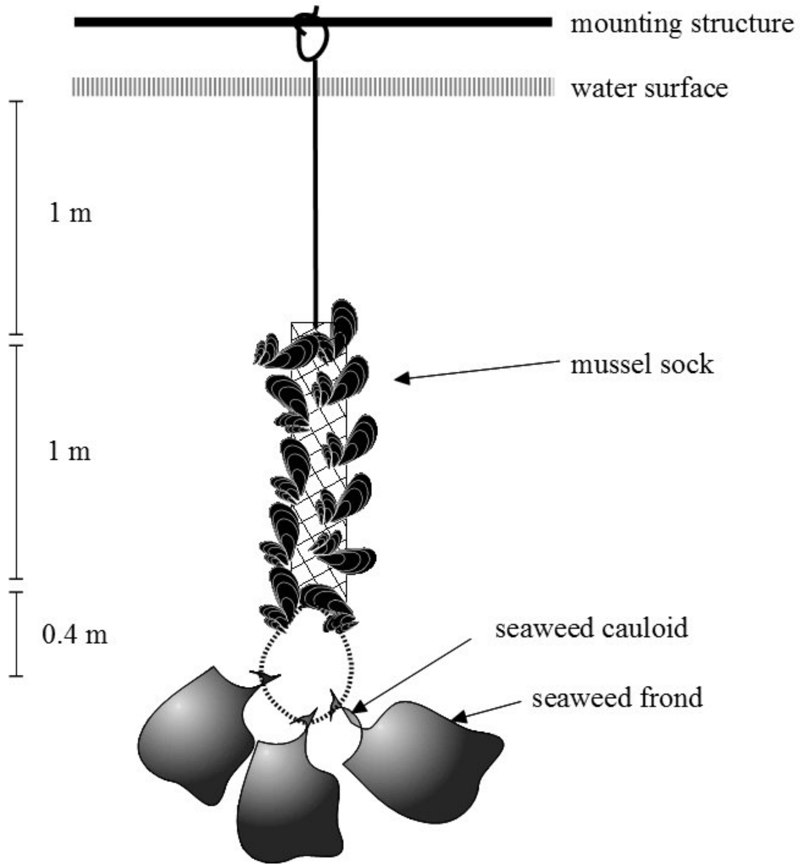

Fig. 2: Experimental mussel and seaweed culture system (EMSC)

During winter 2010/ 2011, Eider ducks destroyed the EMSC by depletion of all mussels on modules at ECK and KW. Due to this substantial loss of bivalves at two stations, further measuring was impossible. Therefore, all EMSC modules were renewed in February 2011 with mussels originating from suspended ropes at the trout farm (SL = 42.2 ± 4.4 mm, n = 24). Due to the larger shell sizes in February (mussels from the same cohort like in October 2010), mussels were filled into plastic mesh bags (mesh size 15 mm) despite using cotton bisected mussel socks. These mesh bags assured a sufficient feed and oxygen supply by larger mesh size but hindered mussels from moving out to avoid another loss of individuals by predatory ducks. Besides mussel restocking, seaweed units were renewed by cutting the seaweed fronds 10 cm above the cauloid to avoid damage or loss during harsh winter conditions and adding of one replicate.

Growth and condition of seaweed and mussels

Seaweed growth was determined monthly by length measurements of the fronds from the end of the cauloid to the apical end of the frond. Seaweed growth rates (SGR) were calculated as follows:

$SGR = L_2 - L_1 * (t_2 - t_1)^{-1}$

Where L_1 is the length of frond at day 1 (t_1) and L_2 is the length of frond at the next sampling day (t_2).

As all seaweed fronds were cut 10 cm above their cauloid prior to exposition in the field and again in February 2011, L_1 was similar at the measurement in November and March at all locations.

At the end of the monitoring period, in May 2011, seaweed was harvested completely. Each individual was cut 20 cm and again 10 cm above cauloid to gain a representative sample of the phylloid that was generally clean (not fouled) and did not belong to the meristematic tissue. Seaweed samples were freeze-dried (CHRIST, Alpha 1-4) to constant weight to determine seaweed biomass. Carbon and nitrogen content of the dried, ground seaweed samples were determined using a C/N Elemental Analyser (Euro EA 3000, EuroVector).

At monthly sampling intervals, randomly chosen mussels (n= min. 15 per replicate) were sampled from the outside of mussel socks or from mesh bags and analysed concerning shell length [mm], biomass [g] (fresh and dry weights of meat and shell), as well as carbon and nitrogen contents [mg g DW^{-1}]. Mussel individuals were frozen at -20 °C prior to dissection. After mussel shell lengths and fresh weights were determined individually, meat was pooled per replicate and freeze dried (CHRIST, Alpha1-4) to constant weight to determine dry mass for estimation of condition index.

Mussel condition index (MCI) was calculated according to (Lucas and Beninger 1985):

$MCI = DW_{meat}\,[g] * DW_{shell}\,[g]^{-1} * 100$

Where DW_{meat} and DW_{shell} were the mean dry weight of meat and shell of all mussels per replicate.

Carbon and nitrogen content of the dried, ground mussel meat were determined using a C/N Elemental Analyser (Euro EA 3000, EuroVector).

Statistics

Statistical analysis was performed with Graph Pad Prism software 5.03 (GraphPad Software, Inc., USA). Seaweed growth rate (SGR) was analysed by linear regression over time. Differences in slopes with a significance level of $p < 0.05$ indicated significant differences between locations. Carbon and nitrogen contents of seaweed samples were pooled and analysed using a linear regression over seaweed biomass to illustrate the relationship between nitrogen, carbon, and biomass. The regression was performed for all locations and additionally excluding KW due to its substantial higher biomass and carbon content.

Mussel growth was presented as an increase of shell size over time by linear regression separately for both sampling periods (before and after restocking in February 2011). Mussel condition as well as nutrient allocation and C/N-ratio was analysed using a non parametric Kruskal-Wallis ANOVA followed by a Dunn's post hoc test.

It was tested, whether the slopes (F_S) or the elevations of the y-intercepts (F_E) were significantly different, for all performed linear regressions. If the slopes were different, it was not possible to test whether the y-intercepts differed significantly.

Results

Hydrography and nutrient observation

In Eckernförde (ECK) the water temperature was characterised by high weekly variability in winter months and an occasional inflow of cold water, probably caused by upwelling events (Fig. 3). Interestingly, no short time cold water inflow was measured at the eastern locations which were only approximately 30 km away. At the algae/mussel farm (KW), the water temperatures changed most evenly throughout the experimental period and showed only low fluctuations. On the contrary, at the opposite side of the Kiel Fjord at the trout farm (KE), water temperatures were elevated and changed with the highest frequency among all observed locations. We relate this effect to the nearby power plant which releases cooling water into the fjord. Additionally to the increased temperature at this location, the inflow of the small river Schwentine caused a decrease of approximately one PSU in salinity compared to KW (Tab. 1). At the most eastern location in Marina Wendtorf (MW), the sheltered exposition of the site (small harbour) basically influenced hydrography. Due to the overall low current velocity, water temperatures remained constantly low during winter, resulting in sea ice development. After the sea ice disappeared, water temperature increased faster compared to ECK or KW reaching 10 °C already in the end of March 2011.

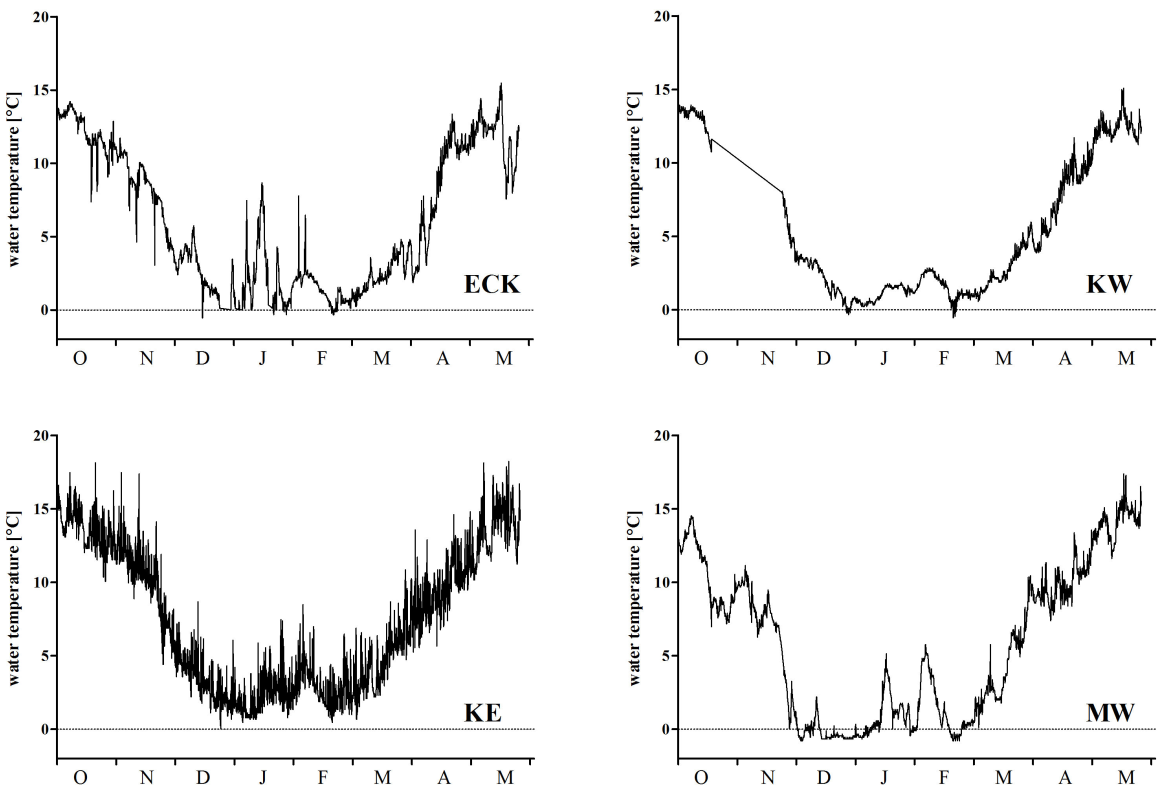

Fig. 3: Water temperature [°C] at 1 m depth at the study sites during the observation period from October 2010 until May 2011

Although ECK tended to be more saline and KE less saline, no significant salinity gradient from eastern to western locations was observed (Tab. 1).

Tab. 1: Salinity during observation period from November 2010 until May 2011 at all study sites (LLUR 2010)

location	ECK	KW	KE	MW
Mean ± SD	17.10 ± 2.09	15.11 ± 2.56	15.50 ± 2.40	16.65 ± 1.36
min	14.47	11.45	11.85	15.03
max	20.74	18.72	18.19	18.64

The study sites differed significantly concerning light regimes (Fig. 4), showing highest irradiance at KW with light intensity exceeding light inhibition threshold of 150 µmol m^{-2} s^{-1} (Lüning et al. 1990) in April and May 2011. At all other sites much less light was available.

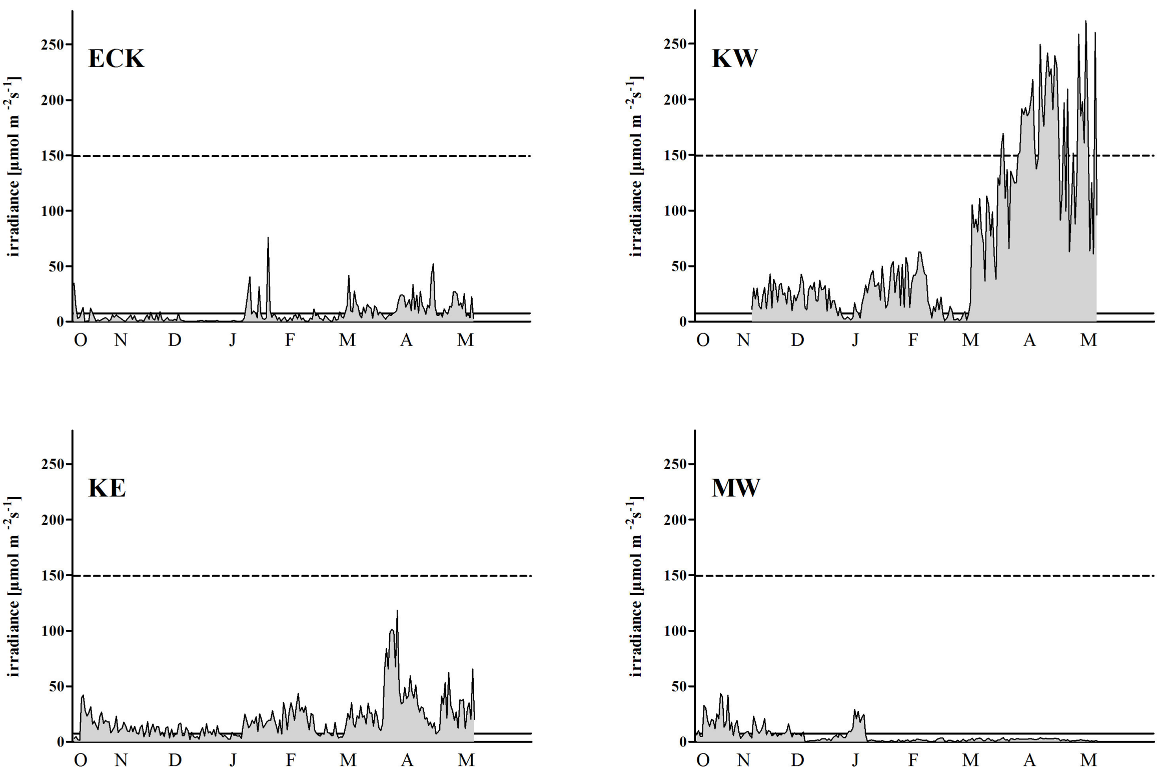

Fig. 4: Quantum irradiance [µmol $m^{-2}s^{-1}$] at 1 m below surface at the study sites during the observation period from October 2010 until May 2011. Continuous line show the irradiance at light limitation of 8 µmol $m^{-2}s^{-1}$ and dashed line show irradiance of 150 µmol m^{-2} s^{-1} at light inhibition.

Dissolved nitrogen showed a decreasing trend in KE and MW, whereas ECK and KW exhibited constantly low nitrogen concentrations. Dissolved phosphorus was decreasing throughout the monitoring period. The N/P ratio was used to estimate the limiting nutrient for plant growth.

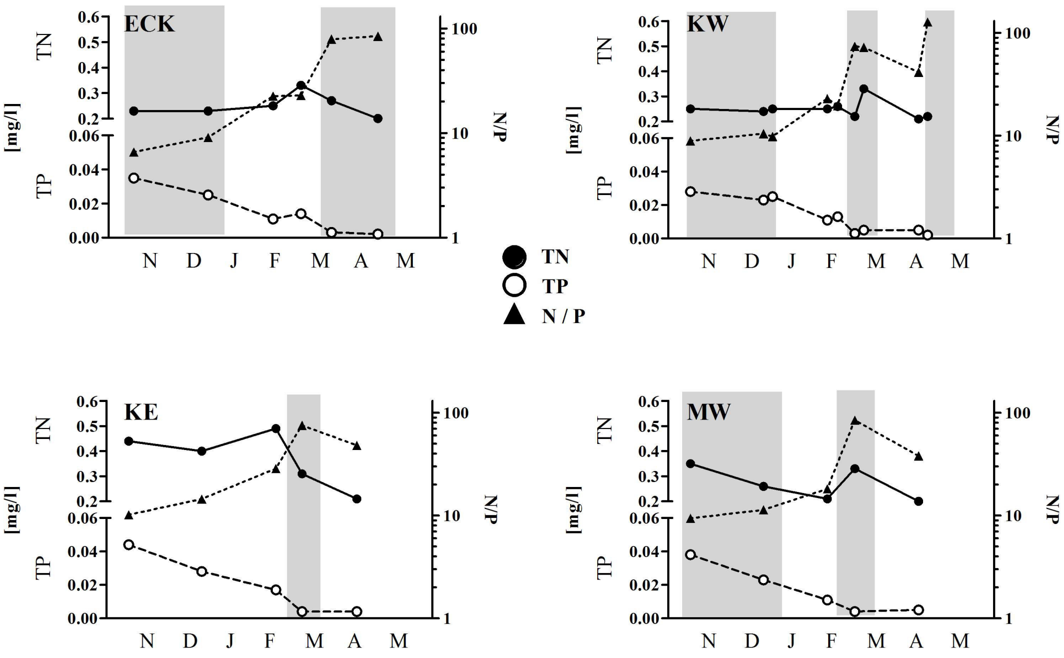

Fig. 5: Total nitrogen (TN) [mg l⁻¹], total phosphorus (TP) [mg l⁻¹], and N/P ratio during monitoring period at all study sites (LLUR 2011). Shaded areas represent periods of nutrient limitation.

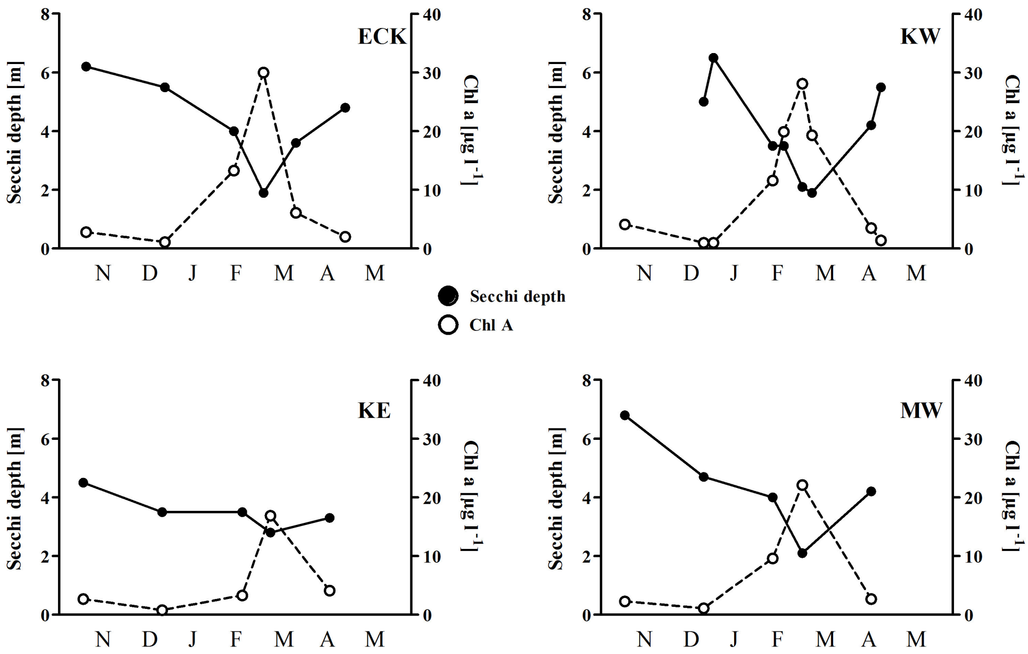

Fig. 6: Particulate load of water represented as Secchi depth [m] and phytoplankton occurrence represented as Chl a [µg l⁻¹] at all study sites (LLUR 2011).

Accordingly, an N/P ratio lower than 10 or above 50 identified nitrogen or phosphorus to be limiting, respectively. Hence phosphorus was limiting between March and May at all locations. Regarding nitrogen, all locations except KE were limited during winter months (Fig. 5).

Beginning in February 2011, phytoplankton bloom peaked at all locations in March, although the intensity of the bloom depended on the site (Fig. 6). Besides chlorophyll a content, phytoplankton bloom is described by water transparency and thus Secchi depth. As the chlorophyll a content was only elevated in March, the constantly low Secchi depths at KE were caused by a higher particulate load rather than by phytoplankton abundance. Thus the inflow of the river Schwentine at KE not only caused a decrease in salinity but probably carried a high amount of silt and sand. The decreased water transparency presumably influenced light availability and subsequently created the lowest chlorophyll peak among locations, although nutrients were not limiting in KE.

Seaweed growth, biomass and C/N biochemistry

Seaweed growth rate (SGR) differed significantly between study sites, showing the most explicit difference at the algae / mussel farm in the Kiel Fjord (KW) (Fig. 7). At this location a constant mean SGR of 0.5 ± 0.1 mm day^{-1} was observed. At all other locations, SGR increased monthly from November 2010 with 0.1 ± 0.1 mmday^{-1} until April 2011 with maximum growth rates at the trout farm (KE) of 0.8 ± 0.1 mm day^{-1} and showed a slight decrease in May 2011. Linear regression of SGR within time was significant for all locations except KW. All monitoring sites exhibited different SGRs (F_S = 17.2, p_S < 0.0001), even when data of KW were excluded from calculations (F_S = 5.0, p_S = 0.009). At KE, seaweed individuals grew fastest and largest of all locations. At ECK, lowest SGR was observed and although KW showed no increase of SGR over time, algae grew larger at the west coast of the Kiel Fjord compared to the Eckernförde Bay. In MW, medium SGR was observed.

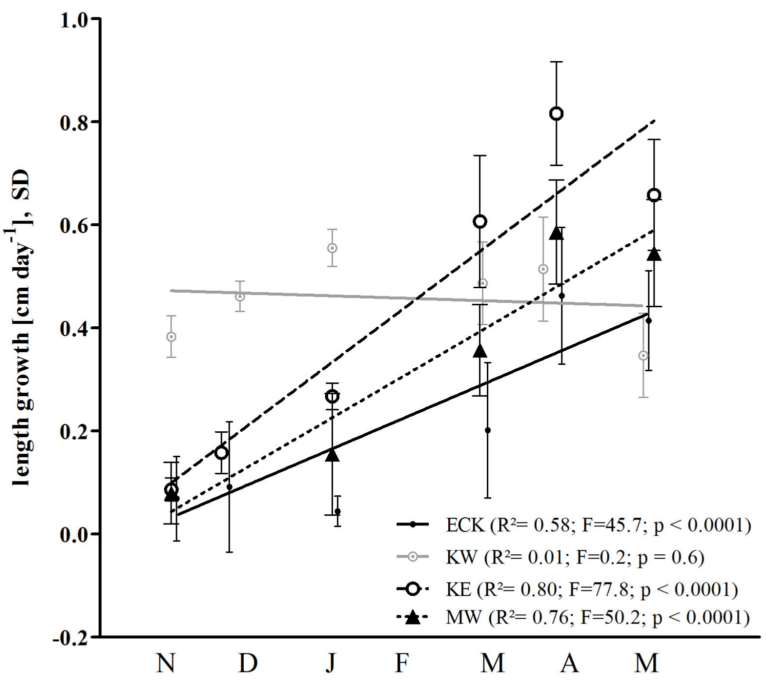

Fig. 7: Seaweed growth rate (SGR) [cm day^{-1}] ± SD at all sites from November 2010 until May 2011. Lines show linear regressions.

The relationship of carbon and nitrogen towards seaweed biomass was diverse. Whereas carbon content and biomass were positively correlated, nitrogen content was reduced with increasing biomass (Fig. 8). This relationship was calculated from pooled data and thus consisted of biochemical data from all locations. KW exhibited the highest biomass with concurrently highest carbon content and lowest nitrogen content. If KW-data were excluded from the former calculation, the formerly observed linear, opposed relationship between carbon, nitrogen and biomass disappeared (F = 0.13; p = 0.72).

