

# Aquaculture of *Fucus* species in the Baltic Sea by means of vegetative reproduction

## **Dissertation**

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## Summary

Species of the brown algal genus *Fucus* are important ecosystem engineers of northern hemisphere coasts. Their biomass is harvested commercially for the production of cosmetic extracts and food supplements, e.g., at the coasts of France, Ireland and Iceland. However, environmental change (e.g., warming, eutrophication) poses increasing risk on wild *Fucus* stocks and has already caused some population declines, for instance in the Baltic Sea, where harvesting of wild stocks is prohibited as consequence. Commercial cultivation of *Fucus* would therefore be a useful alternative to the harvesting of wild stocks especially in the light of an increasing interest in algal products. But so far, no cultivation method for the commercial production of *Fucus* species has been developed. Therefore, a theoretical vegetative cultivation approach has been described which includes the following steps: thallus pieces with apical meristems (“seedlings”) are cut from wild plants and reared unattached in baskets/ net cages deployed in the sea; after a season of growth, the biomass is harvested and new seedlings are cut from the harvest which are used as initial biomass for the next growth season. This process is then repeated after each growth season.

The aim of this doctoral thesis was to test various aspects of this cultivation approach in culture experiments. The experiments were performed in an experimental farm located in the Kiel fjord, western Baltic Sea. Two local *Fucus* species (*F. vesiculosus*, *F. serratus*) were used.

First, it was tested if *Fucus* thalli survive long-term culture with the described cultivation method and which role the formation of sexual organs (receptacles) plays in the cultivation process. It could be shown that principally thalli survive and grow continually in culture with the described method. However, the formation of receptacles and their natural degradation after gamete shedding reduced the growth potential significantly. Therefore, the question was raised how culture biomass with low fertility (i.e., a low percentage of thallus apices with receptacles) can be obtained. In order to answer this question, two approaches were adopted: first, it was tested if thalli from attached wild populations with naturally high fertility acclimatize towards lower fertility if they are cultivated unattached. Second, it was tested if thalli from an unattached wild population in Glücksburg (Flensburg fjord), which morphologically resembles *F. vesiculosus* and exhibits low fertility in the wild, retain their low fertility in culture. Thalli from attached high-fertility populations retained their high fertility in culture and did not acclimatize towards lower fertilities by unattached cultivation. On the other hand, thalli from the unattached population in Glücksburg retained their low fertility over one year of cultivation. Consequently, only thalli from unattached low-fertility populations are potentially useful for the envisaged vegetative cultivation method.

Second, the question was raised how season and cultivation density influence the growth rate and yield in culture. In cultivation experiments, the growth rates varied with season as expected (low growth rates in winter and high growth rates in summer); the yield-density relationship was found to be parabolic for *F. vesiculosus* and asymptotic for *F. serratus*. Based on the measured growth rates, growth was modelled over one year in culture for different cultivation scenarios (different initial stocking densities and harvest densities) in order to estimate potential annual yields and the required number of harvests. Maximum annual yields of 7.71 kg m<sup>-2</sup> were modelled for *F. vesiculosus* (requiring a cultivation from 2 kg m<sup>-2</sup> to 3.25 kg m<sup>-2</sup> and 6 harvests) and of 6.35 kg m<sup>-2</sup> for *F. serratus* (requiring a cultivation from 3.75 kg m<sup>-2</sup> to 5 kg m<sup>-2</sup> and 5 harvests). Further it was tested if the growth rates underlying the model, which were measured in small-scale cultivation baskets, apply also for culture in commercial-scale net cages (1.2 m<sup>2</sup>). For *F. serratus*, this was found to be true while improved net cages with a smaller mesh size are necessary for the smaller *F. vesiculosus* thalli.

Third, the question was raised if epizoan fouling, which was frequently encountered on the culture biomass, can be reduced by regular desiccation during the cultivation process. In order to answer this

question, the effect of different desiccation regimes (different frequencies and intensities of desiccation) on weight and abundance of the most common epizoans (*Electra pilosa* (Bryozoa), *Amphibalanus improvisus* (Crustacea, Cirripedia), *Mytilus* sp. (Mollusca, Bivalvia)) as well as on the growth rate of the cultivated *Fucus* species was tested. Desiccation regimes affected weight and abundance of epizoans as well as *Fucus* growth rates at varying degrees. Frequent mild desiccations (*F. vesiculosus*: 3x week<sup>-1</sup> to 80% of wet weight, *F. serratus*: 3x week<sup>-1</sup> to 90% of wet weight) proved most effective as they reduced epizoan fouling significantly but had no effect on *Fucus* growth. By applying these regimes, the epizoan share of the final harvest could be reduced from 13.0 ± 4.8% in undesiccated controls to 1.8 ± 0.2% for *F. vesiculosus* and from 19.1 ± 2.7 to 1.0 ± 0.1% for *F. serratus*.

In conclusion, *Fucus* aquaculture with the described vegetative cultivation approach is possible if the cultivated biomass can be kept at low fertility (which seems to be possible by using seedling material from unattached low-fertility populations) and if epizoan fouling is reduced by regular desiccation. Future studies should investigate the genetic control of fertility in *Fucus* and the influence of environmental factors on fertility since low fertility is a crucial factor for long-term cultivation success. Alternative cultivation techniques (e.g., in land-based tanks) might enable a cultivation without epizoan fouling and should also be subject of future research.

## Zusammenfassung

Braunalgenarten der Gattung *Fucus* sind wichtige Ökosystemingenieure an Küsten der nördlichen Hemisphäre. Ihre Biomasse wird kommerziell für die Herstellung von Kosmetikrohstoffen und Nahrungsergänzungsmitteln geerntet, z.B. an den Küsten von Irland, Frankreich und Island. Umweltveränderungen (z.B. Erwärmung, Überdüngung) stellen für die *Fucus*-Bestände jedoch eine zunehmende Bedrohung dar und haben schon zum Rückgang einiger Bestände geführt, z.B. in der Ostsee, wo die Algen entsprechend unter Schutz gestellt wurden. Kommerzielle Kultivierung würde eine sinnvolle Alternative zur Beerntung von Wildbeständen darstellen, besonders angesichts eines generell steigenden Interesses an Algenprodukten. Allerdings wurde bisher keine Kultivierungsmethode zur kommerziellen Produktion von *Fucus*-Arten entwickelt. Deshalb wurde ein vegetativer Kultivierungsprozess theoretisch beschrieben, welcher die folgenden Schritte beinhaltet: Thallusstücke, die ein Apikalmeristem enthalten („Setzlinge“), werden von Wildpflanzen abgeschnitten und freitreibend in Körben/ Netzkäfigen im Meer kultiviert; nach einer Wachstumsperiode wird die Biomasse geerntet und neue Setzlinge werden von der geernteten Biomasse abgeschnitten und als Ausgangsmaterial für die nächste Wachstumsperiode genutzt. Dieser Prozess wird dann nach jeder Wachstumsperiode wiederholt.

Das Ziel dieser Doktorarbeit war es, verschiedene Aspekte dieses Kulturansatzes in Kultivierungsexperimenten zu testen. Die Experimente wurden in einer Testfarm in der Kieler Förde (westliche Ostsee) durchgeführt. Dazu wurden zwei lokale *Fucus*-Arten (*F. vesiculosus* und *F. serratus*) verwendet.

Als erstes wurde untersucht, ob *Fucus*-Thalli eine langfristige Kultivierung mit der beschriebenen Methode überleben und welche Rolle die Bildung sexueller Organe (Rezeptakel) im Kultivierungsprozess spielt. Es konnte gezeigt werden, dass *Fucus*-Thalli die Kultur mit der beschriebenen Methode prinzipiell überleben und auch kontinuierliches Wachstum zeigen. Allerdings führte die Bildung von Rezeptakeln und ihre Degeneration nach der Gametenabgabe zu signifikanten Wachstumseinbußen. Deshalb wurde die Frage gestellt, wie Kulturbiomasse mit niedriger Fertilität (d.h. niedrigem prozentualen Anteil an Thallusspitzen mit Rezeptakeln) erzeugt werden kann. Um diese Frage zu beantworten, wurden zwei Ansätze gewählt: erstens wurde untersucht, ob Thalli von an Substrat angehaftet lebenden Wildpopulationen mit natürlicherweise hoher Fertilität sich zu niedrigerer Fertilität akklimatisieren, wenn sie abgelöst vom Substrat kultiviert werden. Zweitens wurde untersucht,

ob Thalli einer unangehaftet wachsenden Wildpopulationen in Glücksburg (Flensburger Förde), die morphologisch *F. vesiculosus* ähnelt und natürlicherweise eine niedrige Fertilität zeigt, ihre niedrige Fertilität in Kultur behalten. Thalli von angehafteten Populationen behielten ihre hohe Fertilität in Kultur und akklimatisierten nicht zu niedrigeren Fertilitäten. Thalli der unangehaftet wachsenden Population in Glücksburg behielten ihre niedrige Fertilität über ein Jahr in Kultur. Folglich sind nur Thalli von unangehaftet wachsenden Populationen potentiell für die Kultur mit der beschriebenen Methode geeignet.

Als zweites wurde die Frage gestellt, wie sich Jahreszeit und Kultivierungsdichte auf Wachstumsrate und Ertrag der Kultur auswirken. In Kultivierungsexperimenten variierte die Wachstumsrate wie erwartet mit der Jahreszeit (niedrige Wachstumsraten im Winter und hohe im Sommer); der Ertrag-Dichte-Zusammenhang konnte für *F. vesiculosus* als parabolisch und für *F. serratus* als asymptotisch beschrieben werden. Basierend auf den gemessenen Wachstumsraten wurde das Wachstum über ein Jahr in Kultur für verschiedenen Kulturszenarien (verschiedene Besatz- und Erntedichten) modelliert um potentielle jährliche Erträge und die dafür benötigten Ernten abschätzen zu können. Der modellierte maximale jährliche Ertrag für *F. vesiculosus* war  $7.71 \text{ kg m}^{-2}$  (bei einer Kultivierung von  $2 \text{ kg m}^{-2}$  bis  $3.25 \text{ kg m}^{-2}$  und 6 benötigten Ernten) und  $6.35 \text{ kg m}^{-2}$  für *F. serratus* (bei einer Kultivierung von  $3.75 \text{ kg m}^{-2}$  bis  $5 \text{ kg m}^{-2}$  und 5 benötigten Ernten). Außerdem wurde untersucht, ob die Wachstumsraten, die der Modellierung zugrunde lagen und in kleinskaligen Kulturkörben gemessen wurden, auch in Netzkäfigen kommerzieller Größe ( $1.2 \text{ m}^2$ ) messbar sind. Das traf für *F. serratus* zu, für die kleineren *F. vesiculosus*-Thalli sind verbesserte Netzkäfige mit kleinerer Maschenweite nötig.

Als drittes wurde die Frage gestellt, ob der häufig auftretende Bewuchs der Kulturbiomasse durch Epizoen mithilfe von regelmäßiger Trocknung reduziert werden kann. Um diese Frage zu beantworten, wurde der Effekt von verschiedenen Trocknungsbehandlungen (verschiedene Intensitäten und Häufigkeiten der Trocknung) auf Gewicht und Abundanz der häufigsten Epizoen (*Electra pilosa* (Bryozoa), *Amphibalanus improvisus* (Crustacea, Cirripedia), *Mytilus* sp. (Mollusca, Bivalvia)) sowie auf die Wachstumsrate der kultivierten *Fucus*-Arten untersucht. Die Trocknungsbehandlungen beeinflussten Gewicht und Abundanz der Epizoen sowie die Wachstumsrate der *Fucus*-Arten in unterschiedlichem Grad. Häufige milde Trocknungen (*F. vesiculosus*:  $3 \times \text{Woche}^{-1}$  auf 80% des Frischgewichtes, *F. serratus*:  $3 \times \text{Woche}^{-1}$  auf 90% des Frischgewichtes) waren am effektivsten, da sie den Bewuchs signifikant reduzierten und gleichzeitig die Wachstumsrate der *Fucus*-Thalli nicht beeinflussten. Durch Anwendung dieser Trocknungsbehandlungen konnte der Anteil von Epizoen an der Ernte für *F. vesiculosus* von  $13.0 \pm 4.8\%$  in unbehandelten Kontrollen auf  $1.8 \pm 0.2\%$  und für *F. serratus* von  $19.1 \pm 2.7$  auf  $1.0 \pm 0.1\%$  reduziert werden.

Zusammenfassend kann gesagt werden, dass *Fucus*-Aquakultur mit der beschriebenen Methode möglich ist, wenn die Kulturbiomasse bei einer niedrigen Fertilität gehalten werden kann (was durch die Nutzung von Thalli aus unangehaftet wachsenden Populationen mit niedriger Fertilität möglich scheint) und der Bewuchs durch regelmäßige Trocknung reduziert wird. Zukünftige Studien sollten sich der genetischen Kontrolle der Fertilität in *Fucus* sowie dem Einfluss von Umweltfaktoren auf die Fertilität widmen, da eine niedrige Fertilität Voraussetzung für einen langfristigen Kulturerfolg ist. Alternative Kulturmethoden (z.B. in landbasierten Tanks) können eventuell zu einer ganz epizoenfreien Kultur verhelfen und sollten deshalb ebenfalls in Zukunft getestet werden.

## Author Contributions

This thesis contains three manuscripts/ published articles:

### **Chapter II: Vegetative aquaculture of *Fucus* in the Baltic Sea- obtaining low fertility biomass from attached or unattached populations?**

Rafael Meichssner, Peter Krost, Rüdiger Schulz

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### **Chapter III: Modelling of annual yield in vegetative *Fucus* aquaculture based on experimental testing of density- and season-dependent growth**

Rafael Meichssner, Peter Krost, Rüdiger Schulz

### **Chapter IV: Control of fouling in the aquaculture of *Fucus vesiculosus* and *Fucus serratus* by regular desiccation**

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Chapter II and IV have been published as research articles in *Journal of Applied Phycology* and Chapter III is currently under revision at the same journal. The already published articles are reproduced with the kind permission of Springer. In the following table, the contributions of the authors are given.

	<b>Chapter II</b>		<b>Chapter III</b>		<b>Chapter IV</b>	
<b>Study outline and design</b>	RM:	90%	RM:	90%	RM:	80%
	RS:	10%	RS:	10%	PK:	10%
					RS:	10%
<b>Experimental work</b>	RM:	100%	RM:	100%	NS:	40%
					AC:	10%
					DS:	20%
					MB:	10%
					HM:	10%
					RM:	10%
<b>Data analysis</b>	RM:	90%	RM:	90%	RM:	100%
	PK:	10%	PK:	10%		
<b>Manuscript preparation</b>	RM:	80%	RM:	80%	RM:	80%
	PK:	10%	PK:	10%	PK:	10%
	RS:	10%	RS:	10%	RS:	10%

# **Chapter I - Introduction**

## History and status of seaweed aquaculture

Red, green and brown marine macroalgae are commonly summarized as “seaweeds” (Hurd et al. 2014). This term is not of taxonomic nature as red, green and brown macroalgae represent evolutionary distinct groups of photosynthetic eukaryotes with profound differences in cellular structure and endosymbiosis history (Graham et al. 2009, Burki 2014). “Seaweeds” is rather a functional term, used to summarize three groups of organisms which have evolved similar macroscopic appearances in response to the environmental conditions of their shared habitat. In this sense it will be used in the following.

Seaweeds have been used by humans since at least 14,000 years as remains of nine seaweed species at the archaeological site of Monte Verde in southern Chile document (Dillehay et al. 2008, Erlandson et al. 2015). Around the world, coastal communities have traditions of using seaweeds for food, traditional medicine, feed for livestock and fertilizer in agriculture (Buschmann et al. 2017, Buchholz et al. 2012). For a long time, the used seaweeds were exclusively collected from the wild and no cultivation was performed (Buchholz et al. 2012). During the Sung Dynasty (960-1279 AD), first primitive cultivation measures were undertaken in China: rocks were cleaned during a specific time of the year to encourage the settlement of propagules of the red alga *Gloiopeltis furcata* (Tseng 1933, 1994). This rock-cleaning method was used in a similar way for the “cultivation” of the red alga *Pyropia haitanensis* in China since about 1800 AD (Tseng CK 1994). During the Tokugawa Era (1603-1868 AD), Japanese fisherman started to deploy substrates (e.g. bamboo) in the sea to cultivate naturally settling seaweeds (Tseng 1984, Buchholz et al. 2012). However, all these efforts only made use of the naturally occurring seaweed propagules and did not require an understanding of the seaweeds’ life cycle. In the 1940s and 1950s, major breakthroughs were made in the understanding of seaweed life cycles and the cultivation of different life cycle stages: the conchocelis stage of the red algal genus *Porphyra/Pyropia* and its connection to the harvestable gametophytes via conchospores was discovered (Drew 1949, Tseng and Chang 1955), and an optimized culture of microscopic stages of the brown alga *Saccharina japonica* was developed (Tseng et al. 1955). Thus, the cultivation of the entire life cycle of these seaweed species became feasible, which enabled the development of their large-scale cultivation in the following decades. In the 1960s and 1970s, cultivation of the red algal genera *Kappaphycus* and *Eucheuma* was initiated in the Philippines and Indonesia (Doty 1973, Hurtado et al. 2014). A number of other seaweed species has also been put to culture in the last decades, e.g. species of the red algal genus *Gracilaria/Agarophyton* (Santelices and Doty 1989) and the brown alga *Undaria pinnatifida* (Peireira and Yarish 2008).

Today, ca. 220 seaweed species are subjects of human use (Zehmke-White and Ohno 1999) of which the majority is still collected from the wild in mostly very small volumes and only few species are produced at large scales in aquaculture. The current worldwide seaweed production of ca. 32,4 million t yr<sup>-1</sup> (reached in 2018, FAO 2020) is almost entirely generated from the culture of six genera (*Saccharina*, *Undaria*, *Porphyra/Pyropia*, *Gracilaria/Agarophyton*, *Kappaphycus*, *Eucheuma*) which make up 98% of the aquacultured biomass (Buschmann et al. 2017), which in turn represents 97.1% of the total seaweed production (FAO 2020). However, many uncultured species undergo cultivation trials in recent years and increasing market demand fosters the development of cultivation techniques for more species (e.g. *Gracilaria dura* (Mantri et al. 2020), *Gracilariopsis longissima*, *Gracilaria bursa-pastoris*, *Chondracanthus teedei* (Bermejo et al. 2020)). At the same time, first specific cultivars of already cultivated species have been bred or obtained from wild populations (Guillemin et al. 2008, Robinson et al. 2013, Su et al. 2020). Thus, many seaweeds are in the midst of an ongoing domestication process which is part of the modern time phenomenon of the domestication of marine animals and algae which succeeds the domestication of land animals and plants by 2,000 to 11,000 years (Duarte et al. 2007, Valero et al. 2017).

The majority of seaweed aquaculture takes place in East Asia and South East Asia, apart from that, larger industries exist in Chile and Zanzibar (FAO 2020). The European seaweed aquaculture industry is still in its infancy: the largest number of seaweed farms exists in Norway and France, mainly for the production of *Saccharina latissima* (Stévant et al. 2017, Araújo et al. 2021). Few farms exist also on the Faroe Islands, in Sweden, France and some other countries (Araújo et al. 2021, V. Sandow, pers. comm.). Some of these farms are fully commercial while others operate on an experimental to semi-commercial scale (Stévant et al. 2017). In Germany, only one farm for the combined production of blue mussels (*Mytilus edulis*, *Mytilus trossulus*) and sugar kelp (*Saccharina latissima*) exists in the Kiel fjord, western Baltic Sea (Kieler Meerefarm GmbH & Co. KG, Stauffenberger T., pers. comm.). Some initiatives for the land-based production of seaweeds have been launched in Germany, however, none of them has reached commercial level so far (Weinberger et al. 2020).

### Reproduction techniques used in seaweed aquaculture

There are two principal ways to reproduce seaweeds in aquaculture: in the first, microscopic stages (spores or gametes) are generated from adult thalli by treatments inducing their release. The single cell stages are seeded on cultivation substrates (e.g. ropes) which are deployed in the sea, where the seaweeds grow to harvest size. This general scheme can have several modifications depending on which generation of the seaweed life cycle (gametophyte, sporophyte) represents the harvestable stage, and if intermediate stages (e.g. the conchocelis stage in *Porphyra/Pyropia* or the microscopic gametophytes in *Saccharina*) have to be cultivated. The approach requires land-based hatcheries where the seeded substrata are generated and pre-cultured. Typical examples for this first reproduction method are the cultivated brown algae *Saccharina japonica* and *Undaria pinnatifida* as well as red algae of the genus *Porphyra/Pyropia*.

The second way to reproduce seaweeds is to make use of the ability of many species to reproduce vegetatively. Reproduction is in these cases performed by cutting small pieces (“seedlings”) from cultivated thalli which are used to start the next cultivation cycle. This vegetative reproduction method is used in the culture of the red algae *Gracilaria/Agarophyton* sp., *Kappaphycus* sp. and *Euclima* sp. It can be performed directly at the cultivation site and no land-based hatcheries are required (Hurd et al. 2014).

Overviews over reproduction techniques applied in the culture of the main commercial seaweed species are given in several text book chapters (Lüning 1985, Graham et al. 2009, Buchholz et al. 2012, Hurd et al. 2014).

### The brown algal genus *Fucus*

#### Systematics and basic ecology

Among the ca. 2000 species of brown algae (Phaeophyceae), ca. 600 belong to the order Fucales, of which 9 currently accepted species form the genus *Fucus* (Coyer et al. 2006, Guiry and Guiry 2021). *Fucus* species are a prominent part of the rocky intertidal of northern hemisphere coasts (Lüning 1985), where they form almost monospecific belts along the environmental gradient of the intertidal in accordance to the specific adaptations of the single species to desiccation and other biotic and abiotic factors (Lubchenco 1980, Karez and Chapman 1998, Fig. 1). *Fucus* species occur also in atidal regions like the Baltic Sea, where the sea level is determined rather by wind conditions than by the tidal cycle. Here, members of the genus *Fucus* can be found at irregularly emerged locations as well as at permanently submerged locations (Pearson et al. 2000, own observation, Fig. 2). *Fucus* beds represent an important coastal habitat with a rich associated fauna and flora living either directly attached to the seaweeds’ surface (epizoans: e.g. bryozoans, bivalves, barnacles, ascidians, sponges etc.; epiphytes: ephemeral green, red and brown algae, sessile chain-forming diatoms) or freely around the alga (e.g.



isopods, amphipods and snails, which all in part graze on *Fucus*, further fishes, crustaceans etc.) (Kersen et al. 2011).

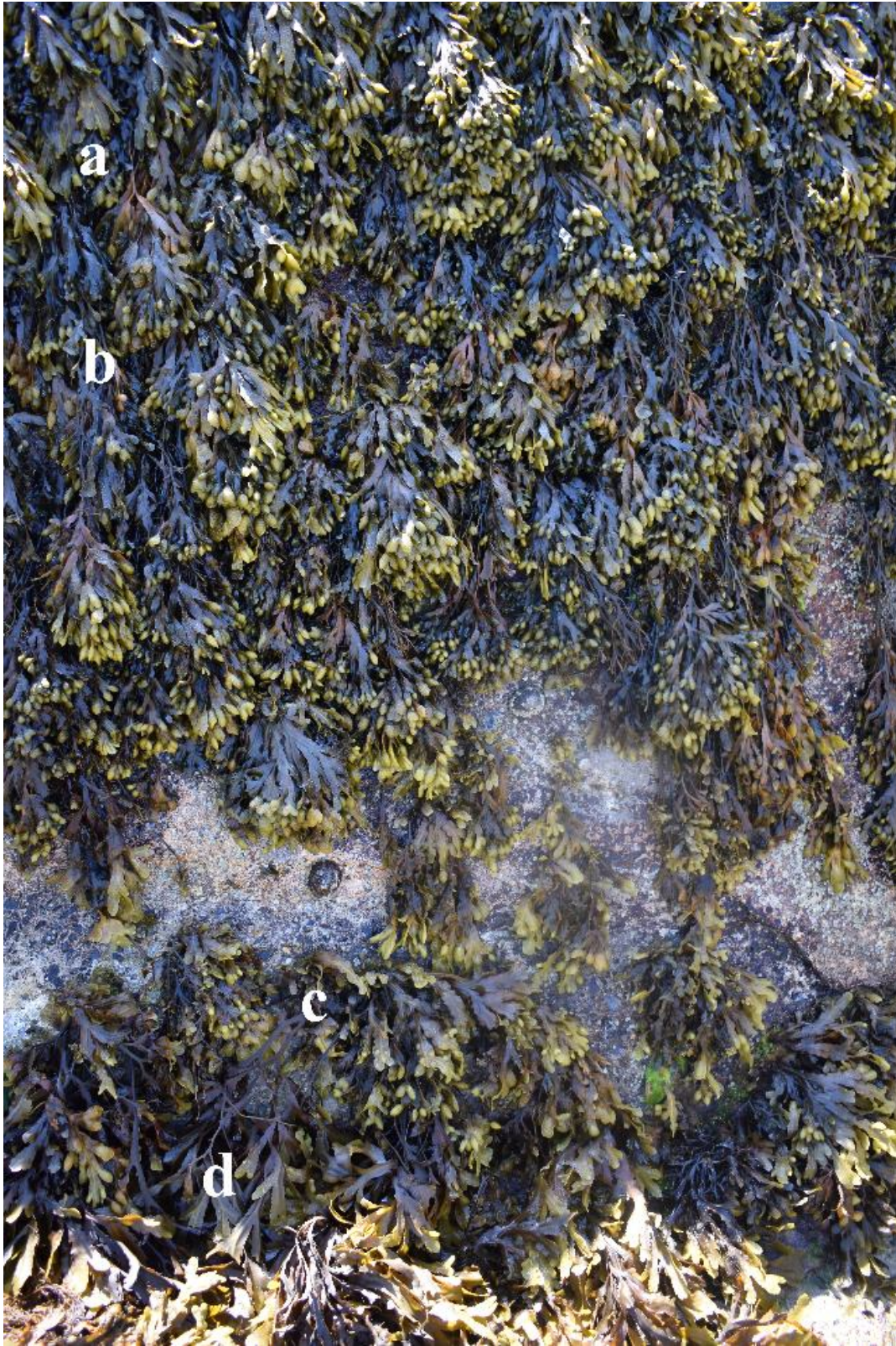


Fig. 1: *Fucus* species sequence along the intertidal environmental gradient at a harbor wall in Roscoff, Brittany: a) *F. spiralis*, b) *F. guiryi* (those two species can hardly be distinguished in the field), c) *F. vesiculosus*, d) *F. serratus*.





Fig. 2: *Fucus* species in Bülk, Kiel fjord, western Baltic Sea: *F. vesiculosus* (on the temporarily air-exposed rock) and *F. serratus* beneath the water surface in the center and at the left side.

#### Morphology and reproduction

*Fucus* plants live attached to the substrate by a discoid holdfast. From the holdfast, flattened fronds are erected by a rather rigid stipe (Figs. 3 and 4). The apex of a frond contains a huge apical cell and adjacent layers of smaller meristematic cells which together form the apical meristem (Moss 1967). This apical meristem gives rise to new branches by dichotomous branching initiated by a division of the apical cell (Moss 1967). In most *Fucus* species, one side of the dichotomy has a higher growth potential than the other, this dominance alternates from forking to forking resulting in a monopodial appearance of the overall thallus (Knight and Park 1950). Thallus apices of the side of the dichotomy with lower growth potential usually form sexual organs called receptacles (Knight and Park 1950, own observations). When a receptacle is initiated at a thallus apex, the growth of the apex is terminated and receptacles are formed in which antheridia and oogonia produce male (sperm) and female (egg) gametes. When the receptacle has matured, the gametes are released in the surrounding sea water and fuse to a zygote, which, after settlement on substrate, germinates into a new plant. After gamete release, the receptacle degenerates, including the thallus parts beneath, down to the next branch that bears a vegetative apex (Knight and Park 1950). Some *Fucus* species are dioecious (e.g. *F. vesiculosus*), i.e. a single plant produces only male or female gametes, while others are monoecious (e.g. *F. spiralis*), i.e. each plant produces male and female gametes (Coyer et al. 2006). Reproduction is mostly focused on certain seasons, e.g., western Baltic Sea *F. serratus* reproduce during late autumn to spring, the receptacles for this reproductive season are already initiated in late summer (own observation). In contrast, *F. vesiculosus* occurs with two genotypes in the western Baltic Sea, one reproduces only in spring, the receptacles for this reproductive season are initiated in late autumn/ winter, the second reproduces in spring and in addition in late summer, the receptacles are initiated in July (Berger et al. 2001, own observations).





Fig. 3: Morphology of typical attached-growing *F. vesiculosus* plant. a) Entire plant, b) frond with reproductive apices (“receptacles”) and one vegetative apex (not swollen), c) stipe and holdfast attaching the plant to the substrate.



Fig. 4: Morphology of typical attached-growing *F. serratus* plant. a) Entire plant, b) frond with two reproductive apices (“receptacles”, left forking) and two vegetative tips (right forking), c) stipe and holdfast attaching the plant to the substrate.

*Fucus* plants do not always grow attached to hard substratum like rocks and boulders; at some locations, *Fucus* populations exist on soft sediment where the thalli are found embedded in mud, entangled between salt marsh angiosperms (*Spartina* spp.), or hold by byssus filaments of blue mussels (*Mytilus* spp.) (Norton and Mathieson 1983). In these cases, it is assumed that previously substrate-attached *Fucus* thalli were detached (e.g., by wave forces) and drifted to the present location where they are kept from sinking to unfavorable depths and beach casting by the abovementioned means. The arriving thalli are assumed to reproduce via vegetative reproduction, i.e., breaking thallus parts continue to grow and

are also kept at the location. Thus, larger populations are formed (Norton and Mathieson 1983). These unattached populations usually show specific morphologies like dwarfism and very bushy growth (Norton and Mathieson 1983, Wallace et al. 2004). The most characteristic features are the absence of a holdfast and in the majority of cases a very reduced fertility, i.e., only few or no receptacles are formed and reproduction seems to be exclusively vegetative (Norton and Mathieson 1983, Mathieson and Dawes 2001, Maczassek 2014).

In summary, *Fucus* species show two modes of reproduction depending on the populations considered: sexual reproduction via gametes and zygotes in populations growing attached to hard substratum and vegetative reproduction via breaking thallus pieces in populations growing unattached on soft sediment.

### **Commercial use of *Fucus* species**

*Fucus* species are not only important parts of coastal ecosystems, they are also of high commercial interest: *Fucus* biomass is used to produce for example extracts for the cosmetic industry and food supplements (Caterino et al. 2018, Ferreira et al. 2019, Torres et al. 2020). These applications make use of the high content of bioactive compounds in *Fucus* biomass. Examples are the accessory photosynthesis pigment fucoxanthin, which shows anti-oxidant, anti-diabetic and anti-obesity activities (Maeda et al. 2005, Abidov et al. 2010, Mikami and Hosokawa 2013, Gammone and D’Orazio 2015), sulfated fucose-containing cell wall polysaccharides (called “fucoidans”), which show for example anti-coagulant, anti-oxidant, anti-cancer, anti-viral and anti-skin aging activities (Cooper et al. 2002, Li et al. 2008, Jeon et al. 2011, Wang et al. 2017, Dörschmann et al. 2019, Neupane et al. 2019, Bittkau et al. 2019), and phlorotannins, a brown algal-specific class of polyphenols (Ragan and Glombitza 1986), which exhibit anti-oxidant, anti-allergic, anti-inflammatory and UV-protective activities (Pavia et al. 1997, Kim and Himaya 2011, Sathya et al. 2013). Other bioactive constituents of *Fucus* biomass are iodine, laminarin, unsaturated fatty acids and tocopherols (Catarino et al. 2018).

### **Need for *Fucus* aquaculture**

Currently, *Fucus* biomass for commercial use is solely obtained by harvesting wild stocks along northern hemisphere coasts, e.g., in France, Portugal, Ireland, Iceland etc. (netalgae.org). Harvests are usually performed manually during low tide, but in some cases also from boats or by diving (netalgae.org). The biomass is then sold dried or frozen to processing companies (Woldmann S., pers. comm.). However, exploiting wild stocks is not without concern as *Fucus* species, similar to other perennial brown macroalgae, have experienced stock declines in recent years due to environmental change (Kautsky et al. 1986, Nicastro et al. 2013, Valdoza et al. 2017, Butler et al. 2020). One example for this trend is the Baltic Sea, where *Fucus* populations have experienced dramatic declines in the second half of the 20<sup>th</sup> century (Kautsky et al. 1986, Vogt & Schramm 1991). *F. vesiculosus* from the eastern Baltic Sea, which occurred down to depths of 11.5m in the 1940s, was found at maximum depths of only 8.5m in 1984 (Kautsky et al. 1986). Similar observations were made in the western Baltic Sea, where a population decline of 95% was recorded for *Fucus* spp. between field surveys performed in 1950/51 and 1987/88 (Waern 1952, Vogt and Schramm 1991). Eutrophication and the concomitant reduced light penetration of the water column has been identified as most important cause for the declines of the *Fucus* stocks, another discussed reason is the historical intense stone fishery in the Baltic Sea (Vogt and Schramm 1991, Torn et al. 2006, Rohde et al. 2008). As a result of the described declines, Baltic Sea *Fucus* stands are under protection legislation in Germany and listed in the Red list (Bundesamt für Naturschutz 2013). Consequently, the harvesting of wild stocks is prohibited.

Therefore, German companies which sell *Fucus*-based products cannot generate biomass from the Baltic Sea but have to rely on biomass from other countries. However, there is interest to use regional biomass from the Baltic Sea, because “regionality” is a desired claim in the targeted markets (L. Piker, pers.



comm.). Aquaculture could be a promising alternative for the generation of regional biomass in the Baltic Sea. Apart from the local interest in Germany, *Fucus* aquaculture could be a promising alternative to wild harvesting also in other countries where *Fucus* stocks like Portugal (Nicastro et al. 2013). Besides, aquaculture of *Fucus* species might become necessary in the future anyways, if the market demand for *Fucus* biomass rises, which can be expected based on the increasing demand for algal products in general (Cherry et al. 2019), but so far, there is no established aquaculture of *Fucus* species worldwide.

Therefore, the objective of this thesis was to develop a method for the commercial cultivation of *Fucus* species, which can be implemented in the Baltic Sea. To the knowledge of the author, there are only two published studies dealing with culture of *Fucus* species for applied purposes (neglecting publications regarding lab culture of *Fucus* for physiological experiments etc.): Haglund and Pedersen (1988) cultivated *F. vesiculosus* in land-based spray culture. Ryzhik et al. (2014) cultivated *F. serratus* and *F. distichus* plants bound to ropes as biofiltration system for the treatment of oil spills with seaweed associated bacteria. Both publications do not present methods useful for the production of greater amounts of biomass as needed in the food supplement or cosmetic industry. Therefore, another method for the production of *Fucus* biomass has to be developed.

### ***Fucus* species in the western Baltic Sea**

The study area of this thesis is the Kiel fjord located at the east coast of the German federal state Schleswig-Holstein in the western Baltic Sea (54.3564 N, 10.1614 E). By help of Kieler Meeresfarm GmbH & Co.KG it was possible to establish an experimental farm in the fjord for cultivation experiments with local *Fucus* species. The western Baltic Sea harbors three *Fucus* species: *F. vesiculosus*, *F. serratus* and *F. distichus* subsp. *evanescens* (Fürhapter et al. 2008). *F. vesiculosus* and *F. serratus* are native to the brackish Baltic Sea with *F. vesiculosus* ranging far into the low-salinity eastern parts (Gulf of Bothnia, Gulf of Finland) and *F. serratus* reaching eastwards to the south east coast of Sweden (Malm et al. 2001). In contrast, *F. distichus* subsp. *evanescens* is originally a species of the Northeast Pacific and started to colonize the western Baltic Sea at the beginning of the 20th century, today, established populations are found at several locations, e.g. in the Kiel canal (Schueller and Peters 1994, Steinhagen et al. 2019). All *Fucus* species of the western Baltic Sea grow attached to hard substrate and reproduce by sexual reproduction. In the Flensburg fjord, ca. 100 km north of the Kiel fjord, the only known unattached population of the western Baltic Sea is found on soft sediment. This population seems to reproduce only by vegetative reproduction and exhibits a strikingly low fertility (i.e. receptacle production) (Maczassek 2014). It morphologically resembles *F. vesiculosus* but the precise species affiliation is unclear. As *F. distichus* subsp. *evanescens* is a non-native species, a cultivation would be prohibited by governmental authorities. Therefore, the focal species of this thesis are *F. vesiculosus* (Fig. 3), *F. serratus* (Fig. 4), and the population from Glücksborg, which is called “Glücksborg population” due to the uncertain taxonomical status.

### **Potential vegetative aquaculture method for *Fucus* species**

As described above, *Fucus* species have the potential to reproduce vegetatively via thallus fragments which continue growing as well as sexually via sperm and egg, which, after fusing to a zygote, germinate into a new plant. Thus, both general aquaculture methods described in the beginning (culture involving sexual reproduction, culture involving vegetative reproduction) might be used in the culture of *Fucus* species. For the development of a cultivation method for the Baltic Sea, the second approach (vegetative reproduction) was chosen as it does not require expensive land-based hatcheries, and was seemingly easier to establish. Seaweeds which are already cultivated by use of vegetative reproduction (*Gracilaria/Agarophyton* spp., *Kappahycus* spp., *Eucheuma* spp.) are usually reared in the following manner: fragments (“seedlings”) are tethered to ropes or pegged in the sediment. After a period of growth, the

biomass is harvested, from the harvest, new fragments are obtained and used to start the next cultivation season (Hurtado et al. 2014). This approach works only under calm weather conditions as storms are expected to loosen the thalli from the ropes or the sediment. First trials with *Fucus* thalli tied to a swimming structure in the Kiel fjord showed that the thalli have a high probability of getting lost under the local conditions. Therefore, an alternative cultivation approach was drafted: thallus pieces are cultivated freely floating in baskets/ net cages which are deployed in the sea. After a period of growth, the biomass is harvested and vegetative thallus apices are cut from the harvested thalli to be used as seedlings which are put back into the baskets/ net cages to serve as starting biomass for the next cultivation season (Hurtado et al. 2014). This cultivation approach is sketched in Fig. 5.

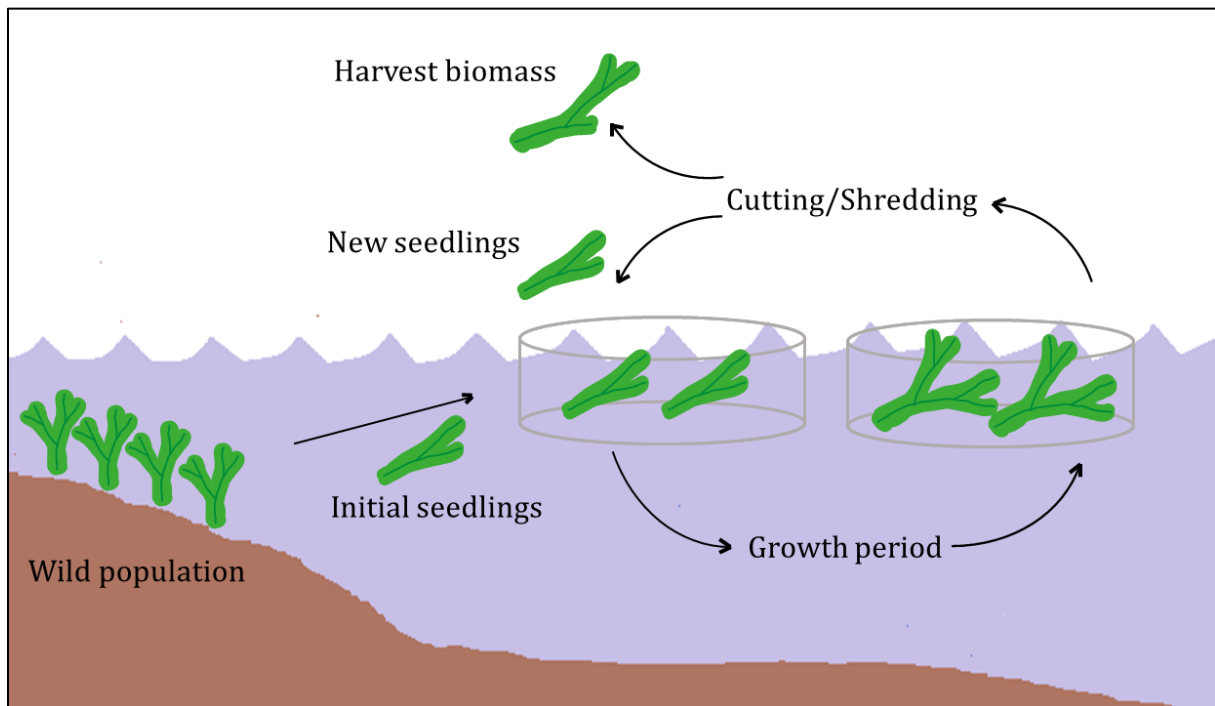


Fig. 5: Initial idea of cultivation cycle of vegetative *Fucus* aquaculture.

### This thesis

The goal of this thesis was to experimentally examine basic questions concerning the envisaged vegetative cultivation process described above. The targeted questions will be explained individually in the following:

1. Do *Fucus* thalli survive long-term cultivation in freely floating aquaculture?

It is known that detached *Fucus* thalli travel over long distances with the currents (Rothäusler et al. 2015). However, it is not known how long thalli survive unattached culture in baskets/ net cages and if they retain their growth potential under these conditions.

2. How does fertility (receptacle formation) affect the cultivation process?

As described above, thalli from attached *Fucus* populations regularly produce receptacles for sexual reproduction. As the receptacle tissue and the tissue below the receptacle disintegrates after gamete shedding, biomass losses during the cultivation process can be expected due to receptacle degeneration. The extent of these losses and the corresponding impact on the culture process are unclear.

### 3. Are attached or unattached populations better suited for cultivation, especially with respect to fertility?

Unattached populations growing on soft sediment usually show low fertilities or no receptacle formation at all (Norton and Mathieson 1983) and should therefore be advantageous for the envisaged cultivation process as no or only little biomass losses by receptacle degeneration can be expected. However, it is unclear if this feature is the result of an adaptation process (i.e. it is genetically fixed) or an acclimatization to the specific conditions of their specific habitat (i.e. it is not genetically fixed) (Norton and Mathieson 1983, Mathieson and Dawes 2006). If it arose from acclimatization, a back-acclimatization towards high fertilities during the cultivation process is possible. At the same time, an acclimatization of high-fertility populations from attached origin towards low fertilities under culture conditions might also be possible (Maczassek 2014). It is therefore unclear, if source biomass from attached or unattached populations should be preferred for cultivation and if any of the two source biomasses can be cultivated without fertility-related biomass losses.

Questions 1-3 were addressed in cultivation experiments, the results of these experiments are described in **Chapter II**, titled “Vegetative aquaculture of *Fucus* in the Baltic Sea- obtaining low fertility biomass from attached or unattached populations?” which has been published in *Journal of Applied Phycology*.

### 4. How does the seasonal fluctuation of the environmental conditions affect growth rates with the envisaged cultivation method?

In order to estimate achievable yields of *Fucus* cultures, growth rates at different seasons have to be known. Seasonal growth cycles are well studied in wild *Fucus* populations (Brenchley et al. 1998, Lehvo et al. 2001) but have never been examined in culture.

### 5. How does the initial stocking density affect growth rates and yields in culture?

It has been shown that initial stocking density has a significant effect on growth rates and yields of crop plants in agriculture (Assefa et al. 2016), and mathematical models have been developed to describe this yield-density relationship (Holliday 1960, Bleasdale and Nelder 1960). However, it is not known how initial stocking density affects growth rates and yields of *Fucus* cultures.

### 6. Which annual yields can be reached under which cultivation scenarios?

In order to estimate the commercial potential of the envisaged cultivation method, potential annual yields need to be known. Annual yields will of course vary depending on the used cultivation conditions, but not all conditions can be controlled by the farmer. When the cultivation set up (basket type, basket depth, position in the fjord etc.) and the growth variation imposed by seasonal fluctuations of environmental conditions are assumed as given, initial stocking density and harvest density are the two parameters that can be determined by the farmer. It would be helpful to know which combination of initial stocking density and harvesting density will result in the maximum annual yield and how many harvests will be necessary in such a scenario.

### 7. Are growth rates measured in small scale experimental culture transferable to commercial-size culture?

Conditions in larger cultivation devices differ from conditions in small-scale experiments. It is important to know if growth parameters determined in small small-scale cultivation devices do apply in commercial-scale net cages.

Questions 4, 5, 6 and 7 were addressed in cultivation experiments. The results of these experiments are shown in **Chapter III**, which is under revision at *Journal of Applied Phycology*.

## 8. Can epizoan fouling on cultivated thalli be reduced by regular desiccation of the cultivated biomass?

Wild *Fucus* plants are subject to colonization by epiphytes and epizoans (Kersen et al. 2011) and so are cultivated thalli. From the beginning of the culture experiments in the Kiel fjord, fouling appeared to be a serious problem and the culture biomass seemed to suffer from even stronger colonization than *Fucus* plants in the natural habitat (own observation). While ephemeral algae are only a seasonal problem in culture and disappear after their growth season (Colina 1981), epizoans (e.g. barnacles, blue mussels, bryozans) remain on the cultivated biomass once they have settled. Therefore, a method for the reduction of epizoan foulers is necessary for successful long-term cultivation of *Fucus* thalli. One potential way to combat epizoan colonization is the regular desiccation of cultivated biomass by air exposure. As *Fucus* species colonize intertidal habitats, they are adapted to times of emersion and the concomitant desiccation (Schramm 1968, Schonbeck and Norton 1979). If the settling larvae of epizoan colonizers are less resistant to desiccation than the *Fucus* thalli, desiccation might reduce the number of successfully settling larvae as well as the growth of already established individuals. Thus, desiccation could be an effective measure to reduce the epizoan biomass. However, it is important that growth rates of the cultivated *Fucus* thalli are not negatively affected by the applied desiccation treatments.

Question 8 was addressed in a cultivation experiment. The results of this experiment are presented in **Chapter IV**, titled “Control of fouling in the aquaculture of *Fucus vesiculosus* and *Fucus serratus* by regular desiccation“. The chapter has been published in *Journal of Applied Phycology*.

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**Chapter II –  
Vegetative aquaculture of *Fucus* in the  
Baltic Sea - obtaining low-fertility biomass  
from attached or unattached populations?**



# Vegetative aquaculture of *Fucus* in the Baltic Sea—obtaining low-fertility biomass from attached or unattached populations?

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## Abstract

An experimental farm has been installed in the Kiel Fjord, western Baltic Sea, aiming at the development of a sustainable production process for *Fucus* species (*Fucus vesiculosus*, *Fucus serratus*). The envisaged cultivation method includes the unattached rearing of thalli in baskets deployed in the sea and their vegetative reproduction. Fertility (i.e., receptacle formation) is expected to be problematic for this approach, because receptacles are terminated in growth and degrade after gamete release. In culture experiments, natural fertility led to only minimal overall growth in *F. vesiculosus* and even weight loss in *F. serratus*. Therefore, we tested if long-term unattached cultivation of formerly attached thalli leads to a lowering of fertility by an acclimatization process. However, fertility after 1 and 2 years of unattached cultivation was statistically equal and still comparable to the high fertility of attached populations. Furthermore, we tested if the only known naturally unattached population in the western Baltic Sea near Glücksborg, which remains largely infertile in the wild, keeps its low fertility if put under culture conditions. During an experimental 1-year cultivation, thalli from this population remained almost entirely vegetative ( $2.0 \pm 3.1\%$  fertile apices). Hence, the Glücksborg population is a promising source of aquacultural seedling biomass. Yet, further tests are necessary to check, if the fertility remains low over several years of cultivation. If unattached populations are used as source for commercial cultures, the collection of seedling material should always be accompanied by strong measures to ensure the continued integrity of these valuable habitats.

**Keywords** *Fucus vesiculosus* · *Fucus serratus* · Phaeophyceae · Aquaculture · Ecad · Vegetative reproduction · Fertility

## Introduction

The brown algal genus *Fucus* is known for its high content in bioactive ingredients like phlorotannins and fucoxanthin (Catarino et al. 2018). *Fucus* biomass is therefore used for a variety of products like cosmetic extracts and food supplements (Ferreira et al. 2019; Torres et al. 2020). Currently, the entire market demand is satisfied by biomass harvested from wild populations, e.g., in France and Ireland (netalgae.eu). In Germany, harvesting from wild populations is prohibited and *Fucus* stands listed as protected habitat (Bundesamt für Naturschutz 2013; HELCOM 2013). Yet, German companies desire regional biomass, because

“regionality” is a valuable claim in the targeted markets (L. Piker, pers. comm.). The only means to obtain regional biomass from the German Baltic Sea coast is therefore aquaculture. *Fucus* aquaculture could potentially become necessary in other countries, too, if the market pull increases (Cherry et al. 2019), or environmental change puts wild populations at risk (Nicastro et al. 2013). Consequently, an experimental *Fucus* farm has been established in the Kiel fjord at the German Baltic Sea coast aiming at the development of a method for the production of local *Fucus* species (*Fucus vesiculosus*, *Fucus serratus*) in aquaculture.

A key step in the development of a commercial cultivation process is the establishment of a successful reproduction method. Commercially cultivated seaweeds can be placed in two categories with respect to reproduction: The first is produced in a process involving sexual reproduction (e.g., *Saccharina latissima*, *Undaria pinnatifida*, *Pyropia* spp.), including the rearing of different life stages (gametophyte, (tetra-)sporophyte, in red algae also carposporophyte), the production of seeded ropes in land-based hatcheries, and their later deployment in the sea for the outgrowth of the germlings

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to harvest size (Yarish and Pereira 2008; Graham et al. 2009; Hurd et al. 2014; Azevedo et al. 2019). The second category is produced in a process involving vegetative reproduction (*Eucheuma* spp./*Kappaphycus* spp., *Gracilaria* spp.), where thalli are reared either tethered to ropes, pegged to the sediment, or freely floating in tanks or baskets. Fragments for the next growing season (“seedlings”) are obtained by cutting small pieces from the cultivated thalli. No hatchery is needed for vegetative reproduction and only one stage of the life cycle is cultivated (Yarish and Pereira 2008; Graham et al. 2009; Hurd et al. 2014). Due to the ease of the process, vegetative reproduction was envisaged for *Fucus* aquaculture. The overall cultivation method comprises the cutting of fronds from wild specimens, which grow unattached in floating baskets until harvest size (Fig. 1). From the harvest, small pieces containing apical meristems are generated, e.g., by automated shredding, which are used as seedlings for the next growing season, and so forth.

Typical wild *Fucus* populations produce high numbers of sexual structures (receptacles) at their thallus apices. During receptacle formation, the activity of the apical meristem is terminated and, after shedding of the gametes, the receptacle including the former meristem and the tissue below the receptacle degrades down to the next branch that bears a vegetative

apex (Knight and Parke 1950; Moss 1967). During a typical reproductive season, 70–100% of the apices turn into receptacles and degrade later (Knight and Parke 1950; Bäck et al. 1991; Graiff et al. 2017), which causes attached *Fucus* populations to lose significant amounts of biomass (*F. vesiculosus*: ca. 25% (Berger et al. 2001), *F. serratus*: 30–50% (Arrontes 1993; Brenchley 1996)). This process is expected to lead to severe growth reductions in unattached cultures which consist only of fronds. However, these fertility-related growth reductions have not been precisely determined yet, and it is unclear how detrimental they are for successful cultivation. In general, culture biomass with constantly low fertility is expected to be advantageous for effective biomass production with the described cultivation method.

Interestingly, there are some *Fucus* populations occurring in certain habitats like estuaries, salt marshes, or blue mussel banks, which naturally produce no or only few receptacles and would thus be very suitable for vegetative cultivation (Norton and Mathieson 1983; Mathieson and Dawes 2001). These populations are not found attached to hard substrata but live entangled in salt marsh angiosperms, embedded in mud, or are held by the byssus filaments of blue mussels (Baker and Bohling 1916; Nienburg 1925; Nienhuis 1970; Norton and Mathieson 1983). It is usually assumed that they initially

**Fig. 1** **a** Package of cultivation baskets for experiment 1 (bottom, quadratic) and cultivation baskets for experiment 3 (top, round). **b** White boxes with side windows installed in the Jetfloat system. **c** Packages of cultivation baskets for experiment 2 which were inserted in the white boxes shown in **b**



had a holdfast, but were pulled off from their substrate and drifted to their current location, where angiosperms, mud, or blue mussel byssus filaments prevented them from being washed ashore (Norton and Mathieson 1983). Being trapped this way, they are supposed to have reproduced by vegetative fragmentation, thus forming larger populations (Mathieson et al. 2006). They share some typical features like absence of holdfast, low fertility, i.e., almost no receptacles, often small size, and atypical, curling growth. Due to these features, they are termed “ecads” to indicate that their morphology is a result of the environmental conditions (e.g., Baker and Bohling 1916). However, there is some confusion if the unusual morphological features, especially the low fertility, arose from acclimatization to the specific conditions of their current growth locations, or if it required adaptation, i.e., only certain genotypes, which show low fertility or are able to quickly shift towards low fertility, have successfully colonized these locations after arrival (Wallace et al. 2004; Mathieson et al. 2006).

Some unattached populations have been shown to be hybrids of *F. vesiculosus* and *F. spiralis* (Wallace et al. 2004; Coyer et al. 2006; Mathieson et al. 2006; Sjøtun et al. 2017), or result from backcrosses between these hybrids and *F. vesiculosus* (i.e., introgression, Wallace et al. 2004), or they are polyploids of *F. vesiculosus* (Coyer et al. 2006; Sjøtun et al. 2017). As hybridization and polyploidy are known to often cause sterility in land plants, it can be speculated that these genetic changes are causal for the low fertilities of these populations, which in turn can be regarded as a result of adaptation and not acclimatization. However, in the study of Mathieson et al. (2006), thalli of the unattached *F. spiralis* ecad *lutarius*, found in the Gulf of Maine, were genetically equal to neighboring attached *F. vesiculosus* or *F. spiralis*, which indicates an acclimatization origin of these thalli, but it was questioned how long they might persist as “ecads.”

From the western Baltic Sea, only one unattached population is known from Glücksburg in the Flensburg fjord (Maczassek 2014), where thalli morphologically similar to *F. vesiculosus* are found embedded in mud, showing a strikingly low number of receptacles. So far, the genetic affinities of this population are unresolved. Maczassek (2014) first described it as *F. vesiculosus* f. *mytili*, although the genetic relationship between the unattached population from Glücksburg and *F. vesiculosus* f. *mytili*, an unattached population described from the Wadden Sea (Nienburg 1931), is unclear. Therefore, we use the term “Glücksburg population” in the following. Maczassek (2014) hypothesized that it arose through acclimatization of local attached *F. vesiculosus* thalli and that the acclimatization process was induced by detachment from the substrate. Following this reasoning, the long-term cultivation of local attached *F. vesiculosus* thalli with the envisaged cultivation protocol, which includes the detachment of thalli from the substrate and their unattached rearing, could

potentially induce an acclimatization towards the morphology of the Glücksburg population, including the low fertility.

If the Glücksburg population itself is put under culture conditions, it will retain its low fertility if this feature is genetically fixed. If the low fertility is the result of an acclimatization process, it could also be retained depending on the culture conditions. In any of the two scenarios, the Glücksburg population itself could potentially be a useful seedling source for aquaculture. For successful vegetative cultivation, it is crucial to know, if biomass which continuously remains at a state of low fertility, can be obtained via acclimatization from attached populations and/or if the unattached Glücksburg population can be used, because it retains its low fertility in culture. From a quantitative perspective, the acclimatization of former attached thalli would be advantageous, because most of the 402 km of the western Baltic Sea coastline of Germany (Schleswig-Holstein) are colonized by attached *F. vesiculosus* and *F. serratus* populations (Führhapter et al. 2008), while the Glücksburg population only covers an area of about 50 m<sup>2</sup> in the Flensburg fjord and thus represents only a limited source for seedling material.

Based on these considerations, three questions were addressed in this study:

- (1) How strong is the fertility-related reduction of growth in unattached culture, and is it detrimental for commercial cultivation (Experiment 1)?
- (2) Do *Fucus* thalli from attached populations (*F. vesiculosus*, *F. serratus*) survive long-term unattached cultivation, and does this long-term unattached cultivation reduce the fertility? Specifically, are less receptacles formed in the second year of unattached culture than in the first year of unattached culture (Experiment 2)? *F. serratus* was included in this experiment, although the acclimatization hypothesis of Maczassek (2014, see above) only concerned *F. vesiculosus*.
- (3) Do thalli from the Glücksburg population grow in culture and does their fertility change under culture conditions (Experiment 3)?

## Materials and methods

All experiments were conducted at the premises of Kieler Meeresfarm (GmbH & Co. KG), located in the northwestern part of the Kiel fjord (54.381975 N; 10.162034 E). For experiments 1 and 2, individuals from local attached populations were collected, *Fucus vesiculosus* from Kiel/Holtenau (54.368966 N; 10.154112 E) and *Fucus serratus* from Büllk (54.455146 N; 10.198858 E). For experiment 3, individuals from the Glücksburg population were collected from its only known growth location in the Flensburg fjord (54.837671 N;



9.521850 E, Fig. 2). For all experiments, whole plants were transported in seawater to the cultivation site and stored in buckets overnight. The following day, thallus pieces of 3–10 cm in length (*F. vesiculosus* and Glücksburg population) and 5–15 cm in length (*F. serratus*) were cut from the plants and used to start the experimental cultures. The sizes represent typical fronds of the species, when cut above the stipe. The size difference was accepted, because no inter-species comparisons were intended. Measured response variables during the experiments were wet weight, total number of apices, and fertility. Wet weight was regularly assessed by weighing the cultured biomass with a lab scale (Kern EMB 1200) under wind protection after 15 s of drying with a salad spinner. The total number of apices was assessed by counting all apices in the experimental cultures. Apices were counted as “1,” if the gap between two dividing apices was smaller than 1 mm, and as “2,” if it was larger than 1 mm. Fertility was calculated as the percentage of fertile apices (receptacles) of the total number of apices. An apex was considered as fertile as soon as receptacle formation was initiated, discernable by a commencing swelling of the apex and the formation of high numbers of conceptacles (visible when thalli were held up against the light).

### Experiment 1—effect of fertility on growth in culture

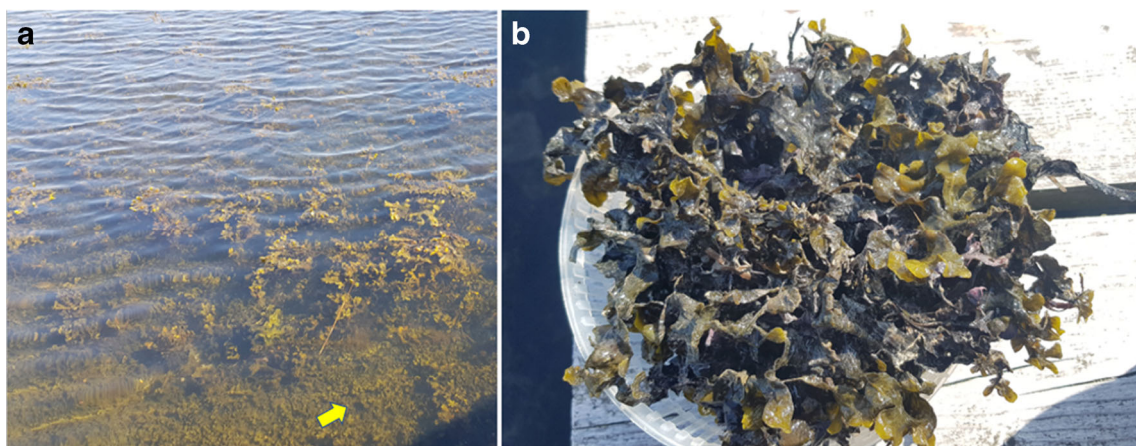
In order to test the impact of fertility on the growth of cultivated *Fucus* thalli, culture biomasses with different initial fertilities were created by assembling thalli with ripe fertile and vegetative apices. The following groups were prepared: *F. vesiculosus*: 100% receptacles, 80% receptacles, 0% receptacles; *F. serratus*: 100% receptacles, 70% receptacles, 0% receptacles.

For *F. vesiculosus*, 80% receptacles represents the estimated fertility during the spring reproductive season (Graiff et al. 2017, own pre-experiments). The experiment for this species

was started during this season (14 May 2019) and lasted until 04 September 2019. Measurements of response variables were performed ca. weekly due to the high growth rates during summer. For *F. serratus*, 70% receptacles represents the estimated fertility during its reproductive period in late autumn/winter (own pre-experiments). The experiment for this species was therefore started on 20 December 2019 and lasted until 1 April 2020 with measurements every 2–4 weeks due to the low growth rates during winter. The difference in season was accepted, because a direct comparison of the two species was not intended.

The same experimental setup was used for both species: nine black plastic baskets (BAUHAUS; Oase Pflanzkorb; edge length: 28 cm; volume: 14 L) served as cultivation devices, allowing for permanent inflow of seawater by holes of ca.  $0.3 \times 0.3$  cm at all sides. The baskets were arranged in three packages of three and covered with transparent plastic nettings to avoid loss of culture material (Fig. 1). Each of the three groups (100%; 80%/70%; 0%) was represented with one replicate in each of the three packages, at a different position in each package. Thus, position effects could be excluded. The packages were deployed in the sea with polyethylene foam pipe insulation as floating bodies (Fig. 1). The packages were protected from wave activity by a pipe frame ( $2 \times 4$  m). Cultures were started with 20 g biomass of the respective fertility per basket. The total number of apices at the beginning of the experiments was not equal among the groups, because receptacles are heavier than vegetative thallus apices. Therefore, the percentual change of the total number of apices was calculated as an additional response variable using the following formula:

$$\text{Percentual change of total number of apices} = ((\text{TNA}_{t_{\text{end}}}/\text{TNA}_{t_0})/\text{TNA}_{t_0}) = \times 100$$



**Fig. 2** **a** Glücksburg population growing embedded in mud (see arrow) in the understory of larger *F. vesiculosus* thalli with receptacles. **b** Thalli

from Glücksburg population after collection, showing no receptacles and a dark coloration of the thallus parts that were embedded in anoxic mud

where TNA is the total number of apices,  $t_0$  is the beginning of the experiment, and  $t_{\text{end}}$  is the end of the experiment.

The weight data were of main interest and are presented here; the total number of apices, the fertility over the course of the experiment, and the percentual change of the total number of apices are shown in the supplementary material (Fig. 3, Fig. S1, Tab. S1).

### Experiment 2—fertility of attached populations under long-term unattached culture

In order to investigate the growth and fertility of formerly attached thalli during long-term unattached culture, *F. vesiculosus* and *F. serratus* thalli were cultivated unattached for 24 months (24 May 2018–29 May 2020) in the same type of plastic baskets used in experiment 1. The baskets were not submerged directly into the sea as in experiment 1, but inserted into white boxes (glass fiber reinforced plastic, shape: turned truncated pyramid, upper opening: 80 × 80 cm, basal area: 64 × 64 cm, height: ca. 40 cm, Fig. 1). The white boxes had a window (35 × 37 cm) at each of the four side faces to allow water inflow. They were hung in the gaps of a swimming platform of black Jetfloat elements (Jetfloat International, double elements: 100 × 50 × 40 cm, single elements: 50 × 50 × 40 cm, Fig. 1). Thus, the baskets were deployed in the sea, but with less water flow-through than under direct deployment, resulting in significantly less epizoans colonizing the thalli (e.g., barnacles, blue mussels, bryozoans), than if baskets were directly deployed in the sea (own pre-experiments). Using this setup it was possible to conduct a long-term cultivation experiment without the necessity of frequent cleaning of the thalli from epibionts, which might have caused considerable damage to the thalli (own pre-experiments). Six white boxes were available, containing a package of eight black baskets each, resulting in a total of 48 experimental units. The white boxes were only used for installation and hence not considered as entities; thus, the 48 baskets were considered as independent experimental units. The position of the baskets within the white boxes was changed regularly to avoid position effects. Of the 48 baskets, 36 were used for other experiments and 12 were used for this experiment, yielding 4 groups of 3 replicates each:

- *F. vesiculosus*: unattached cultivation starting on 24 May 2018
- *F. vesiculosus*: unattached cultivation starting on 24 May 2019
- *F. serratus*: unattached cultivation starting on 24 May 2018
- *F. serratus*: unattached cultivation starting on 24 May 2019

The experimental cultivation was started with 20 g of vegetative thalli per basket and lasted until 29 May 2020. Thus, for each *Fucus* species, one group was cultivated unattached for 2 years and the second group was cultivated unattached for 1 year, parallel to the second year of the first group (Figs. 4 and 5). Weight was recorded during the entire experiment. The total number of apices (data not shown) and fertility was recorded three times during the first year and every 2–4 weeks during the second year. Thus, it was possible to compare the fertility of the group cultivated for 2 years between year 1 and year 2. Furthermore, it was possible to compare the fertility of the group cultivated for 2 years with the fertility of the group cultivated for 1 year at the same time points and under the same environmental conditions during year 2. A higher measurement frequency was chosen during year 2 to ensure the detection of even small fertility differences between the two groups cultivated in parallel.