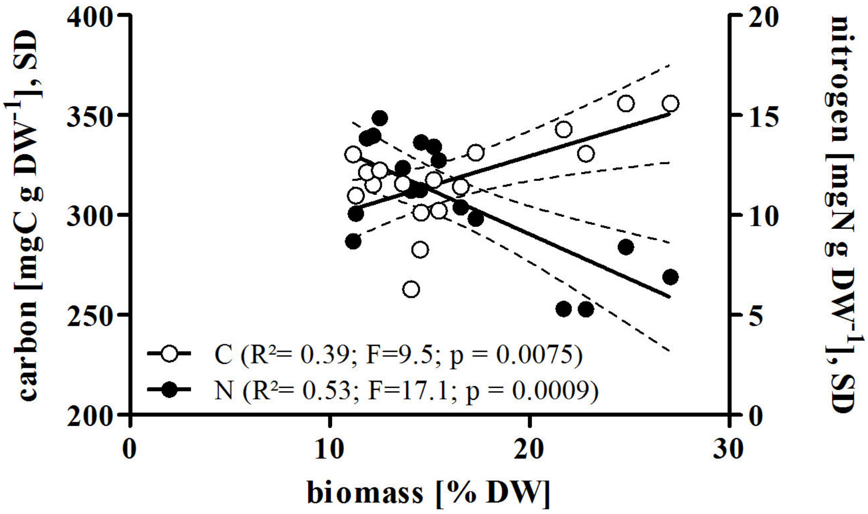

Fig. 8: Relationship of carbon (R^2= 0.39; F=9.5; p = 0.0075) and nitrogen (R^2= 0.53; F=17.1; p = 0.0009) content [mg gDW^{-1}] to seaweed biomass [% DW]. Values originated from pooled data of all seaweed replicates. Continuous lines show linear regressions, dashed lines show the respective 95% confidence intervals.

Although the colour and texture of seaweeds were not quantified in this investigation, it was noteworthy that seaweeds also differed in phenotypes. Seaweed fronds from KW were very thin (but still tough), amber coloured, and translucent. Whereas seaweed fronds at all other locations were tight, “crispy” and opaque with dark brown colour.

Mussel growth and condition

Mussels grew comparably at all locations (Fig. 9) and linear regressions showed no differences in shell growth before (F_S = 2.67, p_S = 0.07; F_E = 2.17, p_E = 0.11) and after (F_S = 1.03, p_S = 0.39; F_E = 0.51, p_E = 0.68) restocking of the experiment. Initial shell length increased significantly at all locations by approximately 60 % to 28.2 ± 1.5 mm (mean ± SD) from October 2010 to January 2011. After restocking, mussels grew only at KE (trout farm). The final shell length of 44.1 ± 2.2 mm (mean ± SD) was comparable between all locations in May 2011.

The condition index of the mussels (MCI) showed a seasonal pattern with relatively high values during winter 2010/ 2011 in young mussels and lowest MCI in March 2011 just before spawning period (Fig. 10). Locations exhibited no differences between MCI and meat content before restocking. After restocking, MCI increased differently between locations. At the eastern location in the Kiel Fjord (KE), MCI increased significantly in April compared to ECK and KW (*F*= 10.6, p= 0.0141). At ECK, KE and KW, MCI increased in May significantly different to MW (*F*= 16.7, p= 0.0011), where it remained consistently low during spring 2011.

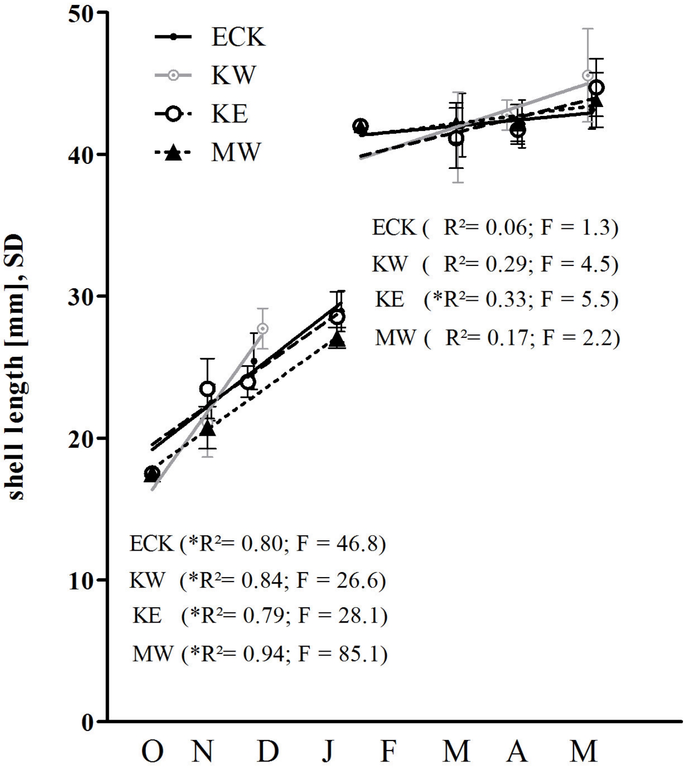

*Fig. 9: Mussel shell length [mm] ± SD at all sites from November 2010 until January 2011 and from February until May 2011. Lines show linear regressions (*p < 0.05).*

The amount of protein (major nitrogen reserve) was presented by nitrogen content and the amount of glycogen (major carbon reserve) was described by carbon content of the mussel tissue. The C/N ratio decreased during winter month at all locations, showing a reduction in carbon and a slight increase in nitrogen content (Fig. 11). Significantly lowest carbon contents were observed in ECK in March 2011 (Tab. 2). After restocking of the mussels in spring 2011, C/N ratio increased significantly with a concurrent increase in carbon and a significant decrease in nitrogen content at both Kiel locations. In ECK and MW no such decrease in nitrogen or increase in carbon were observed and thus the C/N ratio (4.1 ± 0.1) remained relatively even throughout springtime 2011.

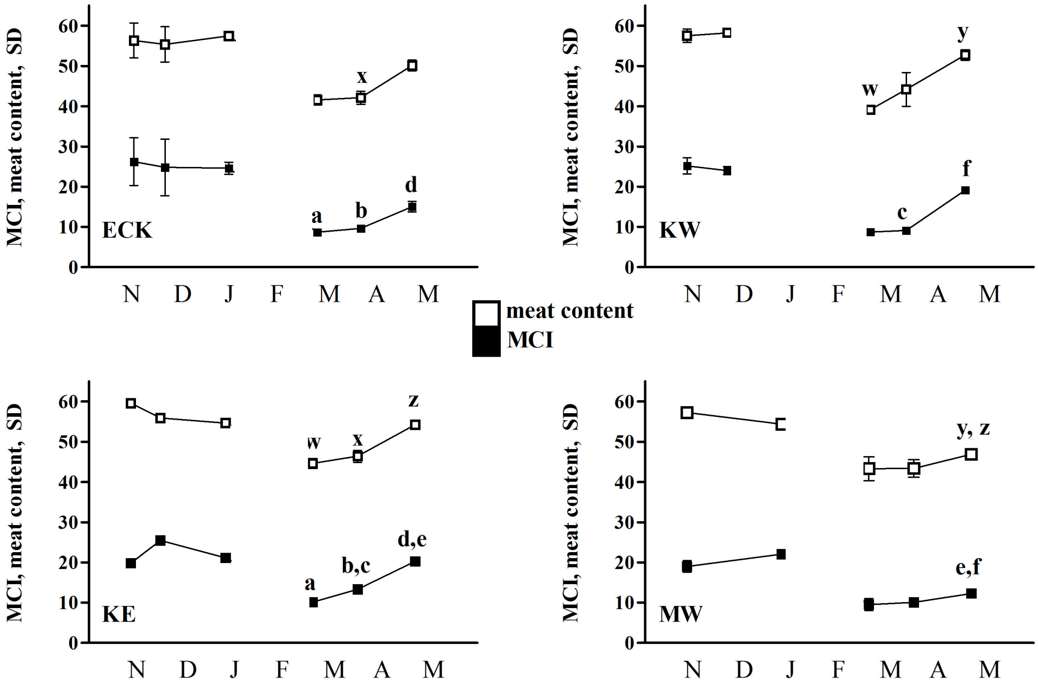

Fig. 10: Mussel Condition Index (MCI) [DWmeat DWshell^{-1} 100] and meat content [%] ± SD during monitoring period from November 2010 to January 2011 and from February to May 2011. Similar letters mark significant differences (p < 0.5) between sites at each sampling date.

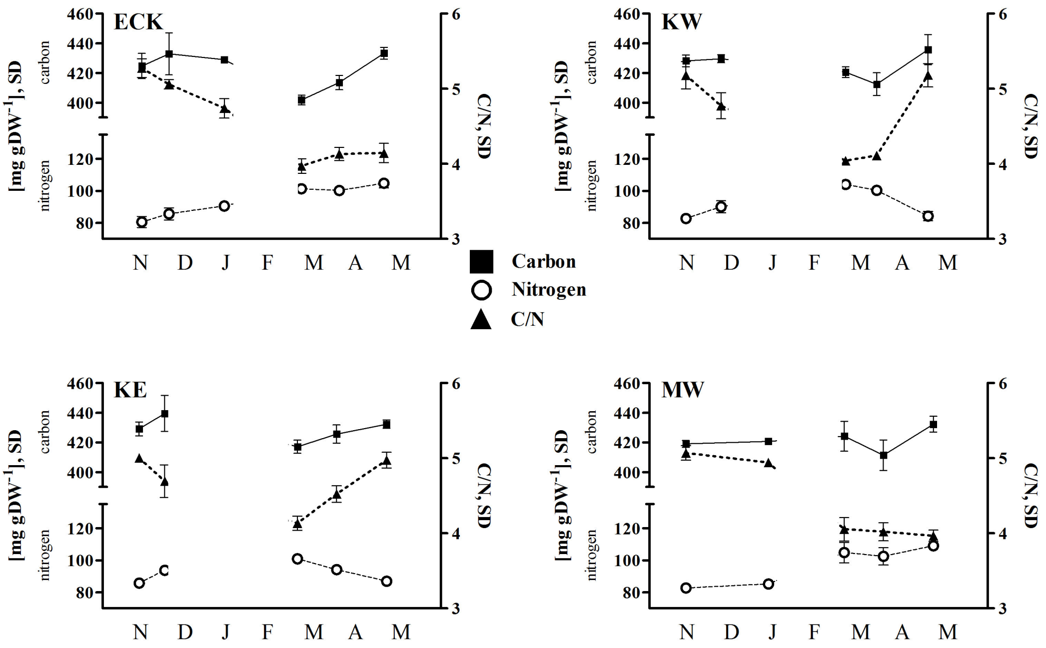

Fig. 11: Carbon and nitrogen content [mg gDW^{-1}] and C/N ratio of mussels as mean ± SD at all study sites during monitoring period from November 2010 to January 2011 and from February to May 2011.

Tab. 2: Test statistic of the non parametric Kruskal-Wallis ANOVA and multiple comparison (Dunn's post test) of carbon and nitrogen content and C/N ratio of mussel meat during monitoring period. Significant differences(p < 0.05) are marked with an asterisks.

Kruskal Wallis ANOVA	**sampling date**	**Kruscal Wallis Statistic**	**No. groups**	**p**	**Dunn's Post test multiple comparisons**	**ECK vs KW**	**ECK vs KE**	**ECK vs MW**	**KW vs KE**	**KW vs MW**	**KE vs. MW**
carbon content	November 2010	4,791	4	0,1877	Diff. Rank sum	-1,667	-2,333	4,000	-0,667	5,667	6,333
					Significant ? 'p < 0,05	No	No	No	No	No	No
	Dezember 2010	0,970	3	0,6158	Diff. Rank sum	1,333	-1,333		-2,667		
					Significant ? 'p < 0,05	No	No		No		
	Januar 2011				Diff. Rank sum						
					Significant ? 'p < 0,05						
	März 2011	13,660	4	0.0034 **	Diff. Rank sum	-9,500	-8,250	-11,170	1,250	-1,667	-2,917
					Significant ? 'p < 0,05	* Yes	No	* Yes	No	No	No
	April 2011	6,729	4	0,0811	Diff. Rank sum	1,250	-7,750	1,500	-9,000	0,250	9,250
					Significant ? 'p < 0,05	No	No	No	No	No	No
	Mai 2011	0,175	4	0,9815	Diff. Rank sum	0,179	1,429	0,429	1,250	0,250	-1,000
					Significant ? 'p < 0,05	No	No	No	No	No	No
nitrogen content	November 2010	6,720	4	0,0814	Diff. Rank sum	-1,583	-7,250	-0,917	-5,667	0,667	6,333
					Significant ? 'p < 0,05	No	No	No	No	No	No
	Dezember 2010	6,315	3	0.0425 *	Diff. Rank sum	-2,733	-6,067		-3,333		
					Significant ? 'p < 0,05	No	* Yes		No		
	Januar 2011				Diff. Rank sum						
					Significant ? 'p < 0,05						
	März 2011	4,464	4	0,2155	Diff. Rank sum	-6,375	0,375	-3,875	6,750	2,500	-4,250
					Significant ? 'p < 0,05	No	No	No	No	No	No
	April 2011	9,614	4	0.0221 *	Diff. Rank sum	0,250	9,500	-2,250	9,250	-2,500	-11,750
					Significant ? 'p < 0,05	No	No	No	No	No	* Yes
	Mai 2011	15,690	4	0.0013 **	Diff. Rank sum	8,893	6,393	-5,107	-2,500	-14,000	-11,500
					Significant ? 'p < 0,05	No	No	No	No	** Yes	* Yes
C / N ratio	November 2010	6,896	4	0,0753	Diff. Rank sum	2,250	7,583	4,250	5,333	2,000	-3,333
					Significant ? 'p < 0,05	No	No	No	No	No	No
	Dezember 2010	7,515	3	0.0233 *	Diff. Rank sum	5,333	5,667		0,333		
					Significant ? 'p < 0,05	No	No		No		
	Januar 2011				Diff. Rank sum						
					Significant ? 'p < 0,05						
	März 2011	5,892	4	0,1170	Diff. Rank sum	-4,188	-8,063	-4,646	-3,875	-0,458	3,417
					Significant ? 'p < 0,05	No	No	No	No	No	No
	April 2011	11,350	4	0.01 **	Diff. Rank sum	2,125	-8,125	5,375	-10,250	3,250	13,500
					Significant ? 'p < 0,05	No	No	No	No	No	** Yes
	Mai 2011	15,390	4	0.0015 **	Diff. Rank sum	-9,036	-6,536	4,714	2,500	13,750	11,250
					Significant ? 'p < 0,05	No	No	No	No	** Yes	* Yes

Discussion

The experiment was conducted during the major period of combined production of Baltic *S. latissima* and *M. edulis* at temperatures below 15 °C (optimum temperature for *S. latissima* (Lüning 1990)) from October 2010 until May 2011.

Seaweed growth, as main performance parameter, is influenced by physical parameters including water temperature, nutrients, and light (Lüning et al. 1990). Algal nutrient allocation depends on photosynthetic efficiency and thus reflects both, nutrient supply (Gerard 1997) and light conditions (Chapman et al. 1978). The results of this investigation indicated that seaweed varied biochemically and phenotypically among different sites. Whereas at three locations algae growth increased within seasonal light availability, seaweed from the west coast of the Kiel Fjord (KW) exhibited low constant growth rates and the highest biomass with a concurrently high carbon content. The light brown, translucent phenotype of algae from KW indicated a low concentration of pigments, photosynthetic enzymes, and thus, tissue nitrogen content. This may be explained by nutrient limitation (Chapman et al. 1978), in particular the low concentrations of dissolved nitrogen 0.3 mg l^{-1} were assumed to be limiting at all locations except KE. Seaweeds of all locations except KE were expected to be affected by nitrogen limitation. It appears that growth was proceeded at the expense of internal nitrogen reserves (Chapman and Craigie 1977) at ECK, KW and MW. Nevertheless, in November, seaweed growth at ECK, KE, and MW was comparable, even though nitrogen was suspected to be limiting at ECK and MW.

Besides low nitrogen, light limitation may have also affected seaweed growth. However, light limitation was due to the overall fewer hours of light available during winter months and probably also due to an additional shading effect by module structures (piers, pontoons). Shading by a longline (+ buoy), like at KW, is much lower, compared to a shading from a pier or fish farm pontoon. As light limitation is suspected to be more important than nitrogen limitation during winter (Chapman et al. 1978), plants from all locations except KW might have been light limited until March 2011.

High levels of irradiance were suspected to enhance growth during winter at KW and were probably also related to the reduced seaweed growth rate at KW during spring 2011. In particular, *Laminaria* growth rate is reduced at an irradiance >70 µmol $m^{-2}s^{-1}$ and moreover photosynthesis is saturated at >150 µmol $m^{-2}s^{-1}$ and thus might have caused light inhibition (Lüning et al. 1990). Nevertheless, besides the effect of light, reduced growth of algae from KW in spring was also expected to be due to nitrogen limitation.

The relation of carbon content, C/N ratio and nitrogen content determine distinctive nitrogen content levels (Mizuta et al. 1992, Mizuta et al. 1997 in *S. japonica*), namely subsistent (1.3 %) and critical (2.1 %) nitrogen values. In this study, the relational pattern of tissue composition was comparable to those described by (Mizuta et al. 1997), though the overall nitrogen content was much lower in Baltic

S. latissima. According to this significant low nitrogen content in Baltic seaweed, distinctive nitrogen levels were shifted to the lower concentrations (Fig. 12). Thus the re-estimated subsistent and critical nitrogen values for Baltic *S. latissima* were 0.85 % and 11.5 %, respectively.

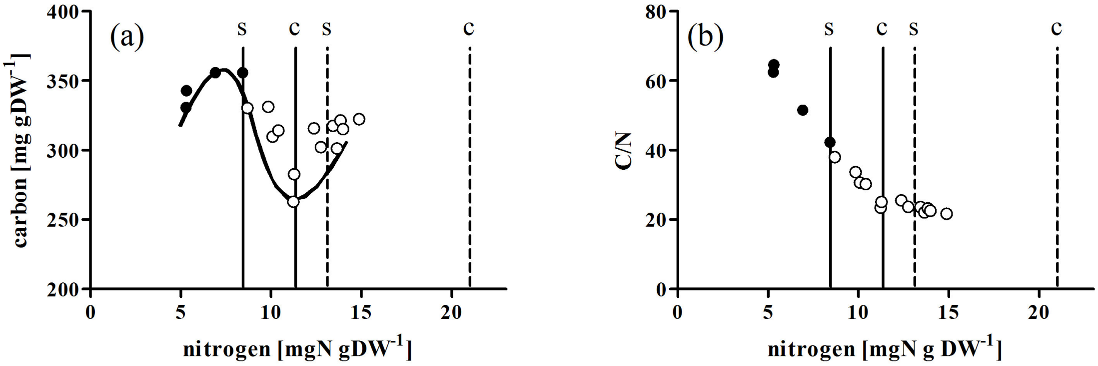

Fig. 12: Relationship of carbon content (a) and C/N ratio (b) to nitrogen content of seaweed. Values originated from pooled data of all seaweed replicates. Closed circles show seaweed samples at KW, open circles show seaweed samples from all other locations. The modified sine curve in (a) was drawn manually to highlight the relation of carbon and nitrogen. Continuous vertical lines show the estimated (for Baltic S. latissima) and dashed vertical lines show nitrogen thresholds for S. japonica (Mizuta 1997), with s= subsistent and c= critical nitrogen contents.

The low nitrogen content of Baltic S. latissima that ranged from 0.5 – 1.5 % of dry weight was unique and has not been reported before. Most calculations for nutrient retention by macroalgae were based on substantially higher nitrogen concentrations ranging from 1 to 3 % dry weight (Sjøtun 1993, Gevaert et al. 2001, Sanderson 2006, Broch and Slagstad 2012). Analyses of seaweed from the Kiel Fjord with 1 to 2 % in young and 0.6 ± 0.02 % in adult sporophytes (own unpublished data) supported the low nitrogen concentrations reported here. The considerable biochemical difference to S. latissima from other regions might be explained by genetic variability, intraspecific differentiation due to geographical separation or different habitat conditions (Gerard 1997). Moreover, maximum growth rates of 0.8 cm day^{-1} are lower than growth rates from Helgoland with approximately 1.5 cm day^{-1} (Lüning 1979) or from other North Atlantic populations (Kain 1979). This might be explained by the suboptimal growth conditions in the Baltic Sea, low salinity, low current velocity.

Unlike the growth rates of seaweed, mussel growth rates were comparable to growth rates of other regions; for example, Jade (North Sea, Walter 2006) or the Black Sea (Karayücel et al. 2010), but considerably higher compared to less saline waters of the Baltic Sea near Rostock (Böttcher and Mohr 1992) and even higher than those in Sweden, about 70 km south of Stockholm (Kautsky 1982a). This reflects the influence of salinity on mussel growth which was described by (Almada-Villela 1984) with significantly reduced growth rates in *Mytilus edulis* at salinities below 12.8 PSU.

Mussel grew similarly in shell length at all locations. However, growth was only measured until

January, before restocking of the experiment. Thereafter no significant increase in size was detectable, except at KE. However, the final size of mussels was comparable between locations, indicating comparable growth conditions between sites.

Nevertheless, seasonally and regionally differences occurred for mussel condition indices. The overall condition of Baltic Sea mussels in this study was comparable to that of the Irish waters (Aldrich and Crowley 1986) with a condition index of 7.0 ± 2.2 (intertidal) to 26.6 ± 8.4 (raft culture), Galician waters (Camacho et al. 1995) with condition indices from 15 to 20, and the Jade in the North Sea (Walter 2006) with condition indices from 20 to 40.

In this study, highest mussel condition indices occurred in winter months and lowest in March. In case of both locations from the Kiel Fjord, low condition and carbon contents recovered until May with a concurrent loss of nitrogen that indicated spawning. Although in ECK the mussel condition increased in May with a concurrent increase in carbon, condition and C/N ratio were still lower than in Kiel. In MW mussels did not recover from poor condition and exhibited an almost constant nitrogen and carbon content in tissue. Mussel condition and nutrient allocation is influenced by environmental and nutritional conditions of the location and internal reproduction cycle (Jansen et al. 2012) and reflect mussel metabolic activity. Generally, high condition indices reflect somatic growth and accumulation of energy storage in mussel tissue (generally in form of glycogen) (Bayne et al. 1982, Dare and Edwards 1975), whereas low conditions and low carbon content indicate starvation and consumption of stored energy of mussels (Pieters et al. 1980). The reduced nitrogen content in mussel tissue is related to spawning, when high amounts of gametes are released into the water and thus protein is lost in mussel meat (Schlüter and Josefsen 1994).