During year 1, a harvest was performed in September 2018 to avoid limitation of growth by too high densities. Density limitation occurs above ca. 100 g basket<sup>-1</sup> (i.e., ca. 2.5 kg m<sup>-2</sup>) (own pre-experiments). During this harvest, all sexual thalli were removed and the cultures were continued with 20 g of vegetative thalli. After 1 year of cultivation (24 May 2019), the biomass was again harvested and cultivation was continued with the vegetative biomass present in the cultures at this point (*F. vesiculosus*: 6 g; *F. serratus*: 11 g), with the parallel groups of year 2 being added to the experiment (*F. vesiculosus*: 6 g; *F. serratus*: 11 g).

*Fucus vesiculosus* shows two reproductive periods in the western Baltic Sea, the first from November to May (spring reproductive season (SRS)) and the second from July to November (autumn reproductive season (ARS)). Two genotypes occur in the Kiel Bay, from where the thalli were collected; the first genotype is fertile only during the SRS (“only spring bloomers”) and the second genotype shares this reproductive period but is fertile also during the ARS (“autumn and spring bloomers”) (Berger et al. 2001, F. Weinberger, pers. comm.). Vegetative thalli of these two genotypes cannot be distinguished in the field. Therefore, thalli were randomly picked and initially both genotypes were present in the culture biomass in the ratio of the natural field populations. The ratio of the two genotypes changed unintentionally during year 1, due to the intermediate harvests (see “Results”).

*Fucus serratus* has only one long reproductive period from July to March (winter reproductive season (WRS)) in the western Baltic Sea and no genotypic variants with respect to reproductive behavior have been observed in the sampling area (pers. observation, see also Malm et al. (2001) for similar observations at the Swedish Baltic Sea coast).

### Experiment 3—fertility of unattached Glücksburg population in culture

In order to investigate if biomass from the unattached Glücksburg population retains its low fertility under culture conditions, thalli were cultivated for 1 year. Four larger black plastic baskets (BAUHAUS; Oase Pflanzkorb; round; diameter: 40 cm; volume: 35 L) were used as cultivation devices and tethered individually to the 2 × 4-m pipe frame using the same floating bodies as in experiment 1 (Fig. 1). The experiment was started on 29 June 2019 with 55–62 g of thalli, which was cultivated until 29 June 2020. Weight, total number of apices, and fertility were recorded regularly. No intermediate harvests were necessary, because the limiting density was not reached in this experiment. Epibionts had to be carefully removed in November 2019, since the thalli were heavily colonized. This was not necessary in experiments 1 and 2.

### Statistics

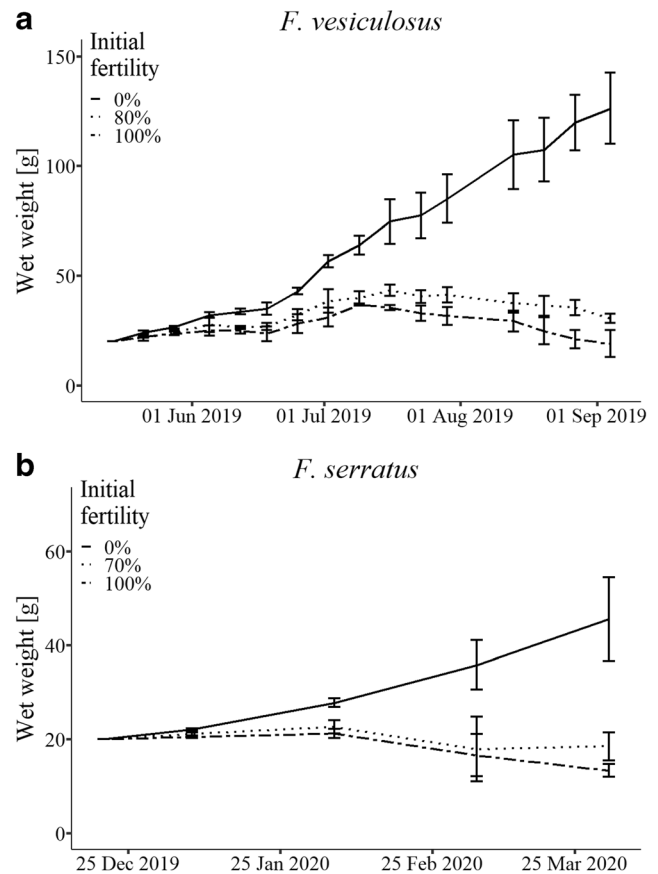
Statistical analysis was performed using the R software package (R Core Team 2013). For experiment 1, weight and fertility of experimental groups at  $t_{\text{end}}$  as well as percentual change of the total number of apices over the experimental time were compared by one-way ANOVA and Tukey's HSD post hoc test. For experiment 2, the fertility of the experimental groups was compared by Welch's two-sample  $t$  test at defined time points of the experimental cultivation period. Experiment 3 did not require statistical analysis. Normality of residuals was tested by Shapiro-Wilk's test and homogeneity of variances was tested by Fligner's test.  $p$  values below 0.05 were considered statistically significant. The detailed results of the statistical analysis are depicted in the supplementary material (Tab. S2–S5).

## Results

### Effect of fertility on growth in culture (experiment 1)

Both species showed strong growth when the initial culture biomass consisted only of vegetative thalli, but showed only marginal growth or even weight loss, when the biomass consisted of thalli with the typical fertility found in attached wild populations (*F. vesiculosus*: 80%, *F. serratus*: 70%), or when the biomass consisted of thalli with 100% fertility (Fig. 3). Detailed results are given in the following:

For *F. vesiculosus*, thalli of the purely vegetative treatment (0% fertility) increased in weight from 20 g to  $126 \pm 16$  g during the experimental time, while thalli in the other two treatments, after an initial weight increase ending at mid of July, showed only slight growth or even weight decreases



**Fig. 3** Effect of fertility status at the beginning of experimental cultivation (termed “initial fertility,” measured as percentage of receptacles of total number of apices) on wet weight of *F. vesiculosus* (a) and *F. serratus* (b). Data displayed as mean  $\pm$  SD,  $N = 3$  baskets. The total number of thallus apices and the fertility during the subsequent cultivation period are shown in the supplementary material (Fig. S1)

ending at  $30 \pm 2$  g for the 80% treatment and at  $19 \pm 6$  g for the 100% treatment (one-way ANOVA of weight at  $t_{\text{end}}$ :  $p = 2.3 \times 10^{-5}$ , significant differences in post hoc Tukey's HSD between 100 and 0%; 80 and 0%, Fig. 3).

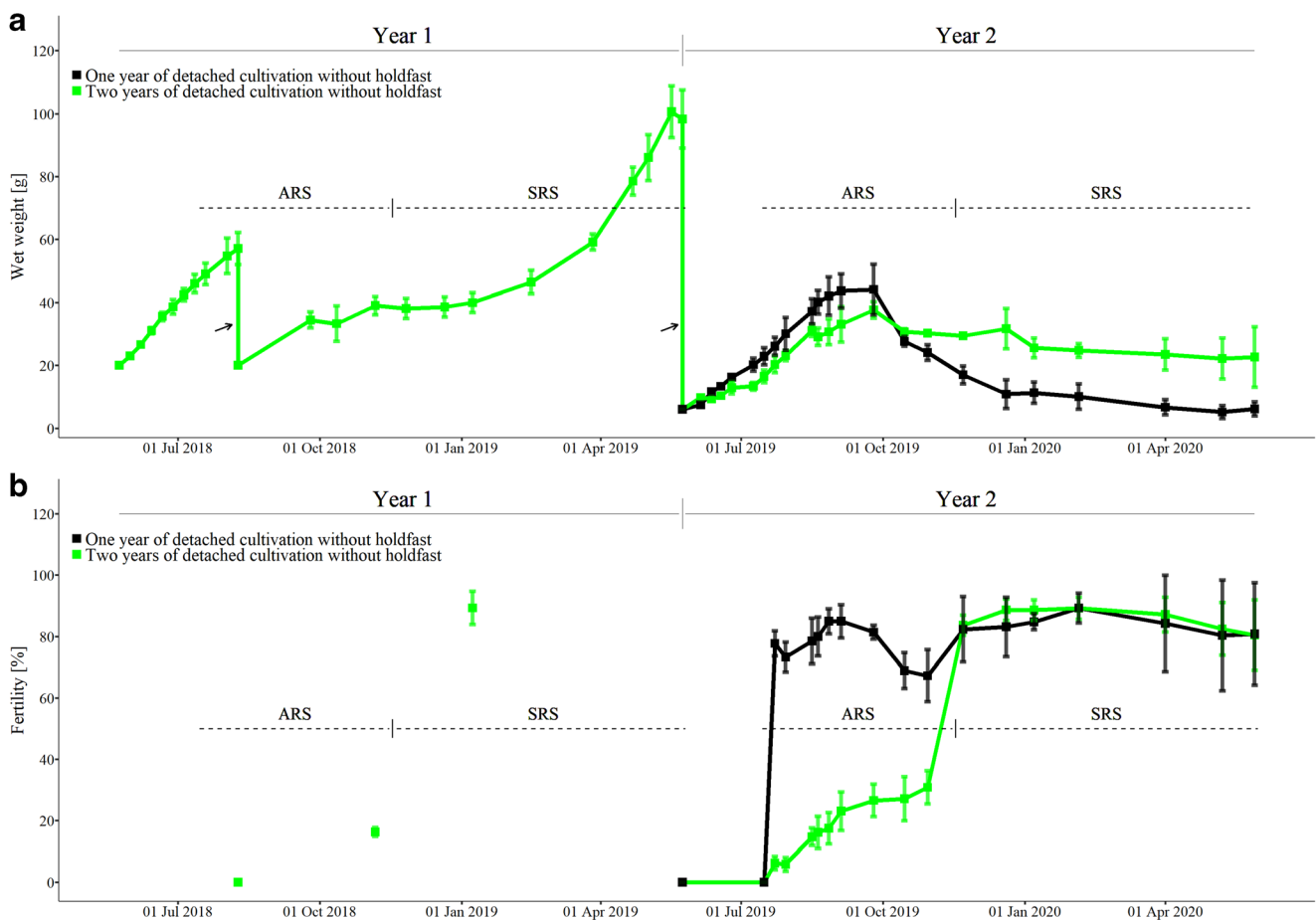
For *F. serratus*, the thalli of the 0% treatment showed a weight increase from 20 to  $42 \pm 9$  g during the time frame of the experiment (Fig. 3). At the same time, the thalli of the 70% treatment decreased in weight from 20 to  $18 \pm 3$  g and the thalli of the 100% treatment from 20 to  $13 \pm 1$  g by slow degradation of the receptacles and the thallus tissue below the receptacles (one-way ANOVA of weight at  $t_{\text{end}}$ :  $p = 0.0021$ , significant differences in post hoc Tukey's HSD between 100 and 0%; 80 and 0%). The weight results of both species were paralleled by the total number of apices (Supplementary material, Fig S1). The fertility during the experiment matched the expectations with an increasing share of vegetative thalli over time in the groups that contained vegetative apices and no change in the 100% group (Supplementary material, Fig. S1).

## Fertility of attached populations under long-term unattached culture (experiment 2)

It was possible to cultivate both *Fucus* species for 2 years as unattached thalli. They remained viable and produced new biomass from the vegetative apices present in the cultures (Figs. 4 and 5). The fertility of both species was not reduced during the 2 years of cultivation. It was statistically equal between year 1 and year 2 of the same culture and between cultures of different ages (first vs. second year in culture) during the same year (Figs. 4 and 5). As it remained at the high levels typical for wild attached populations, no acclimatization towards low fertility, which is typical for wild unattached populations, could be observed. However, for both species, unattached cultivation over time led to disoriented, twisted growth (Fig. 6), resulting in a morphology similar to that of unattached populations in the wild (Norton and Mathieson 1983). In addition, *F. vesiculosus* thalli showed a reduction in width, which has also been observed in wild

unattached populations (Mathieson and Dawes 2001; Mathieson et al. 2006). Species-specific results will be given in the following:

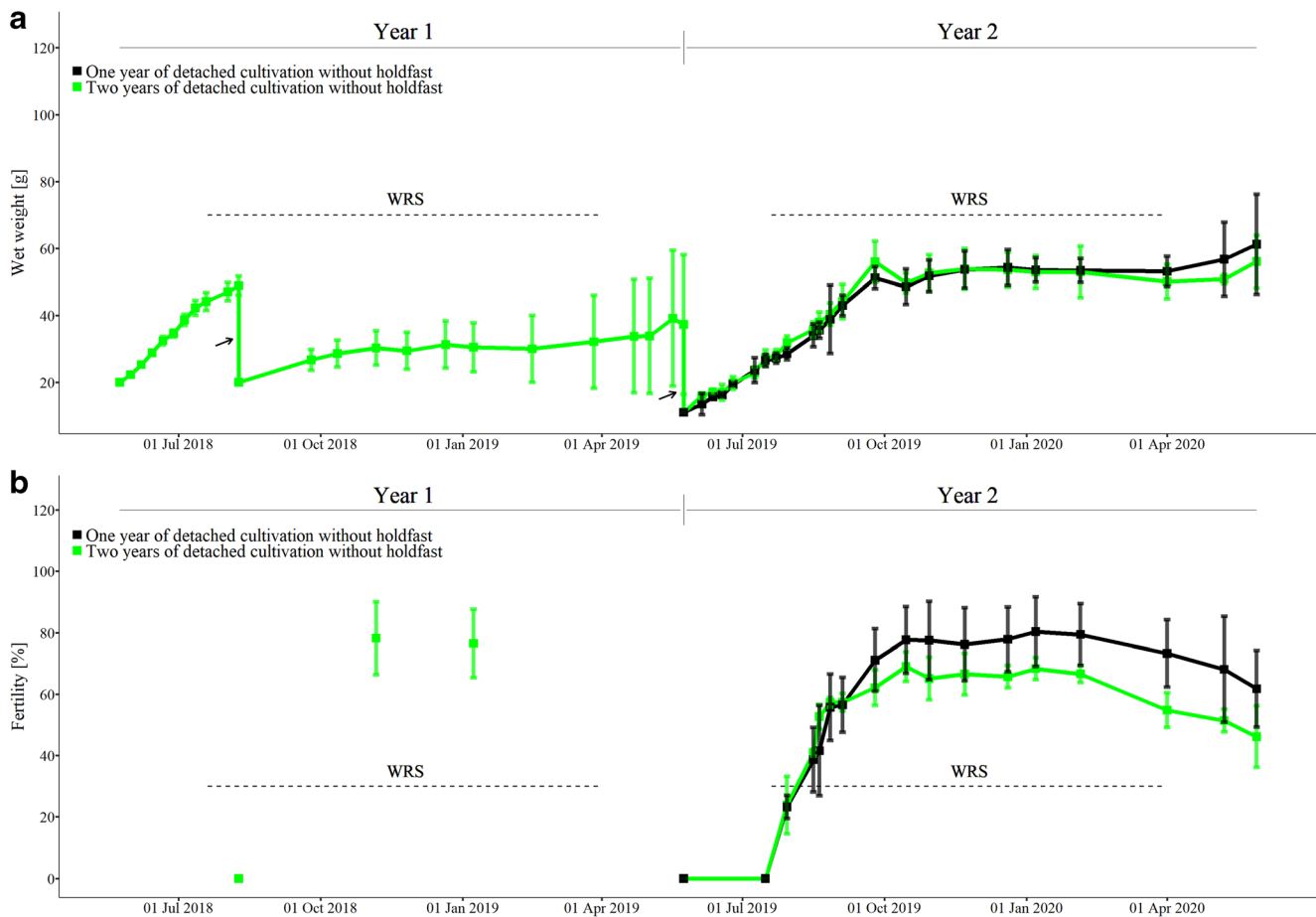
*Fucus vesiculosus* showed fast growth during the first months of cultivation reaching  $57 \pm 5$  g after 2.5 months; therefore, a harvest to 20 g was performed on 9 August 2018 to avoid reduction of growth by density limitation (Fig. 4). During this harvest, which fell in the ARS (autumn reproductive season), all fertile thalli (which were the vast majority but not counted at that point) were removed and the 20 g, which were used for the continuation of the culture, consisted only of vegetative thalli. Thus, the ratio of “spring and autumn bloomers” to “only spring bloomers” in the cultivated biomass was probably shifted from its natural value in the field strongly towards “only spring bloomers.” This was reflected in the fertility during the continuing ARS 2018 as well as during the ARS 2019, when only few sexual organs were formed (6 November 2018:  $16.2 \pm 1.4\%$ ; 30 October 2019:  $30.8 \pm$



**Fig. 4** Unattached cultivation of formerly attached *F. vesiculosus* thalli over 2 years (24 May 2018–29 May 2020). Comparison between first and second year of cultivation of the same thalli (first vs. second year of the light green group) and thalli cultivated unattached for 1 and 2 years at the same time period (black vs. light green group during year 2). **a** Wet

weight. **b** Fertility. During year 1, two intermediate harvests have been performed (09 August 2018, 24 May 2019, indicated by arrows). Data displayed as mean  $\pm$  SD,  $N = 3$  baskets. ARS, autumn reproductive season (July to November); SRS, spring reproductive season (November to May)





**Fig. 5** Unattached cultivation of formerly attached *F. serratus* thalli over 2 years (24 May 2018–29 May 2020). Comparison between first and second year of cultivation of the same thalli (first vs. second year of the light green group) and thalli cultivated unattached for 1 and 2 years at the same time period (black vs. light green group during year 2). **a** Wet

weight. **b** Fertility. During year 1, two intermediate harvests have been performed (09 August 2018, 24 May 2019, indicated by arrows). Data displayed as mean  $\pm$  SD,  $N = 3$  baskets; WRS, winter reproductive season (July to March)

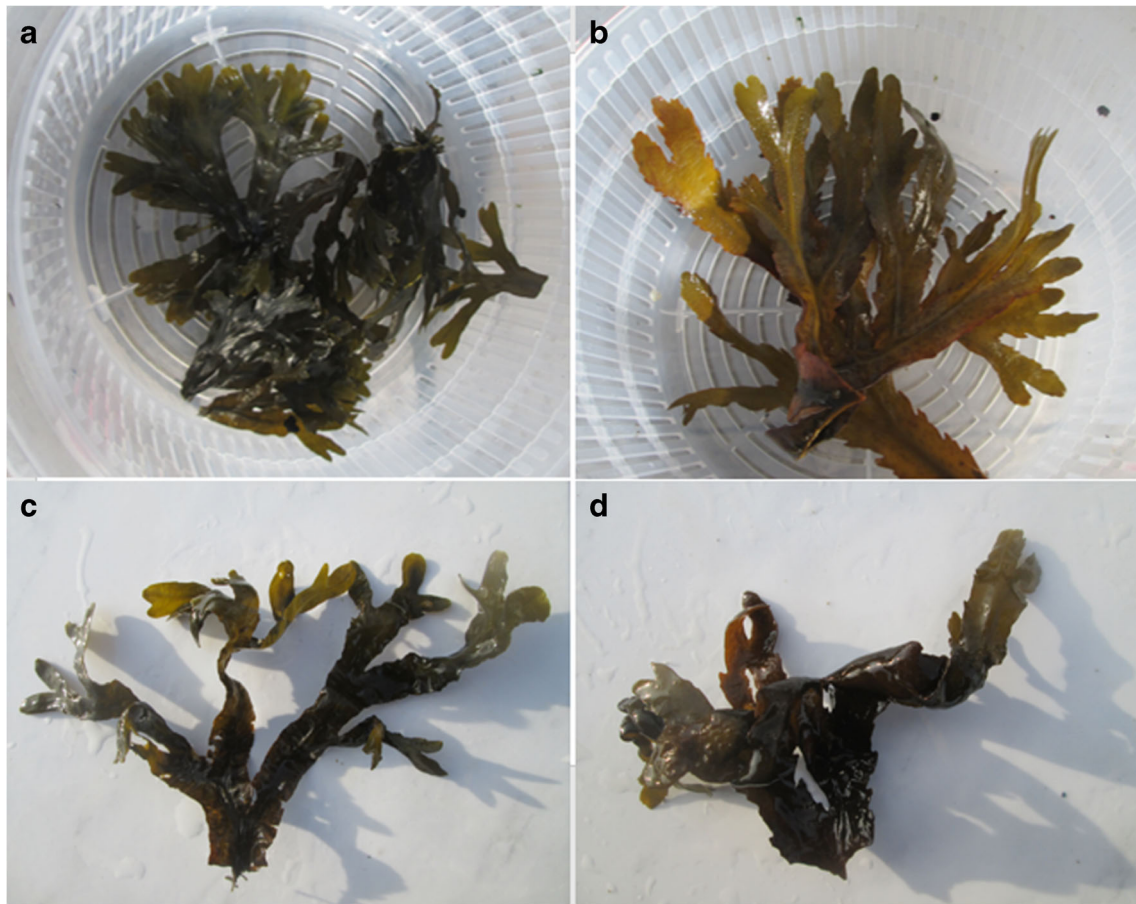
5.4% fertility). The parallel group, which was added in year 2, consisted of the natural mixture between the two genotypes and produced up to  $67.2 \pm 8.5\%$  receptacles during ARS 2019. Thus, the difference in fertility during ARS 2019 has to be considered as artifact caused by the shift of the genotype ratio. This artificial difference led to different growth patterns between the two groups: the thalli in the first cultivation year showed a marked weight decrease after the ARS 2019 due to many degrading receptacles. The thalli in the second cultivation year produced less receptacles during the ARS 2019 and did consequently not show this marked weight decrease (Fig. 4).

A valid comparison of fertility was possible during the SRS 2020, when both genotypes were expected to produce receptacles. During this reproductive season, the fertility of thalli which were in the first year of cultivation (7 January 2020:  $84.8 \pm 2.6\%$ ) was statistically equal to the fertility of thalli which were in the second year of cultivation (7 January 2020:  $88.6 \pm 3.4\%$ ; Welch's two-sample  $t$  test:  $p = 0.203$ ). The group cultivated for 2 years showed

statistically equal fertilities during the SRS of both years (8 January 2019:  $89.3 \pm 5.4\%$ , 7 January 2020:  $88.6 \pm 3.4\%$ ; Welch's two-sample  $t$  test:  $p = 0.445$ ).

The frequent measurements during year 2 revealed very rapid increases in fertility for both groups. The actual fertility increase might have been slightly more gradual, but small differences in the timing of the receptacle initiation of individual apices were not detectable by the measurement method.

For *F. serratus*, no difference in fertility was observed between year 1 and year 2 (6 November 2018 vs. 30 October 2019: Welch's two-sample  $t$  test:  $p = 0.138$ ), as well as between thalli cultured for 1 and 2 years during year 2 (30 October 2019: Welch's two-sample  $t$  test:  $p = 0.234$ , Fig. 5). The typical reproductive season in winter was clearly observable. Although the same harvests as for *F. vesiculosus* were performed during year 1, no artificial change in reproductive behavior could be observed, because *F. serratus* only occurs with one known genotype with respect to the reproductive season. The weight loss due to the degradation of receptacles



**Fig. 6** a, b *F. vesiculosus* and *F. serratus* thalli from the attached origin after collection from the field with typical flat and well-oriented shape. c,

d *F. vesiculosus* and *F. serratus* thalli showing disoriented, twisted shape after ca. 14 months of unattached culture

was partly compensated by new biomass from vegetative apices, which was well visible at the end of year 1 and year 2.

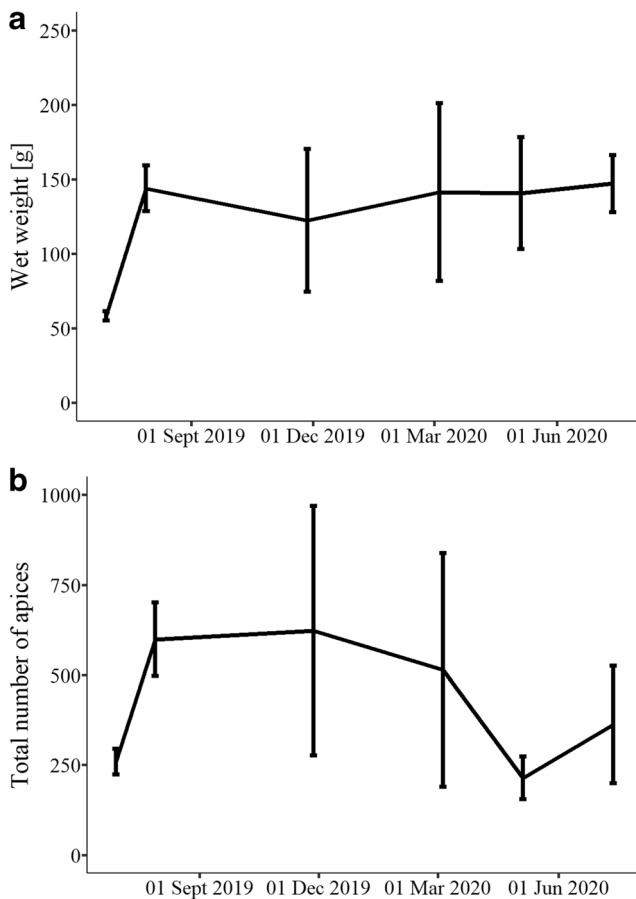
### Fertility of unattached Glücksburg population in culture (experiment 3)

Thalli of the Glücksburg population remained almost entirely infertile during 1 year of cultivation; only very few receptacles were formed during spring 2020 leading to a maximum fertility of  $2.0 \pm 3.1\%$  at 29 June 2020 ( $t_{\text{end}}$ ). Within the first month of cultivation, the weight increased from  $58 \pm 3$  to  $144 \pm 15$  g (Fig. 7a). However, growth did not continue in the following, probably due to decreasing light and temperature in autumn and commencing grazing. Beginning in autumn 2019 and especially during spring 2020, the number of apices was strongly reduced by intense grazing by amphipods (*Gammarus* sp.) and isopods (*Idotea baltica*), which accumulated in the baskets during the experiment (Fig. 7b). As the grazers in most cases consumed only the fresh apical parts including the meristems, this process did not lead to drastic losses of weight but rather limited further growth. The cultures also

suffered from coverage by filamentous diatoms during spring 2020, which might have led to an additional reduction of growth during this time of the experiment. Intact apices continued to produce fresh tissue during the entire experiment, visible by the light green color of newly formed apical tissue. Therefore, a suppressing effect of the general culture conditions (basket, location) on the growth of the thalli can be excluded.

### Discussion

In this study, we confirmed the expectation that the typical fertility of attached *Fucus* populations (*F. vesiculosus*: ~ 80% receptacles, *F. serratus*: ~ 70% receptacles) is detrimental for unattached vegetative cultivation. As expected from wild populations, degrading receptacles caused significant biomass losses in experimental cultures with natural fertility, leading to only little overall biomass gain (*F. vesiculosus*) or even biomass loss (*F. serratus*, Fig. 3.). The observed growth reduction during reproductive phases was more severe than reported for wild *Fucus* plants (e.g., Arrontes 1993; Berger



**Fig. 7** Cultivation of thalli from unattached Glücksburg population over the course of 1 year. **a** Wet weight. **b** Total number of apices. Data displayed as mean  $\pm$  SD,  $N = 4$  baskets. Heavy grazing is visible by a reduction of the total number of apices in spring 2020

et al. 2001). This was probably due to the fact that fronds were used in the experimental cultures, which sometimes degraded completely, while entire *Fucus* plants are able to retain the stipe from which growth can continue (Knight and Parke 1950). Our experiment underlined the necessity of low-fertility biomass for successful vegetative unattached *Fucus* aquaculture.

In experiment 2, we tested if low-fertility biomass can be obtained from attached thalli through long-term acclimatization in unattached culture. Within a 2-year period, no acclimatization of formerly attached thalli towards reduced fertility was observed (Figs. 4 and 5). Therefore, we conclude that it is impossible to acclimate attached *Fucus* populations to low fertility by unattached culture, which is in contradiction to the hypothesis of Maczassek (2014). Maczassek had assumed that the unattached Glücksburg population resulted from the detachment-induced acclimatization of *F. vesiculosus* thalli towards low fertility. Whether other factors present at the growth location of the population (e.g., reduced salinity, low/high nutrients, low oxygen levels, or others) and not detachment alone are causal for a potential acclimatization is

unclear. If so, acclimatization in aquaculture would be complicated, because usually the conditions of the used water body can hardly be changed in the large-scale culture. If the acclimatization process just requires more than 2 years, seedling production would be very ineffective. However, this is unlikely, because no signs for the initiation of an acclimatization process were visible. Other features typical for unattached populations (unoriented growth, reduced size) were induced by unattached culture (Fig. 6). This suggests that these features can be acquired via acclimatization and probably result from the absence of a fixation to the substrate and the concomitant constant orientation in space (Norton and Mathieson 1983).

In experiment 3, we tested if biomass collected from the unattached Glücksburg population retains its low fertility under culture conditions. During 1 year of cultivation, it remained almost entirely receptacle-free (Fig. 7) showing only the minor fertility which is typical for the wild population (pers. observation, Maczassek 2014). Consequently, biomass losses due to receptacle degradation are expected to be very low and generated seedlings have a high probability to contain and retain viable meristems and produce new biomass. Thus, the Glücksburg population seems to be a good candidate for vegetative culture and reproduction of *Fucus*. Based on the morphology of the Glücksburg population, the produced biomass could be considered as *vesiculosus*-type biomass.

However, based on our data, it cannot be guaranteed that the fertility of biomass from the Glücksburg population remains low for several years, since a slow back-acclimatization towards higher fertilities cannot be excluded. Maczassek (2014) found a maximum of 30% fertility for the Glücksburg population in mesocosm experiments, which suggests that higher fertilities are possible. So far, all observed fertilities were not high enough to be detrimental for aquacultural success. A genetic determination of the low fertility of the Glücksburg population would be desirable for the planning of future aquaculture efforts. However, it is impossible to prove a genetic fixation at the current state of knowledge, because the genes responsible for receptacle formation are not known and no *Fucus* genome has been published yet. Even if hybridization or polyploidy would be shown for the Glücksburg population, as have been for other unattached populations by analysis of microsatellites, mtDNA RFLP, and nuclear DNA content (Coyer et al. 2006; Mathieson et al. 2006; Sjøtun et al. 2017), no permanence of low fertility could be guaranteed, because no causal connection between polyploidy/hybridization and low fertility has been shown for *Fucus* species so far. Therefore, further cultivation trials over longer time spans are necessary to ensure the long-term usability of the Glücksburg population. Based on the results presented here, we assume that biomass from the Glücksburg population retains a low fertility also for longer cultivation periods.

As the focus of this study was on fertility, no optimization of growth was aimed at in the experimental culture of biomass from the Glücksburg population. Grazing by *Gammarus* sp. and *I. balthica* was therefore not sufficiently dealt with, which led to only little growth in the second half of the experimental culture year. If this factor had been controlled, continuous growth could have been expected. Grazing on the thalli from the Glücksburg population seemed to be more intense than grazing on thalli from the attached origin. One explanation is that grazers prefer vegetative material and avoid slimy receptacles (Hemmi et al. 2005). Another explanation is that the Glücksburg population has a comparatively poor grazing defense, which is not known so far, but could be tested in grazer preference experiments (Goecker and Käll 2003). A reduction of grazing might be possible by regular air exposure or fresh water treatment of the cultivated biomass. Air exposure can in addition be used to reduce the number of fouling organisms (Meichssner et al. 2020). For commercial-scale culture, we suggest baskets of about 1 m<sup>2</sup> area, similar to baskets for *Eucheuma* cultivation shown in Hurtado et al. (2008). This size would allow for easy handling and air exposures could potentially be performed. Plastic or other solid material should be used for the base of the baskets to create a plane area where the biomass can be well spread. If too flexible material is used, the biomass tends to aggregate at the lowest point, which limits growth (own observation). An alternative option would be cultivation in land-based tanks similar to *Gracilaria* tank cultures (Friedlander and Levy 1995). In this case, grazing could be excluded by pre-filtering the used seawater.

The principle of using unattached *Fucus* populations for vegetative aquaculture can of course be applied at other locations, too. Unattached populations with low fertility are known from other parts of the Baltic Sea as well as from many coasts of the North Atlantic and the North Pacific (Luther 1981; Norton and Mathieson 1983; Neiva et al. 2012). Some populations are genetically affiliated to *F. serratus*, *F. gardneri*, *F. ceranoides*, and *F. spiralis*; thus, biomass other than the *vesiculosus* type can potentially be produced (Norton and Mathieson 1983; Neiva et al. 2012). However, the principle might also be used for the culture of other genera of the Fucales, as unattached populations with low fertility are also known from *Ascophyllum*, *Hormosira*, and *Sargassum* (Norton and Mathieson 1983).

A main concern with the commercial utilization of unattached populations is that they are not very large in most cases and therefore prone to extinction, not only by environmental change but also by random events. Unattached populations from the Baltic Sea, like the one from Glücksburg, are therefore listed by HELCOM as endangered habitats (HELCOM 2013). The continued existence of these populations should therefore always be prioritized before commercial utilization. In our opinion, a use of unattached populations as seedling source could be

approved by governmental authorities, if the following conditions are fulfilled: (1) a maximum of 5–10% of the standing stock is collected and (2) the collection of material is accompanied by regular monitoring of the population, which guarantees the maintenance of the population size. We suggest that only little amounts are collected as stock, from which larger amounts are produced to start commercial culture. Optimally, seedling biomass has to be taken only once and can be generated from culture in subsequent growing seasons.

## Conclusion and outlook

Low fertility has proven to be crucial for unattached vegetative aquaculture of *Fucus* species. Based on our results, biomass with this feature can only be obtained from unattached populations, like the Glücksburg population in the western Baltic Sea. For successful implementation, the fertility has to remain low over consecutive years in culture, and grazing has to be controlled. Both require further studies. The relatively easy cultivation protocol presented here offers not only a possible solution for the production of *Fucus* biomass but could also be applied for the cultivation of other species of Fucales for scientific and commercial purposes.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10811-021-02419-x>.

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**Author contributions** Rafael Meichssner designed and conducted the experiments, performed the statistical analysis, and wrote the manuscript. Peter Krost assisted in data analysis and Rüdiger Schulz in the study conceptualization. Peter Krost and Rüdiger Schulz reviewed and edited the manuscript.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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## Chapter II - Supplementary material

## Supplementary data from experiment 1

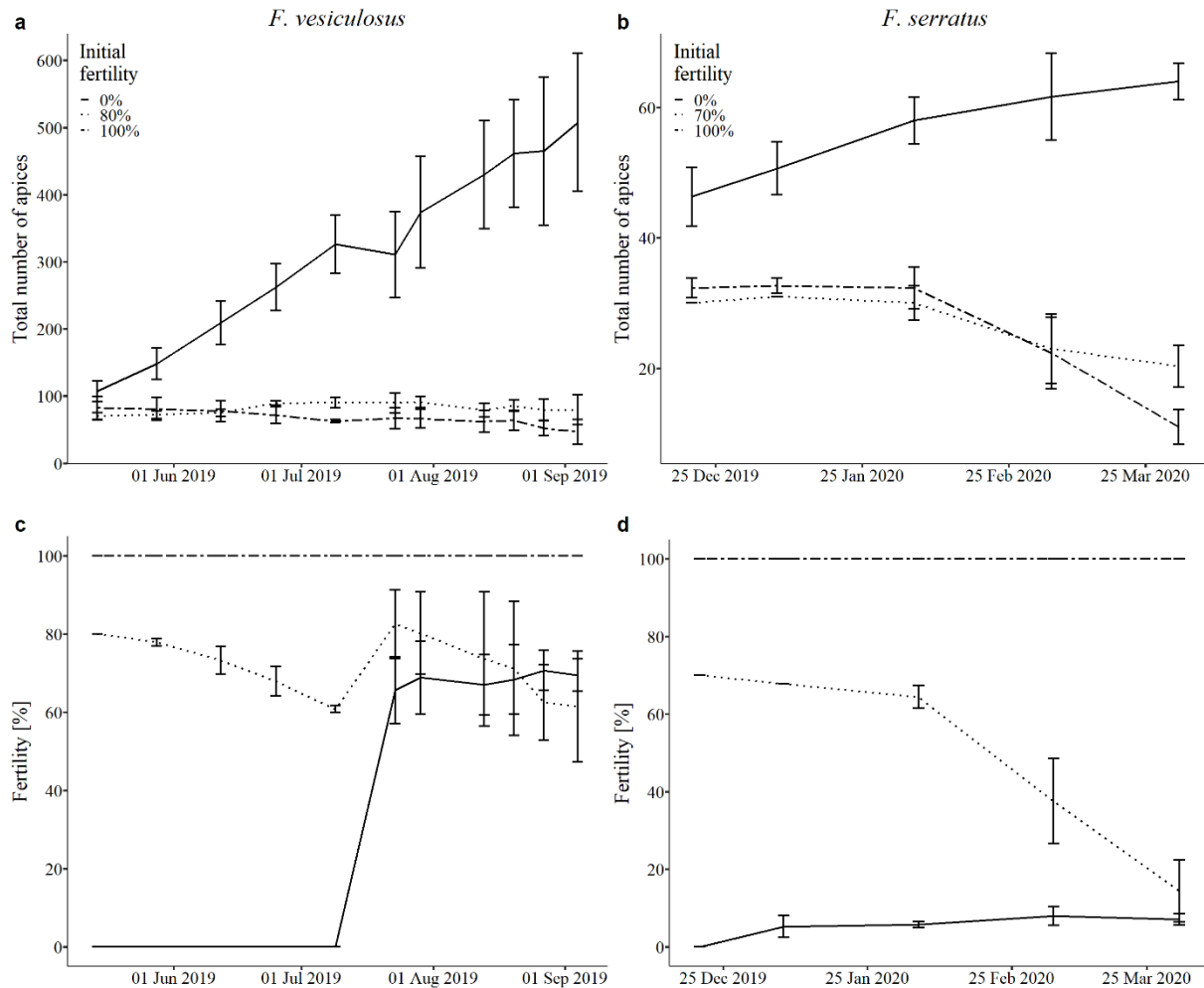


Fig. S1: Effect of fertility status at the beginning of experimental cultivation (termed “Initial fertility”, measured as percentage of receptacles of total number of apices) on total number of apices (a,b) and fertility (c,d) of *F. vesiculosus* (a,c) and *F. serratus* (b,d). Data displayed as mean  $\pm$  SD, N=3 baskets. For *F. vesiculosus*, the fertility increased at the end of July because many vegetative thalli entered into the autumn reproductive season.

Table S1: Change in total number of apices from  $t_0$  to  $t_{end}$  [%] for the experimental cultures of *F. vesiculosus* and *F. serratus* in experiment 1, mean  $\pm$  SD, N=3 baskets. Different letters indicate statistically significant differences (one-way ANOVA and post-hoc Tukey’s HSD). The statistical analysis was performed for each species separately.

	Treatment	Change in total number of apices from $t_0$ to $t_{end}$ [%]
<i>F. vesiculosus</i>	0%	375.5 $\pm$ 76.4 <sup>a</sup>
	80%	12.5 $\pm$ 26.4 <sup>b</sup>
	100%	-43.6 $\pm$ 14.1 <sup>b</sup>
<i>F. serratus</i>	0%	38.5 $\pm$ 12.9 <sup>a</sup>
	70%	-32.2 $\pm$ 10.7 <sup>b</sup>
	100%	-65.8 $\pm$ 9.5 <sup>c</sup>

## Statistical Analysis

### Experiment 1

Table S2: *F. vesiculosus*: results of one-way ANOVA and post-hoc Tukey's HSD.

	Sum of squares	df	Mean Square	F	p
<b>Weight at t<sub>end</sub> (04.9.2019)</b>					
ANOVA	20825	2	10413	102.5	2.3*10 <sup>-5</sup>
Tukey's HSD					
100%-0%					0.0000308
80%-0%					0.0000599
80%-100%					0.3962274
<b>Percent change in total number of apices</b>					
ANOVA	1.4513	2	0.7257	33.91	0.000537
Tukey's HSD					
100%-0%					0.0005011
80%-0%					0.0030114
80%-100%					0.1351001
<b>Fertility at t<sub>end</sub> (04.9.2019)</b>					
ANOVA	2490.9	2	1245.4	17.13	0.00331
Tukey's HSD					
100%-0%					0.0109893
80%-0%					0.5150369
80%-100%					0.0034912

Table S3: *F. serratus*: results of one-way ANOVA and post-hoc Tukey's HSD.

	Sum of squares	df	Mean Square	F	p
<b>Weight at t<sub>end</sub> (01.04.2020)</b>					
ANOVA	1359.7	2	679.8	33.75	0.00125
Tukey's HSD					
100%-0%					0.0023225
70%-0%					0.0063995
70%-100%					0.5518485
<b>Percent change in total number of apices</b>					
ANOVA	13175	2	6587	57.25	0.000358
Tukey's HSD					
100%-0%					0.0002978
70%-0%					0.0018553
70%-100%					0.0275963
<b>Fertility at t<sub>end</sub> (01.04.2020)</b>					
ANOVA	14757	2	7379	284.6	7.07*10 <sup>-6</sup>
Tukey's HSD					
100%-0%					0.0000150
70%-0%					0.3339184
70%-100%					0.0000135

## Experiment 2

Table S4: Results of Welch two-sample tests comparing fertility of the *F. vesiculosus* experimental groups.

	<b>t</b>	<b>df</b>	<b>p</b>
Year 1 vs. Year 2 of the same culture (08.01.2019 vs. 07.01.2020)	0.19221	3.3733	0.8586
1 <sup>st</sup> year in culture vs. 2 <sup>nd</sup> year in culture during the same time point (08.01.2020)	1.5392	3.7805	0.2026

Table S5: Results of Welch two-sample tests comparing fertility of the *F. serratus* experimental groups.

	<b>t</b>	<b>df</b>	<b>p</b>
Year 1 vs. Year 2 of the same culture (06.11.2018 vs. 30.10.2019)	1.6393	3.2223	0.1934
1 <sup>st</sup> year in culture vs. 2 <sup>nd</sup> year in culture during the same time point (30.10.2019)	-1.4753	3.0934	0.2339

**Chapter III –  
Modelling of annual yield in vegetative  
*Fucus* aquaculture based on experimental  
testing of density- and season-dependent  
growth**



## Modelling of annual yield in vegetative *Fucus* aquaculture based on experimental testing of density- and season-dependent growth

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### Abstract

In the Kiel fjord, western Baltic Sea, an experimental culture of *Fucus vesiculosus* and *Fucus serratus* has been established in order to develop a sustainable method for biomass production of these species. The cultivation method includes the unattached rearing of thalli in drifting baskets and their vegetative reproduction by cutting of small vegetative apices. In this study, we modelled growth with this cultivation method over one year based on experimental growth data for different seasons and for different initial stocking densities. Using the modelling approach, it was possible to compare different cultivation scenarios and address the following questions: How much annual yield can be expected? Which combination of initial stocking density and harvest density is optimal and generates maximum annual yields? How many harvests are required per year in different cultivation scenarios?

For *F. vesiculosus*, the model calculated a maximum annual yield of 7.71 kg m<sup>-2</sup> for a cultivation from an initial stocking density of 2 kg m<sup>-2</sup> to a harvest density of 3.25 kg m<sup>-2</sup>, requiring 6 harvests per year. For *F. serratus*, the calculated maximum annual yield was 6.35 kg m<sup>-2</sup> for a cultivation from an initial stocking density of 3.75 kg m<sup>-2</sup> to a harvest density of 5 kg m<sup>-2</sup>, requiring 5 harvests per year. For both species, the model calculated similar annual yields for a lower number of harvests at slightly changed combinations of initial stocking density and harvest density. Higher growth rates measured in other studies suggest that even higher yields are possible under improved cultivation conditions. In a first practical upscaling attempt, we could show that growth rates in 1.2-m<sup>2</sup> net cages are similar to growth rates in the used small-scale experimental cultures, which suggests that the model results are applicable to commercial-scale culture.

We conclude that the modelling results offer a valid and helpful orientation for future efforts to produce *Fucus* species in commercial culture. Possible improvements of the used culture conditions and solutions for commercial-scale culture are discussed.

### Introduction

Today's world-wide seaweed production is dominated by farmed biomass with 97.1% of the total marketed biomass (32.4 million t) origination from aquaculture (FAO 2020). Yet, a variety of species is still only produced by harvesting of wild stocks (Mac Monagail et al. 2017, Lotze et al. 2019, netalgae.org). These species are often used for low-volume products like specialized cosmetics, food supplements, biostimulants etc. (Zemke-White & Ohno 1999, Lotze et al. 2019, netalgae.de). The two brown algal species *Fucus vesiculosus* and *Fucus serratus* are typical examples for this practice. Collection takes place in the intertidal zone of the coasts of France, Ireland and other countries at the North Atlantic coast and the biomass is sold frozen or dried to processing companies (Zemke-White & Ohno 1999, netalgae.org). As typical products, cosmetic extracts and food supplements are fabricated from *Fucus* biomass due to its high content of bioactive ingredients like phlorotannins and fucoxanthin (Caterino et al. 2017, Ferreira et al. 2019, Torres et al. 2020). However, harvesting of wild *Fucus* stocks is not possible everywhere; in the Baltic Sea, *Fucus* stocks have experienced a dramatic decline in the second half of the 20<sup>th</sup> century, which has been attributed mainly to eutrophication and stone fishery (Kautsky et al. 1986, Vogt & Schramm 1991, Rohde et al. 2008). As a consequence, the stocks of the German Baltic Sea coast are protected by nature protection legislation and their harvesting for

commercial purposes is prohibited (Bundesamt für Naturschutz 2013). However, a regional production in the Baltic Sea would be advantageous for German companies, as “regionality” is a desired claim in the targeted markets (L. Piker, pers. comm.). Under these circumstances, aquaculture is the only legal means for biomass production in the Baltic Sea. Aquaculture of *Fucus* species might also be preferable in other regions where wild populations are increasingly threatened by environmental change, like the Atlantic coasts of southern Europe (Jueterbock et al. 2013, Nicastro et al. 2013)

Hitherto, no commercial *Fucus* farm exists worldwide and only little related literature is published (Haglund & Pedersen 1988, Ryzhik et al. 2014, Meichssner et al. 2020, 2021). Therefore, in 2015 an experimental *Fucus* aquaculture has been established in the Kiel fjord, western Baltic Sea, aiming at the development of a sustainable and regional production method for *F. vesiculosus* and *F. serratus*. The following approach has been chosen for the experimental cultivation: thalli (fronds cut above the stipe) are reared unattached in baskets or net cages installed in the fjord. After a growth period, the thalli are harvested and parts of the harvested thalli (vegetative apices) are used as seedlings for the next growing season, i.e. vegetative reproduction. Thus, the intended method is similar to *Kappaphycus/ Eucheuma* basket culture in Indonesia (Hurtado et al. 2014). Meichssner et al. (2021) could show, that the method only works, if the culture biomass retains low fertility, because fertile apices (receptacles) degrade after gamete shedding, which causes severe biomass losses. The necessary low-fertility biomass can be achieved by using unattached *Fucus* populations with naturally low fertility as initial source of seedlings (Meichssner et al. 2021). Besides, a successful method for the reduction of fouling on the culture biomass has been developed (Meichssner et al. 2020).

However, so far it is unclear, how much annual yield can be expected from this cultivation method. It is further unknown, which initial stocking density and which harvest density should be chosen in order to obtain maximum yields. Therefore, we aimed at an estimation of the annual yield for different combinations of initial stocking density and harvest density. For this purpose, growth rates at different seasons and different initial stocking densities were examined in small-scale culture experiments (“Seasonal growth experiment”, “Density experiment”). Based on the results, growth in culture was modelled over the course of one year for different initial stocking densities and different harvest densities in order to answer the following questions: 1) What is the maximum annual yield? 2) Which initial stocking density and which harvest density has to be chosen to reach the maximum annual yield? 3) How many harvests have to be performed in the different cultivation scenarios?

Finally, we tested if the growth rates, which have only been measured in small-scale experiments (20x20cm baskets), are transferable to commercial-scale net cages (1,2 m<sup>2</sup>, “Upscaling experiment”). If so, the model results can be used as an estimation of yields in commercial-scale culture cages.

## Materials and methods

### Location of the experimental farm

The cultivation experiments were conducted at premises of Kieler Meeresfarm GmbH & Co KG at the northwestern shore of the Kiel fjord in the western Baltic Sea. During the main part of the Density experiment in 2017, the experimental farm was located at 54.3758 N, 10.1620 E, later it had to be moved about 700m along the shore to 54.3820 N, 10.1620 E, where all other experiments took place (see below). The moving of the farm was performed during a time when no experiments were conducted.

### Algal material

All experiments were conducted for both *Fucus* species (*F. vesiculosus*, *F. serratus*) in parallel. *F. vesiculosus* was collected from Kiel Holtenau (54.3690 N; 10.1541 E) and *F. serratus* from Bülk (54.4552 N; 10.1989 E). A sufficient number of individuals (>5) was collected to achieve genotypically mixed experimental biomasses. The collected individuals were transported as whole plants in sea water to the experimental site where they were stored in buckets overnight. The next day, fronds of 3-10cm (*F. vesiculosus*) and 5-15cm (*F. serratus*) were cut from the plants (referred to as “thalli” in the following) and used for the start of the experimental cultures. The size range is typical for fronds found in the field when cut above the stipe. In order to ensure an even distribution of thallus sizes and genotypes among the experimental units, a bulk of thalli was prepared from which random portions of the desired weight were taken to inoculate the experimental units. In a parallel experiment (results published in Meichssner et al. 2020), this method for the assembly of biomass yielded  $4.5 \pm 1.3$  thalli per 10g of experimental biomass with  $43 \pm 7$  meristems for *F. vesiculosus* (N=14) and  $3.4 \pm 0.7$  thalli per 10g of experimental biomass with  $15 \pm 2$  meristems for *F. serratus* (N=9). The maximum observed relative deviation of the normalized meristem number (meristems (10g biomass)<sup>-1</sup>) from the mean was 27% for *F. vesiculosus* and 12% for *F. serratus*. Deviations of single replicates from the mean number of meristems seemed to have no significant impact on the growth behavior: no correlation was observed between the relative deviation of the normalized meristem number from the mean of the treatment group and the relative deviation of the relative daily growth rates, measured over the following 14 days, from the mean of the treatment group (see Supplementary material, Fig. S1). Thus, this method of assembling the experimental biomass is in our opinion close to aquacultural practice and allows for valid measurements of growth at the same time. The size difference between *F. vesiculosus* and *F. serratus* was accepted as no direct comparison between the species was intended.

For the Seasonal growth experiment and the Density experiment, only vegetative thalli were used. For the Upscaling experiment, the natural mixture of vegetative and fertile thalli found in the field populations was used, because plenty of biomass was needed. As reproductive thallus apices degrade after gamete shedding (Knight & Parke 1950), a mixture of reproductive and vegetative thalli has a lower growth rate than purely vegetative biomass (Meichssner et al. 2021). Thus, the growth rates in the Upscaling experiment were probably not optimal, but optimal growth was not needed here, since the objective of this experiment was only the comparison between small-scale and commercial-scale culture cages. Meichssner et al. (2021) have shown that low-fertility biomass from unattached *Fucus* populations is needed for successful long-term cultivation, because fertility-related biomass losses are otherwise too strong. Unfortunately, the experiments for the present study were initiated before the results of Meichssner et al. (2021) were obtained. Therefore, the experiments presented here were still conducted with biomass originating from attached populations. However, as only vegetative apices were used in the Seasonal growth experiment and the Density experiment, and the experimental time was very short, i.e. only beginning receptacle initiation and no receptacle degradation did occur during the experiments, we consider the growth in these experiments as vegetative and thus comparable to growth of biomass from unattached low-fertility populations.

### Cultivation baskets

For the Seasonal growth experiment and the Density experiment, black plastic baskets (BAUHAUS; Oase Pflanzkorb; edge length: 28cm; volume: 14l) were used for cultivation, which allowed for water inflow by holes of ca. 3x3mm size (Fig 1a). The baskets were covered with transparent plastic nettings to avoid the loss of culture material. Eight baskets were connected to packages of 3 x 3 baskets with the central position left empty. The packages were inserted into white boxes (glass fiber reinforced plastic, shape: turned truncated pyramid, upper opening: 80x80cm, basal area: 64x64cm, height: ca.

40cm, Fig. 1b), which were deployed in the fjord using the gaps of a Jetfloat® system (Jetfloat® International, double elements: 100x50x40cm, single elements: 50x50x40cm, Fig. 1c). The white boxes had four side windows (35x37cm) allowing for water inflow. Using this construction, the baskets were lowered to a depth of ca. 10cm into the fjord, resulting in an 20x20cm submerged cultivation area per basket. The position of the baskets within the white boxes was changed regularly to avoid position effects. Six white boxes were available, yielding 48 baskets as experimental units.

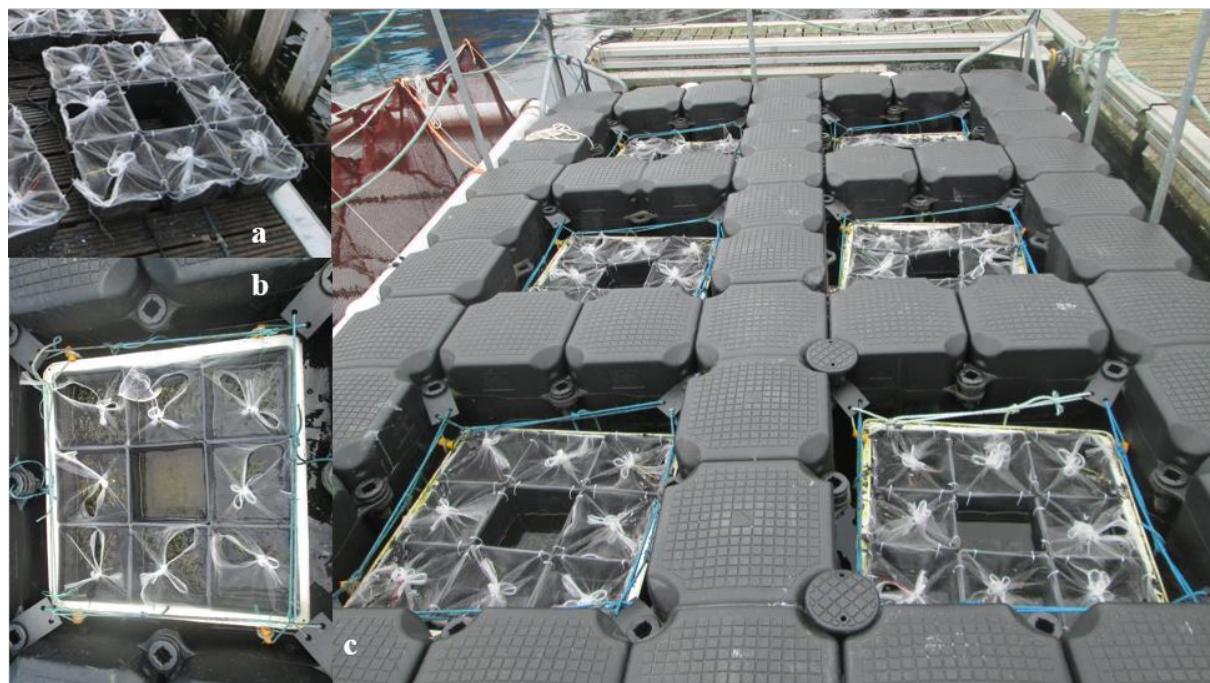


Fig. 1: Experimental cultivation baskets used in the Seasonal growth experiment, the Density experiment, and as control group in the Upscaling experiment. Eight baskets were connected to one package (a), and inserted in a white box (b), which allowed for water flow through by four side windows. Six boxes equipped with cultivation baskets were installed in the fjord by help of a Jetfloat®-system (c).

#### Weight measurements

In all experiments, growth was measured as change in wet weight. All weights given in the following represent wet weights. For comparative purposes, the following dry matter contents can be assumed: *F. vesiculosus*:  $21 \pm 2\%$ , *F. serratus*:  $24 \pm 1\%$  (own pre-experiments). Wet weight measurements were performed with a lab scale (Kern EMB 1200) under wind protected conditions at the experimental site. Before weighing, the thalli were spun with a salad spinner for 15s in portions of maximum 150g to remove attached water.

#### Environmental data

Environmental data are provided for the Seasonal growth experiment. Water temperature within the cultivation baskets was logged every 60min with a Hobo® pendant data logger. Daily totals of photosynthetically active photon flux density (PPFD) were recorded in the Botanical garden of Kiel University, 4.87 km from the experimental site as described in Pescheck and Bilger (2019) and Meichsner et al. (2020). The mesh used to cover the baskets reduced the incoming irradiance by  $4 \pm 2\%$ . This mesh-related irradiance reduction increased to  $12 \pm 4\%$  after 3-4 weeks when the mesh was colonized by microalgae. Nutrient data (concentrations of  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  in 1m depth) from the Mönkeberg measurement station (ca. 3 km from the cultivation site within the Kiel fjord: 54.3538

N; 10.1647 E) were kindly provided by LLUR (Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig-Holstein). Additional nutrient data (concentrations of  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  in 1m depth) with a slightly higher temporal resolution were available from the Boknis Eck time series station (ca. 30 km from the cultivation site in the Kiel bay: 54.5167 N; 10.0333 E, Lennartz et al. 2014). They were kindly provided by Kastriot Qelaj and Hermann Bange. Even though the actual concentrations at the experimental site might vary slightly from the concentrations measured at Mönkeberg and Boknis Eck, the data provide a good indication of the ambient nutrient dynamics, nutrient availabilities and potentially limiting nutrient species. The environmental data are shown in the Supplementary material (Figs. S2, S3, Tabs. S1, S2).

#### Seasonal growth experiment

Growth was measured over one year (15 December 2018 – 15 December 2019) in eight periods of ca. 1.5 months each. The cultures of each period (N=3) were started with 20 g thalli basket<sup>-1</sup> (i.e. 0.5 kg m<sup>-2</sup>). The relative daily growth rates over the 1.5 months were calculated by the following formula:

$$\text{RDG} = \ln(D_t/D_0) * (100/t) \quad (\text{Equation 1})$$

RDG: relative daily growth rate in percent day<sup>-1</sup>, D<sub>t</sub>: density at a given date in kg m<sup>-2</sup>, D<sub>0</sub>: initial stocking density in kg m<sup>-2</sup>, t: number of days between D<sub>0</sub> and D<sub>t</sub>

#### Density experiment

Eight different initial stocking densities (40, 80, 100, 120, 140, 160, 180, 200 g basket<sup>-1</sup>, N=3) were compared corresponding to 1, 2, 2.5, 3, 3.5, 4, 4.5 and 5 kg m<sup>-2</sup>. The experiment lasted from 24 May 2017 to 04 July 2017. As only 48 experimental units were available, three more initial stocking densities were tested in the same time frame in 2018 (0.125, 0.25, 0.5kg m<sup>-2</sup> resp. 5, 10, 20g basket<sup>-1</sup>) to widen the range of tested densities. Since the experimental farm had to be moved between 2017 and 2018 (see above), location and cultivation year of these three groups differed from those of the other eight groups. Nonetheless, the data of the eleven groups were combined to one dataset and analyzed together, because the two locations were in the same water body and the data of the two years complemented each other as expected (see “Results”).

For all groups, relative daily growth rates (RDG) were calculated over the entire experimental time (41 days) using Equation 1, in addition, the yield (Y) in kg m<sup>-2</sup> was calculated:

$$Y = D_t - D_0 \quad (\text{Equation 2})$$

#### Fitting of data from the Density experiment

Fitting was performed with the program Qtiplot using the Levenberg-Marquardt method (Kelley 1999). There are at least three reasonable options for the fitting of RDG-D<sub>0</sub> relationships: First, a fit equation derived from the traditional yield-density equation for land plants (Li et al. 2016, see Supplementary material), which assumes that the maximum RDG at x=0 is approached in a steep manner and the minimum RDG at high densities in a rather asymptotic manner; second, a linear fit equation; third, a sigmoidal fit equation assuming that the highest RDGs at low densities as well as the lowest RDGs at high densities are approached in an asymptotic manner. All three options yielded similar R<sup>2</sup>-values (*F. vesiculosus*: 0.91-0.93, *F. serratus*: 0.84-0.86). A sigmoidal fit equation seemed in our opinion most appropriate, due to the following reasons: i) RDGs from 0.125 kg m<sup>-2</sup> to 1 kg m<sup>-2</sup> were not significantly different for both *Fucus* species (one-way ANOVA over all density groups: p<0.0001, df=10; Tukey’s HSD for all possible group comparisons between 0.125 and 1 kg m<sup>-2</sup>:



$p > 0.05$ ), probably because there was no relevant competition at low densities. This corresponds to a sigmoidal approximation towards  $x = 0 \text{ kg m}^{-2}$ . ii) RDGs from  $3.5 \text{ kg m}^{-2}$  to  $5 \text{ kg m}^{-2}$  were not significantly different for both *Fucus* species (Tukey's HSD for all possible group comparisons between  $3.5$  and  $5 \text{ kg m}^{-2}$ :  $p > 0.05$ ). This corresponds to a sigmoidal approximation towards a theoretical infinite density. iii) The RDG cannot be  $0 \text{ \% d}^{-1}$  or lower at a theoretical infinite density and should asymptotically approach a minimum at high densities. Therefore, we decided to use a sigmoidal Boltzmann fit equation for the RDG- $D_0$  relationship:

$$\text{RDG} = A_2 + (A_1 - A_2) / (1 + \exp((D_0 - x_0) / dx)) \quad (\text{Equation 3})$$

$A_1$  represents the initial value, i.e. the fitted maximum RDG at the minimum density,  $A_2$  represents the final value, i.e. the fitted minimum RDG at the maximum density.  $x_0$  is the turning point, i.e. the density at which the change in RDG is highest,  $dx$  is the time constant, it can be used to calculate the slope at  $x_0$  by the following formula:  $\text{slope} = (A_1 - A_2) / 4dx$ .

For fitting the yield-density relationship, we used an equation deduced from the fit equation for the RDG (Equation 3). For this purpose, Equation 3 was inserted into the following Equation 4, which describes the calculation of the yield from the RDG:

$$Y = D_0 * \exp((100/t) * \text{RDG}) \quad (\text{Equation 4})$$

In case of the Density experiment,  $t = 41$  days. The resulting fit equation for the yield-density relationship was:

$$Y = D_0 * \exp((100/41) * (A_2 + (A_1 - A_2) / (1 + \exp((D_0 - x_0) / dx)))) \quad (\text{Equation 5})$$

In this case, the parameters ( $A_1$ ,  $A_2$ ,  $x_0$ ,  $dx$ ) do not relate to any specific growth features of the cultivated seaweed anymore.

Fitting of the RDG- $D_0$  relationship and yield- $D_0$  relationship with a traditional equation for land plants (see above) is shown in the Supplementary material (Fig. S4).

### Modelling of annual yield

Annual yield was modelled in Microsoft Excel for the period 15 December 2018 to 15 December 2019 (parallel to the Seasonal growth experiment). To create model scenarios, initial stocking densities ( $0.25$ - $4.75 \text{ kg m}^{-2}$ , intervals of  $0.25 \text{ kg m}^{-2}$ ) and harvest densities ( $0.5$ - $5 \text{ kg m}^{-2}$ , intervals of  $0.25 \text{ kg m}^{-2}$ ) were defined. For every possible combination of initial stocking density and harvest density, the annual yield as well as the required number of harvests was modelled. For this purpose, the density for each single day of the year was modelled using the following formula:

$$mD_t = D_{t-1} * (1 + (z * m\text{RDG}_{t-1})) \quad (\text{Equation 6})$$

$mD_t$  is the modelled density at a given day,  $D_{t-1}$  is the density of the day before,  $m\text{RDG}_{t-1}$  is the relative daily growth rate for  $D_{t-1}$ , which was calculated by inserting  $D_{t-1}$  into equation 3.  $z$  is a correction factor, which corrects  $m\text{RDG}_{t-1}$  for the specific period of the year. It was calculated as ratio of the RDG measured during this specific period of the year in the Seasonal growth experiment (used initial stocking density:  $0.5 \text{ kg m}^{-2}$ ) and the modelled RDG for a  $D_0$  of  $0.5 \text{ kg m}^{-2}$ .

If  $mD_t$  reached the defined harvest density, the density of the next day was set to the defined initial stocking density, which was considered as harvest. The annual yield was then calculated by the following formula:

$$\text{Annual yield} = \text{No. of harvests} * (dD_h - dD_0) + (mD_{365} - dD_0) \quad (\text{Equation 7})$$

$dD_h$  is the defined harvest density,  $dD_0$  is the defined initial stocking density,  $mD_{365}$  is the modelled density of day 365 of the modelled year.