The constantly low nitrogen content in mussels from ECK and MW was probably due to a later spawning or no spawning at the respective locations. Spawning in mussels is known to be related to either temperature (Podniesinski and McAlice 1986, Bonardelli et al. 1996) and / or occurrence of phytoplankton (Thorson 1950, Newell et al. 1982, Starr et al. 1990). Regarding this study, both parameters alone were not exclusively responsible, because differences in spawning were not explained by phytoplankton occurrence, as the peak of phytoplankton bloom occurred similarly at all locations. Furthermore, at both Kiel locations spawning was indicated by a reduction in tissue nitrogen, regardless of temperature differences and fluctuations. Maturation and reproduction of mussels was not physiologically determined within this study, and it is possible that in the case of ECK and MW the spawning shifted to June or July, when sampling had already been finished. It is known that mussel spatfall occurs from May until July in the Baltic Sea (Kautsky 1982b) and in some cases delays under unfavourable environmental conditions, like observed in this study.

During the long and cold winter period of 2010/2011 sea ice covered the water surface in many areas including the monitoring locations. In MW the monitoring station was covered with ice for more than one month.

Potential of aquaculture in The Baltic Sea

Despite the overall reduced algae production in the Baltic Sea, the most promising potential for Baltic seaweed aquaculture was shown for the east coast of the Kiel Fjord (KE and MW) originally the least favourable sites (elevated / fluctuating temperature, low current velocity, high sedimentation). The Baltic sub-optimal growth conditions were either compensated for the east coast (high nutrient availability), or were even less appropriate (nitrogen limitation) on the west coast. Both assumptions suggested nutrients to be most important factors and local hydrographical characteristics to be rather secondary. Nevertheless, the response of seaweed to nutrient deficiencies may also depend on other variables (herbicides, pollution).

However, seaweed target markets require different biochemical compositions. The high nitrogen content in seaweed reflects a high amount of pigments and proteins (Rosell and Srivastava 1985), which are highly valuable in the cosmetic and pharmaceutical industry (Wang et al. 2012). In contrast, low nitrogen with a concurrently high carbon content, and high seaweed biomass is more favourable for bioenergy and biofuel production (Broch and Slagstad 2012).

The potential for Baltic mussel aquaculture was also highest in the Kiel Fjord and in ECK, but to a lesser extend in MW. Considering the potential of both species for nutrient retention in coastal areas, seaweed and mussels contribute to nutrient depletion (Tab. 3 and 4). Hence, in regions where mussel production for human consumption (currently the most valuable mussel use) is less likely due to low shell growth, bivalves still provide an environmental service of nutrient retention. The financial acknowledgement of the nutrient retention could serve as an additional income to the use of small sized mussels in animal nutrition. Therefore, mechanisms such as payment for ecosystem services (PES) should be adopted for supporting this industry.

The nutrient assimilation of *S. latissima* is limited, considering the relatively low growth rates and the low nitrogen content (Tab. 3). Although other perennial seaweed species (like *Fucus sp.* or *Chondrus crispus*) exhibit slightly higher nitrogen content (Ilvessalo and Tuomi 1989, Pavia and Toth 2000, Lehvo et al. 2001, Holdt et al. 2009), they are also characterised by rather low production under reduced salinity as in the Baltic Sea (Carlson 1991, Lehvo et al. 2001). Hence the exploitation of high productive ephemeral algae, such as *Ulva* or *Cladophora* that are adapted to low salinity and exhibit high nitrogen contents, might be more relevant regarding nutrient retention by marine plants (Pedersen and Borum 1996, Lehvo and Bäck 2001).

Tab. 3: Estimation of nitrogen (N) and carbon (C) retention in Baltic S. latissima at the study sites (DW = dry weight, FW = fresh weight).

		ECK	KW	KE	MW
N content [kg / t DW]	Mean	12.12	6.48	12.76	10.86
	SD	2.47	1.49	1.09	1.33
	n	6	4	4	3
C content [kg / t DW]	Mean	313.64	346.37	295.98	320.44
	SD	16.70	12.13	23.29	9.46
	n	6	4	4	3
DW [%]	Mean	12.26	24.10	14.83	15.84
	SD	1.22	2.35	0.63	1.92
	n	6	4	4	3
kg N /t FW		**1.49**	**1.56**	**1.89**	**1.72**
kg C /t FW		**38.44**	**83.48**	**43.89**	**50.76**

Furthermore, shellfish cultivation provides an ecosystem service by reducing the amount of phytoplankton through mussel filtration (Lindahl et al. 2005, Gao et al. 2008, Schernewski et al. 2012, Tantanasarit et al. 2013). Although mussels also release nitrogen compounds as metabolic residues like ammonia excretion and faeces production, more nitrogen is retained in mussel tissues when growing (Ferreira et al. 2011) and thus mussel harvest removes nitrogen from seawater. Values of this investigation of 0.5 – 0.9 % nitrogen removal from the marine ecosystem by mussel harvest supported

Tab. 4: Estimation of nitrogen (N) and carbon (C) retention in Baltic M. edulis during general harvesting season from autumn to spring. (DW = dry weight, FW = fresh weight) Values are calculated from pooled data of all sites.

		November	December	January	March	April	May
N content [kg / t DW_{meat}]	Mean	82.77	89.04	89.17	102.31	99.47	97.53
	SD	2.92	4.66	3.22	3.22	3.95	10.87
	n	13	11	4	19	20	19
C content [kg / t DW_{meat}]	Mean	425.19	433.79	426.83	412.45	415.32	433.38
	SD	6.40	11.26	4.41	10.66	8.29	5.50
	n	13	11	4	19	20	19
DW_{meat} [%]	Mean	18.31	18.18	16.86	12.57	13.28	15.62
	SD	1.70	3.01	1.43	0.69	1.41	1.42
	n	14	11	9	20	20	19
Mussel meat content [%]	Mean	57.48	56.28	55.52	42.04	43.64	50.86
	SD	2.80	3.05	1.77	2.42	2.74	2.86
	n	14	11	9	20	20	19
kg N /t FW_{meat}		15.16	16.19	15.03	12.86	13.21	15.23
kg N / t FW_{total}		**8.71**	**9.11**	**8.35**	**5.41**	**5.76**	**7.75**
kg C /t Fw_{meat}		77.87	78.85	71.95	51.85	55.14	67.69
kg C / t FW_{total}		**44.76**	**44.38**	**39.95**	**21.79**	**24.06**	**34.43**

previous findings of (Orban et al. 2002) and (Lindahl et al. 2005), although nutritional data were only obtained from meat and thus mussel nutrient retention was underestimated (Tab. 4).
Concerning Baltic seaweed farming, the combination with mussels it is recommended to compensate seaweeds nutrient limitation through mussel ammonia excretion in periods of nitrogen depletion and to enhance water transparency and thus might compensate light limitation of seaweed in areas of high particulate load.
In this study we have demonstrated that the western Baltic Sea offers suitable conditions for mussel and seaweed growth. Both organisms considerably contribute to the nutrient retention of the Baltic Sea. However, the growth of seaweed and its nitrogen content is lower compared to more saline waters, while, mussel growth (until 40 mm shell size) is comparable with those found in more saline waters. Nevertheless, despite environmental limitations, the use of *S. latissima* and *M. edulis* as extractive components in IMTA still provide a ecologically sustainable approach for Baltic aquaculture. Other sources of payment through identifying the advantages to the environment and its services to the ecosystem (e.g. PES) could supplement income for farmers and thus, contribute to economical sustainability.

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- Chapter III -

Foul play in IMTA – how seaweed can counteract settlement of mussel juveniles

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Abstract

Larvae of blue mussels (*Mytilus edulis*) usually avoid the vicinity of *Saccharina latissima* in natural habitats. However, in Integrated Multi-Trophic Aquaculture (IMTA), both species are cultivated in combination. This study aimed to observe the interaction of seaweed *S. latissima* and *M. edulis* under field conditions at two locations in a Baltic Sea Fjord.

The experiment took place during major spatfall. Mean larvae abundances of above 10 000 Ind·m^{-3} indicated a sufficient supply of mussel spat. Mussel larvae settlement was observed on two-dimensional mussel spat collectors that were either directly inoculated with crude algae extract or installed in the vicinity of young or adult algae sporophytes in different water depths. The settlement of mussels differed within water depth and algae treatments, whereas the impact of the latter changed over time. During the first experimental week mussel settlement was lower on algae treatments compared to the control. This repelling effect was compensated and even reversed after four weeks of exposure in the case of young sporophytes, that exhibited an attracting effect on mussel larvae.

Though not persistently, mussel settlement was influenced by seaweed and this study therefore emphasises important implications for the design of IMTA.

Key words:
IMTA, mussel settlement, *Mytilus edulis*, *Saccharina latissima*, species interaction

Introduction

Sessile organisms adapt to the turbulent marine environment by colonisation of solid substrates. The establishment of fouling communities is an extremely complex process, that is determined by species, substrates, and the marine habitat (Bakus et al. 1986, Wahl 1989). Generally, fouling succession starts with the development of a biofilm; firstly containing macromolecules, followed by bacteria and diatoms, later spores and larvae, and finally the establishment of macroscopic organisms (Wahl 1989). In marine habitats, all solid substrates (natural and artificial) are chemically and biologically covered within a short period of time. Hence, life in the sea is challenging for species like algae that need to protect their surface from fouling (Williams and Seed 1992).

Seaweeds display several strategies to deal with fouling (reviewed in Egan et al. 2012). Besides a possible symbiotic relationship with the fouling organisms (Hayward 1980), fouling is controlled by the release of reactive oxygen species like superoxide, hydrogen peroxide, or hydroxyl radicals (Weinberger 1999, Weinberger and Friedlander 2000, Küpper et al. 2001, Küpper et al. 2002). Furthermore, seaweeds prevent fouling by the production, accumulation, and release of antifouling metabolites like phenols or terpenes (Ragan 1976, Hay and Fenical 1988, Pohnert 2004). The possible use of natural antifoulants as an alternative to artificial toxic antifoulants has fostered the exploration of seaweed secondary metabolites (Holmström and Kjelleberg 1994, Schmitt et al. 1998, Bianco et al. 2009, Hellio 2010).

Blue mussels (*Mytilus edulis*) are prominent macro foulers (Woods Hole Oceanographic Institute 1952). Their reaction to artificial or natural antifouling agents has been explored in various investigations (Post et al. 1997, Hellio et al. 2001, Hellio et al. 2004, Maréchal and Hellio 2011, Da Gama et al. 2002, Deal et al. 2003). Macroalgae and their respective biofilm can either enhance or reduce mussel settlement. Filamentous algae like *Cladophora rupestris or Codium fragile* attract mussel larvae and also serve as a natural substrate (Bayne 1964a, Antsulevich et al. 1999, Bulleri et al. 2006). Whereas other, mostly perennial algae are able to prevent fouling by mussels. For example, a diterpene of the brown algae *Bifurcaria bifurcata* prevented mussel byssus formation by the inhibition of the phenyloxidase of *Mytilus edulis* (Hellio et al. 2001). Furthermore, extracts from the two brown seaweeds *Ishige sinicola* and *Scytosiphon lomentaria* resulted in a repulsive activity of the mussel foot (Cho et al. 2001). Moreover, *Saccharina latissima* is known to be naturally avoided by mussels (Dobretsov 1999, Dobretsov and Wahl 2001, Dobretsov and Wahl 2008).

Defence mechanisms are suspected to be costly in algae (Hayward 1980, Williams and Seed 1992). Therefore, algae health and composition is expected to determine the intensity and strategy of seaweed defence (Weinberger 2007, Wahl et al. 2010). Light and temperature influence energetic metabolisms (Lüning et al. 1990, Schaffelke and Lüning 1994, Heinrich et al. 2012) and defence mechanisms of algae (Laycock 1974, Williams and Seed 1992, Maréchal and Hellio 2011, Wahl et al. 2010). Both factors simultaneously also affect substrate selection of mussel larvae (Bayne 1964b, Crisp 1974, Rumohr 1980). Mussel settlement is additionally affected by exudates from biofilms and organisms (Satuito et al. 1997, Dobretsov 1999, Dobretsov and Wahl 2001, Alfaro et al. 2006,Bao et al. 2007, Dobretsov and Wahl 2008, Toupoint et al. 2012, Wahl et al. 2012).

The success of shellfish farming is seriously dependent on sufficient spatfall and settlement of young mussels. Therefore, it is important to understand and foresee the factors that initiate or repel mussel larval settlement. Recent investigations in mussel recruitment for shellfish culture have predominantly been concerned with the suitability of different materials and techniques (Pulfrich 1996, Walter et al. 1999, Buck 2007, Walter and Leeuw 2007, Brenner and Buck 2010) or habitats (Walter and Walter 2007, Buck 2007). As Integrated Multi-Trophic Aquaculture (IMTA) moves into focus of sustainability in aquaculture (Chopin et al. 2001, Neori et al. 2004, Troell et al. 2009), the importance of blue mussels and kelp as possible extractive organisms in temperate regions (Lander et al. 2013, Sanderson 2006) increase. Although it is known, that seaweed secondary metabolites potentially influence mussel settlement, the particular mutual interaction between both species in IMTA remains unclear.

Accordingly, the aim of this investigation was to observe the impact of macroalgae (*S. latissima*) on blue mussel (*M. edulis*) settlement under different hydrographical conditions at two locations in a Baltic Sea Fjord.

Material and Methods

This investigation aimed to observe the particular influence of the macroalgae *Saccharina latissima* on mussel (*Mytilus edulis*) larvae settlement in integrated aquaculture, based on the ecological interaction of seaweed and mussels.

Study location

The historical background concerning mussel cultivation (Möbius 1870), and the two commercial aquaculture sites (algae / mussel farm, trout farm) assume a high potential for integrated aquaculture in the Kiel Fjord. Therefore, the settlement of mussels was observed at both aquaculture sites, weekly from 3rd until 31st July 2012, during major spatfall. The first study site was located at an algae/mussel farm (AMF) on the west coast of the Fjord (54°22' N, 10° 9' E) with an average water depth of 10 m. The second site, with a water depth of 6 m, was located at the east coast of the fjord nearby a trout farm (TF) (54°20' N, 10°10' E), close to the Kiel power plant. The power plant uses seawater for cooling processes and releases it with elevated temperature in the fjord. The fjord water current passes parallel to the coastline. On the west coast, marine water inflow passes the algae/mussel farm from north to south, whereas on the opposite side of the fjord the outflow passes the trout farm from south to north. All parameters were observed at both locations in 1, 3, and 5 m depth and at the algae / mussel farm additionally in 9 m depth during the whole study period.

Hydrography and light

Water temperature and light illuminance were continuously measured every hour with HOBO ® pendant temperature / light data loggers. With respect to the spectral sensitivity of the plants, the illuminance [LUX] was converted into quantum irradiance [$\mu mol \cdot m^{-2} s^{-1}$] by multiplying with 0.02 according to Lüning (Lüning 1981). Due to missing tidal currents, the current velocity is generally wind driven in the Kiel Fjord. Current velocity was measured hourly in 2 m water depth with a current meter (SD6000, sensordata) at the algae / mussel farm.

Larvae abundance

The abundance of mussel larvae within the water column was investigated in triplicates. Water samples with a volume of 1 – 3 litres were taken with a sampling device (PWS, Niskin type) into clean plastic canisters and transported to the lab. After the determination of the salinity (WTW test probe), the water was gently filtered through a sieve with a mesh size of 125 µm. The residue was transferred into clean falcon tubes and stored cool and dark. Immediately after processing, samples were examined at 180x magnification using a binocular microscope (Wild-Heerbrugg, M3Z). Mussel larvae were counted and their mean abundance per cubic metre was calculated.

Larvae settlement

In order to analyse the influence of seaweed (*Saccharina latissima*) on the success of mussel (*Mytilus edulis*) larvae settlement, three seaweed treatments: adult algae (> one year old), juvenile algae (< one year old), and algae crude extract) were observed and compared with a control treatment (no algae). Therefore a special experimental design was developed (Fig. 1).

The basic unit of the experimental installation was a weighted mooring rope, that ranged over the whole water column. Stainless steel rings were used as application spots for the experimental materials that were directly attached to the ring. The ring diameter of 50 mm assured a distance between the sampling material and the mooring rope to avoid interferences. Additionally, the ring constantly marked the sampling depth and hence facilitated the on site installation and sampling.

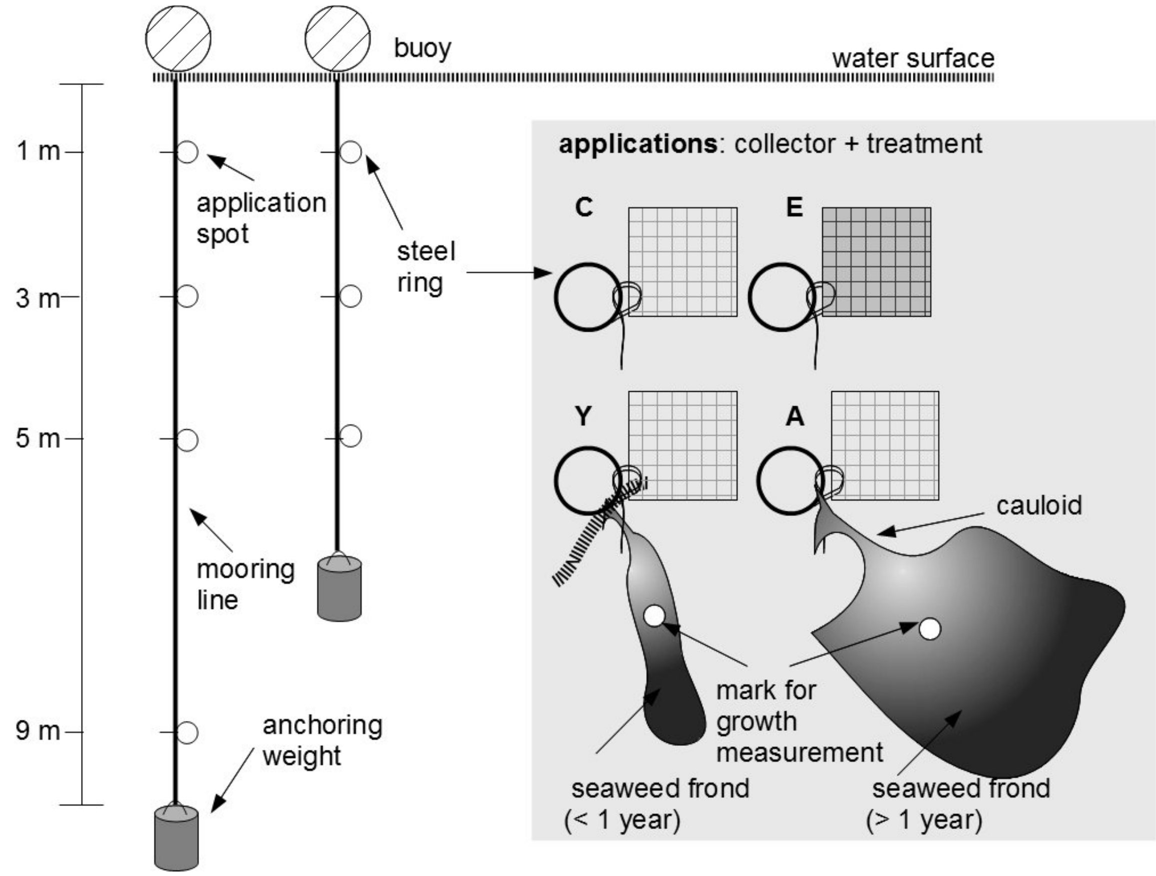

Fig. 1: Experimental set up of the on site settlement experiment. Shaded area highlights the treatment application of (C) control treatment, (E) extract treatment, (Y) young algae, and (A) adult algae and the collector material.

The mussel settling substrate was a commercially approved mussel spat collector (mussel farms in Germany, Sweden, and Denmark). The woven structure of the collector provided a smooth, diverse and thus favourable surface for mussel larvae. At the same time, the two dimensional shape of this substrate simplified the analytic observation. Each 1 mm thick, white polypropylene, 50 x 50 mm collector was directly fixed at the application spot at the mooring rope.

All algae individuals, as well as the raw material for the crude extract, originated from the algae / mussel farm. The control treatment was represented by collectors without any algal unit or extract. Stipes of adult sporophytes were directly fastened to the steel ring at the application spot. Rhizoids of juvenile sporophytes were plaited into a thin twisted rope, which was tightened to the steel ring. Algae extract was produced of 20 mg of adult algae powder that was suspended in one litre of salt water (16 PSU).

The collectors treated with extract were inoculated for 24 hours in the algae suspension at room temperature. The collectors of the live algae and the control treatments were incubated in 10 µm filtered seawater at room temperature for 24 hours before installation at the study sites, to allow accumulation of marine biopolymers on the collector surface.

Seaweed individuals were exposed during the complete experimental period. All collector materials, including algae extract treatment, were exchanged by new collectors, weekly at sampling events. After the transport into the lab, the surfaces of the collectors were examined using a binocular microscope (Wild Heerbrugg, M3Z). All settled mussel larvae were counted in four fields of view (area of collector material diameter = 10 mm) and the mean larvae abundance per square centimetre was calculated.

Algae growth

All algae leafs were marked five centimetres above the cauloid with a small hole, to allow measurement of length growth in the end of the experiment. The algal growth was observed by measuring the distance from the hole to the beginning of the cauloid and subtracting five centimetres.

Statistics

All treatments were installed in four replicates. Statistical analyses were performed with Graph Pad Prism software 5.03 (GraphPad Software, Inc., USA). Due to the low number of replicates, neither the data for larval abundance nor the data for mussel settlement were distributed normally. Therefore significant differences between mean larvae abundances were statistically analysed between water depths using a non parametric Kruskal-Wallis ANOVA followed by a Dunn's post hoc test with a significance level of $p < 0.05$.

The observation of mussel settlement was analysed by a Two-Way ANOVA using algae treatments and water depth as factors, followed by a Bonferroni Multi Comparisons post hoc test. All results were displayed as bar charts ± standard deviation.

Locations and sampling events were always regarded separately and were not compared with each other.

Results

Hydrography and light

The mean water temperature was slowly rising throughout the experimental period (Fig. 2), exhibiting significant differences between the different water depths (One Way ANOVA, F_{AMF}= 163.3, p_{AMF}< 0.0001; F_{TF}= 441.8, p_{TF}< 0.0001). At both study sites, the algal optimum temperature of 15 °C was exceeded throughout the water column until the end of July. At the trout farm, the upper water layer was always warmer than 18 °C and even exceeded the algal lethal temperature of 20 °C for some days. The salinity ranged from 15.1 to 16.2 PSU (algae / mussel farm) and from 14.3 to 15.8 PSU (trout farm) in all water depths.

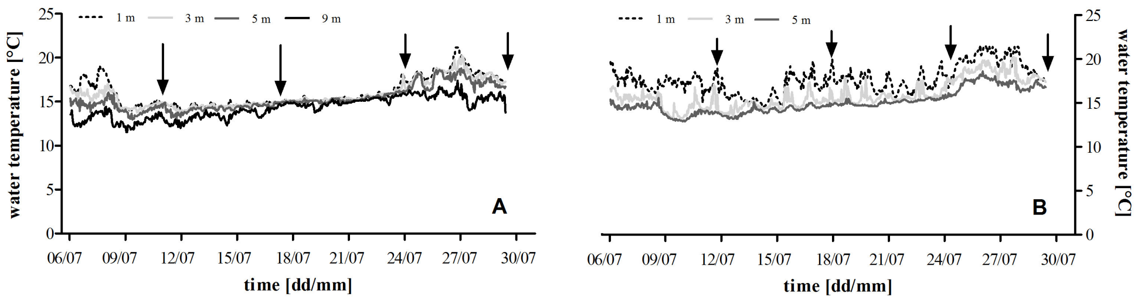

Fig. 2: Water temperature at the (A) algae / mussel farm (AMF) and (B) trout farm (TF) in different water depths during the experimental period (July 2012). Arrows mark the sampling events.

The mean current velocity was 2.4 ± 1.4 cm · s^{-1}, ranging from 0.0 to 8.6 cm · s^{-1}.

The irradiance decreased exponentially within water depth at both locations. A light induced inhibition of the algal photosynthesis occurred at the algae / mussel farm and also sometimes at the trout farm in one metre water depth when light intensity exceeded 150 µmol·$m^{-2}s^{-1}$ (Tab. 1), although the mean daily irradiance was lower. Net photosynthesis was possible at both locations, except at the algae / mussel farm at 9 m water depth where the mean irradiance undercut the light compensation point of 5-8 µmol · $m^{-2}s^{-1}$.

Tab. 1: Range of irradiance at the locations [µmol m^{-2} s^{-1}]

location	water depth [m]	1 m	3 m	5 m	9 m
	mean ± SD	144.5 ± 47. 3	44.8 ± 17.4	21.3 ± 9.8	3.7 ± 2.0
algae / mussel farm	maximum	212,0	84,4	47,3	9,1
	minimum	19,8	6,9	1,7	0,2
	mean ± SD	80.4 ± 36.6	18.5 ± 7.1	10.8 ± 5.6	-
trout farm	maximum	142,3	28,7	20,7	-
	minimum	19,6	2,8	1,0	-

Larval abundance

During the whole study period high abundances of up to 40 000 larvae · m^{-3} (algae / mussel farm) and 57 000 larvae · m^{-3} (trout farm) were observed. The planktonic larvae were relatively evenly distributed over the different water depths at the algae / mussel farm (Fig. 3A). Only at the 24th and 30th July significant differences in larvae abundance occurred between 3 m / 9 m and 5 m / 9 m, respectively (Tab. 2).

At the trout farm, the persistent water stratification was reflected by higher mussel larvae abundances at 5 m water depth (significant for the 18/07/12 and 24/07/12). The 30th July, when the water column was mixed, larvae abundances were distributed equally within water depths (Fig. 3B). Similarly to the location at the algae / mussel farm, no migration of mussel larvae through the water column with a preference towards increasing light into the upper water layer was detectable.

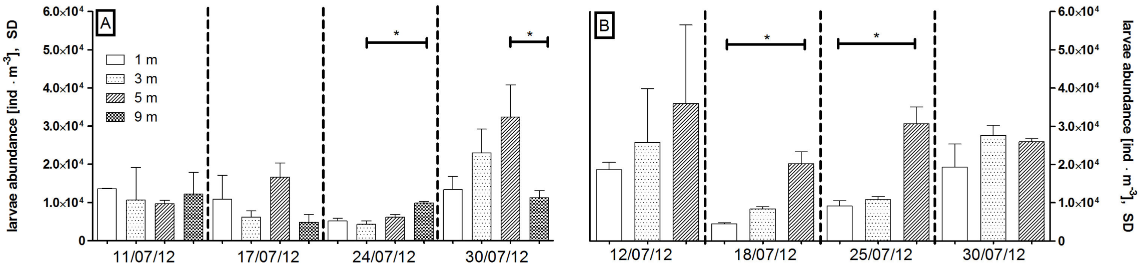

Fig. 3: Mussel larvae abundance [Ind.· m-³] ± SD (n = 3) in the water column at the (A) algae / mussel farm and (B) trout farm at all sampling events. Lines with asterisks mark significant differences with $p < 0.05$.

Tab. 2: Test statistic for mussel larvae abundance at two locations (algae / mussel farm and trout farm) in 1 m, 3 m, 5 m, and at the algae / mussel farm also in 9 m depth at 4 sampling events (n = 3). (Kruskal Wallis ANOVA and Dunn's post test)

Kruskal Wallis ANOVA location	sampling date	Kruscal Wallis Statistic	No. groups	p	Dunn's Post test multiple comparisons	1 m vs 3 m	1 m vs 5 m	1 m vs 9 m	3 m vs 5 m	3 m vs 9 m	5 m vs 9 m
algae / mussel farm	11/07/2012	1.9030	4	0.5929	Diff. Rank sum	2.500	3.667	2.000	1.167	-0.500	-1.667
					significant?	No	No	No	No	No	No
	17/07/2012	7.5130	4	0.5720	Diff. Rank sum	3.333	-2.333	5.000	-5.667	1.667	7.333
					significant?	No	No	No	No	No	No
	24/07/2012	8.8130	4	0.0319 *	Diff. Rank sum	1.833	-2.833	-6.333	-4.667	-8.167	-3.500
					significant?	No	No	No	No	Yes	No
	30/07/2012	8.9930	4	0.0294 *	Diff. Rank sum	-3.667	-6.167	1.833	-2.500	5.500	8.000
					significant?	No	No	No	No	No	Yes *
trout farm	12/07/2012	0.5556	3	0.7575	Diff. Rank sum	0.000	-1.333		-1.333		
					significant?	No	No		No		
	18/07/2012	7.2000	3	0.0273 *	Diff. Rank sum	-3.000	-6.000		-3.000		
					significant?	No	Yes *		No		
	25/07/2012	6.4890	3	0.0390 *	Diff. Rank sum	-2.333	-5.667		-3.333		
					significant?	No	Yes		No		
	30/07/2012	5.9560	3	0.0509	Diff. Rank sum	-5.333	-3.667		1.667		
					significant?	No	No		No		

Larvae settlement

Mussel settlement was observed during the whole study period in all water depths on mussel collector material (Fig. 4). Abundance of settled mussel larvae ranged from 0.4 to 78 ind. cm^{-2} with a mean settlement of 17.5 ± 14.5 ind. cm^{-2} (algae / mussel farm) and from 1.0 to 131.1 ind. cm^{-2} with a mean settlement of 21.8 ± 20.5 ind. cm^{-2} (trout farm). The intensity of larvae settlement was influenced by algae treatment, as well as by water depth (Fig. 5).

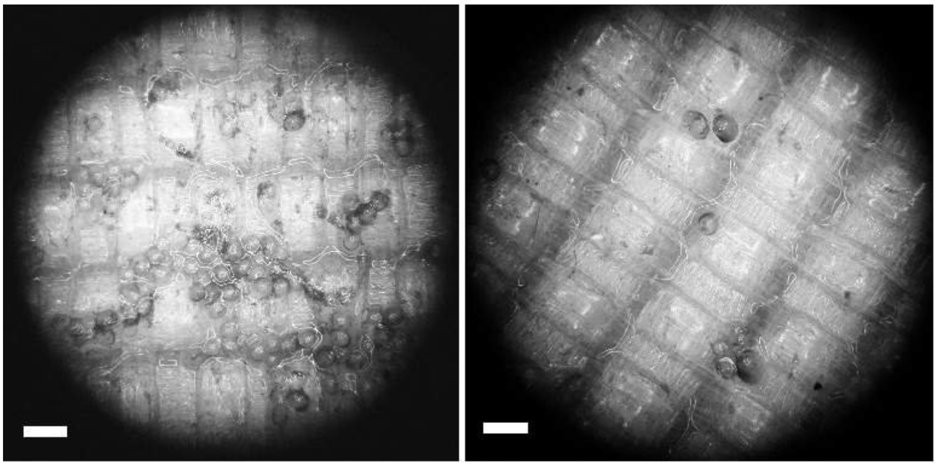

Fig. 4: View through binocular microscope at 100 fold magnification. Collectors after one week of exposure in the field. White bar represents 1 mm.

Although the Two-Way-ANOVA discovered significant effects, the inconclusive Bonferroni Multi Comparisons (BMC) post hoc test was unable to detect the particular differences regarding different treatments and depths (Tab. 3).

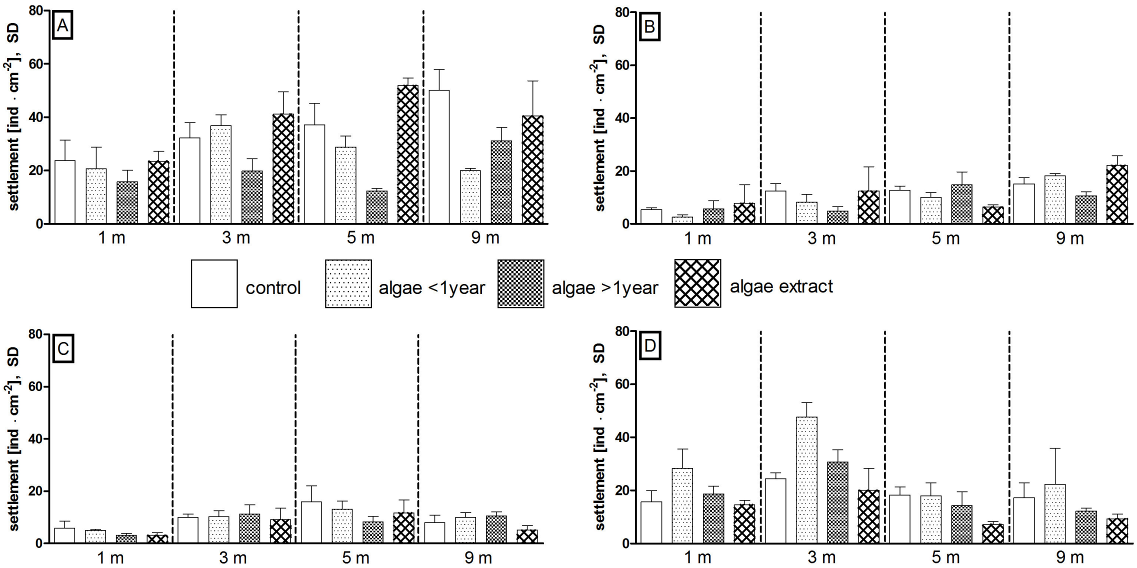

Fig. 5: Mussel settlement at the algae / mussel farm in [Ind.·cm^{-2}] ± SD at sampling events: (A) 11/07/12, (B) 17/07/12,(C) 24/07/12, and (D) 30/07/12. (n = 4, except algae < 1 year at 1 m and algae > 1 year at 5 m with n = 3)

At the algae / mussel farm, live seaweed treatments exhibited both, a reducing and an enhancing effect on mussel settlement (Fig. 5). Juvenile sporophytes reduced mussel settlement during the first week in 9 m water depth (Fig. 5A), resulting in significantly less mussel larvae on collectors compared to the control ($t_{BMC} = 3.353$, $p < 0.05$). During the same week, adult seaweed sporophytes also tended to reduce mussel settlement in 5 m water depth, although this was not proven significant in the BMC post hoc test. In the second and third week of the experiment, a comparably low mussel settlement was observed in all treatments and water depths (Fig. 5 B, C). During the last week of the study, juvenile sporophytes tended to enhance mussel settlement in 3 m water depth (Fig. 5D). This was only observed for juvenile sporophytes. The collector material of the control treatment and of the crude

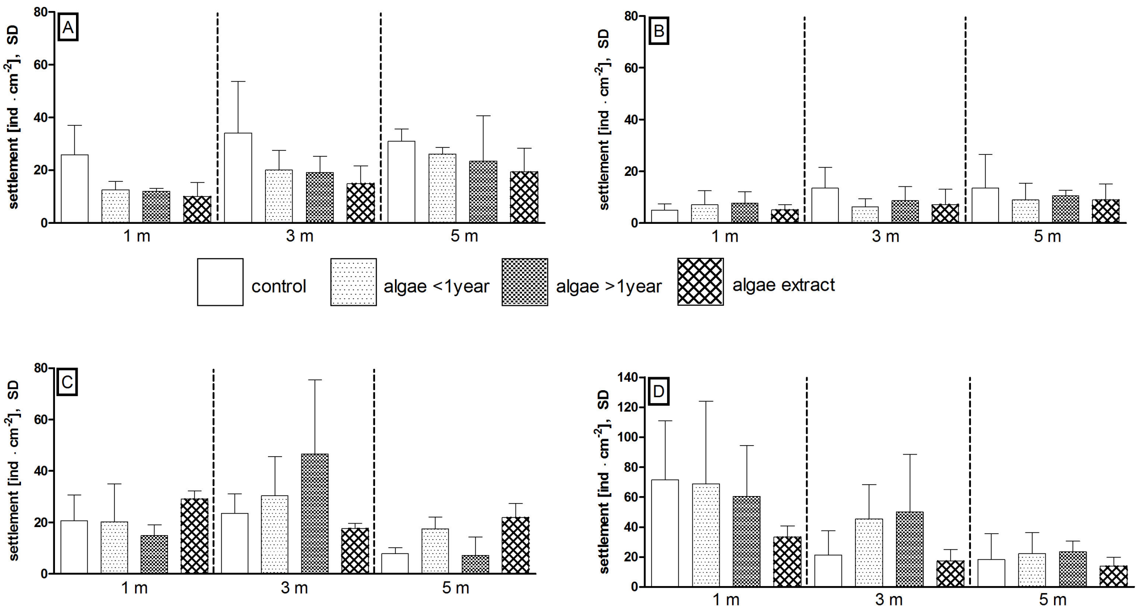

Fig. 6: Mussel settlement at the trout farm in [Ind.·cm^{-2}] ± SD at sampling events: (A) 12/07/12, (B) 18/07/12,(C) 25/07/12, and (D) 30/07/12. (n = 4, except algae > 1 year at 3 m with n = 3)

extract treatment were occupied comparably by mussel larvae during the whole study period.

At the trout farm, all seaweed treatments tended to reduce mussel settlement only during the first week of the experiment (Fig. 6A). Later observations did not show any effect of seaweed on mussel settlement at this site.

Tab. 3: Test statistic for mussel settlement. Effect of algae treatment (algae < 1 year, algae > 1 year and algae extract) and water depth (1m, 3m, 5m, and for the algae / mussel farm 9m) compared to control treatments (2-way ANOVA).

location	sampling date	factor	Df	SS	MS	*F*	p
algae / mussel farm	11/07/2012	algae treatment x water depth	9	2690.0	298.8	1.8450	0.0841
		algae treatment	3	3765.0	1255.0	7.7490	0.0003 *
		water depth	3	1996.0	665.3	4.1080	0.0113 *
		Residual	48	7773.0	161.9		
	17/07/2012	algae treatment x water depth	9	554.1	61.6	1.1100	0.3747
		algae treatment	3	100.7	33.6	0.6050	0.6150
		water depth	3	1025.0	341.7	6.1590	0.0013 *
		Residual	47	2608.0	55.5		
	24/07/2012	algae treatment x water depth	9	152.7	17.0	0.4887	0.8746
		algae treatment	3	71.7	23.9	0.6884	0.5638
		water depth	3	540.9	180.3	5.1940	0.0036 *
		Residual	46	1597.0	34.7		
	30/07/2012	algae treatment x water depth	9	758,5	84,28	0,6961	0,7089
		algae treatment	3	2166	722,1	5,965	0,0016 *
		water depth	3	2544	847,9	7,004	0,0005 *
		Residual	47	5690	121,1		
trout farm	12/07/2012	algae treatment x water depth	6	126.9	21.2	0.2327	0.9631
		algae treatment	3	1618.0	539.2	5.9340	0.0021 *
		water depth	2	824.7	412.3	4.5380	0.0175 *
		Residual	36	3271.0	90.9		
	18/07/2012	algae treatment x water depth	6	105.4	17.6	0.4579	0.8344
		algae treatment	3	93.4	31.2	0.8123	0.4957
		water depth	2	147.3	73.6	1.9200	0.1617
		Residual	35	1342.0	38.4		
	25/07/2012	algae treatment x water depth	6	2374.0	395.7	3.5380	0.0077 *
		algae treatment	3	260.4	86.8	0.7761	0.5152
		water depth	2	1957.0	978.5	8.7500	0.0008 *
		Residual	35	3914.0	111.8		
	30/07/2012	algae treatment x water depth	6	2650.0	441.7	0.6259	0.7084
		algae treatment	3	4387.0	1462.0	2.0720	0.1217
		water depth	2	12506.0	6253.0	8.8610	0.0008 *
		Residual	35	24699.0	705.7		

At the algae / mussel farm, water depth always exhibited a significant impact on mussel settlement (Tab. 3). Whereas larvae tended to settle more in deeper water during first three weeks, vice versa was observed during the last week of the experiment.

At the trout farm, impact of water depth was only observed during first and last week (Fig. 6A, D). In contrast to the observed trend towards deeper water during the first week, larvae settled more in the upper water layer, during the last week of the experiment. Although the ANOVA discovered a significant effect of depth during the third week, both factors (algae treatment and water depth) significantly interacted within the statistical analysis (Tab. 3). Therefore, the impact of water depth on mussel settlement was inconclusive to interpret in this week.

Algae growth

At both locations algae increased in length during the study, although temperatures exceeded their optimum range (Tab. 4). Young algae grew approximately 5 cm during four weeks of the experiment at the algae / mussel farm. At the trout farm they grew even more, especially in one metre water depth, where they grew approximately 9 cm. Adult algae grew less during the experiment and increased only 2 to 3 cm in length at both locations and all depths.

*Tab. 4: Seaweed growth [cm· month^{-1}] ± SD, n = 4 (*n = 3)*

location	Seaweed age	1 m	3 m	5 m	9 m
algae / mussel farm	< 1 year	*1.33 ± 4.04	4.75 ± 0.50	4.25 ± 2.36	5.50 ± 3.32
	> 1 year	2.75 ± 0.50	2.00 ± 0.82	*2.67 ± 0.58	1.75 ± 0.50
trout farm	< 1 year	9.50 ± 3.11	2.75 ± 0.96	5.00 ± 1.63	-
	> 1 year	2.00 ± 0.82	*2.00 ± 0.00	3.50 ± 1.73	-

Discussion

In the Baltic Sea the spatfall of mussels happens yearly in early summer when temperatures reach 10-12 °C (Sunila 1981, Kautsky 1982). Occurrence and abundance of mussel larvae in this study confirm observations of Kautsky (1982), who found similar amounts of mussel larvae in the east of the Baltic Sea. Although the amount of pelagic larvae varied in this study between water depths and locations, it was assumed not to be limiting throughout the experiment. Unfortunately, only few data is available about larvae settlement on a comparable collector material. Nevertheless, the here observed mean settlement of 17500 and 21800 Ind. m^{-1} collector at the algae / mussel farm and the trout farm, respectively, was regarded as sufficient for mussel recruitment in shellfish culture.

The abundance of pelagic larvae was not correlated to the amount of settled spat. This might have been due to the fact that in addition to the "ready to settle" pediveliger larvae also younger larvae stages occurred in the samples and have not been distinguished. According to the mesh size of 125 µm of the sampling sieve, all larvae larger than 175 µm were caught quantitatively. Therefore veliconcha larvae (without eye spot) and eyed veliger larvae were abundant in the samples. The typical vertical migration of pelagic mussel larvae towards light and gravity (Bayne 1964b), was not observed in this study.

As reviewed by Crisp (1974), larvae settlement and substrate occupation is triggered by biological cues (e.g. originating from seaweed) to a significant extent. *S. latissima* is less overgrown by epiphytes (Schmidt and Scheibling 2006) and is even avoided by mussel larvae (Dobretsov 1999, Dobretsov and

Wahl 2001). Confirming these previous ecological investigations, mussel settlement was influenced by the seaweed in this study. There are two main assumptions regarding the influence of the algae abundance on mussel larvae settlement.

Firstly, the hydrodynamics might have changed due to the specific seaweed leaf structure. Single bladed algae like *S. latissima* are known to cause turbulences or swirl on their fronds and in their direct vicinity (Hurd and Stevens 1997, Hurd 2000, Eckman et al. 1989, Eckman and Duggins 1991). Moreover, a large canopy of the marine vegetation can significantly reduce current velocity (Nepf et al. 1997). Turbulent water movement is suspected to influence mussel settlement behaviour (Dobretsov and Wahl 2008). However, it is doubted that a single algae (like in this study) has a significant impact on water flow or turbulence (Koehl et al. 2003), especially in habitats with a low current velocity like the Kiel Fjord. Therefore it is assumed, that the application of algae specimens in this investigation, had a minor or even no effect on water flow close to mussel spat collectors.

Secondly, the release of secondary metabolites originating from *S. latissima,* was suspected to interact with larvae settlement. The family of *S. latissima, Laminariaceae,* is known for its exudation of secondary metabolites like reactive oxygen species (Küpper et al. 2001, Küpper et al. 2002, Rickert 2007) and Mannitol (Salaün et al. 2012)). Generally, spreading and mixing of molecules are explained by physical parameters. The concentration within a certain diffusion path is proportionally dependent on its quantity and on time. Thus, the impact of any chemical cue is directly depending on its source specific biochemistry and on the location specific hydrography. The low current velocity of the Kiel Fjord amplified the importance of algal metabolites.

Whereas the second interpretation appears to be more likely, the first assumption cannot be excluded completely. However the impact of flow cannot explain the differences between the effect of the algae treatments on the mussel settlement within time in different depths.

The age of algae sporophytes determines their content and composition of pigments (Lüning and Dring 1985, Hanelt et al. 1997) and thus light exploitation within depth. Young algae contain more chlorophyll c and less xanthophylles like carontenoids or fucoxanthin compared to older sporophytes (Hanelt et al. 1997). Juvenile *S. latissima* are also known to have a higher spectral quantum yield of gross photosynthesis (Lüning and Dring 1985). Hence, they might have been more photosynthetically active and thus defended against fouling in deep water, resulting in significantly lower mussel settlement at the algae / mussel farm. The age dependent light utilisation was also reflected by the different growth behaviour of young and adult sporophytes during the experimental period. Adult algae grew much less compared to young algae. *S. latissima* from Helgoland is known to reduce growth in the end of July (Lüning 1979). In this study adult plants seemed to have reduced their growth rate already, particularly when compared to the young sporophytes. In contrast to living sporophytes, algae extract did not influence the mussel settlement at the algae / mussel farm. Hence, at this site the species interaction tended to be related to an active defence metabolism rather than to a passive exudation of metabolites.

At the trout farm, all three algae treatments exhibited a similar impact on mussel settlement in one and three metres water depth during the first experimental week. Therefore it was likely, that the impact on mussel settlement corresponded to a signal that passively originated from all three treatments to a similar extent. Exuded metabolites like polyphenols (Sieburth and Jensen 1969, Geiselman and McConnell 1981, Ragan 1976) or terpenes (Nys et al. 1996, Hellio et al. 2001, Maréchal et al. 2004) are known to repel larvae from settlement. Regarding *S. latissima*, one such signal could be the algae sugar alcohol Mannitol and its (bacterial) disintegration. At high temperatures, like in the upper water layers of the trout farm, photosynthetic production as well as the major enzymes of the Calvin Cycle (GADPH and RuBisCO) are reduced in Laminaria (Davison and Davison 1987). At the same time, Mannitol content increases (Davison and Davison 1987). Mannitol is a major energy reserve of brown algae and occurs in living sporophytes as well as in the extract. The Mannitol content of Laminaria is often higher than 20 % of the algae dry weight (Schaffelke 1993) and its release by the algae is suspected to either serve as a bacterial substrate or to discourage bacterial adhesion (Salaün et al. 2012).