#### Upscaling experiment

To check if growth parameters observed in small scale experiments do apply for commercial scales, growth in the abovementioned small cultivation baskets was compared to growth in 1.2-m<sup>2</sup> net cages. For this purpose, rectangular net cages (base area: 1.2x1m, height: 1m) were constructed, using a scaffold of steel threaded rods and aquaculture nets with a mesh size of 0.8cm. The net cages were attached to a 2x6m frame of plastic tubes as floating aid (Fig. 2). Thalli were cultivated in parallel in the net cages (N=3) and the small cultivation baskets (N=5) starting with a density of 0.5 kg m<sup>-2</sup> from 04 October 2018 to 16 November 2018. RDG was calculated as described above.



Fig. 2: Net cages (1.2 m<sup>2</sup> area) used in the upscaling experiment.

#### Statistical analysis

Statistical analysis was performed with R (R Core Team 2013). For the Seasonal growth experiment and the Density experiment, the RDGs of the groups were compared by one-way ANOVA and post-hoc Tukey's HSD test (in part to validate the used fitting method in the Density experiment, see above). For the Upscaling experiment, the RDGs of the two groups were compared by Welch's two-sample t-test. Normality of residuals was tested by Shapiro-Wilks test and homogeneity of residuals by Fligner's test.

## Results

### Seasonal growth experiment

*F. vesiculosus* showed a typical seasonal growth curve with highest RDGs in summer ( $2.43 \pm 0.35$  %  $d^{-1}$  in July) and lowest RDGs in winter ( $0.33 \pm 0.09$  %  $d^{-1}$  in January, one-way ANOVA over all groups:  $p < 0.0001$ ,  $df=7$ , Fig. 3). *F. serratus* also showed seasonal differences (one-way ANOVA over all groups:  $p < 0.0001$ ,  $df=7$ ), but without a pronounced peak in summer. In contrast, average growth rates were rather constant from April to October (Tukey's HSD:  $p > 0.05$  for all possible group comparisons between April and October) and ranged between  $1.38$  %  $d^{-1}$  and  $1.46$  %  $d^{-1}$ . The lowest growth rates of *F. serratus* were observed in January ( $0.34 \pm 0.03$  %  $d^{-1}$ ).

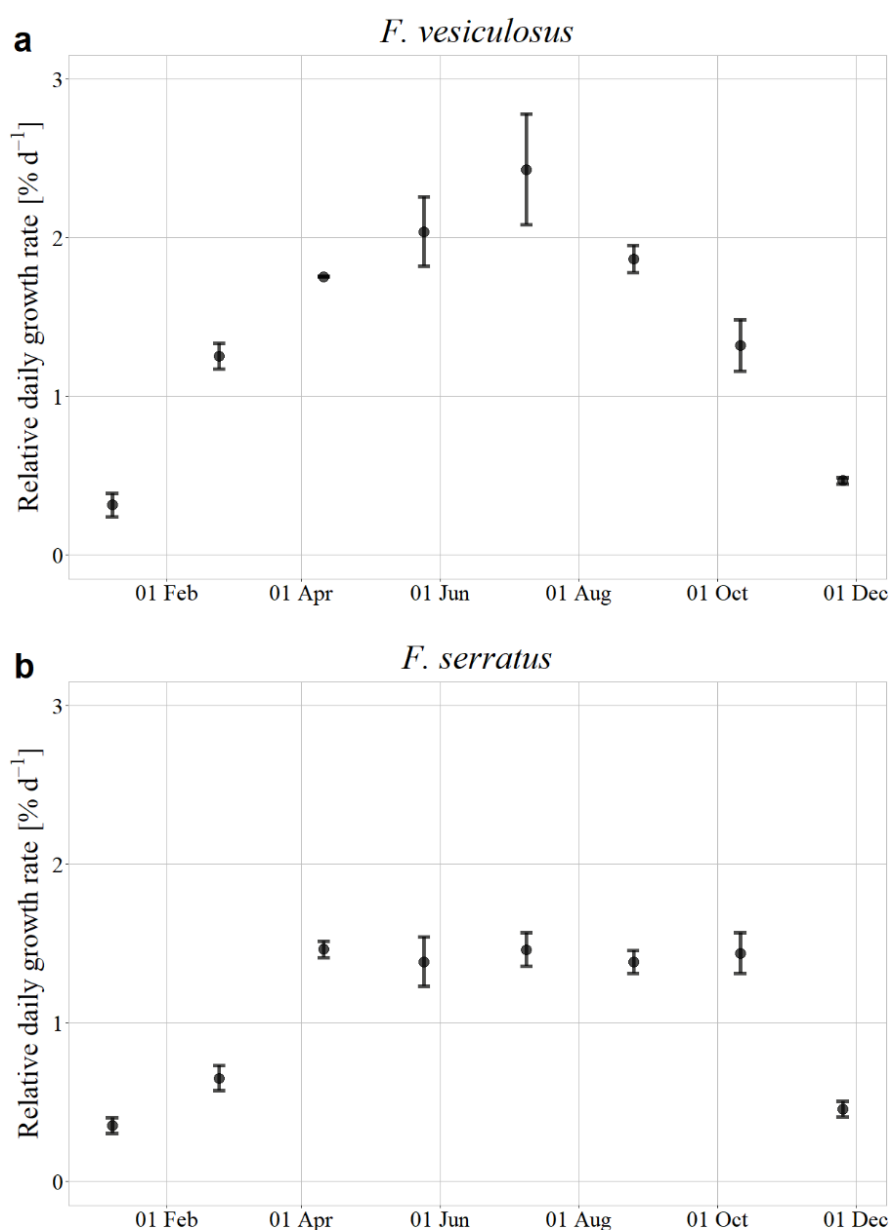


Fig. 3: Relative daily growth rate in %  $d^{-1}$  of *F. vesiculosus* (a) and *F. serratus* (b) in culture over the course of one year (15 December 2018 – 15 December 2019), measured in eight intervals of ca. 1.5 months. The RDG was measured for an initial stocking density of  $0.5$   $kg\ m^{-2}$ , it is displayed as mean  $\pm$  SD ( $N=3$ ) at the middle day of each cultivation period.

### Environmental data during Seasonal growth experiment

During the Seasonal growth experiment, the water temperature varied between a short-time minimum of 0°C in February 2019 and a short-time maximum of 26°C in July 2019 (Supplementary material, Fig. S2). The daily total of the photosynthetically active photon flux density (PPFD) varied between 0.7 mol m<sup>-2</sup> d<sup>-1</sup> at 16 November 2019 and 60.1 mol m<sup>-2</sup> d<sup>-1</sup> at 22 June 2019 (Supplementary material, Fig. S3). Nutrient concentrations of the sea region showed the typical annual cycle with maximal concentrations in winter and lower concentrations in summer (Supplementary material, Tabs. S1, S2). N:P ratios of the measured inorganic nutrient species varied between 0.7 and 15.2 (with one outlier of 82) which indicates that in case of nutrient limitation, nitrogen would be the limiting element (N:P ratio of uptake: 30-40 (Gordillo et al. 2002), tissue N:P ratio: ca. 30 (Harrison and Hurd 2001, Pedersen et al. 2010)). This corresponds to the general observation that nitrogen is the limiting nutrient for seaweed growth in temperate sea regions (Hurd et al. 2014, Roleda and Hurd 2019).

### Density experiment

The average measured RDGs decreased with increasing initial stocking density for both species, for *F. vesiculosus* from 2.04 ± 0.25 % d<sup>-1</sup> at 0.125 kg m<sup>-2</sup> initial stocking density to 0.38 ± 0.23 % d<sup>-1</sup> at 5 kg m<sup>-2</sup> initial stocking density, and for *F. serratus* from 1.67 ± 0.22 % d<sup>-1</sup> to 0.54 ± 0.01 % d<sup>-1</sup> (Fig. 4 a, b). This stronger decrease for *F. vesiculosus* was reflected in a significantly steeper fit curve than for *F. serratus*. The obtained fit parameters and R<sup>2</sup>- values of the fit curves are given in Fig. 4.

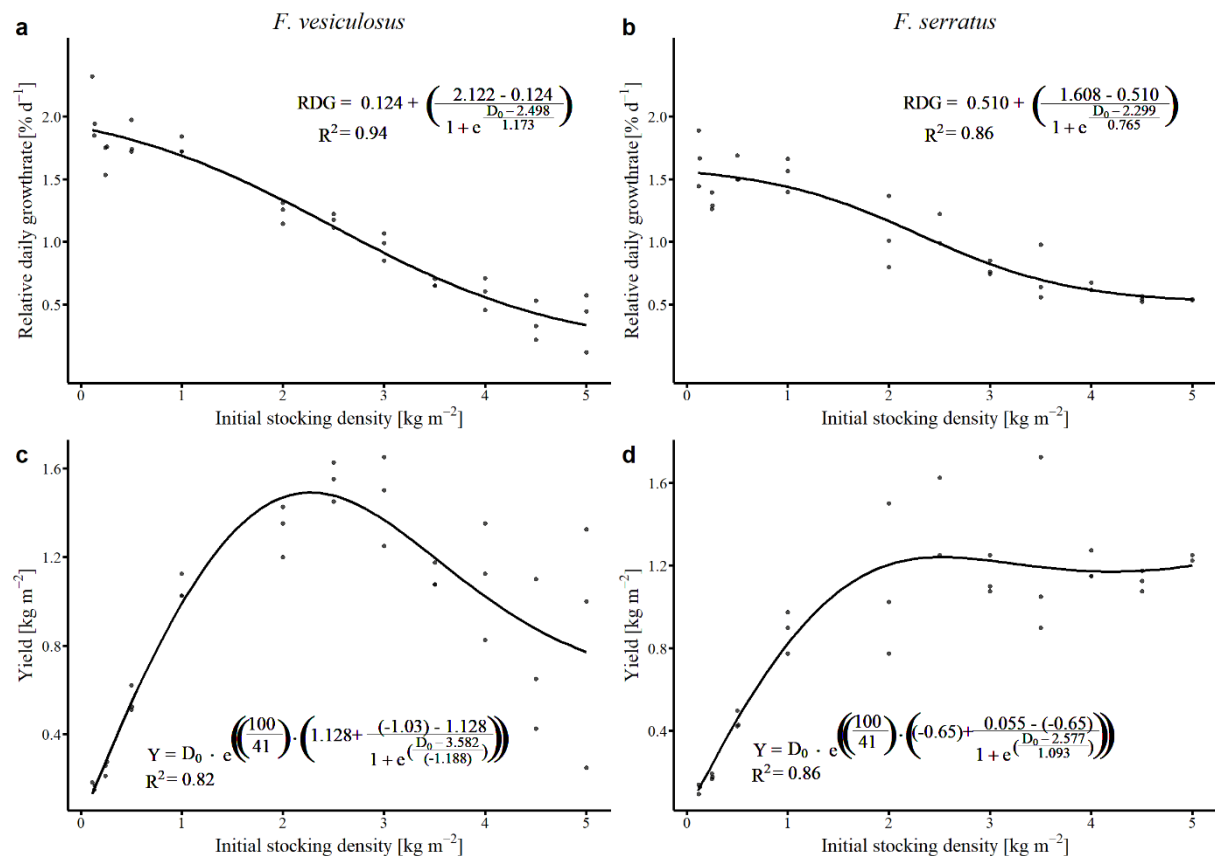


Fig. 4: a, b) Relative daily growth rate in % d<sup>-1</sup> of *F. vesiculosus* and *F. serratus* at 11 different initial stocking densities from 0.125 kg m<sup>-2</sup> to 5 kg m<sup>-2</sup> (N=3) after an experimental cultivation of 41 days (24 May – 04 Jul 2017/2018). The data were fitted with sigmoidal Boltzmann equations. Resulting equations with parameters and R<sup>2</sup>-values are given in the graphs. c, d) Yield of *F. vesiculosus* and *F. serratus* in kg m<sup>-2</sup> obtained in the same experiment after 41 days of cultivation. The fit equation for the

yield was obtained from the sigmoidal Boltzmann fit equation for the RDG (see “Material and methods”).

The measured yields after 41 days of cultivation ranged between 0.15 kg m<sup>-2</sup> and 1.65 kg m<sup>-2</sup> for *F. vesiculosus* and between 0.095 and 1.72 kg m<sup>-2</sup> for *F. serratus* (Fig. 4 c, d). Average yields increased linearly with increasing initial stocking densities for both species. For *F. vesiculosus*, the maximum average yield (1.54 ± 0.08 kg m<sup>-2</sup>) was found at an initial stocking density of 2.5 kg m<sup>-2</sup>, for *F. serratus*, the maximum average yield (1.43 ± 0.27 kg m<sup>-2</sup>) was also found at an initial stocking density of 2.5 kg m<sup>-2</sup>. At initial stocking densities above 3 kg m<sup>-2</sup>, the yields decreased for *F. vesiculosus* and remained rather stable for *F. serratus*, resulting in a parabolic fit curve for the first species and a rather asymptotic curve for the latter (see “Discussion”). The fit parameters and R<sup>2</sup>-values are given in Fig. 4.

#### Modelled annual yield

The modelled annual yields for *F. vesiculosus* varied between 1.81 kg m<sup>-2</sup> and 7.71 kg m<sup>-2</sup> (Fig. 5). Highest annual yields were obtained if initial stocking densities ranged between 1.75 kg m<sup>-2</sup> and 2.25 kg m<sup>-2</sup> and harvest densities between 3 kg m<sup>-2</sup> and 3.5 kg m<sup>-2</sup>. The maximum annual yield of 7.71 kg m<sup>-2</sup> was obtained for the combination of 2 kg m<sup>-2</sup> initial stocking density and 3.25 kg m<sup>-2</sup> harvest density. In this scenario, 6 harvests had to be performed during the modelled year. Slightly different cultivation scenarios produced similar annual yields with less harvest (Fig. 5, Tab. 1).

Tab. 1: Maximum annual yields for 1-6 required harvests and the corresponding initial stocking density and harvest density. The overall maximum annual yield obtained from the model is indicated in bold.

No. of harvests	Max. annual yield [kg m <sup>-2</sup> ]	Initial stocking density [kg m <sup>-2</sup> ]	Harvest density [kg m <sup>-2</sup> ]
<b><i>F. vesiculosus</i></b>			
1	6.91	1.25	4.75
2	7.39	1.5	4
3	7.54	1.75	3.75
4	7.6	1.75	3.5
5	7.64	2	3.5
6	<b>7.71</b>	2	3.25
<b><i>F. serratus</i></b>			
1	6.25	1.75	5
2	6.32	2.75	5
3	6.31	3.25	5
4	6.32	3.5	5
5	<b>6.35</b>	3.75	5
6	6.34	4	5



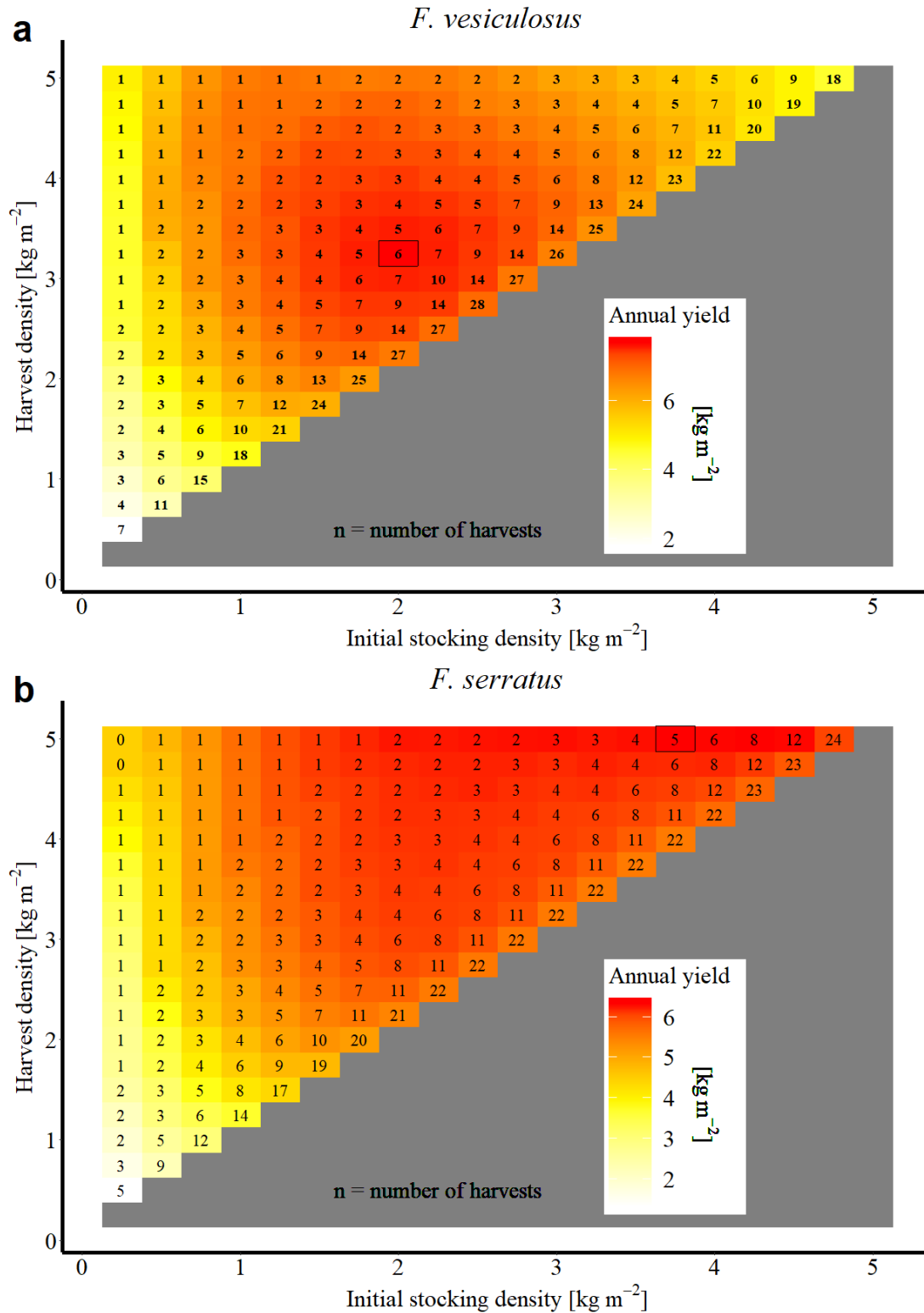


Fig. 5: Modelled annual yield (indicated by color) and necessary number of harvests (indicated by numbers) for *F. vesiculosus* (a) and *F. serratus* (b). Yield and necessary number of harvests were modelled for all possible combinations of initial stocking density and harvest density in the range of 0-5  $\text{kg m}^{-2}$  (intervals of 0.25  $\text{kg m}^{-2}$ ). The obtained maximum annual yield (*F. vesiculosus*: 7.71  $\text{kg m}^{-2}$ , *F. serratus*: 6.35  $\text{kg m}^{-2}$ ) is marked by a rectangle.

For *F. serratus*, the annual yields obtained from the model varied between 1.34 kg m<sup>-2</sup> and 6.35 kg m<sup>-2</sup>. Highest annual yields were found at higher cultivation densities than for *F. vesiculosus*, i.e. for initial stocking densities between 2 kg m<sup>-2</sup> and 4.5 kg m<sup>-2</sup> and a harvest density of 5 kg m<sup>-2</sup>. The maximum annual yield of 6.35 kg m<sup>-2</sup> was obtained for an initial stocking density of 3.75 kg m<sup>-2</sup> and a harvest density of 5 kg m<sup>-2</sup>, requiring 5 harvests. Similar annual yields could be obtained with less harvests, e.g. 6.32 kg m<sup>-2</sup> with only two harvests (Fig. 5, Tab. 1). Annual yields higher than the modelled maximum annual yield were obtained, if harvest densities of more than 5 kg m<sup>-2</sup> were allowed in the model. However, a validation of these model scenarios by real data was not possible as densities above 5 kg m<sup>-2</sup> were not tested in the Density experiment, therefore, these scenarios were excluded from the model.

### Upscaling experiment

For *F. vesiculosus*, the Upscaling experiment yielded no valid results, because it was impossible to keep the thalli well spread within the 1.2 m<sup>2</sup> net cages. The thalli aggregated within a short time at the lowest point of the flexible net cage bottom, where this aggregation led to losses of single thalli, which were pressed through the mesh by overlying thalli. Therefore, no meaningful comparison with growth in the small experimental baskets (20x20cm) was possible. For *F. serratus*, the thalli remained well spread within the net cages, because the serrated thallus margins prevented a drifting of the thalli towards the lowest point of the net cage. Besides, the *F. serratus* thalli were too large to get lost through the mesh. For *F. serratus*, growth rates in the net cages ( $0.43 \pm 0.07$  % d<sup>-1</sup>) were statistically equal to growth rates in the experimental baskets ( $0.46 \pm 0.13$  % d<sup>-1</sup>, Welch's two-sample t-test:  $p=0.72$ , Fig. 5).

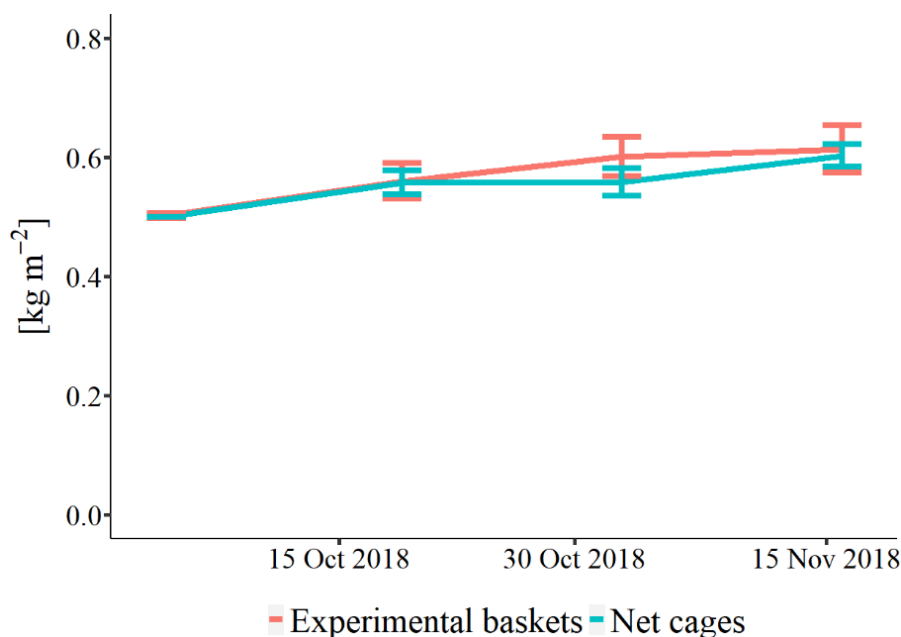


Fig. 6: Growth of *F. serratus* thalli in experimental baskets (20x20cm) and net cages (1.2x1m), the measured weights are normalized to an area of 1 m<sup>2</sup>. Weights are given as mean  $\pm$  SD (Experimental baskets: N=5, Net cages: N=3).

## Discussion

In this study we modelled the potential annual yield of *Fucus vesiculosus* and *Fucus serratus* in vegetative aquaculture based on experimentally determined growth rates during different seasons and at different initial stocking densities. The model results (Fig. 5) offer a useful estimation of maximum annual yields and the corresponding cultivation scenarios (*F. vesiculosus*: 7.71 kg m<sup>-2</sup> maximum annual yield for a cultivation from 2 kg m<sup>-2</sup> to 3.25 kg m<sup>-2</sup>, requiring 6 harvests; *F. serratus*: 6.35 kg m<sup>-2</sup> maximum annual yield for a cultivation from 3.75 kg m<sup>-2</sup> to 5 kg m<sup>-2</sup>, requiring 5 harvests). The model also calculated cultivation scenarios with only slightly lower annual yields at only 2 or 3 harvests per year (Fig. 5, Tab. 1), which might be the more relevant for commercial culture. Further, we tested if the growth parameters underlying the model, which were measured in small-scale culture baskets, are transferable to commercial-scale net cages. The growth rates measured in net cages (1.2 m<sup>2</sup>) were comparable to growth rates in small-scale baskets for *F. serratus* (Fig. 6), which suggests that the experimentally determined growth rates and thus the model results are transferable to commercial-scale culture. However, the unsuccessful cultivation of *F. vesiculosus* in the Upscaling experiment revealed a need for further optimization of the used net cages.

In the following, we will discuss the results of the single experiments and the modelling in the sequence in which they have been presented in the “Results” part.

In the Seasonal growth experiment, a marked seasonality of the growth rates was found with low growth rates in winter and high growth rates in summer (Fig. 3), which reflects the seasonal growth cycle of *Fucus* species in the wild (e.g. Brenchley et al. 1998, Lehvo et al. 2001). Growth rates remained at levels >0.3% d<sup>-1</sup> during winter, which supports the concept of year-round culture. Interestingly, *F. serratus* growth seemed to be limited during summer with rather constant growth rates between April and October, while *F. vesiculosus* showed a clear peak in July. How to explain this difference? Growth in general is the result of intrinsic growth regulation as well as all abiotic (e.g., temperature, light availability, nutrient concentrations) and biotic (e.g., grazing, fouling) factors acting jointly on the cultivated thalli. Intrinsic regulation leading to a growth limitation in summer has never been observed in *Fucus* species and can be excluded as cause for the limited growth of *F. serratus* during summer. As no severe grazing or fouling was observed in the experiments, biotic effects are also unlikely to be causal for the observed difference. Temperature might be an explanatory factor as the growth rate decrease at temperatures above the optimum of 15°C is slightly stronger for *F. serratus* than for *F. vesiculosus*, at least for North Sea individuals (Fortes and Lüning 1980). Unfortunately, there are no studies comparing the temperature-growth relationship of Baltic Sea populations of both species (but see Graiff et al. for *F. vesiculosus*). Light stress is unlikely to be the reason for the observed pattern as the growth limitation of *F. serratus* did not occur specifically during the time of highest irradiances (June-July, Supplementary material, Fig. S3) and higher growth rates have been observed in other experiments in which the thalli experienced stronger light exposure (Meichssner et al. 2020, see below). The timing of the growth limitation of *F. serratus* coincided with the time of lowest ambient nutrient concentrations (Supplementary material, Tabs. S1, S2) suggesting that growth of *F. serratus* might have been nutrient limited. *Fucus* species are able to store nutrients during times of excess nutrient concentrations (typically occurring in winter) and utilize them during summer when ambient nutrient concentrations are low. Limitation commences as soon as internal reserves are used up, usually in late summer (Perdersen and Borum 1996, Lehvo et al. 2001). It is, however, unclear if/how the conditions in the cultivation baskets have altered the exploitability of available nutrients. The reduced water motion within the baskets might have led to an increased thickness of the diffusive boundary layer and thus a less efficient nutrient uptake (Lichtenberg et al. 2017). It is further unclear if the used Baltic Sea populations of the two *Fucus* species differ in their nutrient storage capacities and in their requirements of nitrogen, which was the limiting element

according to the ambient nutrient concentrations (DIN:DIP < 15 (Supplementary material, Tabs. S1, S2), tissue N:P of both species ca. 30 (Harrison and Hurd 2001, Pedersen et al. 2010, Gordillo et al. 2002)). The mentioned reduced water motion within the experimental baskets might have had additional negative effects, like reduced DIC uptake or reduced removal of metabolic waste products, which might have caused the limited growth of *F. serratus* during summer. In conclusion, the cause(s) for the observed growth limitation of *F. serratus* during summer remain unclear. It might also have been different causes during different times of the limitation period, e.g. temperature stress during July/August (Fortes and Lüning 1980) and nutrient limitation during September (Pedersen and Borum 1996) or combinations of causes.

Both *Fucus* species showed a clear density dependence of measured relative daily growth rates and yields in the Density experiment. The yield-density curves of the two species differed markedly, *F. vesiculosus* showed a clear peak at ca. 2.5 kg m<sup>-2</sup> density with a significant reduction of the yield at higher densities, while *F. serratus* showed no significant reduction of the yield at high densities (Fig. 4 c, d). Thus, the yield-density relationships can be regarded as parabolic for *F. vesiculosus* and asymptotic for *F. serratus* following the definition by Assefa et al. (2016), who describe asymptotic behavior as “yield increase with increasing plant density to a maximum, without decreasing afterward”, and parabolic behavior as “increases in yield with density to a maximum density, with lower yields as density increases further” (see also Holliday 1960, Bleasdale & Nelder 1960, Yahuza 2010, Li et al. 2016). The parabolic behavior of *F. vesiculosus* might be due to the presence of vesicles in a part of the *F. vesiculosus* thalli. Vesiculated thalli drifted at the surface which caused a shading of unvesiculated thalli lying on the ground of the basket. This effect was probably proportionally stronger at high densities, as the same percentage of vesiculated thalli that would e.g. cover 20 % of the surface of a low-density basket would cover almost the entire surface of a high-density basket. Thus, the yields of high-density groups might have been reduced by proportionally stronger shading resulting in a parabolic yield-density curve. This process could also be an explanation for the high yield variation among replicates of *F. vesiculosus* at high densities, as replicates with many vesiculated thalli might have experienced a stronger reduction of growth than replicates with few vesiculated thalli. Unfortunately, the percentage of vesiculated thalli was not quantified and did in addition change during the experiment as the thalli produced new vesicles. *F. serratus* produces no vesicles, thus, no shading by drifting thalli occurred resulting in an asymptotic yield-density curve. We assume that the shape of the *F. vesiculosus* yield-density curve would shift towards asymptotic behavior, if thalli from unattached populations, which usually produce no vesicles (Norton and Mathieson 1983), were used for cultivation (Meichssner et al. 2021).

From the data of the Seasonal growth experiment and the Density experiment, annual yields were modelled (Fig. 5). The model results seem reasonable: i) the yields reflect the measured growth rates integrated over one year. ii) Many harvests were calculated if the difference between initial stocking density and harvest density was small and few harvests were calculated if it was large. iii) The model calculated highest annual yields at intermediate densities for *F. vesiculosus* and at higher densities for *F. serratus*, which reflects the shape of the experimentally determined yield-density curves of the two species. Thus, we conclude that the used model is a suitable tool for the forecasting of annual yields based on our experimentally determined growth rates.

The maximum annual yields obtained were 7.71 kg m<sup>-2</sup> for *F. vesiculosus* and 6.35 kg m<sup>-2</sup> for *F. serratus* based on the used experimental cultures. The modelled yield of *F. vesiculosus* was in the range of the annual biomass production of wild beds, which is 1400-2300 g dry weight m<sup>-2</sup> yr<sup>-1</sup> according to measurements by Lamela-Silvarrey et al. (2012), i.e. 6.6-11 kg wet weight m<sup>-2</sup> yr<sup>-1</sup> (assuming a dry matter content of 21% (own pre-experiments)). For *F. serratus*, the modelled yield was higher than the primary production of wild beds, which has been measured to be 402 g C m<sup>-2</sup> yr<sup>-1</sup>

(Bordeyne et al. 2020), i.e. ca. 5.4 kg wet weight  $\text{m}^{-2} \text{yr}^{-1}$  (assuming 37% carbon in the dry matter (Balina et al. 2016) and a dry matter content of 24% (own pre-experiments)). We assume that even higher annual yields are possible in culture if cultivation conditions are optimized: both underlying experiments (Seasonal growth experiment, Density experiment) were conducted in baskets deployed in the gaps of a Jetfloat system (Fig. 1), where water exchange was limited and light levels were slightly reduced by the surrounding Jetfloat elements. In a parallel cultivation experiment (May-June 2019), where identical experimental baskets were deployed directly in the fjord (i.e., outside the Jetfloat system, Meichssner et al. 2020), the measured daily growth rates were 59% higher for *F. vesiculosus* and 51% higher for *F. serratus* than at the same time in the Seasonal growth experiment. If the modeling was corrected by these higher growth rates, maximum annual yields of 11.92 kg  $\text{m}^{-2}$  for *F. vesiculosus* and 9.46 kg  $\text{m}^{-2}$  for *F. serratus* were obtained (the corrected modeling results for improved growth conditions are shown in the Supplementary material, Tab. S3, Fig. S5). Thus, we believe that the model correctly estimates the annual yields under the given experimental conditions, but the non-optimal experimental cultures have led to an underestimation of the actual potential of vegetative *Fucus* aquaculture. The combination of initial stocking density and harvest density as well as the required number of harvests were only slightly affected if the model was run with higher growth rates. Hence, the principal pattern of the model results remains the same and can be used as an orientation for the planning of *Fucus* cultivation efforts, but higher yields should be expected if optimal cultivation conditions are used.