The impact of algae treatment was not consistent throughout this study, indicating an effect of time. This confirms findings of (Carlsen et al. 2007), who found a time related composition of epibios on *S. latissima*, resulting from environmental stress and the presence of meroplankton in the water. As defence is suspected to be costly for the algae, the success as well as the extent of a chemical defence might be influenced by the health and composition of the seaweed (Hellio et al. 2004, Weinberger 2007). However, this is dependent on the species and the habitat, because environmental stress did not influence the strength of resistance in *Fucus vesiculosus* (Wahl et al. 2010) and in tropical seaweed (Appelhans et al. 2010). The Kiel Fjord is close to the edge of the natural distribution of *S. latissima*, which is adapted to lower temperatures and a higher salinity (as reviewed in Bartsch et al. 2008). Hence, in this study, algae sporophytes were exposed to multiple stress factors, like low salinity and persistent high larvae abundance at concurrently rising temperatures. Thus it was assumed that the biological and physical stress on the seaweed were increasing over time and significantly influenced seaweed defence and thus, the impact on mussel settlement.

At the algae / mussel farm young algae hindered mussel settlement initially but then seemed to encourage mussel settlement at a later point. Although Cronin and Hay (Cronin and Hay 1996) found that young parts of algae are less defended than older parts, an attractive effect of young *S. latissima* on mussel larvae has not been reported before. On the contrary, past investigations reported exclusively that *S. latissima* is avoided by mussels (Al-Ogily 1985, Dobretsov and Wahl 2001). Blue mussels are usually attracted by filamentous algae and their biofilm (Bayne 1964a, Antsulevich et al.

1999, Dobretsov 1999, Dobretsov and Wahl 2001, Bulleri et al. 2006, Dobretsov and Wahl 2008).
The fact that all algae fronds were almost completely overgrown by mussel larvae (Fig. 7 & Fig. 8) at the end of the experiment indicated, that the seaweed were not able to defend themselves, or influence settlement on surrounding substrata. However, healthy fronds of *S. latissima* are seldom overgrown by mussels or other biota during periods of low water temperature (own unpublished data). This fact additionally emphasises the effect of environmental stress on the seaweed.

Generally, fouling succession on substrates influences larvae settlement (Wahl 1989). Biofilm development on mussel seed collectors in the vicinity of seaweed might have been the most relevant factor concerning mussel settlement. Biofilms differ in their species composition and can either consist of bacteria strains that are deterrent (see Armstrong et al. 2000, Bowman 2007, Dobretsov et al. 2013 for review) or attractive towards macrofouling organisms (Salaün et al. 2010, Wahl et al. 2012). Seaweed exudates and their decomposition either discourage bacterial attachments (Salaün et al. 2012) or represent a valuable food source for micro organisms (Laycock 1974, Weinberger et al. 1994, Weinberger and Friedlander 2000). Hence, the seaweed is able to (actively and passively) influence the biofilm formation. Hence, the analysis and characterisation of the biofilm on the mussel substrate represents a further step towards understanding how seaweed and mussel larvae potentially interact in integrated aquaculture.

This investigation shows that even a small amount of algae biomass can influence mussel larvae settlement. It might be doubted that this respective influence persists within increasing space between the farmed organisms in IMTA practice, but it is also possible that the influence of a larger algae biomass might be even greater than observed here. In this case, the relevance of a large algal canopy needs to be considered regarding the impact of turbulence and plume development. Due to the overlapping of seaweed and mussel cultivation during mussel spatfall in the Kiel Fjord, it was recommended to install mussel collectors upstream to the seaweed to avoid negative interferences. The importance of chemical signals originating from seaweed might be even more relevant in regions like Canada or Norway, where environmental conditions allow an almost year-round seaweed production.

Major differences in defence strategies occur between algae families (red, green or brown algae) and even within the same order. More research is needed to define a potentially threat for associated organisms through farmed seaweed, e.g. by observation of other species such as *Palmaria sp.* or *Undaria sp.*. On the one side, the potential use of living seaweed as an alternative to toxic artificial antifouling coatings might become more important, in particular if established in an IMTA system containing solid infrastructure such as fish cages. An alternative view is that specific algae exudates might enhance mussel settlement in areas of low spat fall, e.g. the Wadden Sea (Nehls et al. 2009).

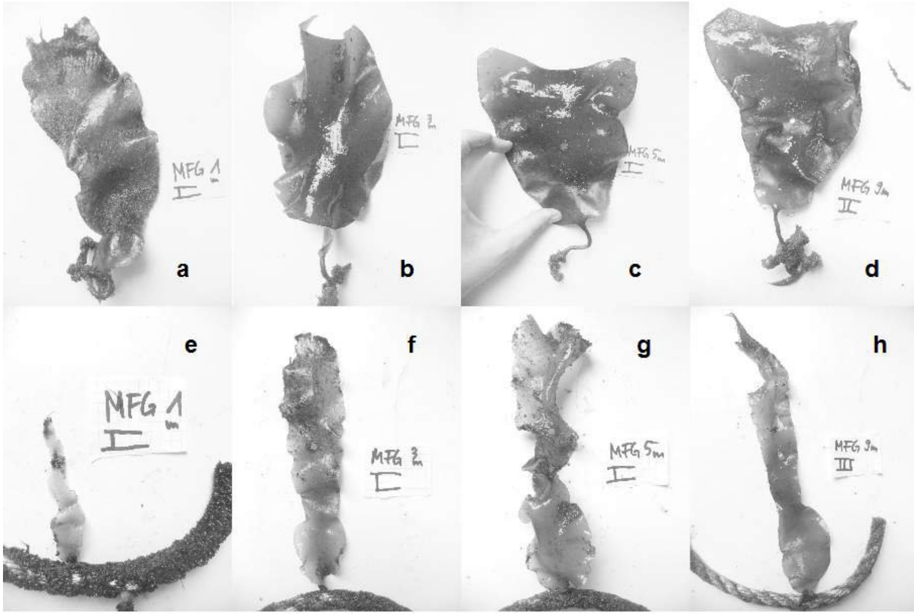

Fig. 7: Algae individuals after one month exposure at the algae / mussel farm (a-d) sporophytes >1 year, (a) 1 m , (b) 3 m, (c) 5 m , (d) 9 m depth; (e-h) sporophytes <1 year;(e)1 m , (f) 3 m , (g) 5 m , (h) 9 m depth.

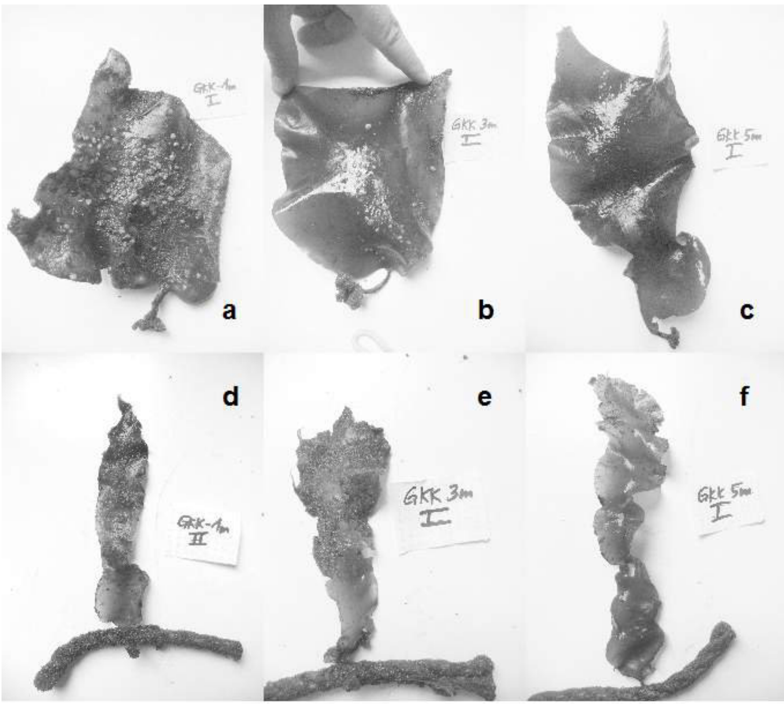

Fig. 8: Algae individuals after one month exposure at the algae / mussel farm (a-c) sporophytes >1 year, (a) 1 m, (b) 3 m, (c) 5 m depth; (d-f) sporophytes <1 year;(d)1 m, (e) 3 m, (f) 5 m depth.

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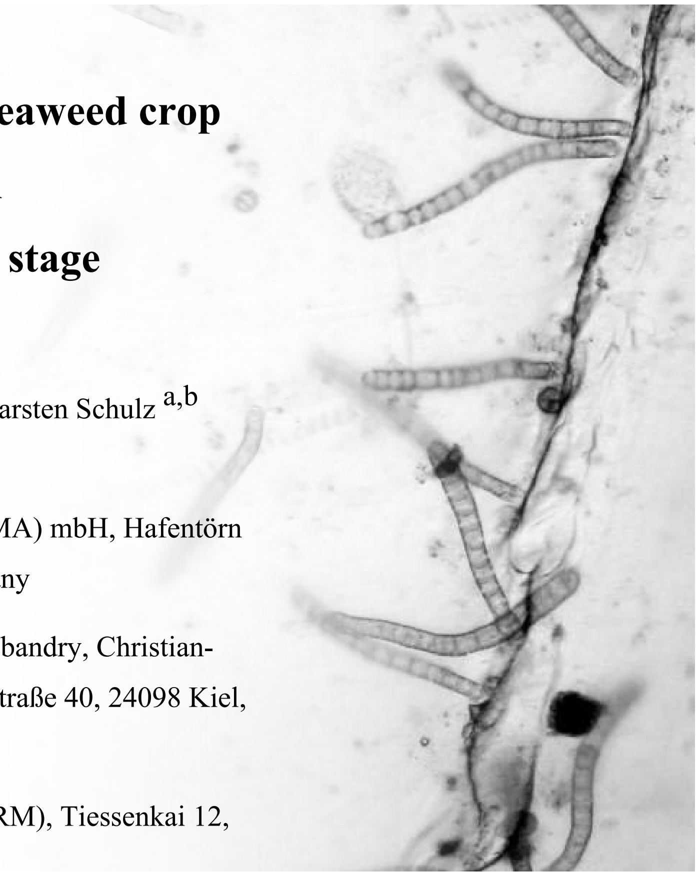

- Chapter IV-
Increasing production of seaweed crop by fertilisation at an early nursery stage

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The results of this investigation were orally presented at the *21st International Seaweed Symposium* the 22nd April 2013 in Bali in the IMTA session and was thereafter submitted to the peer reviewed *Journal of Applied Phycology* as part of the respective conference proceedings (under review).

Abstract

Extractive aquaculture using seaweeds is gaining attention outside of Asia where it is already an established industry. However there are still limitations to viable production, including quantification and optimisation of production yields in addition to site suitability. This investigation presents the specific growth enhancing effect of *Mytilus edulis* on *Saccharina latissima* during early life stages of seaweed in the lab and in the field.

Gametogenesis and juvenile sporophyte development was evaluated with and without blue mussels for nine weeks in the lab. The presence of mussels resulted significantly higher abundance of large multicellular sporophytes. After the lab study, seedling lines were transferred into the field and installed in the direct vicinity and 25 m apart from mussel culture ropes. The previously observed supporting effect of mussels on seaweed growth was still visible after six months of production phase in the sea, resulting in a higher biomass of seaweed of the previous combined lab treatment. As expected, mussels reduced particulate load in the field, but exhibited also shading and mechanical stress in their direct vicinity. The effect of mussels on the habitat was reflected in an elevated biomass development in seaweed, although no significant difference in carbon or nitrogen contents was observed.

This investigation suggests, that mussels have a positive effect on early life stages of seaweed during lab phase, thereby enhancing algal production in the following growth out phase at open sea.

Key words:

IMTA, algae development, *Mytilus edulis*, *Saccharina latissima*

Introduction

Seaweed farming is of increasing interest in the western world (Kain and Dawes 1987, Holdt and Kraan 2011). Despite the use of seaweed for food (McHugh 2003, MacArtain et al. 2007) or cosmetics (Choi et al. 2013, Ruxton and Jenkins 2013), it can be used as a nutrient remediation tool in eutrophic waters (Fei 2004, Schories et al. 2006, Xu et al. 2011) or in intensive aquaculture (Sanderson 2006, Holdt 2009, Holdt et al. 2009). Seaweed is also aimed to be processed as resource for biofuel and bioenergy (Bruton et al. 2009, Hughes et al. 2012, Alvarado-Morales et al. 2013). Extractive seaweed aquaculture can be certified organic by the EU regulation 834/2007 (European Commission 2009) and thus be additionally economically sustainable.

Seaweed cultivation in the Baltic Sea is relatively unexplored (Schories et al. 2006, Blidberg et al. 2012). However, the brown seaweed *Saccharina latissima* is endemic in the Western Baltic Sea. Although it is close to the edge of its geographical distribution, the relatively good growth rates indicate a high potential for seaweed cultivation. In particular, *S. latissima* is a possible candidate for IMTA in the Baltic Sea.

Environmental factors like nutrients, temperature, light intensity, and light quality generally influence growth rates of *S. latissima* (Lüning 1979). Optimal production conditions for *S. latissima* are limited to water temperatures below 15 °C (Lüning et al. 1990). Accordingly, elevated temperatures during summer reduce the production period of *S. latissima* to mid September until the end of June in the Baltic Sea. Additionally, eutrophication of Baltic coastal waters result in high phytoplankton abundances during early spring, when irradiance is increasing and nutrients are available (Wasmund et al. 2011). Due to the high amount of particulate matter in the upper water layers, the phytoplankton creates a shading effect on benthic macroalgae (Wasmund et al. 2011). The overall low salinity, elevated temperatures, and light limitation is suspected to reduce growth *in S. latissima.*

Generally, cultivation of *S. latissima* requires two phases of production (Kain 1991, Brinkhuis et al. 1987). During the first production phase, the sporulation and the alteration of generations from gametophyte to multicellular sporophytes is usually performed under controlled condition in a seaweed hatchery. The first production phase usually lasts 9 to 12 weeks, depending on the nutrient availability and water quality, flow, gas and water exchange rates. Gametophytes and the first stages of sporophytes are sensitive to environmental factors like light, temperature, salinity and nutrients (Han and Kain 1996, Hanelt et al. 1997, Wang et al. 2010). Thus, this is the most vulnerable phase of algae production and a crucial factor for the success of development of a healthy and vigorous sporophyte. As all breeding materials are suspected to be possibly infected with bacteria, viruses, or other challenging organisms, this cultivation period still is most risky. After a successful hatchery period, the juvenile seaweeds are transplanted into the sea, where they remain until harvest. The duration of the grow out period depends on season and is mostly determined by water temperature.

To overcome the challenges of reduced seaweed growth rates in a brackish habitat with an overall short production period, a specific seaweed farm management is required. Fertilisation of seaweed juveniles is suspected to reduce hatchery period and to increase production yield by maximising duration of grow out.

In the case of the organic status of a crop the nutrient source is restricted to the general framework of organic certification. Generally, artificial fertilisers are not allowed in organic production (European Commission 2009). Their application would result in a loss of the certified status, which is not the case if natural fertilizers (e.g. mussels) are applied.

Blue mussels (*Mytilus edulis*) are endemic in the Baltic Sea and occur in high abundances (Remane and Schlieper 1971). Their ammonia excretion and filtration activity indicates a high potential as living fertilisers (Kotta et al. 2009). Hence, the combination of *S. latissima* and *M. edulis* is suspected to reduce the duration of the hatchery phase and consequently, to increase the production yield of seaweed. Moreover, the application of algae culture lines on the same farm infrastructure like mussel culture ropes also exhibit a practical benefit for the farmer, resulting in less effort despite farming two species separately.

The implementation of different trophic levels within one farm site is known as Integrated Multi-Trophic Aquaculture (IMTA). The majority of seaweed studies concerning IMTA focussed on nutrient transformation from fish or shrimp culture (Chopin et al. 2001, Seema and Jayasankar 2005, Matos et al. 2006, Abreu et al. 2011). The relevance of species integration at an early nursery stage has not yet been discussed in recent research.

Accordingly, this investigation aimed to attend the mean and sustained influence of *Mytilus edulis* as living fertilisers on the development and growth of juvenile *Saccharina latissima* in the Western Baltic Sea.

Material and Methods

In this investigation, the impact of blue mussels (*M. edulis*) on the early nursery stage of seaweed (*S. latissima*) was evaluated in a brackish habitat. All mussels and seaweed used in this study originated from a commercially working algae-mussel farm in the Kiel Fjord. According to the seaweeds' characteristic reproduction cycle, the study was divided in two parts. Firstly, seaweed development after sporulation was observed during a nine weeks hatchery period from 09/11/2011 until 17/01/2012. Thereafter, juvenile seaweed sporophytes were transplanted from the hatchery into the sea. Seaweed growth and biochemistry was analysed after six month of grow out on the farm site in the Kiel Fjord.

Seaweed hatchery period

Seaweed development from zoospores to sporophytes was observed on six seedling lines (polypropylene, 2 mm thick, 8 m long). Each seedling line was twisted around a 20 cm long PVC tube (Ø = 50 mm), washed in sterile seawater, scrubbed with a brush, blanched and dried. Prior to inoculation with algae spores, seedling lines were incubated in sterile seawater (10 µm filtered, boiled for 20 minutes) at 10 °C for 24 hours to allow sufficient absorption of sea water and macromolecules. Sorus of adult seaweed was used to generate zoospores. The sorus was scrubbed with a moist, clean brush, gently dried with a tissue and stored cool, moist, and dark for 12 hours. The release of zoospores occurred within 30 minutes under laboratory light conditions (31.2 ± 6.6 µmol m^{-2} s^{-1}) in sterile seawater (10 °C). Motility and quantity of zoospores were estimated at 180x magnification using a binocular microscope (Wild-Heerbrugg, M3Z). At a sufficient amount of motile zoospores (> 10 per field of view), the seedling line was sprinkled equally with the spore suspension using a clean, disposable pipette. Thereafter, all seedling lines were submerged in the spore suspension for 3 hours and swirled every 30 minutes to allow resuspension of motile spores. Finally, the inoculated seedling lines remained in the spore suspension at 10 °C, still, and at long day laboratory light conditions (12/12) for 24 hours.

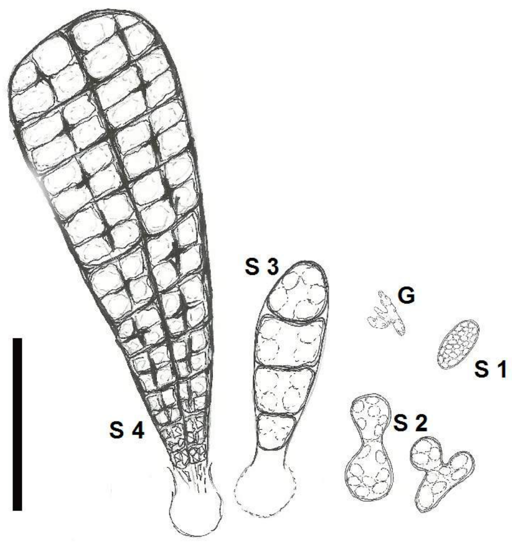

Fig. 1: Seaweed development categories: gametophyte (G), 1-cell sporophyte (S 1), 2-3 cell sporophyte (S 2), small multicell sporophyte (S 3), large multicell sporophyte (S 4). The black line represents 500 µm.

After the incubation, each seedling line was placed randomly in one mesocosm, which contained a volume of 6 litres unfiltered, untreated seawater from the Kiel Fjord. The seawater was completely exchanged every second day and was not aerated. Three mesocosms were additionally equipped with 6 mussels from the algae-mussel farm (55 mm shell length, 17 month old). Due to low air temperatures (< 12 °C) mesocosms were placed outdoor during the hatchery period, which assured natural light and temperature conditions.

The seaweed development was examined using an impression of the seedling lines on coated microscope slides (Polysine OT), 27, 49, 61, and 69 days after sporulation. Algae cells were observed at 100x magnification using a binocular microscope (Wild-Heerbrugg, M3Z). Five different seaweed development stages were categorised (Fig. 1) and their relative amount was determined: gametophytes [G], one-cell sporophyte [S1], two-cell sporophyte [S2], small multi-cell sporophyte (3-8 cells) [S3], and large multi-cell sporophyte (8 ++ cells) [S4].

Hatchery period was completed, as soon as at least one third of all juvenile sporophytes reached development stage S4.

Production period in the sea

All seedling lines were cut into slices of 50 mm length each. These slices were pooled per hatchery pretreatment (with or without mussels) and plaited into a 10 m long, twisted mooring rope (Fig. 2). Each meter of the mooring rope contained 5 slices of seedling line at a distance of 10 cm. Six ropes per pretreatment were produced. Half of the ropes (3 per hatchery pretreatment) was installed 0.5 m apart from suspended mussel culture ropes (0 to 3 m long) on the mussel culture longline.

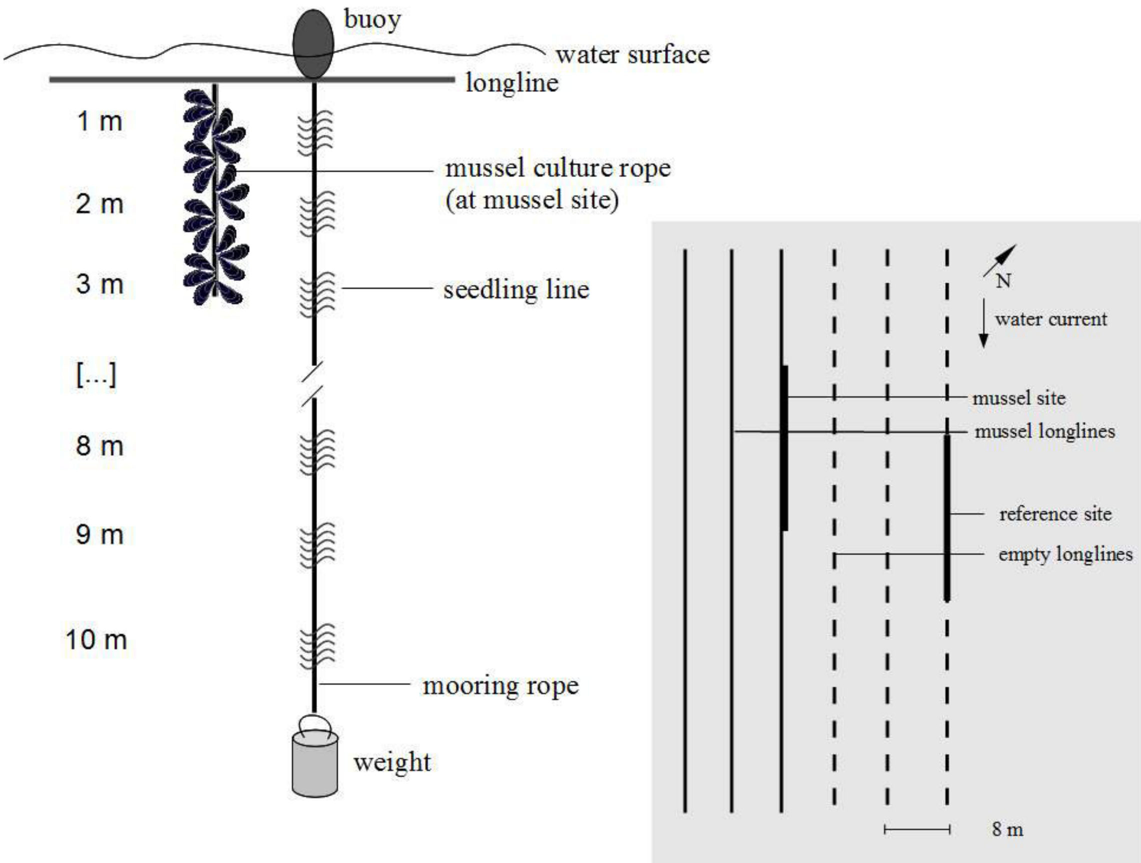

Fig. 2: Experimental set up of the on site settlement experiment. Shaded area highlights the installation of longlines in the sea.

The other half was installed 25 m apart from mussel culture ropes on the reference longline 3 m distant from each other.

Seaweed was harvested 6 month after installation on the farm site. The five slices of seedling lines were pooled per section metre and replicate. All sporophytes were categorised in four length classes (< 1 cm, < 5 cm, > 5 cm, and > 10 cm). Using a binocular microscope (Wild-Heerbrugg, M3Z), the amount of sporophytes per cm and length category was counted. After the determination of sporophyte growth, biomass was analysed by freeze drying (CHRIST, Alpha1-4) to constant weight. Carbon and nitrogen content of the dried, ground seaweed samples were determined using a C/N Elemental Analyser (Euro EA 3000, EuroVector).

The algae-mussel farm is located on the west coast of the Kiel Fjord (54°22' N, 10°9' E) with an average water depth of 10 m. The fjord water current passed parallel to the coast and the longlines of the algae-mussel farm. Water temperature [°C] and light illuminance [LUX] were continuously hourly measured in all water depths with HOBO® pendant temperature / light data loggers. With respect to the spectral sensitivity of the plants, the illuminance [LUX] was converted into quantum irradiance [$\mu mol\ m^{-2}\ s^{-1}$] by multiplying with 0.02 (Lüning 1981).

During the grow out period in the sea, water parameters (salinity, seston, ammonia, and phosphate) were analysed in 3 one litre water samples per site (06/03/12, 16/03/12, 12/04/12, and 14/05/12), using a sampling device (PWS, Niskin type). Salinity was determined at all sampling events using a WTW test probe. 50 ml of each sample were frozen at -20 °C until analysis of dissolved ammonia (Salicylate method 8155, Hach Lange ®) and phosphate (PhosVer 3 method 8048, Hach Lange ®). The seston was quantitatively determined, after filtration of water sample through a cellulose filter (MACHEREY-NAGEL, MN 640w) and drying at 60 °C until constant weight.

Statistics

Statistical analyses were performed with Graph Pad Prism software 5.03 (GraphPad Software, Inc., USA). Due to the low number of replicates, data was not distributed normally.

During the hatchery period, the relative amount of cells per development category was presented in a vertically stacked bar chart. Treatments were compared separately for each category and sampling, using a one sided, non parametric Mann Whitney U-test with a significance level of $p < 0.05$.

After grow out period, seaweed biochemical composition (biomass, carbon and nitrogen content) was analysed using a linear regression over water depth, comparing the difference in slope and elevation of the y-intercept between the pretreatments and the sites. Due to the severe loss of seaweed within the first 3 m at the mussel site, only values below 4 m water depth were compared.

Mean monthly irradiance was analysed by linear regression, using log transformed data. It was tested, whether the slopes or the elevations of the y-intercepts at the sites were significantly different.

Due to the low variation between nutrient and seston analyses within the different water depths, pooled data (whole water column) was compared between sites at each sampling event, using a non parametric, one-tailed Mann Whitney U-test with a significance level of $p < 0.05$.

Results

Seaweed development during hatchery period

In contrast to the relative consistent amount of gametophytes throughout the hatchery period, the amount of larger cell stages changed within time (Fig. 3).

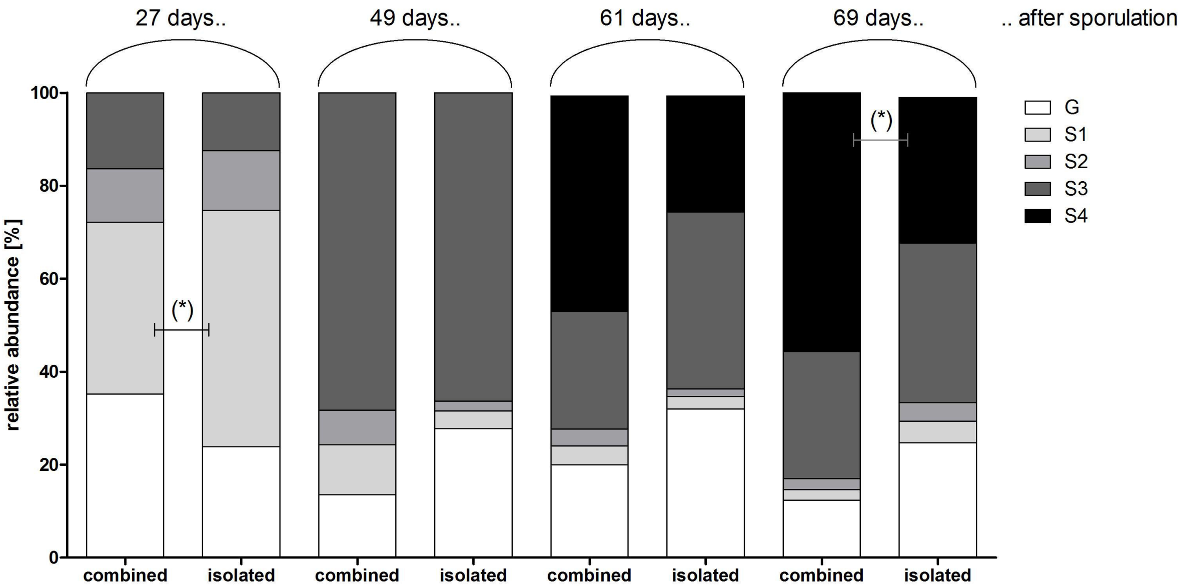

Fig. 3: Algae development during hatchery period. Bars represent the relative amount of the development categories. Asterisks mark significantly differences between treatments ($p < 0.05$).

One month after sporulation, 1-cell sporophytes (S1) were significantly more abundant in isolated treatments ($p < 0.05$), whereas 2-cell sporophytes (S2) occurred to a similar amount in both treatments. At this time, both intermediate cell stages represented more than 40 % of all cells. S1 and S2 sporophytes occurred in low amounts in both treatments until the end of the hatchery period.

27 days after sporulation, the small multicellular sporophytes (S3) represented already more than 10 % of all cells. Three weeks later, more than two thirds of all cells reached this development stage.

The first large multicellular sporophytes (S4) were observed 61 days after sporulation, representing already more than 40 % and 20 % of all cell stages in the combined and isolated treatments, respectively. The hatchery period was completed 69 days after sporulation, when at least one third of all cells was represented by S4 sporophytes in both treatments. Finally, significantly more large multi-cell sporophytes occurred on seedling lines of the combined treatment ($p < 0.05$).

Grow out period on the farm site

Hydrography and light

The mean water temperature ranged from -0.1 to 17.1 °C and from 0.2 to 14.9 °C in 1 and 10 m water depth, respectively. Lowest mean monthly temperatures were observed in February with 3.7 ± 1.0 °C in 1 m water depth (4.2 ± 0.3 °C, in 10 m) and and highest in June with 14.1 ± 0.4 °C in 1 m water depth (12.5 ± 1.5 °C, in 10 m). The seaweed optimum temperature of 15 °C was exceeded for few days in the end of May. Salinity ranged from 13.5 to 20.6 over time and water depths at both locations with a mean values of 16.8 ± 3.9 PSU.

Tab. 1: Mean monthly quantum irradiance at the farm location within the water column in [µmol · $m^{-2}s^{-1}$] ± SD and n as hours of daylight per month. Linear regression of log transformed data including test statistic for slope and elevation of Y-intercept comparison.

		February		March		April		May		June	
Depth [m]		reference lines	mussel lines	reference lines	mussel lines	reference lines	mussel lines	reference lines	mussel lines	reference lines	mussel lines
1	mean ± SD	37,3 ± 41,2	31,6 ± 31,7	95,3 ± 104,4	84,7 ± 85,3	100,2 ± 117,7	89,2 ± 94,0	110,6 ± 125,3	81,3 ± 92,0	83,4 ± 118,5	72,5 ± 97,4
	n	263	245	377	377	433	435	506	491	366	369
2	mean ± SD	22,0 ± 22,9	26,0 ± 25,6	49,4 ± 45,3	48,6 ± 47,8	61,5 ± 62,4	48,9 ± 47,5	64,3 ± 70,8	47,1 ± 49,7	60,8 ± 74,3	47,0 ± 49,8
	n	258	244	373	375	431	359	496	391	361	348
3	mean ± SD	18,0 ± 16,8	23,3 ± 22,0	48,4 ± 48,8	36,6 ± 32,6	54,5 ± 58,0	43,3 ± 40,2	54,9 ± 56,4	37,1 ± 37,6	39,2 ± 50,0	23,0 ± 25,4
	n	259	238	366	362	428	423	495	488	354	338
4	mean ± SD	14,2 ± 14,1	19,5 ± 16,2	29,2 ± 27,7	23,7 ± 21,2	38,8 ± 40,5	26,3 ± 26,3	31,2 ± 38,0	21,7 ± 22,0	22,5 ± 24,4	16,5 ± 18,3
	n	249	232	356	357	418	418	480	480	348	340
5	mean ± SD	9,6 ± 8,8	14,1 ± 12,3	19,9 ± 18,8	18,8 ± 16,5	27,7 ± 27,1	23,3 ± 22,2	21,3 ± 21,4	19,4 ± 19,5	18,2 ± 18,4	14,5 ± 15,7
	n	237	234	352	350	415	408	482	480	344	333
6	mean ± SD	8,2 ± 6,6	8,8 ± 7,6	12,4 ± 11,5	13,5 ± 11,9	17,3 ± 17,7	17,8 ± 16,7	13,9 ± 17,1	13,5 ± 13,7	9,9 ± 9,8	10,0 ± 10,2
	n	237	225	348	342	408	404	462	470	333	332
7	mean ± SD	5,6 ± 5,0	6,5 ± 4,8	10,0 ± 8,7	9,3 ± 8,4	13,5 ± 13,2	12,1 ± 11,4	10,8 ± 12,4	9,8 ± 10,8	8,9 ± 8,6	6,8 ± 6,9
	n	227	217	336	335	400	399	455	459	332	328
8	mean ± SD	4,4 ± 3,4	5,4 ± 4,0	7,2 ± 6,7	6,9 ± 6,2	9,5 ± 9,6	9,2 ± 9,3	7,2 ± 9,1	7,8 ± 8,1	6,4 ± 6,6	5,4 ± 5,7
	n	223	210	331	328	391	392	425	442	319	316
9	mean ± SD	3,5 ± 2,9	3,8 ± 3,0	4,9 ± 4,3	4,2 ± 3,8	7,2 ± 8,1	5,8 ± 5,5	6,5 ± 9,5	5,0 ± 5,6	4,0 ± 4,0	2,9 ± 2,8
	n	219	200	311	319	372	379	382	429	292	303
10	mean ± SD	2,2 ± 1,8	2,5 ± 1,8	3,8 ± 3,5	3,6 ± 3,0	5,9 ± 8,1	4,9 ± 4,7	3,9 ± 4,6	3,3 ± 3,5	2,6 ± 2,6	2,2 ± 2,1
	n	207	194	305	303	365	361	410	398	282	277
linear regression	R²	0,990	0,980	0,990	0,995	0,994	0,989	0,990	0,991	0,992	0,985
	F	1155	394,3	821,20	1502	1245	718,5	791,2	905,4	1001	515,5
	Dfn , Dfd	1 , 8	1 , 8	1 , 8	1 , 8	1 , 8	1 , 8	1 , 8	1 , 8	1 , 8	1 , 8
	p	< 0.0001 *	< 0.0001 *	< 0.0001 *	< 0.0001 *	< 0.0001 *	< 0.0001 *	< 0.0001 *	< 0.0001 *	< 0.0001 *	< 0.0001 *
	slope	-0.127 ± 0.003	-0.125 ± 0.006	-0.154 ± 0.005	-0.150 ± 0.004	-0.139 ± 0.004	-0.135 ± 0.005	-0.158 ± 0.006	-0.145 ± 0.005	-0.165 ± 0.005	-0.161 ± 0.007
	Y-intercept	1.650 ± 0.023	1.706 ± 0.039	2.080 ± 0.033	2.021 ± 0.024	2.119 ± 0.025	2.025 ± 0.031	2.153 ± 0.035	1.998 ± 0.030	2.077 ± 0.032	1.950 ± 0.044
slope	F	0,1185		0,3013		0,4189		3,0000		0,2408	
	Dfn , Dfd	1 , 16		1 , 16		1 , 16		1 , 16		1 , 16	
	p	0,7351		0,5907		0,5267		0,1025		0,6302	
elevation of intercept	F	11,69		4,52		15,51		14,05		17,18	
	Dfn , Dfd	1 , 17		1 , 17		1 , 17		1 , 17		1 , 17	
	p	0,00327 *		0,0485 *		0,001 *		0,0016 *		0,0007 *	

During the grow out period, the mean monthly irradiance increased (Tab. 1) from lowest values in February to highest in May 2012. In February, light intensity was significantly higher at the mussel site. This was reversed in the following months with a significant higher light intensity at the reference site, indicating a shading effect in the vicinity of mussel culture ropes.

Light intensity was not exceeding 150 µmol m^{-2} s^{-1}, therefore a light induced inhibition of the seaweed photosynthesis was not observed, neither at the reference, nor at the mussel site. Although light intensity decreased exponentially within water depth similarly at both study sites, irradiance was sufficient for photosynthesis down to 6 m water depth. In deeper water layers, the mean irradiation exceeded the light compensation point of 5-8 µmol m^{-2} s^{-1} and photosynthesis was only possible for few hours a day.

Water parameters

The seston concentration of the water decreased from March until May, indicating a phytoplankton bloom in the beginning of March (Fig. 4). During the phytoplankton bloom (06/03/12), the particulate load in the water column resulted in higher amounts of seston in the vicinity of mussel lines ($p < 0.001$). Vice versa was observed later in March ($p < 0.0001$) and April ($p < 0.01$), when significantly more particulate matter was observed at the reference lines. Seston concentration was lowest in May, with similar amounts at both observation sites.

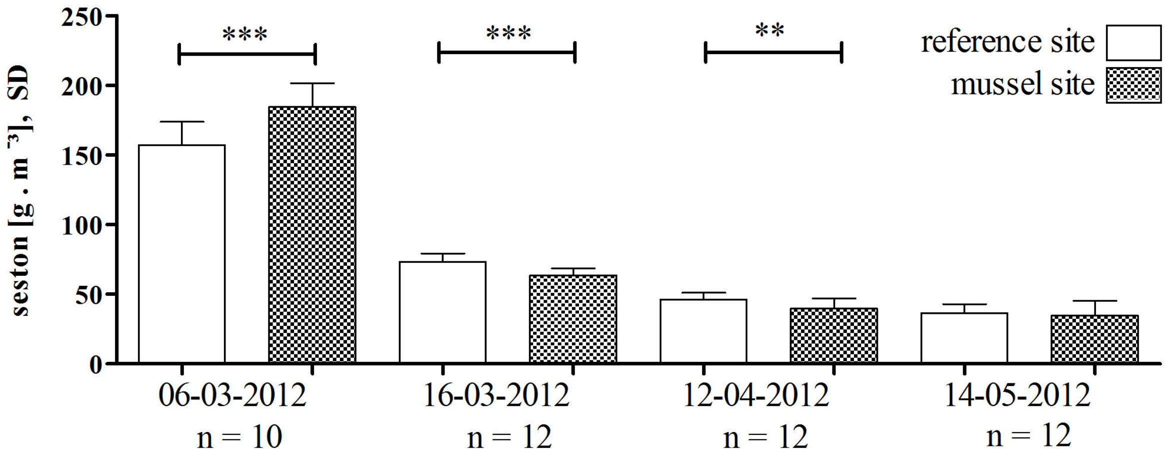

*Fig. 4: Seston concentration in the water column as [mg l^{-1}] ± standard deviation at both study sites. Asterisks mark significant differences (p*** < 0.001, p**<0.01).*

In addition to the high seston concentrations, the low concentrations of phosphate (Fig. 5) and the lack of ammonia in the water (Fig. 6) indicated a nutrient depletion by a phytoplankton bloom in the beginning of march. The concentration of phosphate and ammonia slightly increased comparably at both sites after the bloom. The amount of dissolved ammonia and phosphate was similar at both sites, except in May, with a higher phosphate concentration at the reference site.

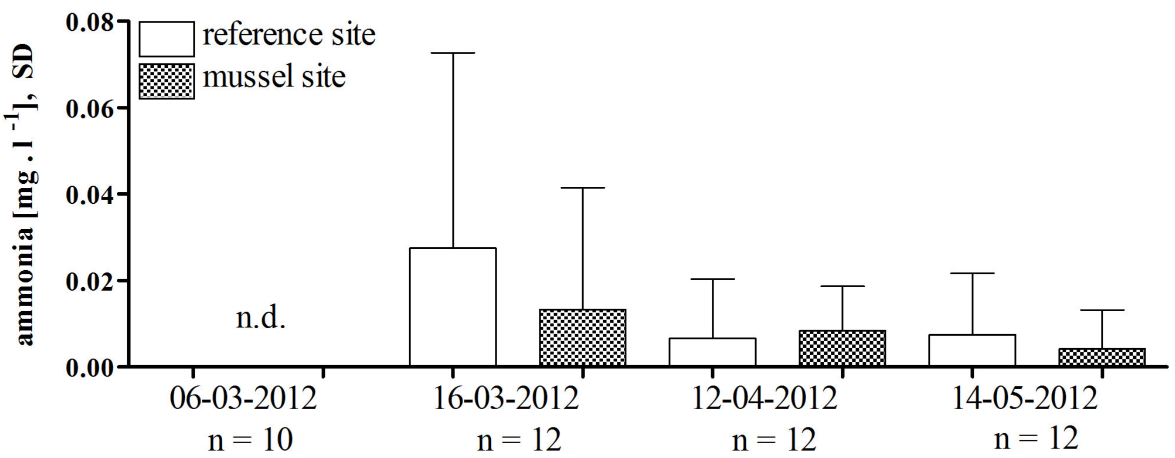

Fig. 5: Ammonia concentration in the water column as [g m^{-3}] ± standard deviation at both locations.

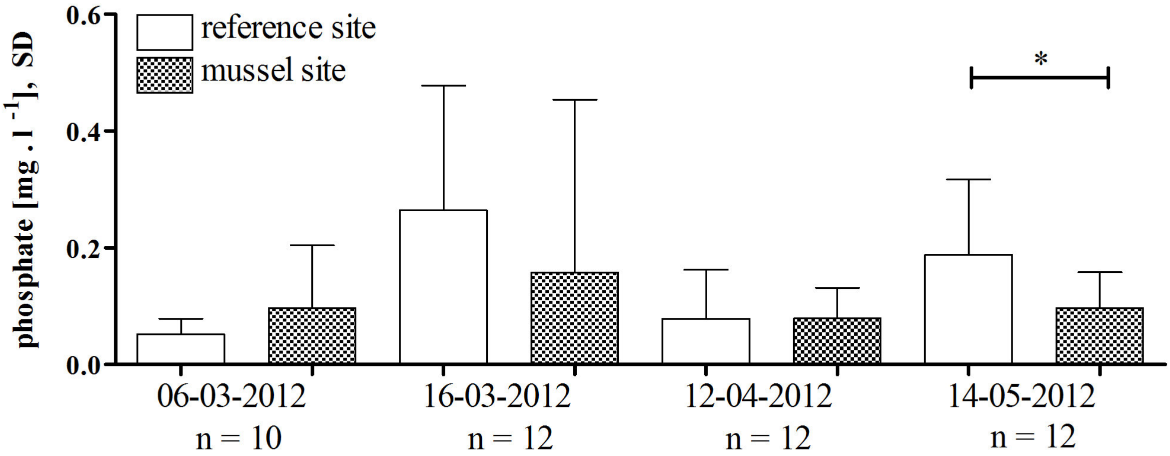

Fig. 6: Phosphate concentration as [mg l^{-1}] ± standard deviation at both study sites. The asterisk mark the significant difference (p< 0.05) in May.*

Seaweed growth

Due to harsh weather conditions during harvest, one replicate of the combined pretreatment was lost at the reference site. At the reference site, sporophytes occurred on seedling lines throughout the whole water column, whereas at the mussel site no sporophytes appeared in the first three meters of water depth (Fig. 7). Although the direct contact of mussels and seaweed ropes was excluded, the fuzzy and destroyed seedling lines indicated a high mechanical stress in the vicinity of the mussel culture ropes.

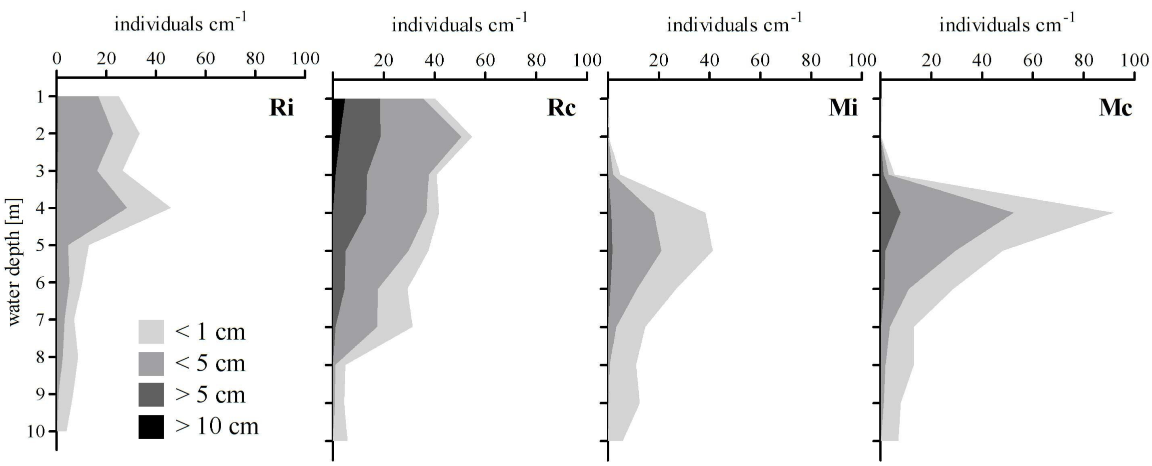

Fig. 7: Mean abundance per centimetre of all seaweed length categories after six month of grow out period in the sea of the isolated (i) and combined (c) pretreatment in the hatchery at the reference site (R) and at the mussel site (M).