For long-term culture, thalli from unattached populations are necessary as they seem to retain their naturally low fertility in culture, thus having the potential to be cultivated continually without the biomass losses related to receptacle degradation (Meichssner et al. 2021). In the present study, we still used vegetative thalli from attached origin as the experiments were initiated before the results of Meichssner et al. (2021) were obtained. We believe that the modeling results of this study in principle do also apply for the culture of unattached populations. However, it has to be considered that the optimal combination of initial stocking density and harvest density for unattached and therefore unvesiculated *F. vesiculosus* thalli (Norton and Mathieson 1983) might shift to higher densities (similar to the *F. serratus* model results), as the yield-density relationship underlying the model is assumed to shift towards asymptotic behavior (see above).

The Upscaling experiment showed that the growth rates and thus the model results generally do also apply to commercial-scale net cages (Fig. 6). However, successful upscaling was only achieved for *F. serratus*, while for *F. vesiculosus*, thalli were either lost through the mesh or aggregated at one point in the cage, which led to reduced growth. We assume that growth rates comparable to those of small-scale cultures could be achieved for *F. vesiculosus*, if the following improvements were implemented: nets with a smaller mesh size (0.3-0.5cm) should be used to avoid the loss of thalli. Cages should have a solid, flat bottom, where the thalli can be well spread to avoid the aggregation of thalli at one point and the concomitant reduction of growth. An even coverage of the net cage bottom has, in our experience, the additional advantage of preventing the colonization of non-covered bottom parts by ephemeral algae. We further suggest a lower depth (only 5-20cm) of the cages and lower cage walls than in the experiment presented here (Fig. 2) in order to achieve optimal light availability in the cages. For practical implementation, we suggest flat net cages/baskets of about 1 $\text{m}^2$  size, similar to basket examples for *Kappaphycus/ Eucheuma* culture shown in Hurtado et al. (2014).

It is unclear if the modelled yields can be transferred to hectare scales as scaling effects like potential nutrient limitation or grazer accumulation can hardly be predicted. If a direct scalability is assumed, annual harvests of 38.5t hectare $^{-1}$  and 31.8t hectare $^{-1}$  could be expected for *F. vesiculosus* and *F. serratus*, respectively (assuming 5,000 1 $\text{m}^2$ -baskets hectare $^{-1}$ , leaving space for boating). Even higher yields might be possible if cultivation conditions are optimized (see above). The calculated yields per



hectare are lower than the typical yields of farmed Laminariales (except for *Alaria esculenta*, Kraan 2010), which was expected considering the comparatively high growth rates of Laminariales. Yet, it has to be remembered that *Fucus* is not produced for its high growth potential but for its high-priced end products. Thus, a cultivation at the modelled yields could still be profitable even at smaller scales. Land-based cultivation, similar to *Gracilaria* tank culture (Friedlander & Levy 1995), might also be profitable if it is combined with biomass processing and valorization (Piker L., pers. comm.). We assume that the cultivation parameters determined in this study (yields, densities, harvests) do also apply to culture in flat land-based tanks.

Open-water *Fucus* cultures might serve as nutrient sink for eutrophic water bodies (Rönneberg et al. 1992, Fei 2004, see also Ryzhik et al. 2014): based on the tissue nitrogen and phosphorous content (ca. 2% and 0.2% of the dry weight (Rönneberg et al. 1992, Pedersen et al. 2010, Perini and Bracken 2014)), 1 ton of harvested *F. vesiculosus* biomass would remove ca. 4.2 kg nitrogen and ca. 420g phosphorous from the water column (assuming a dry matter content of 21% (own pre-experiments)) and 1 ton of harvested *F. serratus* biomass would remove ca. 4.8 kg nitrogen and 480g phosphorous (assuming a dry matter content of 24% (own pre-experiments)). However, it is unclear if the produced biomass would still be useful for high-value products as high epiphyte loads can be expected in nutrient-rich cultures (Rönneberg et al. 1992, Worm and Sommer 2000). The same is true if *Fucus* cultures are intended as nutrient trap for fish aquaculture (Rönneberg et al. 1992). Even though *Fucus* growth can be nutrient limited during short periods of the year even in nutrient-rich sea areas (Pedersen and Borum 1996), fertilization of commercial cultures should be avoided considering the overall eutrophic state of many temperate coastal waters and especially the Baltic Sea.

For any commercial culture attempts with the introduced vegetative cultivation method, biomass from unattached *Fucus* populations is needed (Meichssner et al. 2021). Only small amounts should be collected in order to protect these valuable habitats and collection should be accompanied by regular monitoring of the used populations (Meichssner et al. 2021). In an optimal scenario, only little biomass from the field is initially collected and used to build a larger culture over time (Meichssner et al. 2021). Local populations should be used, as the introduction of culture material from foreign populations bears the risk of introducing i) non-native *Fucus* genotypes and ii) non-native *Fucus*-associated species like crustaceans, snails and epiphytic algae.

An alternative to the introduced vegetative cultivation approach would be a cultivation technique including sexual reproduction, i.e. the production of zygotes by mixing of female eggs and male gametes, and their subsequent seeding on cultivation ropes, which are transplanted to the sea for outgrowth to harvest size. This technique would be similar to the cultivation techniques used for the production of *Saccharina* spp., *Undaria pinnatifida* and *Porphyra/Pyropia* spp. (Graham et al. 2009, Hurd et al. 2014). Protocols for the production of *Fucus* zygotes are well established (e.g. Bogaert et al. 2015) and an implementation in an aquaculture process should be possible. However, it has to be noted that *Fucus* eggs and zygotes are not mobile like the spores of Laminariales. Therefore, the fertilized zygotes have to have the opportunity to sink on and attach to the used ropes/ cultivation structures, which contrasts to the cultivation technique used for Laminariales, where the culture ropes can be submerged in spore solution and the spores actively swim towards the rope (Hurd et al. 2014). A potential low-effort method for the seeding of ropes would be the installation of ropes in natural *Fucus* beds during the reproductive season and the transfer to the cultivation site after zygotes have attached.

## Conclusion and outlook

Based on experimental data it was possible to realistically model annual cultivation cycles and resulting yields of vegetative *Fucus* aquaculture. The model results provide a helpful orientation for the planning of future efforts to establish commercial *Fucus* aquaculture. Future research should focus on large-scale implementation including the development of suitable cultivation devices for either open-water net cage culture or land-based tank culture. Other challenges including the efficient dealing with grazing and fouling as well as the establishment of low-fertility culture populations require further investigations. Apart from vegetative culture, cultivation of *Fucus* by sexual reproduction (i.e. the seeding of zygotes on ropes) could be a promising alternative approach.

### Author contributions

Rafael Meichssner conducted the experimental work, data analysis, modelling and wrote the first draft of the manuscript. Peter Krost assisted in the analysis and interpretation of the data. Rüdiger Schulz assisted in the planning of the study. Peter Krost and Rüdiger Schulz reviewed and edited the manuscript.

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## Chapter III – Supplementary material

### Validation of used experimental approach: effect of variability of meristem number within experimental units in a parallel experiment

In a parallel experiment (data of entire experiment published in Meichssner et al. 2020), the culture biomass was assembled in the same way as in the present study. The cultivated thalli were photographed at several days during the experiment, therefore, it was possible to count the meristem number of the single experimental units and correlate it to the corresponding growth rates. In the experiment, 5 treatment groups with 3 replicates each were cultivated for *F. vesiculosus* and 3 treatment groups with 3 replicates each for *F. serratus*. One of the *F. vesiculosus* groups was not included in this analysis as 1 replicate had been lost. The maximal observed relative deviation of the normalized meristem number (meristems (10g biomass)<sup>-1</sup>) from the group mean was 27% for *F. vesiculosus* and 12% for *F. serratus*. No correlation was found between the relative deviation of the normalized meristem number from the mean of the experimental treatment group and the relative deviation of the corresponding relative daily growth rates (RDGs), measured in the following 14 days (27 May 2019 - 10 June 2019), from the mean of the experimental treatment group (Pearson's correlation test: *F. vesiculosus*:  $p = 0.44$  (N=12, df=10), *F. serratus*:  $p = 0.39$  (N=9, df=7), Fig. S1). This indicates that the relatively small variability of meristem numbers in the experimental biomass, which results from the used assembling method (see "Material and methods"), has no measurable effect on the resulting RDGs.

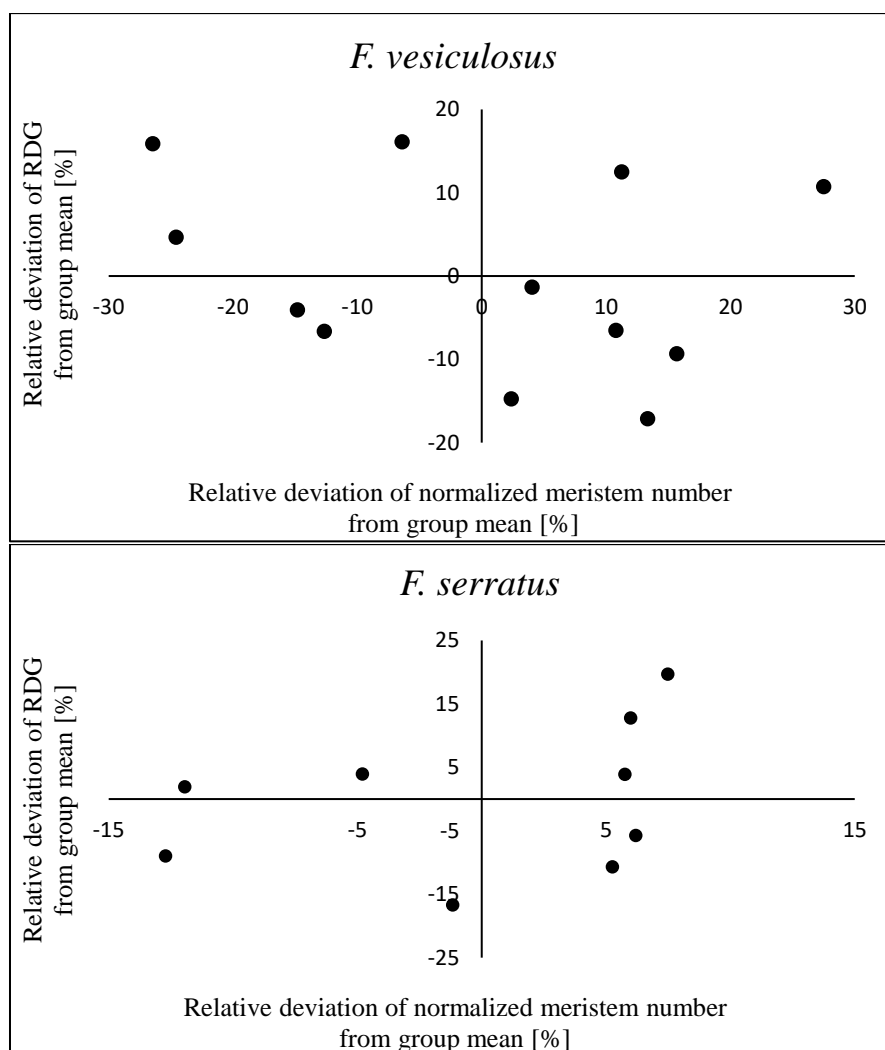


Fig. S1: Relative deviation of normalized meristem number (meristems (10g biomass)<sup>-1</sup>) from group mean plotted against relative deviation of relative daily growth rate (measured over the following 14 days) from group mean. Data from a growth experiment published in Meichssner et al. (2020)

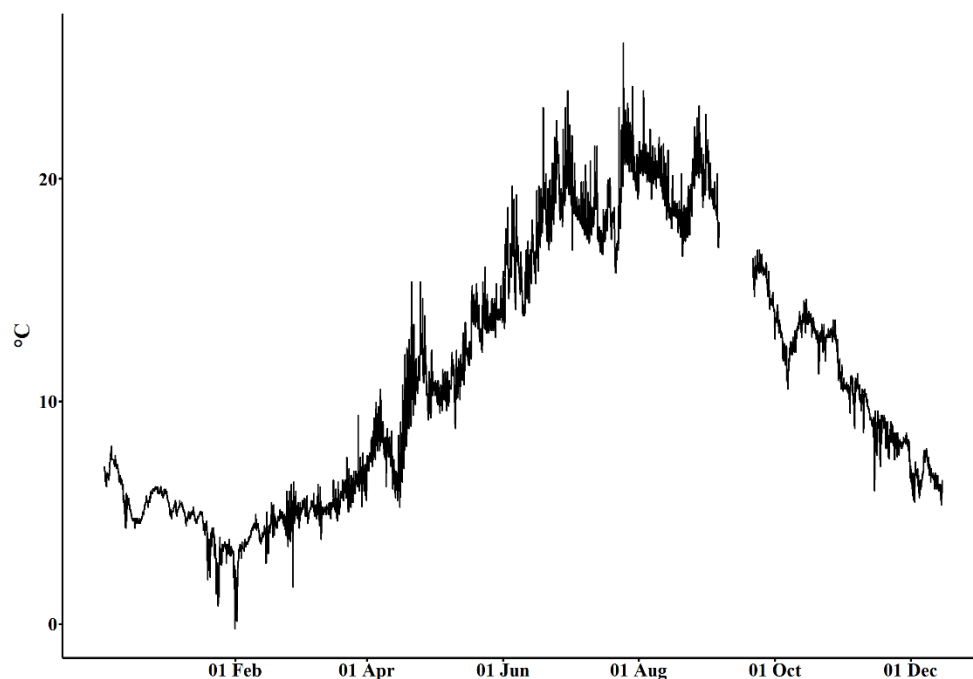
**Environmental parameters during Seasonal growth experiment**

Fig. S2: Water temperature during Seasonal growth experiment (15 December 2018 – 15 December 2019) logged hourly within the cultivation baskets. Due to technical difficulties, the measurements were interrupted for ca. 3 weeks in September 2019.

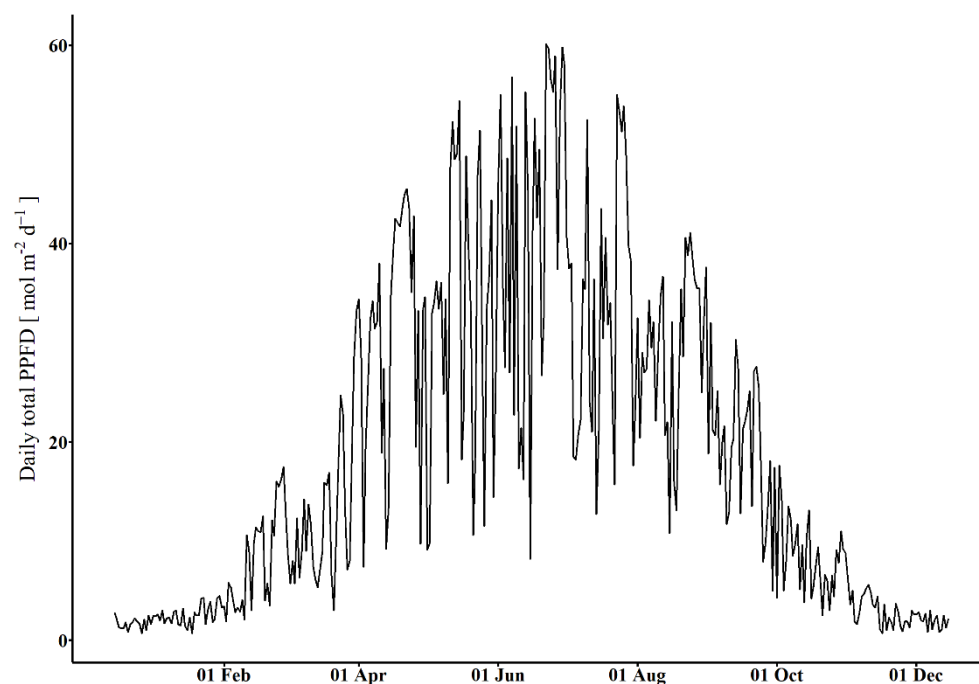


Fig. S3: Daily totals of photosynthetically active photon flux density (PPFD) during the Seasonal growth experiment, measured 4.87 km from the cultivation site in the Botanical garden of Kiel University.

Tab. S1: Concentrations of nitrite, ammonium, nitrate and phosphate and molar N:P ratio during the Seasonal growth experiment as measured at Mönkeberg measurement station, ca. 3km from the experimental site directly within the Kiel fjord (54.3538 N; 10.1647 E).

Date	NO <sub>2</sub> <sup>-</sup> [μmol L <sup>-1</sup> ]	NH <sub>4</sub> <sup>+</sup> [μmol L <sup>-1</sup> ]	NO <sub>3</sub> <sup>-</sup> [μmol L <sup>-1</sup> ]	PO <sub>4</sub> <sup>3-</sup> [μmol L <sup>-1</sup> ]	N:P
21 Jan 2019	not measured	2.68	0.48	0.59	5.4
11 Feb 2019	5.84	2.32	0.30	0.56	15.2
04 Mar 2019	3.29	not measured	0.36	0.31	11.6
01 Apr 2019	10.82	3.55	0.19	0.18	82
13 May 2019	0.46	0.23	0.09	0.19	4.2
09 Sep 2019	0.12	1.00	0.03	0.91	1.3
14 Oct 2019	1.28	7.67	0.35	1.66	5.6
13 Nov 2019	5.29	6.60	0.44	1.41	8.8
09 Dec 2019	8.50	5.45	0.87	1.00	14.7

Tab. S2: Concentrations of nitrite, ammonium, nitrate and phosphate and molar N:P ratio during the Seasonal growth experiment as measured at the Boknis Eck time series station, ca. 30km from the experimental site in the Kiel bay (54.5167 N; 10.0333 E).

Date	NO <sub>2</sub> <sup>-</sup> [μmol L <sup>-1</sup> ]	NH <sub>4</sub> <sup>+</sup> [μmol L <sup>-1</sup> ]	NO <sub>3</sub> <sup>-</sup> [μmol L <sup>-1</sup> ]	PO <sub>4</sub> <sup>3-</sup> [μmol L <sup>-1</sup> ]	N:P
12 Dec 2018	0.19	0.08	0.48	0.57	1.3
26 Feb 2019	0.04	0.00	0.02	0.06	1
20 Mar 2019	0.03	0.01	0.03	0.02	3.5
24 Apr 2019	0.04	not measured	0.05	0.01	9
28 May 2019	0.06	not measured	0.03	0.02	4.5
26 Jun 2019	0.04	not measured	0.02	0.04	1.5
31 Jul 2019	0.01	not measured	0.06	0.07	1.0
27 Aug 2019	0.09	not measured	0.07	0.09	1.8
29 Oct 2019	0.01	0.31	0.02	0.49	0.7
19 Nov 2019	0.24	0.76	1.20	0.87	2.5
10 Dec 2019	0.30	0.58	1.21	0.64	3.3

### Alternative fitting of data from the Density experiment with traditional fit equation for land plants

Data of the Density experiment were alternatively fitted with a classical yield-density fit equation for land plants (Li et al. 2016):

$$Y = a \cdot D_0 / ((1 + b \cdot D_0)^c) \quad (\text{Equation S1})$$

If the equation is applied to land plants,  $D_0$  is defined as number of individuals per unit of ground area. Under this assumption, parameter  $a$  is the “mass of an isolated plant in the absence of competition” and parameter  $b$  is “essentially the area an individual requires to achieve” the value of parameter  $a$  (Li et al. 2016). As  $D_0$  is defined as initial stocking density with the unit “ $\text{kg m}^{-2}$ ” in our study and not as number of individuals, the meanings for  $a$  and  $b$  do not exactly apply here. Parameter  $c$  determines if the curve is asymptotic or parabolic: if  $c \sim 1$ , the curve is considered asymptotic, if  $c > 1$ , it is considered parabolic. The RDG-density fit equation can be deduced from Equation S1, because yield and RDG are connected by a causal mathematic relationship. For this purpose,  $D_t$  is expressed as sum of yield and  $D_0$ , using Equation S1:

$$D_t = Y + D_0 = ((a \cdot D_0) / ((1 + b \cdot D_0)^c)) + D_0 \quad (\text{Equation S2})$$

Subsequently, this resulting expression for  $D_t$  is inserted in the normal equation for the calculation of the RDG:

$$\text{RDG} = \ln(D_t / D_0) \cdot (100 / t) \quad (\text{Equation S3})$$

The resulting fit equation for the RDG-density relationship is:

$$\text{RDG} = \ln((a / ((1 + b \cdot D_0)^c) + 1)) \cdot (100 / t) \quad (\text{Equation S4})$$

Where  $t$  is 41 days in case of the density experiment.

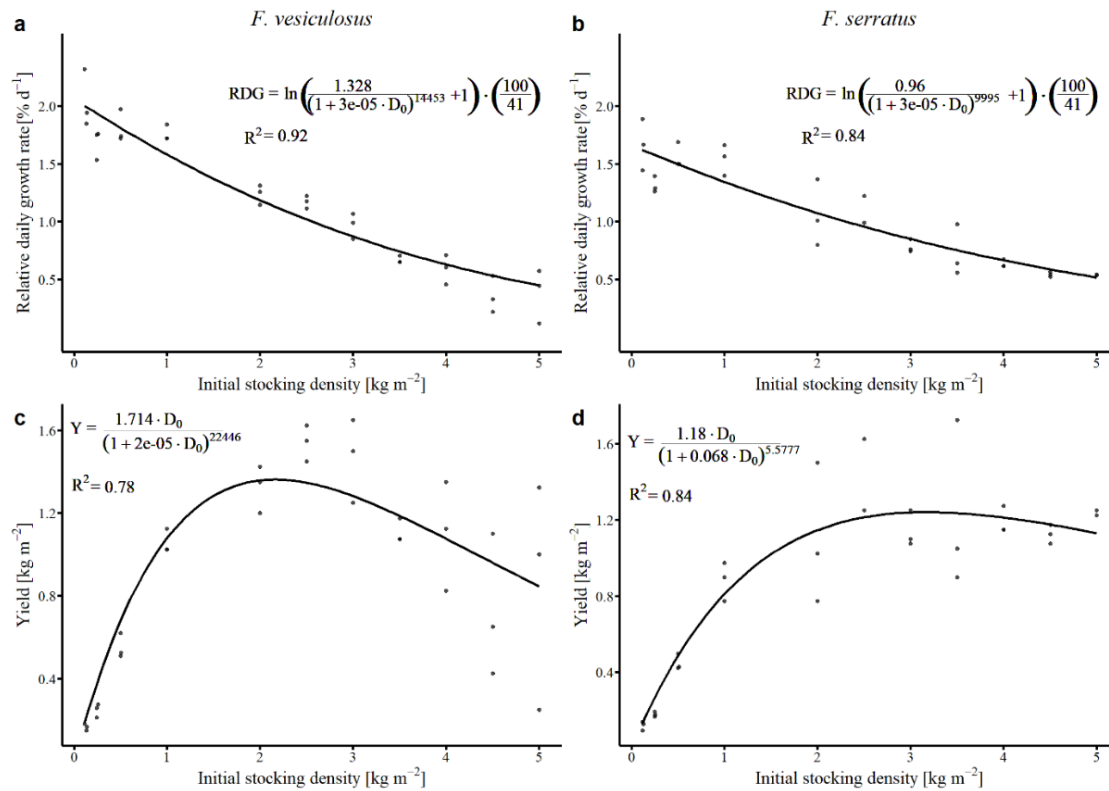


Fig. S4: a, b) Relative daily growth rates of *F. vesiculosus* and *F. serratus* at different initial stocking densities fitted with Equation S4. c, d) Yield of *F. vesiculosus* and *F. serratus* at different initial stocking densities fitted with Equation S1. Fitted equations with parameters and  $R^2$ -values are shown in the graphs.

### Alternative modelling of annual yields with potentially higher growth rates

In a parallel cultivation experiment (May-June 2019), Meichssner et al. (2020) measured relative daily growth rates, which were 59% higher for *F. vesiculosus* and 51% higher for *F. serratus* than at the RDGs measured at the same time in the Seasonal growth experiment of the present study. In the study of Meichssner et al. (2020), the same cultivation baskets were used, but they were deployed directly in the sea and not within a Jetfloat system as in the present study. Therefore, we assume that growth rates and in accordance the modelled annual yields can be higher under optimized cultivation conditions outside the Jetfloat system (see “Discussion”). Here we show the model results when the model was run with higher growth rates. For this purpose, all relative daily growth rates measured in the Density experiment as well as in the Seasonal growth experiment were multiplied by factor 1.59 for *F. vesiculosus* and by factor 1.51 for *F. serratus* (see above). Fitting and modelling were then performed as described in “Material and methods”. The fitted equations for the RDG-density relationship were:

$$F. \textit{vesiculosus}: \text{RDG} = 0.197 + (3.366 - 0.197) / (1 + \exp((D_0 - 2.498) / 1.173))$$

$$F. \textit{serratus}: \text{RDG} = 0.769 + (2.422 - 0.769) / (1 + \exp((D_0 - 2.299) / 0.765))$$

The results of the corrected modeling are depicted in the same form as the modeling results in the main manuscript (Tab. S2, Fig. S5).

Tab. S3: Maximum annual yields for 1-7 required harvests and the corresponding initial stocking density and harvest density from the corrected model. The overall maximum annual yield obtained from the corrected model is indicated in bold.

No. of harvests	Max. annual yield (kg m <sup>-2</sup> )	Initial stocking density (kg m <sup>-2</sup> )	Harvest density (kg m <sup>-2</sup> )
<i>F. vesiculosus</i>			
1	8.59	0.25	4.75
2	10.76	1.0	4.75
3	11.32	1.25	4.25
4	11.66	1.5	4
5	11.84	1.75	3.75
6	11.92	1.75	3.5
7	<b>11.92</b>	2	3.5
<i>F. serratus</i>			
1	8.42	0.75	5
2	9.38	1.75	5
3	<b>9.46</b>	2.25	5
4	9.44	2.75	5
5	9.43	3.25	5
6	9.40	3.5	5
7	9.40	3.75	5



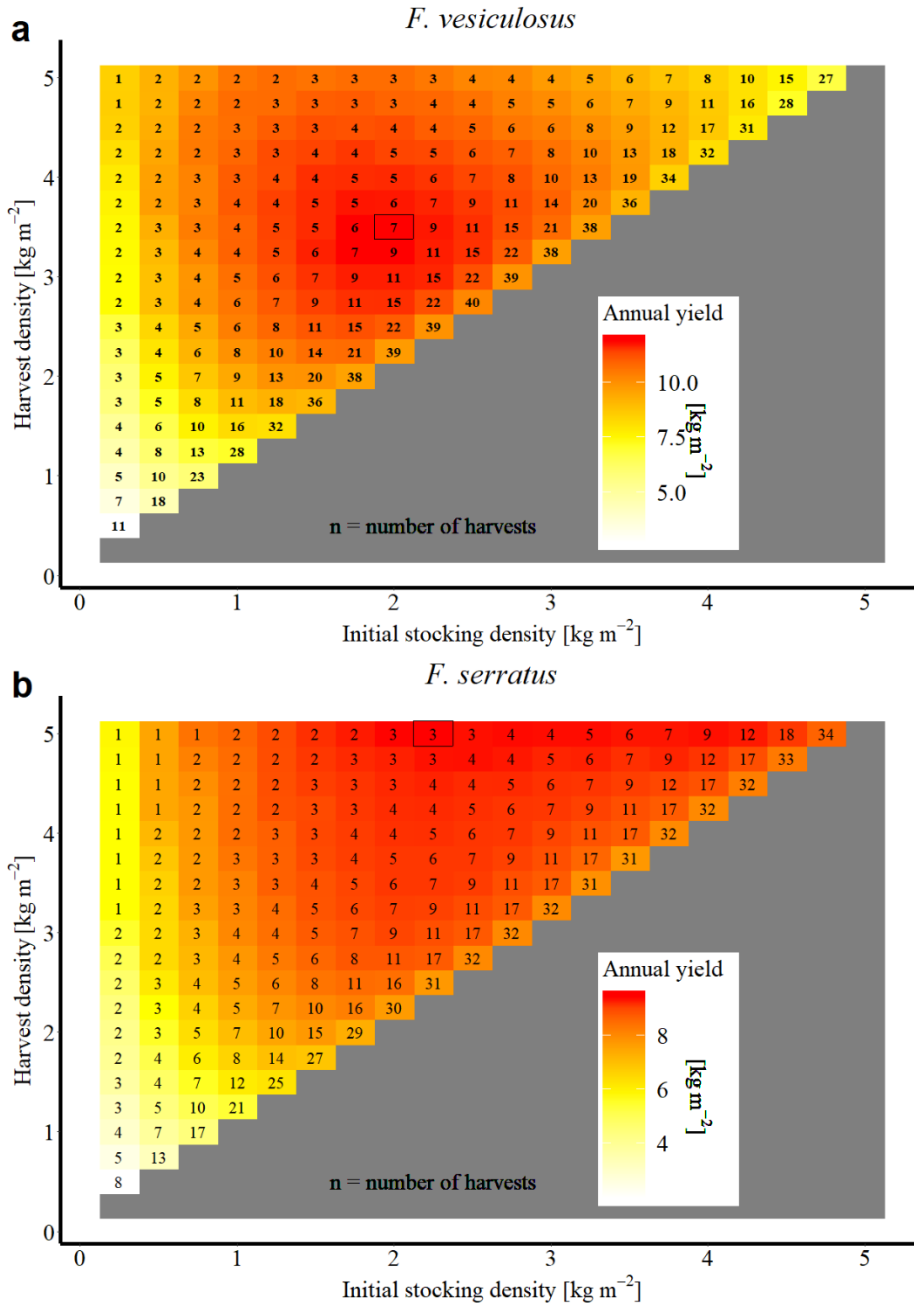


Fig. S5: Annual yield (indicated by color) and necessary number of harvests (indicated by numbers) for *F. vesiculosus* (a) and *F. serratus* (b) calculated by the corrected model. Yield and necessary number of harvests were modelled for all possible combinations of initial stocking density and harvest density in the range of 0-5 kg m<sup>-2</sup> (intervals of 0.25 kg m<sup>-2</sup>). The obtained maximum annual yield (*F. vesiculosus*: 11.92 kg m<sup>-2</sup>, *F. serratus*: 9.46 kg m<sup>-2</sup>) is marked by a rectangle.

**Chapter IV –  
Control of fouling in the aquaculture of  
*Fucus vesiculosus* and *Fucus serratus* by  
regular desiccation**



# Control of fouling in the aquaculture of *Fucus vesiculosus* and *Fucus serratus* by regular desiccation

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## Abstract

Fouling is a major problem in seaweed aquaculture and one of the main obstacles during the domestication process for new culture species. During first attempts to cultivate *Fucus vesiculosus* and *Fucus serratus* in the Kiel fjord (Western Baltic Sea), fouling by the epizoans *Electra pilosa*, *Mytilus* sp., and *Amphibalanus improvisus* rendered the production of marketable biomass impossible. This study tested (1) if regular desiccation by air exposure is effective in decreasing the abundance and size of foulers and (2) if and how regular desiccation affects the growth performance of the cultivated *Fucus* thalli. For this purpose, thalli of *F. vesiculosus* and *F. serratus* were cultivated freely floating in baskets directly deployed in the fjord and desiccated to defined percentages of the wet weight (ww) by air exposure. The treatments comprised controls and desiccations of different intensities (from 90 to 40% of ww) and at different frequencies ( $1 \times \text{week}^{-1}$ ,  $3 \times \text{week}^{-1}$ ). Growth rates of both *Fucus* species were not or only slightly reduced by the desiccation treatments. The final harvested biomass of *F. vesiculosus* under frequent mild desiccations ( $3 \times \text{week}^{-1}$  to 80% of ww) was even higher than the biomass of undesiccated controls. The size of the epizoans *E. pilosa* and *A. improvisus* was significantly reduced by the desiccation treatments and the abundance of all epizoan species was drastically reduced by the desiccation regimes. Frequent mild desiccations (*F. vesiculosus*:  $3 \times \text{week}^{-1}$  to 80% of ww, *F. serratus*:  $3 \times \text{week}^{-1}$  to 90% of ww) proved to be most effective and decreased the epizoan ww share of the total harvest from  $13.0 \pm 4.8\%$  in the control to  $1.8 \pm 0.2\%$  for *F. vesiculosus* and from  $19.1 \pm 2.7$  to  $1.0 \pm 0.1\%$  for *F. serratus*. Thus, desiccation seems to be an effective measure for the production of clean *Fucus* biomass in culture which is necessary for further valorization. A technical solution for the implementation of this procedure in large-scale cultures remains to be developed.