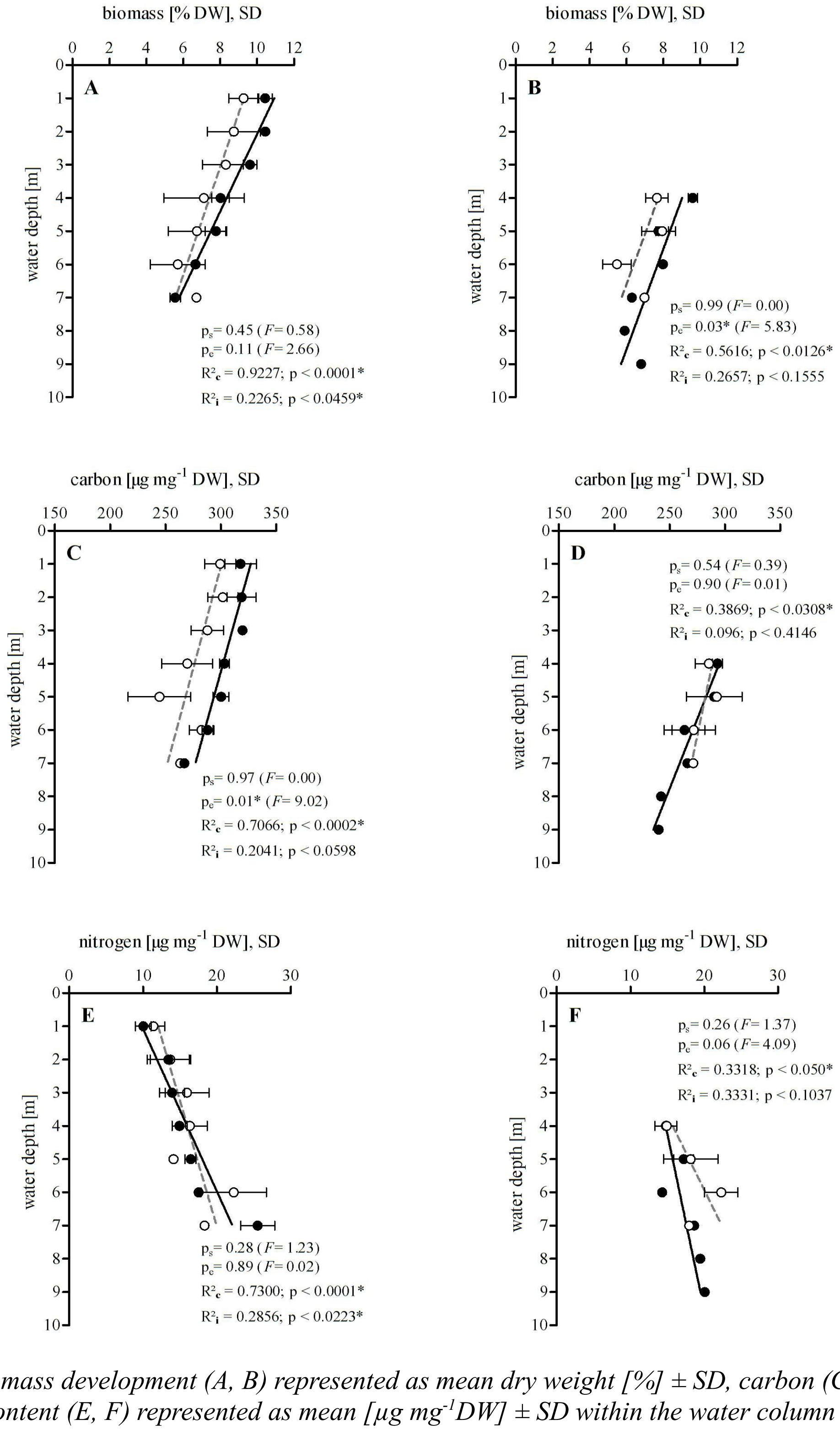

Fig. 8: Biomass development (A, B) represented as mean dry weight [%] ± SD, carbon (C, D) and nitrogen content (E, F) represented as mean [µg mg^{-1}DW] ± SD within the water column [m] at the reference site (A, C, E) and mussel site (B, D, F). Lines show the linear regression of the combined (c, closed circles) and the isolated (i, open circles) pretreatment during hatchery period, each with coefficient of equations (R^2). The slopes (p_s) and elevations of intercept (p_e) show the comparison between hatchery phase pretreatments.

The combination of mussels and seaweed during the hatchery period resulted in an enhanced seaweed length growth on the reference site and in a higher sporophyte abundance of up to 91.78 ± 41.97 individuals per cm at the mussel site (Fig. 7). Although the seaweed density of the combined pretreatment was higher at the mussel site in four metres depth, sporophytes were smaller compared to the reference site in the same depth. Seaweed of the isolated pretreatment was reduced in size and abundance at both sites.

Furthermore, the abundance of mussels was reflected by seaweed growth performance within depth. If combined with mussels during the hatchery, a significant growth was observed until 7 m at the reference site and until 6 m for both pretreatments at the mussel site. In contrast, sporophytes of the isolated pretreatment were already less abundant in 5 m water depth.

Consequently, sporophytes grew in larger size, in higher abundances, and deeper in the water column if combined with mussels, either during the hatchery or during the grow out period.

Juvenile sporophytes exhibited a mean biomass of 7.8 ± 0.3 % DW with a C/N ratio of 19.9 ± 6.7. Similar to growth performance and abundance, seaweed biochemistry exhibited a strong depth dependent pattern (Fig. 8). Whereas seaweed biomass and carbon content decreased within water depth, the nitrogen content increased. Although the depth dependent biochemistry was comparable between pretreatments, the overall biomass development and the carbon content was higher in sporophytes of the combined pretreatment at the mussel and the reference site, respectively. However, this was not reflected in the seaweed nitrogen content.

Discussion

This investigation aimed to determine the effect of *M. edulis* on the cultivation of *S. latissima* during the early development stages of the seaweed.

Mussels significantly enhanced seaweed development during the hatchery period, thereby creating a flow on benefit throughout the production period in the sea. The major assumption regarding the supporting effect on seaweed early nursery stages was active (excretion of ammonia) and passive (depletion of competing microalgae) nutrient allocation by mussels.

The growth enhancing effect of mussels during the initial phase of algal annual succession has been reported by Kotta (Kotta et al. 2009). Although they investigated ephemeral macroalgae, they also referred to mussels as the responsible nutrient source. Besides (pseudo-) faeces (Kautsky and Evans 1987), ammonia is the major metabolic mussel deposit (Dame and Dankers 1988). Nitrogen compounds derived from mussels probably provided an optimal nutrient supply for the seaweed, because ammonia is suspected to be a more important nitrogen source than nitrate in macroalgae (Harrison et al. 1986, Rees 2007).

Ammonia excretion rates of blue mussels depend on hydrography, food conditions as well as on mussel biomass, and range from 0.33 to 2.16 µmol g^{-1} h^{-1} (Jansen et al. 2012) or 0.11 to 2.46 µmol g^{-1} h^{-1} (Schlüter and Josefsen 1994). Consequently, the theoretical ammonia excretion of all mussels (approximately 2.9 g DW_{mussel}/ mesocosm) during the hatchery of this study varied from 0.32 to 7.22 µmol gDW^{-1} h^{-1} and was below the critical concentration for *S. latissima* (Ahn et al. 1998, Yarish et al. 1990). Gametogenesis and sporophyte development is reported to be reduced at high ammonia concentrations (Yarish et al. 1990). This might explain the delayed development of one-cell sporophytes in the combined treatment after the first month of the hatchery period. However, this changed within time and after nine weeks of hatchery, more large multicell sporophytes have developed if combined with mussels. This indicated a successful alternation of generations, including a successful gametogenesis.

Epiphytes are a major problem during seaweed hatchery because they compete for nutrients and light with the juvenile macroalgae (Fletcher 1995, Friedlander and Levy 1995). Different strategies were applied to reduce the amount of epiphytes in tank culture like light reducing (Friedlander and Levy 1995), high density farming (Bidwell et al. 1985), or application of the toxin germanium dioxide (Shea and Chopin 2007). The depletion of epiphytes by mussel filtration has a high potential in organic cultivation of *S. latissima*, because light reduction and high density farming would result in reduced growth rates and the use of GeO2 is not allowed in organic production (European Commission 2009).

Clearance rates of *M. edulis* depend on various exogenous and endogenous parameters (Bayne and Widdows 1978, Smaal and Twisk 1997) and thus, vary seasonally as well as regionally. Blue mussels of the Kiel Fjord with a mean shell size from 4 to 6 cm exhibit a filtration rate of 1.5 to 3 l h^{-1} (Theede 1963). Accordingly, mussels cleared the water in the mesocosms within approximately 30 minutes. Apparently less epiphytes occurred on the mesocosm walls of the combined treatment, however, the amount and composition of epiphytes was not quantified in this investigation.

Whereas a sufficient nutrient supply and epiphyte control by mussels determined seaweed development during the hatchery period, light availability and seaweed physiological status additionally influenced seaweed growth during grow out period. Light availability was determined by water depth and site, whereas seaweed physiological status depended on the previous hatchery treatment. Seaweed growth and biochemistry was significantly different to other regions (Gerard 1990, Sjøtun 1993, Mizuta et al. 1997). However, the high amount of sporophytes indicated a successful transplantation from the hatchery into the sea and sufficient growth conditions during grow out period. Sporophytes of the combined treatments were larger, contained higher biomass and more carbon compared to the isolated pretreatments. The size of juvenile sporophytes was larger after hatchery and consequently, sporophytes were larger after grow out period, compared to the isolated pretreatment.

The difference in biomass development and carbon content might be explained by the size of sporophytes, because larger sporophytes contain more structural polymers like carbohydrates (Broch, Slagstad 2012).

However, it is also possible, that a higher efficiency of photosynthesis resulted in a higher carbon accumulation and hence, biomass production. Light is a crucial factor that determines algae growth especially during early development stages after gametogenesis in the field. According to the exponential decrease of light within the water column, seaweed growth and biochemical composition exhibited a strong depth dependent pattern. As growth of juvenile sporophytes is an indirect measure of photosynthetic efficiency, photosynthesis of sporophytes of the combined pretreatment was suspected to be enhanced due to fertilisation during early nursery stage. The additional nitrogen supply through mussels might have led to a luxury uptake of nitrogen (Chapman et al. 1978, Rees 2007). Nitrogen is stored in proteins of the photosynthetic enzyme complexes (Gerard 1997), thereby creating a large photosynthetic apparatus in excess of demand. This accumulation might have enhanced photosynthesis in sporophytes after out planting in the field. This assumption is supported by the better growth performance within depth of sporophytes of the combined hatchery treatment. Nevertheless, seaweed nitrogen content was comparable between sites and pretreatments after the grow out phase.

Due to the filtration activity of *M. edulis*, water transparency was supposed to be enhanced in the vicinity of mussel culture ropes. Nevertheless, significantly higher seston concentrations occurred close to the mussel lines during the phytoplankton bloom. Farm infrastructure is comparable to a reef (Phillips 1990, Plew et al. 2005) accordingly, reduced current velocity on mussel culture ropes probably caused the accumulation of seston close to the lines. Moreover, mussels reduce their filtration rate at particle concentrations as high as observed in this study (Widdows et al. 1979). Hence, mussels were unable to reduce the amount of particulate matter during the period due to decreased filtration activity. However, mussels significantly decreased the particulate load thereafter.

Although mussels enhanced water transparency after the phytoplankton bloom, they still reduced light intensity due to shading of their direct vicinity. However, the shading occurred only in the first few meters of water depth, where seedling lines have been destroyed anyway. Consequently, the close combination of seaweed and mussel culture ropes is not recommended, because of shading and mechanical stress.

Nevertheless, seaweed grew well below mussel culture ropes. Regardless of pretreatment, algae grew down to 6 m and 7 m at the mussel and at the reference site, respectively.

Although comparable in size, higher amounts of sporophytes occurred at the mussel site in four metres depth, compared to the reference site. This might be explained by nutrient competition between individuals on the expense of smaller individuals at the reference site. *M. edulis* was suspected to provide nutrients in their vicinity, and thus, seaweed probably benefited from ammonia excretion below the mussel culture ropes. However, the concentration of dissolved nutrients and seaweed nitrogen content was was comparable between sites.

In conclusion, the optimisation of seaweed hatchery resulted in a flow-on benefit on the growth performance and biomass development throughout the grow out period. The co-cultivation of seaweed and mussels is a sustainable way to produce algae sporophytes in a shorter time period, at low cost, and with the possibility to be certified organic. Nevertheless, further observations are needed to analyse the specific impact of mussel derived nitrogen on early development stages of the seaweed. For example, transcriptomic analyses on up or down regulated genes of nitrogen uptake related enzymes could be applicable, as shown for temperature and light stress in *S. latissima* by Heinrich et. al (2012).

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General Discussion

Increasing demand for seafood in a situation of stagnating traditional fishery makes aquaculture a sector of immense growing potential (FAO 2012). However, intensive aquaculture is associated with ecological problems such as nutrient discharge, use of medicine, and occurrence of parasites and diseases. This has fostered the development of environmentally friendly techniques.

Integrated Multi-Trophic Aquaculture (IMTA) is one of the most promising approaches for sustainable aquaculture (Chopin et al. 2001, Neori et al. 2004, Abreu et al. 2009, Reid et al. 2010). In IMTA, extractive components like shellfish and seaweeds reduce the impact of nutrient discharge originating from fed components like fish or shrimp (Neori et al. 1996, Seema and Jayasankar 2005). Furthermore, application of shellfish cultivation can reduce the occurrence of parasites and diseases in fish aquaculture (Molloy et al. 2011, Molloy et al. 2012, Bartsch et al. 2013). IMTA has also economic advantages for farmers through the production of high value by-products with diverse use options (Nobre et al. 2010, Neori and Nobre 2012), without much addition of infrastructural investment. In particular, the same farm infrastructure (longlines) can be used for shellfish and seaweed, which facilitates installation and maintenance during farm practice. Products that are produced environmentally friendly (e.g. in IMTA) can be certified as organic in the European Union (European Commission 2009) and thus, generate a profit.

Regarding the Baltic Sea, aquaculture development is limited due to the specific Baltic environmental conditions like low salinity, low water currents, pollution, and eutrophication (Schultz-Zehden and Matczak 2012). Salinity significantly influences species diversity and growth performance (Remane and Schlieper 1971). Accordingly, the mussel production for human nutrition is hydrographically restricted to the western Baltic Sea (Westerbom et al. 2002, Lindahl and Zaiko 2012). Shellfish and seaweed cultivation in the Baltic Sea could potentially be used for purposes other than human consumption. For example, shellfish is used for the manufacturing of animal feed for fish or poultry (Grave 1974, Berge and Austreng 1989, Jönsson et al. 2011, Lindahl 2011, Nagel et al. 2013). Increasing social awareness for a clean environment unveils hidden socio-economic values of extractive aquaculture. Besides food and feed production, shellfish and seaweeds are aimed to be used for nutrient retention in eutrophic waters (Lindahl et al. 2005, Norell 2005, Xu et al. 2011). For example, in 2004, the Swedish coastal town Lysekil bought the ecosystem service from a Swedish mussel producer, to remediate the nitrogen discharge of the towns sewage treatment plant by mussel farming (Lindahl 2011). Additionally, seaweeds are used as pharmaceutics and cosmetics (Choi et al. 2013, Ruxton and Jenkins 2013, Goecke et al. 2012), human food (MacArtain et al. 2007, Holdt and Kraan 2011), animal nutrition (Leonard et al. 2010) or as a resource for bioenergy (Schories et al. 2006, Holdt 2009, Gröndahl et al. 2009, Liu et al. 2011).

Presently, Baltic integrated aquaculture is practised in Denmark (Hjarnø Havbrug and Musholm A/S) and Germany (oceanBASIS GmbH). In 2006, the Danish parliament decided to increase aquacultural fish production from 40.000 to 115.000 t in 2013, while the environmental impact per kg of fish have to be reduced by 40% of nitrogen output per kg fish (Ministeriet for Fødevarer, Landbrug og Fiskeri 2006). This can be achieved by either application of equivalent technology or the implementation of biofilters, like shellfish or seaweed (Ministeriet for Fødevarer, Landbrug og Fiskeri 2009). However, no law or regulation concerning nutrient compensation from open water fish aquaculture exists yet (Per Dolmer 2013). In Germany, in the Kiel Fjord, an integrated mussel (*Mytilus edulis*) and algae farm (*Saccharina latissima*) has been established (see *Chapter I*) since 2009. This farm site was applied as scientific base station for this thesis.

Most IMTA investigations are based on observations of different trophic levels with the purpose to mitigate nutrient discharge from the fed organisms, thereby enhancing the growth of the extractive components. This assumed Win-Win- Situation is suspected to be ecologically and economically sustainable. However, ecological aspects of species combinations and compatibility have not been focussed yet in IMTA research.

Within this thesis, the specific relationships of the extractive organisms were observed in the context of a brackish water IMTA in the Baltic Sea.

Exploitation of the Baltic Sea faces many concerns and thus, its suitability for aquaculture is yet underestimated. Although environmentally friendly approaches in aquaculture suggest a substantial benefit for the coastal industry and also the environment, the development of Baltic Sea aquaculture is still in its infancy. In this Ph.D. study (*Chapter II*), mussels (*M. edulis*) and seaweed (*S. latissima*) exhibited different sensitivity to Baltic Sea environmental conditions during the general combined farming season. Whereas seaweed growth was reduced and biochemistry showed major differences to seaweed from regions with higher salinity, mussel shell growth and condition were comparable. Growth rates of *S. latissima* depended on nutrient concentration and light availability and thus, differed significantly within the observed locations. The condition indices of *M. edulis* changed seasonally and regionally. These results indicated a high potential for IMTA with blue mussels (*M. edulis*) and seaweed (*S. latissima*) in the western Baltic Sea, if species requirements were considered within farm design and site selection. Moreover, during the research period, a commercial mussel farm was developed as an upscaling of the already existing seaweed farm (*Chapter I*). Mussels from the Kiel Fjord were not polluted and algaetoxins were not observed during the project. The microbial quality of the shellfish water was classified as "A" (< 230 cfu/100g mussel meat) during major harvesting season (*Chapter I*). Therefore mussels are sold fresh, as food for human consumption and seaweeds are sold as raw material for cosmetics. Both organisms are certified as organic. The sustainable exploitation of resources represents an alternative to intensive fed aquaculture in a vulnerable ecosystem like the Baltic Sea.

The relationship of extractive organisms in IMTA exhibited advantages and limitations and was therefore not unambiguously described as a solely Win-Win-situation. Hence, the results presented within this thesis demonstrate the importance of farm design for IMTA by exhibiting diverse effects of species interactions. In particular, the interaction between shellfish and algae farm components in IMTA was identified as either a direct, physical impact or an indirect, chemical impact (Tab. 1). The indirect, chemical species interactions were more diverse and rather difficult to measure *in situ*, whereas the direct, physical impact was observed quite obviously.

Tab. 1: Direct and indirect interactions between extractive organisms (M = mussels, A = seaweeds) in IMTA

	direct	**indirect**
Positive	(M) shading / UV protection	(M) Nutrient excretion (M) Filtration → increasing of water transparency → elimination of competing phytoplankton → elimination of epiphyte larvae → elimination of potentially harmful bacteria (A) Depletion of dissolved nutrients (A) Oxygen production (A) Excretion of particulate organic matter (POM) (A) Excretion of antibiotic / antibacterial substances
Negative	(M) shading / less available light (M) mechanical stress/ abrasion (M) fouling (M) habitat for herbivores (M/A) decreasing current velocity	(M) Excretion of faeces / increase of sedimentation (A) Excretion of deterrent agents / antifouling

Excretion of ammonia as well as the filtration activity of mussels was assumed to enhance seaweed development during the early development stages. In *Chapter IV*, the specific growth enhancing effect of *M. edulis* on early development stages of *S. latissima* was observed in the lab and in the field. Mussel abundance resulted in significantly more multicellular sporophytes after alternation of generations. This growth supporting effect of mussels on seaweed juveniles was still visible after six months of field exposure, resulting in a higher biomass of seaweed previously combined with mussels. These results indicated a positive effect of mussels on seaweeds during alternation of generations, thereby enhancing algal production in the following grow out phase in the field. Fertilization of juvenile seaweed by mussel excretion is likely. In contrast to phytoplankton, the assimilation of ammonia by Laminaria is higher, compared to nitrate (Harrison et al. 1986), (Rees 2007)).

Epiphytes are one of the most severe problems in seaweed tank culture (Fletcher 1995, Lüning and Pang 2003) and thus, represent a major threat during lab phase of cultivation of *S. latissima*. Previous research has developed different strategies to decrease this impact by either high density farming (Bidwell et al. 1985), or short day treatment (Lüning 1993, Lüning and Pang 2003). Filtration activity of mussels was assumed to eliminate competing and possibly herbivorous plankton, thereby protecting the juvenile plants from overgrowing or damage. However, as zoospores of *S. latissima* also belong to phytoplankton, they represent potential food source for mussels. To avoid a loss of spores by mussel filtration, bivalves should be applied as natural biofertilizer after spore settlement (*Chapter IV*).
Furthermore, mussels are able to retain also smaller particles than plankton from seawater like bacteria (Vahl 1972, Birkbeck and McHenery 1982, Charles et al. 1992, Canesi et al. 2002). Hence, they may also protect juvenile seaweeds from potential algal pathogens like *Algicola bacteriolytica* known as the origin of the red spot disease in Laminaria (Sawabe et al. 1998) (formerly P*seudoalteromonas bacteriolytica*, Ivanova 2004). However, a new study from (Molloy et al. 2013) suggests that mussels also have the potential to accumulate pathogens and thus, increase the risk of cross infections in IMTA. This might be of high interest concerning especially bacteria that are not sensitive to lysozyme of *M. edulis* like *Staphylococcus aureus* (Birkbeck and McHenery 1982) or infectious pancreatic necrosis virus (IPNV) (Molloy et al. 2013).
Besides elimination of competing and potentially hazardous plankton, mussel filtration enhances water transparency and consequently, directly fertilise seaweed with light. Particle reduction was observed in previous research (Bayne and Widdows 1978, Møhlenberg and Riisgård 1978, Clausen and Riisgård 1996) and was confirmed in the present study with significantly less particulate matter in the direct vicinity of mussel socks (*Chapter IV*). This is of special concern for IMTA in eutrophic areas with high abundance of phytoplankton that competes for light and nutrients with macroalgae.
In environments with high nutrient load, seaweeds contribute to depletion of dissolved nutrients in the seawater, thereby enhancing water quality for the associated organisms. This is important in marine eutrophic waters, where phytoplankton blooms create algal mats, whose decomposition results in anoxic bottom conditions (Lehvo and Bäck 2001, Nausch et al. 2011, Wasmund et al. 2011). Regarding the present study, it is recommended to apply seaweed cultivation close to nutrient sources like a fish farm or river inflow (*Chapter II*). In kelp, luxury uptake of nitrogen in excess of need is known (Chapman et al. 1978, Gerard 1997, Rees 2007). Macroalgae like *S. latissima* can store nitrogen in tissue and utilise it during season of depleted nutrients (Chapman and Craigie 1977). This is a particular advantage of perennial seaweeds compared to phytoplankton which is unable to store nitrogen and thus, requires a high amount of nutrients for growth. Perennial seaweeds therefore attenuate negative impacts of decomposing phytoplankton by nutrient depletion and oxygen excretion (Chopin and Ugarte 2006, Bruton et al. 2009, Xu et al. 2011).