**Keywords** *Fucus vesiculosus* · *Fucus serratus* · Phaeophyceae · Aquaculture · Fouling · Epizoans · Desiccation · Air exposure

## Introduction

Seaweed cultivation has become a large industry in the course of the past decades having reached an annual worldwide harvest of about 30 million t wet weight (ww) in 2016 (FAO 2018a). However, one major impediment limiting quantity as well as quality of seaweed production is the overgrowth of the

cultivated biomass by epiphytic algae and sessile animals, generally referred to as biofouling (Wahl 1989; Bannister et al. 2019). Epiphytic algae (hereafter epiphytes) can be of different systematic origin (red, green and brown algae, cyanobacteria, diatoms, prasinophytes etc.) and can make up to 60% of the harvested biomass (Buschmann and Gomez 1993; Fletcher 1995; Hurtado et al. 2006). Sessile animals (hereafter epizoans) comprise e.g. hydrozoans, ascidians, bryozoans, and barnacles (Park and Hwang 2012; Førde et al. 2016; Bruhn et al. 2016; Rolin et al. 2017). Both epiphytes and epizoans limit the growth of the cultivated seaweeds by reduction of available light and the impairment of nutrient uptake through coverage of the seaweeds' surface (Sand-Jensen and Revsbech 1987; Wahl 1989; Rohde et al. 2008). Moreover, they lead to increased biomass losses due to an intensification of drag forces (Anderson and Martone 2014) and their presence significantly lower the quality of the produced biomass, especially if cultivated for food purposes (Bannister et al. 2019). Biofouling has not only been

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reported for established culture species like *Pyropia* sp./*Porphyra* sp. (Kim et al. 2014), *Kappaphycus alvarezii* (Hayashi et al. 2010; Kasim et al. 2017), and *Gracilaria/Gracilariopsis* sp. (Fletcher 1995) but is also a frequently encountered problem in species that undergo first cultivation trials or have just recently been used in commercial cultivation (Walls et al. 2017).

In 2015, an experimental cultivation of *Fucus vesiculosus* L. and *Fucus serratus* L. has been established at the Kiel fjord in the Western Baltic Sea. Hitherto, *Fucus* biomass has been harvested exclusively from wild stocks e.g. at the Atlantic coast of Ireland and France (Mac Monagail et al. 2017). However, at the German Baltic Sea coast, harvesting of wild *Fucus* biomass for commercial purposes is prohibited (Bundesamt für Naturschutz 2013) due to dramatic stock declines in the twentieth century (Kautsky et al. 1986; Torn et al. 2006; Rohde et al. 2008). Consequently, aquaculture is the only means to sustainably produce *Fucus* biomass in Germany. A local production is desired, because “regionality” is a valuable claim for organic cosmetics and food supplements, the two main target markets for *Fucus* biomass (L. Piker, pers. comm.). The development of a culture method could also encourage the cultivation of this genus in other countries, which might become necessary as the market pull is expected to increase (Cherry et al. 2019). To our knowledge, the experiments in the Kiel fjord are among the first trials to cultivate *Fucus* species for commercial purposes (but see FAO 2018b; Haglund and Pedersen 1988).

The two target species (*F. vesiculosus*, *F. serratus*) are cultivated as freely floating thalli detached from substrate (Meichssner et al. in prep.), floating in net cages or plastic baskets. From the beginning, fouling, especially by epizoans, appeared to be one of the major challenges of this cultivation technique. The cultivated thalli showed a high degree of colonization by barnacles (*Amphibalanus improvisus*), blue mussels (*Mytilus edulis/Mytilus trossulus*), and bryozoans (*Electra pilosa*) in contrast to thalli in local field populations, which show only little colonization by epizoans (Rickert et al. 2015). An effective measure to reduce fouling will thus be crucial for the successful long-term implementation of this cultivation method. Means to prevent fouling in seaweed aquaculture are for example removal by hand, which is only possible if labor costs are low (Fletcher 1995). Secondly, growing and harvesting seasons can be planned in order to avoid the spore/larval fall of epiphytes/epizoans (Anderson et al. 1996). A third method, which is applied in *Pyropia* sp./*Porphyra* sp. culture in Japan, is regular air exposure of the cultivated biomass (Kim et al. 2017). The targeted culture species is able to grow under temporarily aerated conditions, while the settling larvae/germlings of fouling species do not survive this treatment resulting in a strongly reduced amount of epibionts on the cultivated thalli and the cultivation ropes (Mumford and Miura 1988).

Both *Fucus* species cultivated in the experimental farm are able to survive temporal air exposure and desiccation, which enables them to colonize intertidal habitats (e.g. Schonbeck and Norton 1978; Dring and Brown 1982). Among the encountered foulers, *A. improvisus* and *Mytilus* sp. are also able to withstand desiccation and colonize temporally emerged locations (Barnes and Barnes 1957; Luther 1987; Buschbaum and Saier 2001); however, both species do not occur as far up the shore as *F. vesiculosus* in the Baltic Sea (own observation). *Electra pilosa* is not found at temporally air exposed locations in the Baltic Sea at all (own observation). Consequently, we hypothesized that all major fouling organisms observed in the experimental cultures are potentially less desiccation resistant than cultivated *Fucus* biomass. Therefore, regular air exposure (i.e. desiccation) of the cultivated biomass might be an effective method to reduce the abundance of these foulers in the cultures. As a complementary effect, we expected the size of already settled foulers to be affected by such a treatment, because times of emersion cannot be used for filter-feeding.

A pre-experiment in 2018 already revealed a strongly reducing effect of regular desiccation on the abundance of *A. improvisus* on cultivated *Fucus* thalli; however, desiccation was only performed once a week. As larvae and early settlement stages of the epizoans are often more sensitive to desiccation than adult individuals (Foster 1971a; Jenewein and Gosselin 2013), we expected more frequent desiccations to be even more effective, because larvae would experience the treatment early after settlement.

The aim of this study was (1) to test if regular desiccation by air exposure reduces the abundance and size of fouling organisms on the cultivated biomass and (2) to evaluate the effect of regular desiccation on the growth performance of the cultivated *Fucus* species. Different degrees of desiccation were compared as well as different desiccation frequencies. The aim was to find an optimal desiccation regime enabling the production of biomass ready for market demands.

## Material and methods

### Location and timeframe of the experiment

The experiment was located at premises of the Kieler Meeresfarm GmbH & Co. KG in the northwestern part of the Kiel fjord in the Western Baltic Sea (54.381975 N; 10.162034 E). It was performed from 06 May 2019 to 31 July 2019. A pre-experiment in 2018 had shown that this is the season of highest larval pressure, which was confirmed by a weekly quantification of *Mytilus* sp. larvae in the water column ca. 1 km from the experiment site (P. Krost, pers. comm.). The onset of the larval fall at the beginning of July allowed to test the effect of desiccation

on *Fucus* growth rates before the occurrence of epizoans on the thalli, especially since *A. improvisus* and *E. pilosa* did not occur before that time either (Fig. 1). Moreover, former experiments had shown that growth rates of cultivated *Fucus* thalli are highest during this season (see also Lehvo et al. 2001).

### Algal material

*Fucus vesiculosus* and *F. serratus* individuals were collected from the field at 05 May 2019. *Fucus vesiculosus* was collected in Kiel/Holtenau (54.368966 N; 10.154112 E) and *F. serratus* in Bülk (54.455146 N; 10.198858 E). Both were transported in seawater to premises close to the cultivation site where they were stored overnight in seawater. The following day, vegetative thallus apices (hereafter “thalli”) were cut from the collected individuals for use in the experiment. The thalli had lengths of 3–10 cm for *F. vesiculosus* and 5–15 cm for *F. serratus*.

### Cultivation containers

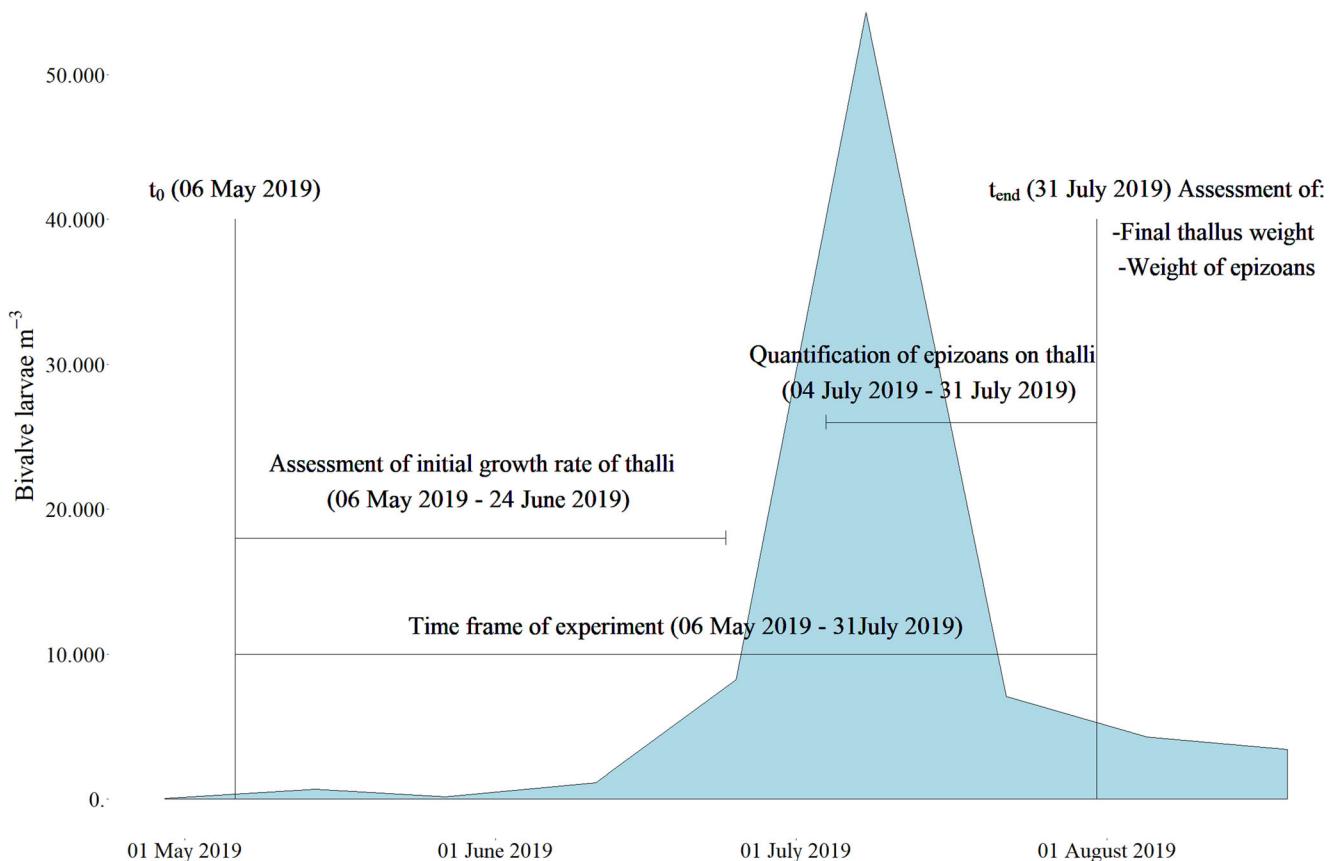
The thalli were cultivated in black plastic baskets (BAUHAUS; Oase Pflanzkorb; edge length: 28 cm; volume: 14 L). The baskets had holes of ca.  $0.3 \times 0.3$  cm at all sides allowing for the permanent inflow of surrounding seawater. Each basket was covered with a transparent plastic mesh to prevent loss of thalli. The baskets were arranged in packages of eight, leaving an empty space in the center (Fig. 2a). The basket packages were kept floating in the fjord by attachment to polyethylene foam pipe insulations. The experimental culture was protected from wave destruction by a pipe frame ( $2 \times 4$  m, Fig. 2a).

Three packages of eight baskets were used for the experiment. Thus, eight experimental groups could be tested, each being represented once in each of the three packages ( $n = 3$ ).

For *F. vesiculosus*, five treatments were applied:

- Control (no desiccation)

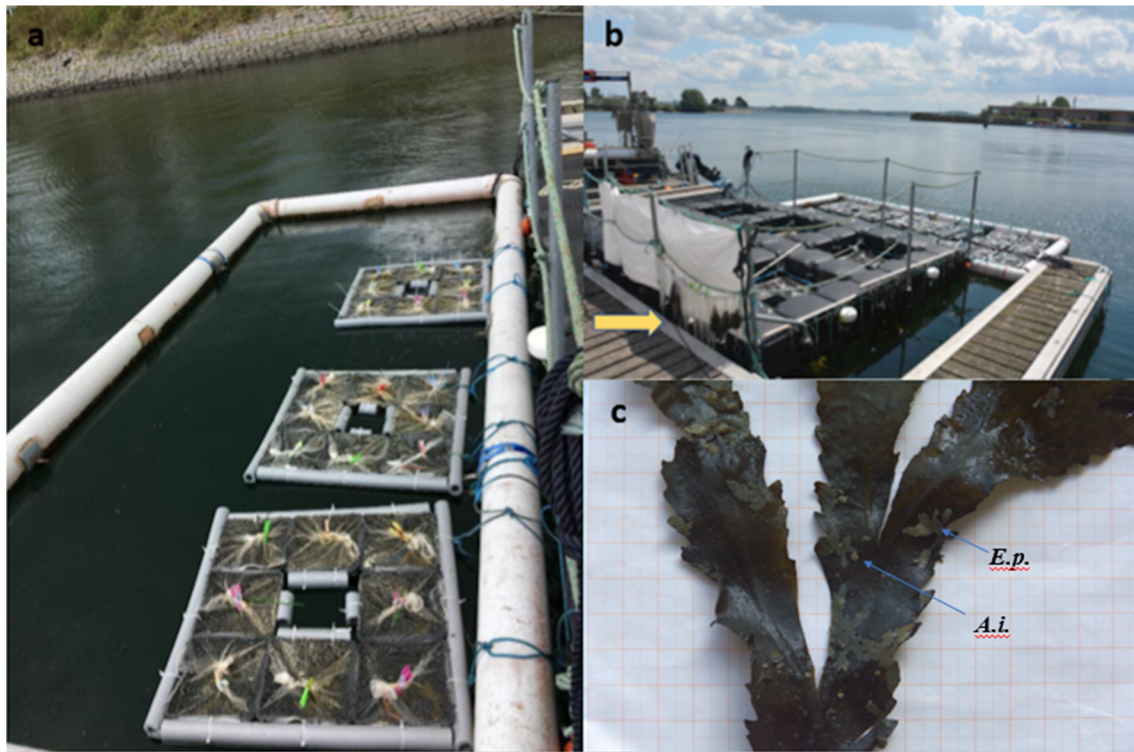
### Experimental time frame and occurrence of bivalve larvae



**Fig. 1** The experiment was arranged around the main bivalve larval peak in July. Weight measurements and screening for epizoans were performed during the entire experiment. Initial growth rates were calculated from unfouled exponential growth at the beginning of the

experiment. First epizoans were encountered after the onset of the peak at the beginning of July and counted from then onwards. For *Electra pilosa* and *Amphibalanus improvisus*, larvae were not quantified, but adults appeared parallel to bivalves on the thalli





**Fig. 2** **a** Cultivation baskets were arranged in three packages of eight baskets each, allowing for eight treatments with three replicates. **b** The cultivation site was located at the northwestern part of the Kiel fjord; thalli

were desiccated in plastic nettings in the shade of an awning (arrow). **c** Fouled *F. serratus* thallus from control treatment with *Electra pilosa* and *Amphibalanus improvisus*

- One desiccation to 80% of ww per week (in the following: 1 × 80%)
- Three desiccations to 80% of ww per week (in the following: 3 × 80%)
- One desiccation to 60% of ww per week (in the following: 1 × 60%)
- One desiccation to 40% of ww per week (in the following: 1 × 40%)

For *F. serratus*, three treatments were applied:

- Control (no desiccation)
- One desiccation to 80% of ww per week (in the following: 1 × 80%)
- One desiccation to 60% of ww per week; from 27.05. onwards: three desiccations to 90% of ww per week (in the following: 3 × 90%)

The treatment 1 × 60% of *F. serratus* was replaced after 3 weeks (27 May 2019) by 3 × 90%, because the thalli were already degrading due to the treatment. The degradation was first visible by a browning of the thallus margins and proceeded with a slow dissolution of the entire thallus. As first epizoans were detected at the beginning of July, a treatment change at this early stage of the experiment was considered possible. The replacing treatment was started at 27 May 2019

using newly collected thalli with the same average weight as the other two *F. serratus* treatments. The treatment 3 × 90% was chosen instead of 3 × 80%, because in pre-experiments, *F. serratus* had been proven to be very sensitive towards desiccation. A direct comparability to the *F. vesiculosus* treatments was lost by this change; however, it was rather the aim to find desiccation regimes suitable for *F. serratus* than to allow for a direct comparison to *F. vesiculosus*. As a consequence, only the controls of the two species were compared directly (see “[Statistical analysis](#)”).

### Cultivation and measurement of thallus growth

The thalli were freely floating in the baskets without any attachment. The cultivation was started with 20 g of thalli per basket. Thallus growth was assessed weekly by weight measurements. Before weighing, the thalli were dried with a salad spinner for 15 s. Weighing was performed with a lab scale (Kern EMB 1200) under wind protected conditions. The relative daily growth rate was calculated using the following formula:

$$\text{DGR}(\% \text{day}^{-1}) = (\ln(W_t/W_0)/t) * 100$$

where *DGR* is the daily growth rate in percent,  $W_t$  is the



weight at a given date,  $W_0$  is the weight at the beginning of the experiment, and  $t$  is the number of days between  $W_0$  and  $W_t$ . DGR was calculated until 24 June 2019 (Fig. 3a), because growth ceased to be exponential after that date for most experimental units, and there was no effect of epizoans on the growth rate up to this date (Fig. 1).

### Desiccation treatment

For desiccation, all thalli of one basket were filled in a plastic netting of known weight. The filled netting was then dipped in seawater for 2 s and hung on a rope for the further drying procedure. The rope was shaded by a white plastic awning to avoid direct sun exposure of the drying thalli. This was done because direct sun had led to very unequal drying of the biomass in a pre-experiment. After letting the thalli drain for 5 min, the weight of the

netting including the thalli was measured. The weight of the netting was subtracted in order to calculate the ww of the thalli. After that, the thalli were regularly weighed until the desired percentage of the ww (90, 80, 60, or 40%) was achieved. The thalli of all experimental units including the controls were filled in nettings three times a week to control for handling effects.

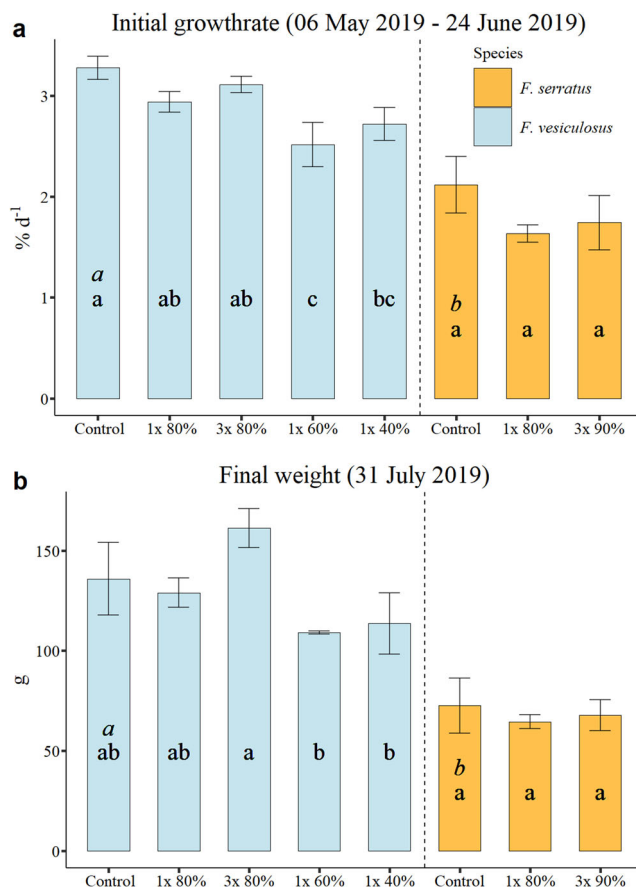
### Quantification of epizoans

The epizoans were counted weekly by screening every single thallus. The first epizoans were encountered at the beginning of July (Fig. 1) which coincided with the bivalve larval fall. A list of all encountered species is given in Table 1. *Clava multicornis* was only encountered on a few thalli and hence not included in the analysis. For *Electra pilosa*, the number of colonies was assessed. All bivalves were combined in one group, because it was impossible to correctly distinguish the young stages. The group was dominated by *Mytilus edulis*/*M. trossulus*, both of which occur at the location and hybridize strongly (Utermann et al. 2018). *Mya arenaria* was only encountered occasionally and represented only a very small portion of the bivalve biomass. *Mya arenaria* individuals larger than 4 mm were never encountered, as they probably died due to the lack of sandy habitat. Bivalves were counted at sizes > 0.5 mm. *Amphibalanus improvisus* was counted if identifiable with the naked eye (diameter ca. 0.7 mm). At the end of the experiment (31 July 2019), ten individuals/colonies of each group were scraped from the thalli of one replicate of each treatment except for *E. pilosa* as ten or more colonies were found only in *F. vesiculosus* control, *F. serratus* control, and *F. serratus* 1 × 80% (*F. vesiculosus* 3 × 80%:  $n = 2$ , 1 × 60%:  $n = 5$ , 1 × 40%:  $n = 0$ , *F. serratus* 3 × 90%:  $n = 7$ ). They were weighed with a fine scale and the average weight of the epizoans was calculated for each treatment individually, because they appeared to have different weights in the different treatments (Fig. 4).

### Calculation of further response variables

Using these average weights of each single treatment, the measured weights of the fouled thalli were corrected by the weights of the counted epizoans, resulting in epizoan-free *Fucus* weights for 31 July 2019 (Fig. 3b). Using these epizoan-free weights, it was possible to normalize the epizoan counts of 31 July 2019 by 100 g of pure *Fucus* biomass (Fig. 5a–c) and calculate the epizoan share of the final harvest (Fig. 5d).

*Fucus* growth curves over the entire experimental time (calculated as epizoan-free, using the epizoan weight data of



**Fig. 3** **a** Initial growth rates of *Fucus vesiculosus* and *F. serratus* thalli during exponential growth phase at the beginning of the experiment when no epizoans were present, calculated as percent ww increase per day. **b** Final weight after 86 experimental days (starting weight: 20 g), calculated as measured final weight minus calculated weight of epizoans. For both graphs, treatments were compared statistically within species (one-way ANOVA, roman letters). For species comparison, controls were compared by Welch's  $t$  test (italic letters). Error bars indicate standard deviation,  $n = 3$ , except for final weight of *F. vesiculosus* 1 × 60%, where  $n = 2$

**Table 1** Epizoan species encountered in the timeframe of the experiment

Species found on the cultured <i>Fucus</i> biomass	Phylum, class
<i>Clava multicornis</i> Forsskal, 1775	Cnidaria, Hydrozoa
<i>Electra pilosa</i> Linnaeus, 1767	Bryozoa, Gymnolaemata
<i>Mytilus edulis</i> Linnaeus, 1758	Mollusca, Bivalvia
<i>Mytilus trossulus</i> Gould, 1850	Mollusca, Bivalvia
<i>Mya arenaria</i> Linnaeus, 1758	Mollusca, Bivalvia
<i>Amphibalanus improvisus</i> Darwin, 1854	Arthropoda (Crustacea), Maxillopoda (Cirripedia)

31 July 2019) and normalized epizoan abundances over time are depicted in the supplementary material (Figs. S3 and S4).

### Environmental data

Irradiance was measured as photon flux density (PFD) in the PAR range in the botanical garden of the Christian-Albrecht University (4.87 km from the experimental site) in parallel to the experiment. For this purpose, a photodiode was placed at a height of 10 cm above the ground at an unshaded location. The photodiode was calibrated against a quantum sensor (LI-190, LI-COR, USA) and PFD was continuously measured every second with averages being stored for 10 min intervals and light sums being calculated for each day (method identical to Pescheck and Bilger 2019). The reduction of PFD within the cultivation baskets caused by the plastic mesh was determined at seven positions within a sample basket by a quantum sensor (LI-250A, LI-COR, USA). New mesh reduced the incoming irradiance by  $4 \pm 2\%$  and mesh colonized by a microalgal biofilm (typically occurring after 3–4 weeks of cultivation) by  $12 \pm 4\%$ . The water temperature within the black cultivation baskets was recorded every 30 min using a Hobo© pendant datalogger. Irradiance and water temperature are depicted in the supplementary material (Figs. S1 and S2).

### Statistical analysis

Statistical analysis was performed with R (R Core Team 2013). The data were square root transformed for analysis. The five groups of *F. vesiculosus* and the three groups of *F. serratus* were compared individually by one-way ANOVA and Tukey's HSD as post hoc test for all measured parameters (initial *Fucus* growth rate; *Fucus* final weight (31 July 2019, epizoan-free); epizoan weight; epizoan abundance; epizoan share of final harvest). In addition, the control groups of the two species were compared by Welch's *t* test. For the group *F. vesiculosus* 1 × 60%, one replicate was lost at the end of the experiment; therefore, the *Fucus* final weight at 31 July 2019, epizoan abundance, and epizoan share of final harvest had to be analyzed with two replicates for this group. For the analysis of the weights of *E. pilosa*, some treatments had reduced numbers of replicates due to the low numbers of

colonies found (see “Quantification of epizoans”). Normality of residuals and homogeneity of variances were tested by Shapiro-Wilk's test and Fligner's test, respectively. In five out of 27 analyses (weight of *A. improvisus* on *F. vesiculosus* and *F. serratus* groups; weight of Bivalvia on *F. vesiculosus* and *F. serratus* groups; *F. vesiculosus* control vs. *F. serratus* control for weight of Bivalvia), the assumptions were not fulfilled due to non-normal residuals. In the four cases of multiple group comparisons, Kruskal-Wallis test with a post hoc Dunn's test was used, while a Wilcoxon-Mann-Whitney test was used for the one case of a pair-wise comparison. *p* values below 0.05 were considered statistically significant.

## Results

### Growth of the cultivated *Fucus* biomass

The *Fucus* thalli of all treatments showed considerable growth during the experiment. Differences between the two *Fucus* species and among the treatment groups will be explained in detail hereafter.

#### Initial growth rate (06 May 2019–24 June 2019)

The control thalli of *F. vesiculosus* showed a significantly higher initial growth rate ( $3.27 \pm 0.12\% \text{ day}^{-1}$ ) than the control thalli of *F. serratus* ( $2.12 \pm 0.28\% \text{ day}^{-1}$ ) (*t* test: *p* = 0.0169, *df* = 2.4162, Fig. 3a), both untreated controls (*F. vesiculosus*, *F. serratus*) showed higher initial growth rates than the desiccation treatments, respectively.

For *F. vesiculosus*, mild desiccations (1 × 80%, 3 × 80%) reduced the growth rate slightly (to  $2.94 \pm 0.10\% \text{ day}^{-1}$  and  $3.11 \pm 0.08\% \text{ day}^{-1}$ ) but not statistically significant compared with the controls. Harsh desiccations (1 × 60%, 1 × 40%) led to a slightly stronger and statistically significant reduction of the growth rates (to  $2.51 \pm 0.22\% \text{ day}^{-1}$  and  $2.71 \pm 0.16\% \text{ day}^{-1}$ , ANOVA: *p* = 0.0007, *df* = 4, Tukey's HSD: *p* = 0.0078 and 0.0007).

For *F. serratus*, mild desiccations (1 × 80%, 3 × 90%) resulted in reduced growth rates ( $1.63 \pm 0.08\% \text{ day}^{-1}$

and  $1.74 \pm 0.27\% \text{ day}^{-1}$ ), the difference was, however, not statistically significant.

Among the mildly desiccated treatments, frequency of desiccation exerted in neither of the species a significant effect.

*Fucus serratus* was more susceptible to desiccation showing similar reductions of the growth performance at mild desiccation regimes as *F. vesiculosus* showed at strong desiccation regimes. As mentioned before, desiccation to 60% had led to degradation of the cultivated *F. serratus* thalli, whereas *F. vesiculosus* grew well at this treatment and even at desiccation to 40% of ww.

### Final weight (31 July 2019)

The average epizoan-free weight of the *Fucus* biomass at the end of the experiment (Fig. 3b) ranged between 109 and 161 g for *F. vesiculosus* and 65 and 73 g for *F. serratus* (averaged over replicates), thus the absolute growth within the experimental time ranged between 378 and 706% for *F. vesiculosus* and between 223 and 264% for *F. serratus*. The controls of *F. serratus* showed a significantly lower final weight than the *F. vesiculosus* controls (average absolute difference: 47%, *t* test:  $p = 0.0088$ ,  $df = 3.9678$ ).

For *F. vesiculosus*, there was no significant difference between the single mild desiccations ( $1 \times 80\%$ ) and the control; however, the  $3 \times 80\%$  treatment on average led to a 19% higher final weight than the control (non-significant, Tukey's HSD:  $p = 0.47$ ). This was not expected, as the highest initial growth rate was found in the control. The effect of harsh desiccations was similar to the effect observed in the initial growth rates but statistically not significant. Similarly, for *F. serratus*, the differences in initial growth rates between the control and the desiccation treatments almost disappeared over the duration of the experiment.

### Fouling

An intense colonization of the cultivated thalli by *E. pilosa*, bivalves, and *A. improvisus* occurred during the experiment from the beginning of July. However, the desiccation treatments led to significant reductions of the average individual weight as well as the abundance of epizoans compared with untreated control thalli. The details will be explained in the following.

### Weight of epizoans

The weight data of all epizoans showed a huge variability reflecting the different sizes of the measured individuals. Among the groups of one *Fucus* species, the maximum measured weights always occurred on control thalli.

### *Electra pilosa*

There was no significant difference in the weight of *E. pilosa* colonies between the controls of *F. vesiculosus* and *F. serratus* ( $0.08$  and  $0.05 \text{ g colony}^{-1}$ , *t* test:  $p = 0.24$ , Fig. 4a). Among the *F. vesiculosus* groups, *E. pilosa* colonies were significantly larger on control than on desiccated thalli, whereas there was no significant difference among desiccation treatments. A desiccated colony (averaged over all desiccation treatments) was 85% smaller than a colony on a control thallus. This trend was already observed during the experiment: the colonies found on the control thalli had sizes of up to several  $\text{cm}^2$  while those on desiccated thalli sometimes consisted only of few ( $< 50$ ) individual zooids. The same pattern was found for *F. serratus*.

On both *Fucus* species, the colonies on thalli desiccated three times a week were the smallest. For *F. vesiculosus* thalli desiccated once a week to 40%, no weights could be determined, because the thalli were completely bryozoan-free.

### Bivalves

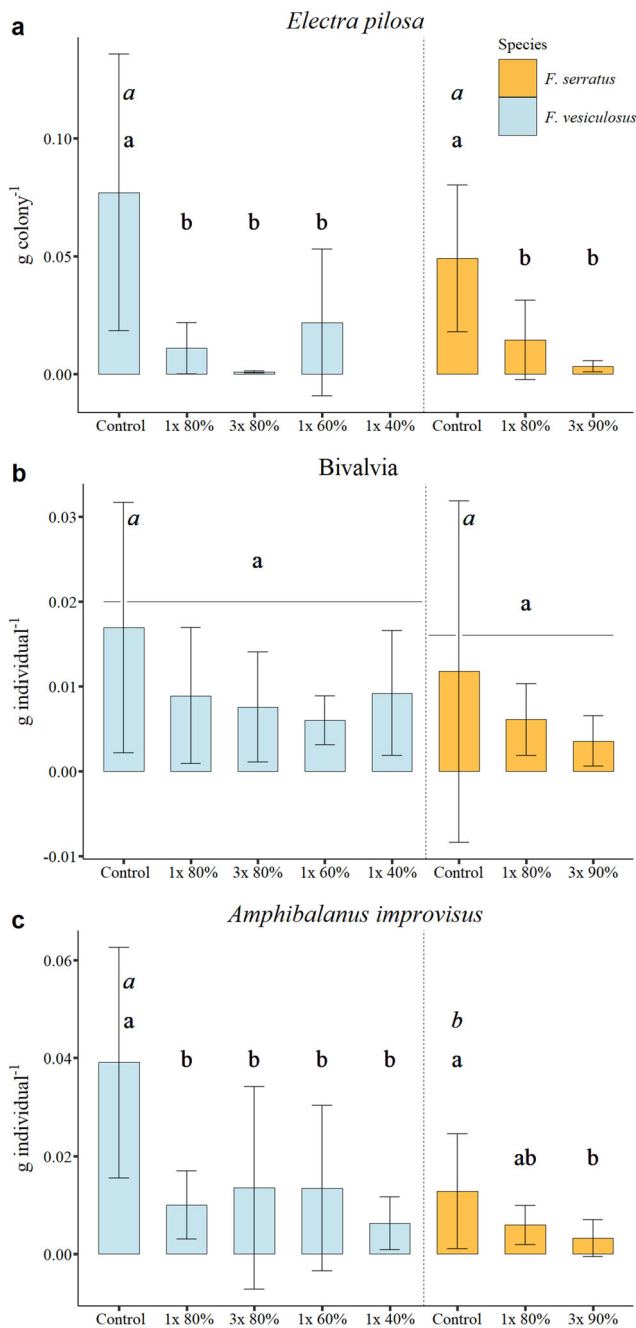
There was no statistically significant difference in the weight of bivalves between the control thalli of the two *Fucus* species (averages: *F. vesiculosus*:  $0.017 \text{ g}$ , *F. serratus*:  $0.012 \text{ g}$ , Fig. 4b). Among the groups, there was no significant difference in the weight of bivalves, but the untreated controls bore by trend heavier bivalves than the desiccated thalli (*F. vesiculosus*: control 53% heavier than average of desiccation treatments; *F. serratus*: control treatment 59% heavier than average of desiccation treatments). There was also a tendency towards smaller bivalves on frequently desiccated thalli compared with thalli desiccated only once a week; however, this trend was also insignificant in both species.