Despite their extractive character, seaweeds also excrete organic matter (Sieburth 1969) which aggregates driven by microbial processes (Alber and Valiela 1994c, Alber and Valiela 1994a). Mussels can incorporate organic aggregates and thus, seaweed excretion might contribute to mussel nutrition in areas of low food availability or quality (Alber and Valiela 1994b). Concerning this study, this is of special interest for areas with a high load of particulate matter, containing low chlorophyll concentrations as at the east coast of the Kiel Fjord (*Chapter II*).

Besides the indirect interaction of seaweed and microbiology through exudates, seaweeds also interact directly with associated microbiology (Egan et al. 2012, Wahl et al. 2012). Seaweeds contain antibacterial substances (Goecke et al. 2012) and are known to exude reactive compounds like iodine (Ashu-Ayem et al. 2012), pheromones (Boland and Mertes 1985), and activated oxygen species (Küpper et al. 2001, Küpper et al. 2002) that control microfauna and potential pathogens (Peng and Kue 1992). Seaweed cultivation therefore potentially influences the microbial fauna of seawater. The direct vicinity of seaweed might either be positive for shellfish cultivation due to less microbial load by antimicrobial substances. On the contrary, seaweeds might also contribute to higher microbial load due to excretion of compounds that are decomposed by bacteria (Sieburth 1969, Alber and Valiela 1994a). The microbiology in IMTA was observed recently (Buer 2012), indicating a mutual effect of mussel and seaweed microbial community. The microbiological quality of shellfish waters is mainly dependent on the amount of coliform bacteria that is determined in mussel meat (European Commission 2007). Although seaweed cultivation potentially reduces the microbial load, the particular influence of seaweed on coliform bacteria in shellfish waters remains unclear.

The development of a biofilm is an obligatory step during a fouling process (Wahl 1989). The excretion of antibacterial agents by seaweed might interact with the biofilm development and thus, exhibit an antifouling character. The antifouling effect was observed in previous research, especially in combination with mussels. (Dobretsov 1999) observed the preferences of *M. edulis* settling as a function of seaweed abundance and biofilm composition. He found that larvae of blue mussels settled significantly less on *S. latissima* in field and laboratory experiments. These results were confirmed by the present study (*Chapter III*). Although a successful mussel settlement was observed over the whole water column at both locations in the Kiel Fjord, the settlement intensity was influenced by seaweed treatment, water depth, and duration. Living algae individuals, as well as the crude extract negatively influenced the mussel settlement only in the beginning of the study. The repellent effect of young algae specimens was reversed after four weeks, indicating an effect of increasing water temperature. This indicated that the algal physiological constitution, which is mainly influenced by water temperature and salinity in the Baltic Sea, influenced algal defence. However, the relationship of stress and defence in seaweed has been discussed controversially (Hemmi et al. 2004, Hellio et al. 2004, Appelhans et al. 2010). Though not persistent, these results indicated a negative effect of seaweed on early life stages of mussels, thereby reducing the success of mussel settlement in an environment with high amount of mussel larvae. In areas with a low mussel larvae abundance, seaweed might additionally reduce mussel settlement success.

Whereas seaweed is suspected to interact mostly indirectly, mussels exhibit a direct impact on associated environment. On the contrary to the bivalent indirect influences, direct impact was almost exclusively negative.

Mussel socks directly shade their surrounding (*Chapter IV*) and also indirectly through increased sedimentation by excretion of faeces. Besides reduced light intensity, shading can also be regarded as protection from ultra violet radiation, which is suspected to harm seaweeds (Dring et al. 1996, Franklin and Forster 1997, Hanelt et al. 1997, Bischof et al. 2000). Furthermore, mussel assemblages can cause mechanical stress, resulting in the abrasion of juvenile sporophytes in their direct vicinity (*Chapter IV*). This physical stress is likely to cause damage on the blades and thalli of seaweeds. However, mussel beds are also known to provide substrate for settlement (Albrecht 1998) and shelter from huge waves (Benedetti-Cecchi et al. 1996) for adapted seaweeds.

As gregarious common fouling organisms, mussels tend to grow on all suitable surfaces (Woods Hole Oceanographic Institute 1952, Crisp 1974, Dobretsov and Wahl 2001), including seaweed. Although only filamentous algae are known to serve as a substrate for mussel settlement (Antsulevich et al. 1999, Bulleri et al. 2006) and *S. latissima* is usually avoided by mussel larvae (Dobretsov and Wahl 2001), all seaweed specimens were overgrown by mussels in the end of the experiment (*Chapter III*).

Shellfish aggregations like mussel socks exhibit a great surface diversity and provide shelter for other invertebrates, like small crustaceans and polychaetes (Möbius 1862, LeBlanc 2002, LeBlanc et al. 2003). In nature, mussel beds are diverse microhabitats (Dittmann 1990, deVlas et al. 2005). Although not quantified, high biomass development and species abundance of associated fauna on mussel socks were observed in this study (*Chapter II*) and during common farm operation (*Chapter I*). The hosted invertebrates might also be herbivorous and thus represent a potential threat for directly associated macroalgae.

Current velocity is a crucial factor for both organisms, mussels and seaweed. However, high species accumulations, like on farm sites, tend to decrease current velocity like artificial reefs (Phillips 1990, Worcester 1995, McKindsey et al. 2011). Hence, shallow bights are less suitable for mussel or seaweed farming due to their overall low current velocity and reduced water exchange.

Summarising direct and indirect interactions of seaweeds and mussels, the mutual relationship exhibits a variety of positive and negative indirect factors. This emphasises that farm components in IMTA needs to be installed distant from each other, placing the seaweed downstream to the mussel components. All *in situ* experiments within this thesis were installed in a practically applied manner, but also showed limitations. The model character of the small scale installations, with the low number of replicates leaves room for a critical discussion of the here presented results.

The aim of this thesis was to observe species interactions as naturally as possible, to compare and extrapolate the results to real farm applications. As mussels and algae are substantially influenced by artificial light and food conditions in the laboratory, only *in situ* observations are useful. Regarding observations in open aquaculture systems, such observations require a direct installation of experiments in the field. Accordingly, numerous factors directly and unpredictably influence the experiment and in particular the interaction of organisms. On the other hand, these are the same factors that influence real farm applications. Hence, the here achieved results reflect the ecological impact that is possible in practical farming. However, the amount of exogenous factors creates a rather blurred image than detailed results, which might have been elucidated by the application of more replicates.

Replicates either clarify results by reducing deviations, but also act as a backup if parts of the installation get lost during the experiment. This was experienced in *Chapter IV*, when one field replicate of three went lost during harvesting process, and also in *Chapter II*, when Eider ducks, storms, and other impacts reduced the number of replicates. Therefore, more replicates should be applied in long term studies, than required for statistical analyses. Also the small scale of the here applied experiments exhibit limitations. High growth rates might be either due to the conditions observed in the experiment, or might be due to an effect of scale. Especially regarding nutrition of organisms, the scale of an installation substantially influences the availability of plankton (for mussels) and light (for seaweed). However, in *Chapter II* it was shown that two similarly scaled installations in the same Fjord (west coast and east coast of the Kiel Fjord) showed different results and thus, reflected conditions of the habitat. Nevertheless, the impact of scale needs to be considered when extrapolating results for use in practise.

Besides the limited significance for huge farm installations, small scale experiments indeed present first, basic insights for site selection in open aquaculture systems. The combination of small scale experiments and officially provided data (e.g. from LLUR) enables evaluation of suitable sites, and facilitates short term decisions for farmers as well as for the official monitoring and licensing. In *Chapter II*, military harbours and small marinas served as monitoring sites. Although these sites were rather suboptimal for aquaculture site acquisition, they offered an opportunity for a save installation of the monitoring modules with low financial investment costs.

Clarification of the biological processes behind species interactions is almost impossible to observe within *in situ* observations and thus, requires lab work in mesocosms. Generally, the release (antifouling from seaweed, ammonia excretion by mussels) and the uptake of substances (dissolved nutrients by seaweed, particulate matter by mussels), as well as the stress response of organisms is regulated by enzymes. Hence, genetic observation of enzyme activity would elucidate metabolic interaction of organisms.

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General Summary

Increasing demand for seafood in a situation of stagnating traditional fishery makes aquaculture a sector of immense growing potential for seafood and biomass production. Associated ecological problems, like eutrophication of coastal ecosystems, have fostered the development of sustainable aquacultural techniques. Presently, Integrated Multi-Trophic Aquaculture (IMTA) is the most promising approach of environmentally friendly aquaculture. In IMTA systems, traditional finfish culture is combined with extractive organisms like algae and shellfish. Hence waste of one species is turned into a value for another, creating a positive mutual and environmental effect among the associated farm components.

Aquaculture in the Baltic Sea faces challenges like reduced salinity, low current velocity and eutrophication. Hence, potential species for Baltic aquaculture need to be adapted to local environmental conditions. As blue mussels (*Mytilus edulis*) and sugarweed (*Saccharina latissima*) are endemic and exhibit good growth rates in the western Baltic Sea, they represent potential candidates for Baltic extractive aquaculture. However, as both species are not occurring naturally in close vicinity, there are reasons to suspect their solely positive mutual relationship.

This study observed the species interaction in different development stages of shellfish (*Mytilus edulis*) and seaweed (*Saccharina latissima*) in an integrated system, and the respective species potential for extractive aquaculture in the western Baltic Sea.

Chapter I, this preliminary, introducing part of the thesis presents the results of a case study about a commercial IMTA in the Kiel Fjord, where mussel cultivation (*Mytilus edulis*) was implemented in an already existing seaweed (*Saccharina latissima*) farm. Mussels from the Kiel Fjord were not polluted and algaetoxins were not observed during the project. The microbial quality of the shellfish water was classified as “A” during major harvesting season. Therefore mussels are sold fresh for human consumption and seaweeds as raw material for cosmetic. Both organisms are certified organic.

In *Chapter II* general criteria for the integrated aquacultural potential of *M. edulis* and *S. latissima* in the western Baltic Sea were evaluated. Biological key criteria like growth and condition of organisms are important parameters determining the success of the aquacultural production. In a brackish system like the Baltic Sea, lower growth rates and poor condition due to reduced salinity and hence permanent osmotic stress were expected. Therefore growth and biomass production of seaweed (*S. latissima)* and mussels (*M. edulis*) were determined monthly in a monitoring study at four locations in the Kiel Bight (Baltic Sea) from October 2010 until May 2011. Seaweed growth performance and biochemical composition strongly depended on location. Mussel shell growth was comparable within sites, but shellfish condition indices reflected regional differences. Besides local characteristics, species

exhibited a different sensitivity to the specific brackish Baltic Sea conditions. Whereas mussel growth and condition was comparable to more saline regions, seaweed production was reduced. Nevertheless, the potential of both species for extractive aquaculture is high, concerning the retention of nutrients in eutrophic coastal waters of the Baltic Sea.

Chapter III exhibited ecological insights into seaweed / mussel interaction in integrated aquaculture, thereby focussing on the period of mussel larvae settlement. Settlement and survival of mussel spat are the most crucial factors determining the success of mussel production. As *Mytilus* larvae are known to avoid the vicinity of *Saccharina latissima*, the seaweed was suspected to deter mussel larvae from settling in integrated aquaculture. Therefore the settlement of mussel spat (*M. edulis*) was observed if associated with young and adult seaweed specimens (*S. latissima*), and seaweed crude extract at two locations in the Kiel Fjord (Baltic Sea) in different water depths.

During this investigation, mussel larvae abundances were high and juvenile mussels settled all over the observed water column. The intensity of settlement was influenced by algae abundance and changed within water depths. Seaweed and seaweed crude extract exhibited a negative effect on mussel settlement in the beginning of the study. However, this repellent effect was reversed for young seaweed specimens after four weeks, resulting in an enhanced mussel settlement. According to the results of this investigation, the aquacultural farm design needs to consider a possible negative impact of seaweed on mussel settlement. Hence, seaweed cultivation components need to be placed downstream in the farm to avoid reduced bivalve larvae settlement.

In *Chapter IV*, the impact of mussels on early nursery stages of seaweeds were observed. The excretion of dissolved nutrients as well as the depletion of competing phytoplankton by mussels was suspected to enhance seaweed growth and development. The cultivation of *S. latissima* generally requires two production phases: hatchery phase in tank culture and grow out phase in the sea. Both production periods were evaluated with and without mussels.

The presence of mussels resulted in significantly more large multicellular sporophytes after lab phase. This supporting effect of mussels on seaweed growth was still visible after six months of field exposure, resulting in a higher biomass, higher carbon content and larger size of sporophytes that were combined with mussels during the hatchery period. Nevertheless, in the field mussels culture ropes caused a shading effect and a significant mechanical stress, resulting in a substantial loss of sporophytes in the direct vicinity of mussel culture ropes. The results of this investigation suggested a positive effect of shellfish on early life stages of *S. latissima* during hatchery, thereby enhancing algal production in the following grow out phase in the field. Consequently, the use of mussels as a biological fertiliser is a sustainable approach to produce seaweed in a shorter time period, at low cost, and with the possibility to be certified organic.

Zusammenfassung

Die steigende Nachfrage nach Meeresfrüchten bei gleichzeitig stagnierenden Erträgen der traditionellen Fischerei führt dazu, dass die Bedeutung von Aquakultur für die Produktion von Seefrüchten und Biomasse enorm gestiegen ist. Möglicherweise damit verbundene ökologische Probleme, wie z. B. die Eutrophierung von Küstengewässern, haben verstärkt zur Entwicklung nachhaltiger Aquakulturtechniken geführt. Derzeit ist die sogenannte Integrierte Multi-Trophische Aquakultur (IMTA) einer der am erfolgversprechendsten Ansätze umweltfreundlicher Aquakultur. In einer IMTA wird die traditionelle Fischzucht mit extraktiven Organismen, wie Algen oder Schalentieren kombiniert. Auf diese Weise wird der Abfall einer Art zum Nährstoff einer anderen, welches sich sowohl zwischen den einzelnen Farmkomponenten und als auch der Umwelt positiv auswirkt. In der Ostsee ist die Aquakultur Herausforderungen, wie z.B. einem niedrigen Salzgehalt, geringer Strömungsgeschwindigkeit und Eutrophierung ausgesetzt. Demzufolge müssen potentielle Arten für eine Aquakultur in der Ostsee an die speziellen Umweltbedingungen angepasst sein. Miesmuscheln (*Mytilus edulis*) und Zuckertang (*Saccharina latissima*) sind in der westlichen Ostsee heimisch und zeigen gute Wachstumsraten. Daher eignen sie sich als Kandidaten für eine extraktive Aquakultur in der Ostsee. Trotzdem gibt es Grund zur Annahme, dass die wechselseitigen Beziehungen zwischen Muscheln und Algen nicht rein positiver Natur ist, denn beide Arten kommen in ihrem natürlichen Lebensraum nicht in enger Vergesellschaftung vor. Diese Arbeit untersuchte die gegenseitige Interaktion von Miesmuscheln (*M. edulis*) und Algen (*S. latissima*) in einem integrierten System und ihr Potential für eine extraktive Aquakultur in der westlichen Ostsee.

Das *Kapitel I* präsentiert als allgemein einleitender Abschnitt dieser Arbeit eine IMTA-Fallstudie, in welcher eine Muschelzucht (*Mytilus edulis*) in eine existierende Algenzucht (*Saccharina latissima*) implementiert wurde. Da die Wasserqualität in der Kieler Förde hinsichtlich Mikrobiologie, Algentoxinen und organischen Kontaminanten als unbedenklich eingestuft wurde, können die Muscheln als Nahrungsmittel für den menschlichen Verzehr und Algen als Kosmetikrohstoff vermarktet werden. Beide Organismen sind ökozertifiziert nach der EG Ökoverordnung (EC 710 / 2009).

In *Kapitel II* wurden generelle Kriterien für das Potential von *M. edulis* und *S. latissima* als Komponenten einer einer extraktiven Aquakultur in der westlichen Ostsee evaluiert. Biologische Schlüsselkriterien wie Wachstum und Kondition von Organismen sind entscheidende Parameter, die den Produktionserfolg der Aquakultur determinieren. Es wurde vermutet, dass die Lebensbedingungen im Brackwasser der Ostsee aufgrund des niedrigen Salzgehaltes und zu reduzierten Wachstumsraten und geringeren Kondition der untersuchten Organismen führte. Daher wurden in einer Monitoring

Studie monatlich das Wachstum und Biomasseproduktion von Algen (*S. latissima*) und Muscheln (*M. edulis*) an vier Standorten entlang der Küste der Kieler Bucht (Ostsee) beginnend im Oktober 2010 bis Ende Mai 2011 durchgeführt.
Das Wachstum und die biochemische Zusammensetzung der Algen war stark regional abhängig. Während das Wachstum der Muscheln relativ vergleichbar war zwischen den Standorten, zeigten sich in der Kondition der Schalentiere regionale Unterschiede. Neben den lokalen Charakteristika zeigten beide Organismen eine unterschiedliche Sensibilität gegenüber den spezifischen brackigen Bedingungen in der Ostsee. Während sich das Wachstum und die Kondition von Muscheln als vergleichbar zu salzhaltigeren Regionen herausstellte, war die Algenproduktivität deutlich geringer.

Trotzdem wird das Potential beider Organismen für eine extraktive Aquakultur als hoch eingeschätzt, besonders im Hinblick auf den durch sie bewirkten Nährstoffrückhalt in den eutrophen Küstengewässern in der Ostsee.

Kapitel III dieser Arbeit widmete sich der Interaktion von Algen und Muscheln in einer integrierten Aquakultur während der Ansiedlungsphase der Muschellarven. Die ausreichende Ansiedlung von Muschellarven und deren Überleben sind entscheidende Faktoren, welche letztendlich über den Erfolg der Muschelproduktion entscheiden. Da Larven von *Mytilus edulis* von Natur aus die Gegenwart von *Saccharina latissima* meiden, wird in einem entsprechenden integrierten System vermutet, dass sich die Anwesenheit von Algen negativ auf die Ansiedlung von Muschellarven auswirkt. Deshalb wurde die Ansiedlung von Miesmuschellarven in Gegenwart von jungen und adulten Sporophyten (*S. latissima*), sowie einem Algen-Rohextrakt an zwei Standorten in der Kieler Förde (Ostsee) in verschiedenen Wassertiefen untersucht. Während des Untersuchungszeitraumes waren große Mengen an Miesmuschellarven im Wasser vorhanden und an beiden Standorten siedelten die Larven über die gesamte untersuchte Wassertiefe. Das Ausmaß der Ansiedlung war wesentlich von der Anwesenheit von Algen und von der Wassertiefe abhängig. Sowohl lebende Pflanzen, als auch der Algenextrakt wirkte sich zu Beginn der Studie negativ auf die Muschelansiedlung aus. Dieser Effekt auf Muschellarven wurde im Fall der jungen Algen nach vier Wochen Versuchszeit umgekehrt und führte dann sogar zu einer vermehrten Muschelansiedlung. Die Untersuchungsergebnisse dieser Studie verweisen auf einem möglichen negativen Einfluss von Algen auf die Ansiedlung von Miesmuscheln, welcher beim Design einer integrierten Aquakultur berücksichtigt werden muss. Folglich sollten die Algenkomponenten im Strömungsschatten der Schalentiere installiert werden, um eine durch Algen reduzierte Muschellarvenansiedlung zu vermeiden.

In *Kapitel IV*, wurde der Einfluss von Miesmuscheln auf die frühen Entwicklungsstadien von Algen evaluiert. Es wurde vermutet, dass die Ausscheidung von gelösten Nährstoffen (Ammonium) und die Abreicherung von konkurrierendem Phytoplankton durch Miesmuscheln einen wachstumsfördernden

Effekt auf Algen (*S. latissima*) haben. Die Zucht von *S. latissima* verläuft generell zwei Produktionsphasen, die Phase der Laboranzucht und die Wachstumsphase im Freiland. Während beider Produktionsphasen wurde die Entwicklung und das Wachstum von Algen mit und ohne Muscheln (*M. edulis*) untersucht. Die Anwesenheit von Muscheln resultierte in signifikant mehr multizellulären Sporophyten nach der Laboranzucht. Dieser wachstumsfördernde Effekt war auch nach sechsmonatiger Freilandphase sichtbar und äußerte sich in einer höheren Biomasseproduktion, einer höheren Kohlenstoffkonzentration und einer größeren Länge der Algen, die zuvor im Labor mit Muscheln vergesellschaftet waren. Allerdings führten Muschelkulturleinen im Freiland durch Abschattung und mechanischen Stress zu einem erheblichen Verlust von Algen in der direkten Umgebung von Muscheln. Die Ergebnisse dieser Studie stellten den positiven Effekt von Miesmuscheln auf frühe Entwicklungsstadien der Algen heraus, der nach der Anzucht zu einem verbesserten Wachstum in der darauffolgenden Freilandphase führte. Demnach ist die Verwendung von Miesmuscheln als biologischer Dünger ein nachhaltiger Ansatz, Algen in einer kürzeren Zeitspanne, mit weniger Kosten und der Möglichkeit einer Ökozertifizierung zu produzieren.

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Curriculum Vitae

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School education

1987 – 1991	Primary School „Rosa- Luxemburg“ in Ilmenau
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Professional education

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University education

10/ 2002 – 06/ 2008	Studies of Life Sciences (Biological Oceanography, Microbiology, Landscape Development) at the Christian-Albrechts University (CAU) in Kiel, Germany

Professional work

09/ 2008 – 10/ 2008	Internship at Coastal Research & Management (CRM) in Kiel
11/ 2008 – 09/ 2009	Biologist (freelancer)
10/ 2009 – 01/ 2010	Diplom Biologist at the Geographical Institute of the Christian-Albrechts University in Kiel
01/ 2010 – 12/ 2012	Research assistant and Ph.D. student at I Gesellschaft für Marine Aquakultur in Büsum and the Institute of Animal Breeding and Husbandry at Christian-Albrechts University Kiel and, Germany with workplace at Coastal Research & Management (CRM), Kiel

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorgelegte Dissertation mit dem Titel „Integrated Multi - Trophic Aquaculture of Mussels (*Mytilus edulis*) and Seaweed (*Saccharina latissima*) in the Western Baltic Sea“ selbständig und ohne unerlaubte Hilfe angefertigt habe und dass ich die Arbeit noch keinem anderen Fachbereich bzw. noch keiner anderen Fakultät vorgelegt habe.

Hiermit erkläre ich, dass gegen mich kein strafrechtliches Ermittlungsverfahren schwebt.

Hiermit erkläre ich, dass die Dissertation nach den Regeln guter wissenschaftlicher Praxis (Standard wissenschaftlichen Arbeitens nach den Empfehlungen der DFG) abgefasst wurde.

Gammelby, den 06.09.2013

Yvonne Rößner