### *Amphibalanus improvisus*

*Amphibalanus improvisus* individuals were significantly heavier on *F. vesiculosus* control thalli ( $0.039 \pm 0.024 \text{ g}$ ) than on *F. serratus* control thalli ( $0.013 \pm 0.012 \text{ g}$ , *t* test:  $p = 0.0027$ ,  $df = 16.507$ , Fig. 4c).

In *F. vesiculosus*, individuals on control thalli were on average 72% heavier than individuals of the desiccation treatments (Dunn's test between control and all desiccation treatments:  $p < 0.01$ ), whereas no significant differences were found among the desiccation treatments.

In *F. serratus*, the barnacles found on control thalli were on average 75% heavier than those on thalli of the  $3 \times 90\%$  treatment (Kruskal-Wallis test:  $p = 0.0013$ ,  $df = 4$ , post hoc Dunn's test:  $p = 0.013$ ), but there was no significant difference



**Fig. 4** a–c Weight of epizoans scraped from one replicate of each treatment at the end of the experiment (31 July 2019). Treatments were compared statistically within species (one-way ANOVA/Kruskal-Wallis test, roman letters). For species comparison, controls were compared by Welch’s *t* test/Wilcoxon-Mann-Whitney test (italic letters). Error bars indicate standard deviation, different letters indicate statistically significant differences,  $n = 10$ , exceptions: *E. pilosa*: *F. vesiculosus* 3 × 80%:  $n = 2$ , 1 × 60%:  $n = 5$ , 1 × 40%:  $n = 0$ , *F. serratus* 3 × 90%:  $n = 7$ . The exceptions were due to the low numbers of *E. pilosa* colonies found on the treated thalli

between the control and the 1 × 80% treatment, although the averaged relative difference was 54%.

## Epizoan abundance

All epizoan species were most abundant on controls, except for *A. improvisus* on *F. vesiculosus*, where a similar number was found in the control and all treatments, except the 3 × 80% treatment (Fig. 5a–c). However, the degree of reduction by the desiccation treatments varied among the epizoan species and will be explained in detail hereafter.

### *Electra pilosa*

There was a significantly lower number of *E. pilosa* colonies per 100 g biomass on *F. vesiculosus* controls than on *F. serratus* controls ( $38.8 \pm 22.5$  colonies vs.  $211.6 \pm 13.9$  colonies; *t* test:  $p = 0.0096$ ,  $df = 2.2954$ , Fig. 5a).

Among the *F. vesiculosus* groups, desiccation reduced the number of bryozoans drastically. Mild desiccations reduced the number significantly from  $38.8 \pm 22.5$  to  $7.6 \pm 4.9$  (1 × 80%) and to  $3.0 \pm 3.0$  (3 × 80%) colonies (100 g seaweed)<sup>-1</sup> (ANOVA:  $p = 0.0004$ ,  $df = 4$ , Tukey’s HSD:  $p = 0.017$  and 0.003). The difference between one and three mild desiccations per week was statistically not significant. An even stronger effect exerted the harsh treatments on the number of bryozoans; in the 1 × 60% treatment, only  $0.5 \pm 0.7$  colonies were found, in the 1 × 40% treatment not a single colony was found in any of the experimental units (Tukey’s HSD:  $p = 0.0014$  and 0.0003).

For *F. serratus*, a similarly strong effect of the desiccation treatments was observed, the number of colonies was reduced from  $211.6 \pm 13.9$  in the control to  $18.0 \pm 3.2$  colonies in the 1 × 80% treatment and  $12.3 \pm 6.8$  in the 3 × 90% treatment (ANOVA:  $p = 1.3 \times 10^{-6}$ ,  $df = 2$ , Tukey’s HSD:  $p < 0.0001$  in both cases).

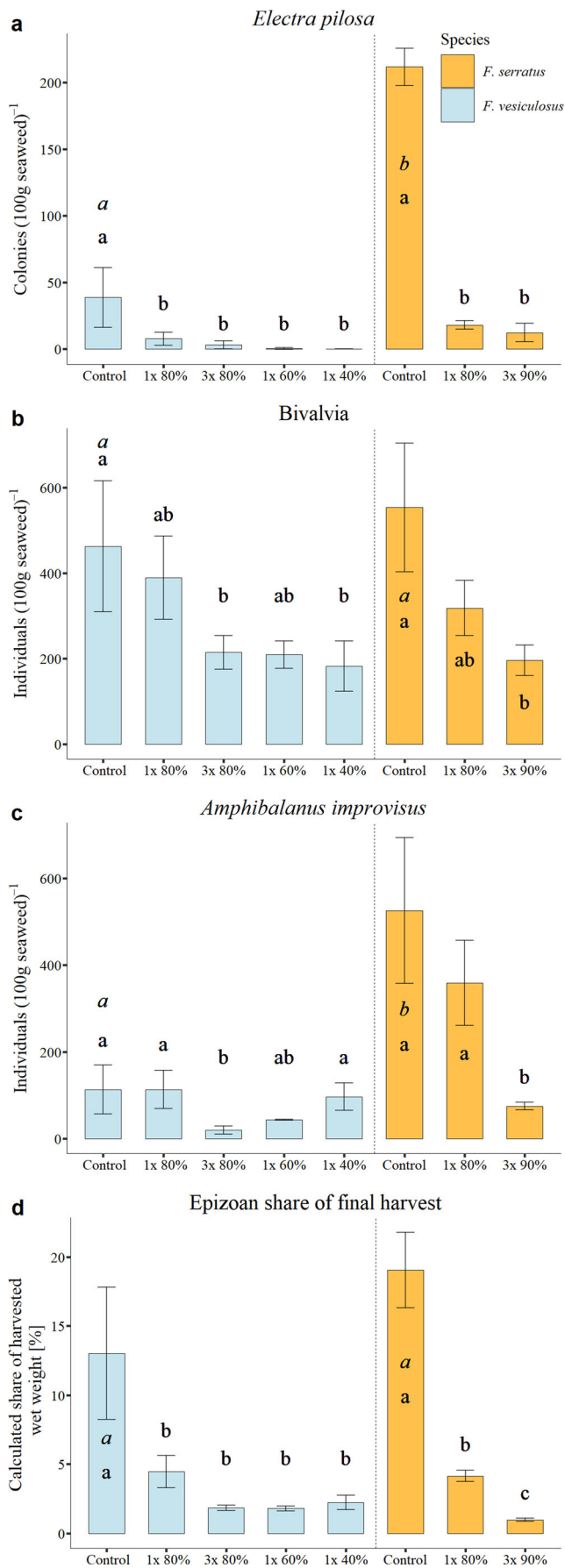
### Bivalves

The colonization by bivalves was fairly strong with at times more than 600 individuals (100 g seaweed)<sup>-1</sup> (Fig. 5b). There was no significant difference between the *F. vesiculosus* control and the *F. serratus* control (*t* test:  $p = 0.50$ ).

For *F. vesiculosus*, very harsh desiccations (1 × 40%) and frequent mild desiccations (3 × 80%) significantly reduced the number of bivalves from  $462 \pm 153$  individuals in the control to  $182 \pm 59$  individuals and to  $214 \pm 39$  individuals, respectively (ANOVA:  $p = 0.0099$ ,  $df = 4$ , Tukey’s HSD:  $p = 0.017$  and 0.043).

In *F. serratus*, desiccation produced similar effects (one-way ANOVA:  $p = 0.007$ ,  $df = 2$ ). The number of bivalves was reduced from  $553 \pm 150$  in the control to  $318 \pm 65$  in the 1 × 80% treatment and to  $196 \pm 36$  individuals in the 3 × 90% treatment (Tukey’s HSD:  $p = 0.055$  and 0.005).





**Fig. 5** a–c Number of epizoans per 100 g of *Fucus* biomass (calculated as epizoan-free) at the end of the experiment (31 July 2019). **d** Percentage of epizoan weight of total harvested weight at the end of the experiment (31 July 2019). For all graphs, treatments were compared statistically within species (one-way ANOVA, roman letters). For species comparison, the controls were compared by Welch's *t* test (italic letters). Error bars indicate standard deviation, different letters indicate statistically significant differences,  $n = 3$ , except for *F. vesiculosus* 1 × 60%, where  $n = 2$  for all graphs

### *Amphibalanus improvisus*

*Amphibalanus improvisus* also appeared in high numbers, especially on *F. serratus* with an average of  $526 \pm 68$  individuals (100 g seaweed)<sup>-1</sup> found in the control (Fig. 5c). This was significantly more than on *F. vesiculosus*, where only  $113 \pm 57$  individuals were found (*t* test:  $p = 0.014$ ,  $df = 3.4954$ ).

In *F. vesiculosus* as well as in *F. serratus*, only the frequent mild desiccations (3 × 80%, 3 × 90%) led to a significant reduction of the number of barnacles in comparison to the controls, on average by 83% and 86%, respectively. Infrequent treatments (1 × per week) were not effective, independent of the harshness of the desiccation.

### Epizoan share of final harvest

The strong fouling intensity is apparent in the high weight percentage of epizoans in the untreated controls (*F. vesiculosus*:  $13.0 \pm 4.8\%$ ; *F. serratus*:  $19.1 \pm 2.7\%$ , *t* test:  $p = 0.155$ , Fig. 5d). However, for both species, all desiccation treatments resulted in a significant reduction of this value (ANOVA: *F. vesiculosus*:  $p = 0.0001$ ,  $df = 4$ , *F. serratus*:  $p = 2.07 \cdot 10^{-6}$ ,  $df = 2$ , Tukey's HSD: all *p* values below 0.005), the frequent desiccation treatments (3 × 80%, 3 × 90%) being most effective resulting in only  $1.8 \pm 0.2\%$  in *F. vesiculosus* and  $1.0 \pm 0.1\%$  in *F. serratus*. For *F. vesiculosus*, there was no statistically significant difference among the desiccation treatments, whereas for *F. serratus*, the epizoan share of the 1 × 80% treatment was significantly larger than the epizoan share of the 3 × 90% treatment (Tukey's HSD:  $p = 0.0015$ ).

### Discussion

In this study, the question was raised if regular desiccation is effective in reducing the abundance and size of epizoans on cultivated *Fucus* biomass. A second question was if regular desiccation affects the growth rate of cultivated thalli. The results show that growth rate as well as epizoan size and number are influenced by regular desiccation and that choosing the right desiccation regime allows for cultivation at growth rates comparable to non-desiccated cultures, but with significantly less epizoan biomass and abundance. In the following

sections, the effects on *Fucus* growth and epizoan size and abundance will be discussed separately.

### **Fucus growth**

The results clearly demonstrate individual differences between the two *Fucus* species with respect to survival and growth performance under desiccation conditions. *Fucus vesiculosus* generally showed significantly higher growth rates (control: 3.27% day<sup>-1</sup>) than *F. serratus* (control: 2.12% day<sup>-1</sup>), which corresponds with data of our other cultivation experiments (Meichssner et al. in prep.).

*Fucus vesiculosus* appeared to be extremely tolerant to desiccation and was able to maintain considerable growth rates at weekly desiccations to 40% of its ww. This is not surprising as this species is adapted to intertidal habitats with regular desiccation (Schonbeck and Norton 1978; Andreev et al. 2012). However, the Baltic Sea populations are only subject to irregular desiccations caused by wind driven sea level changes (pseudo-tidal) and show some adaptation towards lower desiccation resistance compared with e.g. North Sea individuals (Pearson et al. 2000). *Fucus serratus* was less resistant than *F. vesiculosus* and degraded at desiccations to 60% of its ww (Schonbeck and Norton 1979a; Andreev et al. 2012). In the Baltic Sea, this species is air exposed only during extreme weather events. This is in contrast to the rest of its distributional range along North Atlantic coasts, where it forms a belt in the intertidal right below the *F. vesiculosus* belt and experiences desiccation on a daily basis (Schonbeck and Norton 1978; Contreras-Porcia et al. 2017). It seems that the Baltic Sea population has adapted or acclimated to a very low desiccation risk which has not been experimentally investigated as it has been for *F. vesiculosus* by Pearson et al. (2000).

If desiccation regimes applied in the course of the experiment were non-lethal (all but the 1 × 60% treatment for *F. serratus* which was replaced by 3 × 90%), the effects on the growth rates were very small. Only the harsh treatments (1 × 40%, 1 × 60%) applied to *F. vesiculosus* yielded a statistically significant reduction of the growth rate when compared with the control. All other treatments did not result in statistically significant growth reductions compared with their respective control within the 7-week period. Given these results, there seems to be a species-specific threshold of desiccation intensity (percentage of ww desiccated to), below which the thalli die and above which the growth is only slightly impacted, either by minor damages of the metabolism which can be repaired on little costs with respect to growth, or by a slight reduction of the metabolism. Generally, this result supports the applicability of this method for aquacultural purposes. It is also in line with Schramm (1968), who found that Baltic Sea *F. vesiculosus* thalli desiccated to 60% and 30% of ww show very similar relative assimilation rates over time (indicating similar growth rates) while thalli desiccated to less than 30%

of ww show reduced assimilation rates and die after some days. Thus, the threshold seems to lie at about 30% of ww for *F. vesiculosus*. For Baltic Sea *F. serratus*, the threshold is not exactly known, but based on our results, it seems to lie above 60% of ww.

The air temperature during desiccation may also affect survival and growth of *Fucus* thalli (Brinkhuis et al. 1976; Quadir et al. 1979; Gylle et al. 2009). However, as the temperatures during the desiccations were more or less equal for all treatments, we assume that the observed effects on *Fucus* growth resulted from the degree of desiccation rather than the air temperature during desiccation. The data of Schramm (1968), who could show that only air temperatures above 30 °C impact *F. vesiculosus* survival and that photosynthesis rates of thalli at different moderate desiccation regimes are similar at temperatures below 30 °C (which reflects the conditions during our experiment), confirm this assumption (but see Gylle et al. 2009).

The factor season (and hence water temperature, light conditions, and nutrient concentrations) may also impact the desiccation tolerance (Davison and Pearson 1996). Schonbeck and Norton (1979a, b) found higher desiccation tolerance in summer than in winter for *F. spiralis* (see also Haring et al. 2002), yet this has not been investigated for Baltic Sea *F. vesiculosus* and *F. serratus*. Another factor worth investigating in the cultivated *Fucus* species is drought-hardening, i.e. the increased tolerance due to frequent desiccations (Schonbeck and Norton 1979a, b), which was also not within the scope of this study.

Summarizing the effect of desiccation on the growth rate of cultivated *Fucus* thalli, it can be stated that non-lethal regimes reduce growth rates only slightly which allows for the aquacultural use of these regimes.

The appropriate desiccation levels, however, have to be determined for every cultivated species and/or population individually.

### **Epizoans**

In most cases, the desiccation treatments led to significant reductions of epizoan weight as well as epizoan abundance. In this context, the effects on the epizoan weight reflect their growth after they have successfully settled, metamorphosized, and developed on the thalli, while the effects on the abundance reflect the initial larval settlement success and subsequent survival.

The weights of *E. pilosa* and *A. improvisus* were significantly reduced by all desiccation treatments, the bivalve weight was also visibly reduced but not to the same extent as in the other two epizoans and statistically not significant. The strongest effect was observed on the size of *E. pilosa*; the colonies in the 1 × 40% and the 3 × 80% treatment (*F. vesiculosus*) consisted of less than 50 zooids while



colonies in the control treatment had areas of several cm<sup>2</sup>, consisting of hundreds of zooids.

In contrast to the other two epizoans, which are colonizing intertidal locations in the Atlantic and pseudo-tidal locations in the Baltic Sea, *E. pilosa* is a low inter- to subtidal species (Yorke and Metaxas 2011) and hence less resistant to desiccation. This was also clearly visible in the abundance data, where the strongest effects of desiccation were observed on *E. pilosa*. Especially the harsh treatments (*F. vesiculosus*: 1 × 60%, 1 × 40%) reduced the number drastically, in case of 1 × 40% even to zero. However, for *E. pilosa* even mild desiccations seem to be critical for survival, since no significant differences were observed between the different desiccation treatments.

The bivalves were equally affected by intensity and frequency of the desiccation, albeit their overall susceptibility was lower than that of the other two groups. Like *A. improvisus*, the bivalves seem to be most susceptible in the early settlement phase and therefore their number was successfully reduced by frequent desiccations. At the same time, harsh desiccations seem to be lethal even some days after settlement, visible by significantly less individuals in the *F. vesiculosus* 1 × 40% treatment than in the 1 × 80% treatment, which conforms to data of Jenewein and Gosselin (2013).

The abundance of *A. improvisus* was significantly more reduced by frequent mild desiccations than by single harsh desiccations, indicating that only the settling cypris larvae and possibly the early post-metamorphic stages of this species are intolerant to desiccation (Foster 1971a; Essock-Burns et al. 2017). In most of the cases, frequent desiccations every 2–3 days, as in the 3 × 80% (*F. vesiculosus*) and the 3 × 90% (*F. serratus*) treatment, did probably not allow for the sufficient development of structures necessary for desiccation resistance. Single desiccations per week seem to offer enough time for the formation of these structures; the developing adult stages were then able to survive even harsh desiccation treatments.

Epiphytes were scarce on the thalli in our experiment, because their biomass peak on Baltic Sea *Fucus* thalli is in spring (Rickert et al. 2015). Generally, we chose to study the effect of desiccation on epizoans and not on epiphytes, because epiphytes usually disappear after their growth period from the cultivated biomass and thus, despite their negative impact on *Fucus* growth (Rohde et al. 2008), do not have harmful effects on the quality of the biomass. Epizoans, by contrast, remain on the thalli once they have settled and thus drastically lower the quality and usability of the biomass. Our cultivation trials have shown that epiphytism is only a temporary problem while epizoans jeopardize the entire cultivation outcome.

Periodic air exposure and the concomitant desiccation are a determining factor for the distribution of species at intertidal locations (e.g. Foster 1971b; Boese et al. 2005). If the

physiological desiccation resistance of the algal host and the epibiont are not equal, desiccation affects the colonization of the algal host. The data presented here underline this fact and show that this principle can be used in the aquaculture of *Fucus* species in order to reduce the amount of epizoans. Most successful are frequent mild desiccations because they only cause minor growth rate reductions of the cultivated thalli and at the same time have a strongly reducing effect on the weight and abundance of epizoans. Three desiccations to 80% of ww reduced the epizoan share of the total harvest of *F. vesiculosus* from 13.0 to 1.8%, while the thallus growth rate was not significantly impacted. The resulting pure *F. vesiculosus* biomass (calculated as epizoan-free) was therefore higher in the 3 × 80% treatment than in the control (although the difference was not significant). For *F. serratus*, the epizoan share of the harvest was even reduced from 19.0% in the control to 1.0% in the 3 × 90% treatment. Here, the observed differences in the initial growth rate also disappeared in the final weight. These results indicate that the high epizoan load in the controls reduced their growth rates during the last month of the experiment leading to equal final weights at the end of the experiment despite of unequal initial growth rates. Considering these results, desiccation is absolutely necessary in order to maintain maximal growth rates and produce biomass which shows little epizoan colonization and is thus ready for market demands.

Of course, desiccation is not the only abiotic factor determining the amount of epizoans on seaweed thalli. Drag enforced by water motion does also influence the number of successfully settling epizoan larvae (Koehl 2007). In our culture system, the drag forces were almost zero, because the thalli were freely floating and hence able to move with the waves. This may explain the very high abundance of epizoans on the control thalli when compared with local field populations of *F. vesiculosus* and *F. serratus* that grow permanently submerged and show only very little epizoan colonization (Rickert et al. 2015). These populations grow attached by a holdfast, thus each water motion exerts drag forces on objects at the thallus surface possibly dislodging settling larvae.

Due to the absence of strong wave activity in the cultivation baskets, the whiplash effect, i.e. the process by which thalli by “continuous movement [...] clean the adjacent [...] surface and their own surface” (Kiirikki 1996), was also absent, which may have added to the striking difference between culture and field populations.

Apart from abiotic factors, biotic interactions play a crucial role in the colonization of seaweed thalli (e.g. Wahl and Hay 1995; Karez et al. 2000; Wahl et al. 2010). An important difference between the local field populations and the culture populations was that the snail *Littorina littorea*, which is very abundant in the field, was absent in the culture baskets. This snail efficiently grazes and/or bulldozes the epifauna and epiflora at the surface of *Fucus* thalli, probably removing a

huge proportion of settling epizoan larvae (Lubchenco 1983; Watson and Norton 1985; Buschmann 2000). This “cleaning” did not happen in our experiment.

Additionally, the seaweeds and/or associated bacteria produce substances, e.g. diterpenes or phlorotannins, which reduce larval settlement (da Gama et al. 2014; Rickert et al. 2015). At the same time, some epizoans have preferences for specific host species in response to their respective surface chemistry (Matson et al. 2010; Denley et al. 2014). Differences in this anti-fouling/preference interaction may explain the significantly higher abundance of *A. improvisus* and *E. pilosa* on *F. serratus* controls compared with *F. vesiculosus* controls. For *E. pilosa*, neither data on anti-fouling activity by *Fucus* species nor data on preference behavior of the cyphonautes larvae are published. Yet, both *Fucus* species are known to produce phlorotannins against the settlement of *A. improvisus* (Brock et al. 2007). In the study of Rickert et al. (2015), the deterring effect of *F. serratus* surface extracts against *A. improvisus* settlement was stronger than the effect of *F. vesiculosus* surface extracts, which contradicts the results of our experiment. However, the same authors could not find a stringent connection between anti-fouling activity of surface extracts and realized fouling community on the thalli.

Therefore, anti-fouling activity and/or choice behavior could still be an explanation for the huge differences between the colonization of the *F. vesiculosus* and the *F. serratus* control. In general, the influence of the culture conditions of our experiment, as well as the effect of the desiccation regimes on this complex interaction system, is unclear.

From the results presented here, only the effect of the abiotic factor desiccation can be inferred and it remains unclear if this factor acts directly on the epizoans and/or also indirectly via an impact on biotic interactions. However, for the aquacultural application, the actual effect is of greater interest than its precise origin.

### Practical issues

In this experiment, the cultivated biomass was desiccated to the targeted percentage of ww by regular weighing. This is of course not possible in large-scale aquaculture. Therefore, it is necessary to experimentally examine the evaporation of water from the cultivated thalli depending on the vapor pressure deficits that occur under the air temperature, air humidity, and wind conditions present at the cultivation site and can be calculated from those. Once these values have been determined, desiccation times can then be chosen according to the current or forecasted weather conditions. In this experiment, the thalli were desiccated in the shadow of an awning to avoid strong desiccation differences due to microclimatic differences between sun-exposed and shaded thalli. Of course, large aquaculture facilities can hardly be overshadowed

by an awning; therefore, desiccation during the night may be a suitable alternative having also the advantage of less extreme temperature fluctuations and thus more predictable desiccation conditions.

### Conclusion and outlook

The results clearly demonstrate that regular desiccation by air exposure reduces the amount of epizoans on cultivated *Fucus* biomass, on *F. vesiculosus*, and on *F. serratus*. At the same time, the applied desiccation regimes did not lower the growth rates significantly or at least not to levels unacceptable for cultivation purposes. We therefore conclude that the tested approach can be used as an effective measure to reduce epizoan fouling in *Fucus* aquaculture. In this study, we of course only tested the biological basics; a technically feasible large-scale solution for the implementation of this method in a *Fucus* farm was not the aim of this study and requires further development. One possible solution would be the regular elevation of net cages by inflatable floating bodies installed below the cages. The positive effects of desiccation on the fouling of the thalli found in this study can be equally expected on cage and other installation material used in an aquaculture farm. Therefore, a full emersion of entire cages would not only be practical but also beneficial for the cleanliness and thus the water permeability of the cages. During the experiment presented here, no grazing marks were observed on the thalli; however, amphipods, isopods, and snails are usually present in the cultivation baskets and may cause severe biomass losses in *Fucus* aquaculture. As at least amphipods and isopods are very susceptible to desiccation, the emersion of entire cages would probably also be an effective means to reduce grazing pressure in commercial cultures.

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**Author contributions** Rafael Meichssner was responsible for the concept and design of the study, assisted and supervised all experimental work, performed the data analysis, and wrote the first draft of the manuscript. Rüdiger Schulz supervised and supported the study conceptualization and the data analysis. Peter Krost assisted in the study conceptualization and the practical experimental design. RS and PK reviewed and edited the first draft of the manuscript. Anna-Sarah Cosin performed the essential pre-experiments in 2018. Nadja Stegmann performed *Fucus* growth measurements, Dagobert Sachs and Matthias

Bressan performed epizoan quantification and Henriette Marx performed the weight determination of epizoans. All authors read and approved the final manuscript.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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## Chapter IV - Supplementary material

## Environmental data

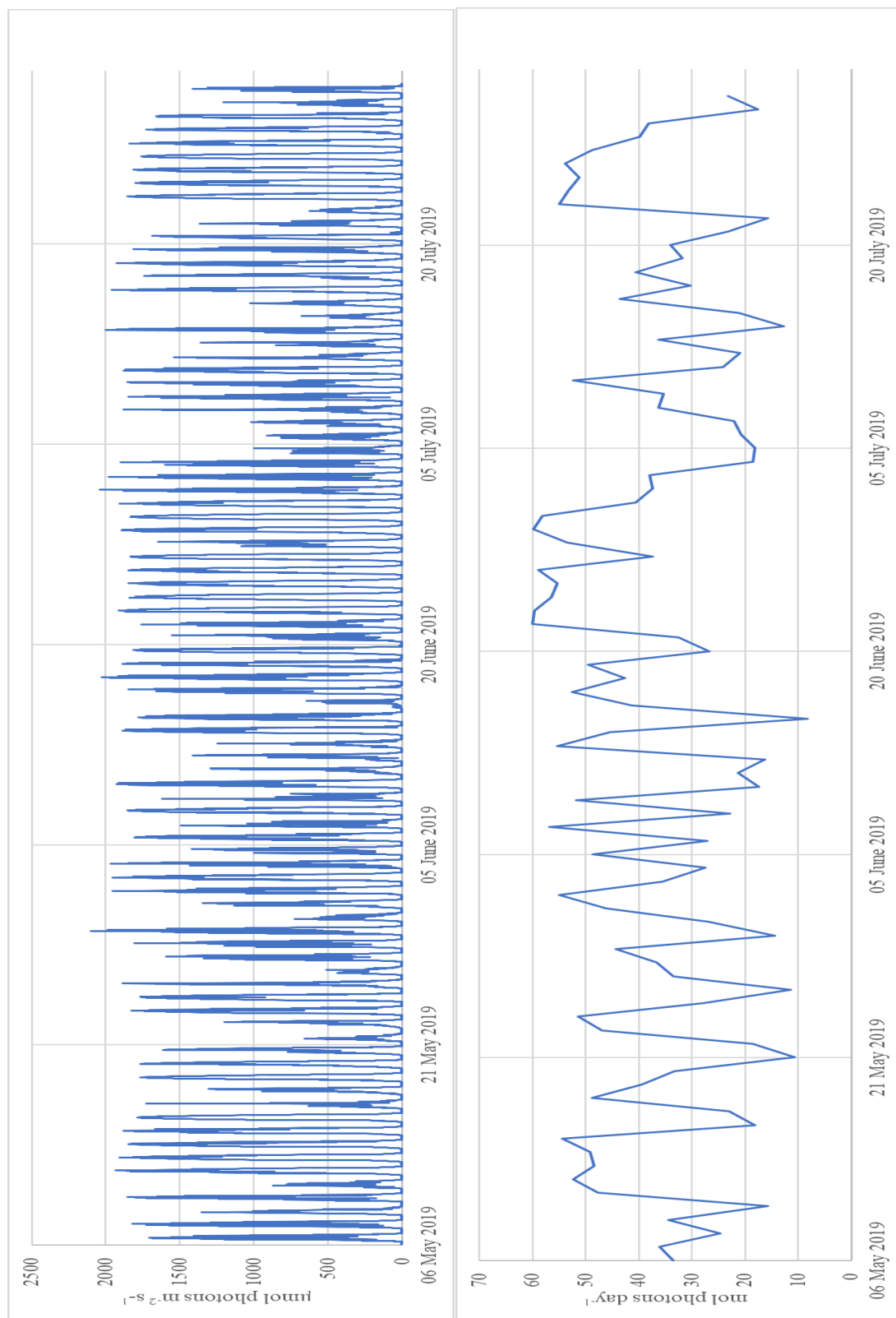


Fig. S1: Irradiance (PAR) during the experiment, measured 4.87km from the experimental site. Left: absolute irradiance ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) given as average of 10min intervals. Right: daily light sum ( $\text{mol photons day}^{-1}$ ), calculated from 10min intervals.

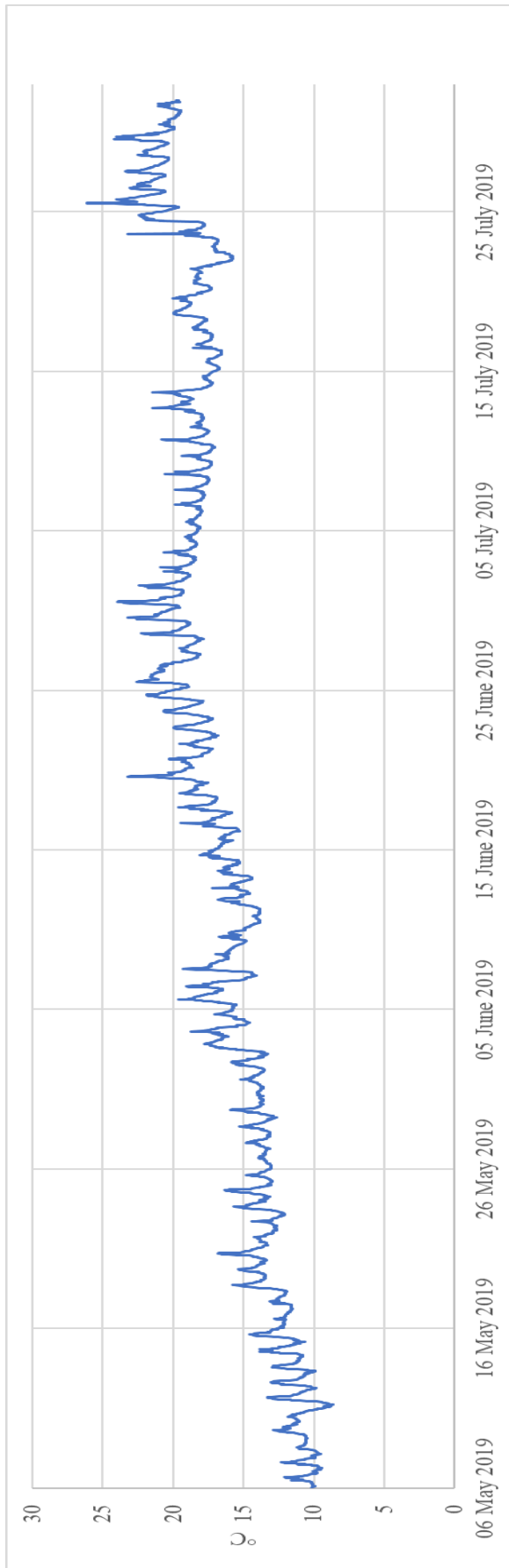


Fig. S2: Water temperature in the cultivation baskets during the experiment.



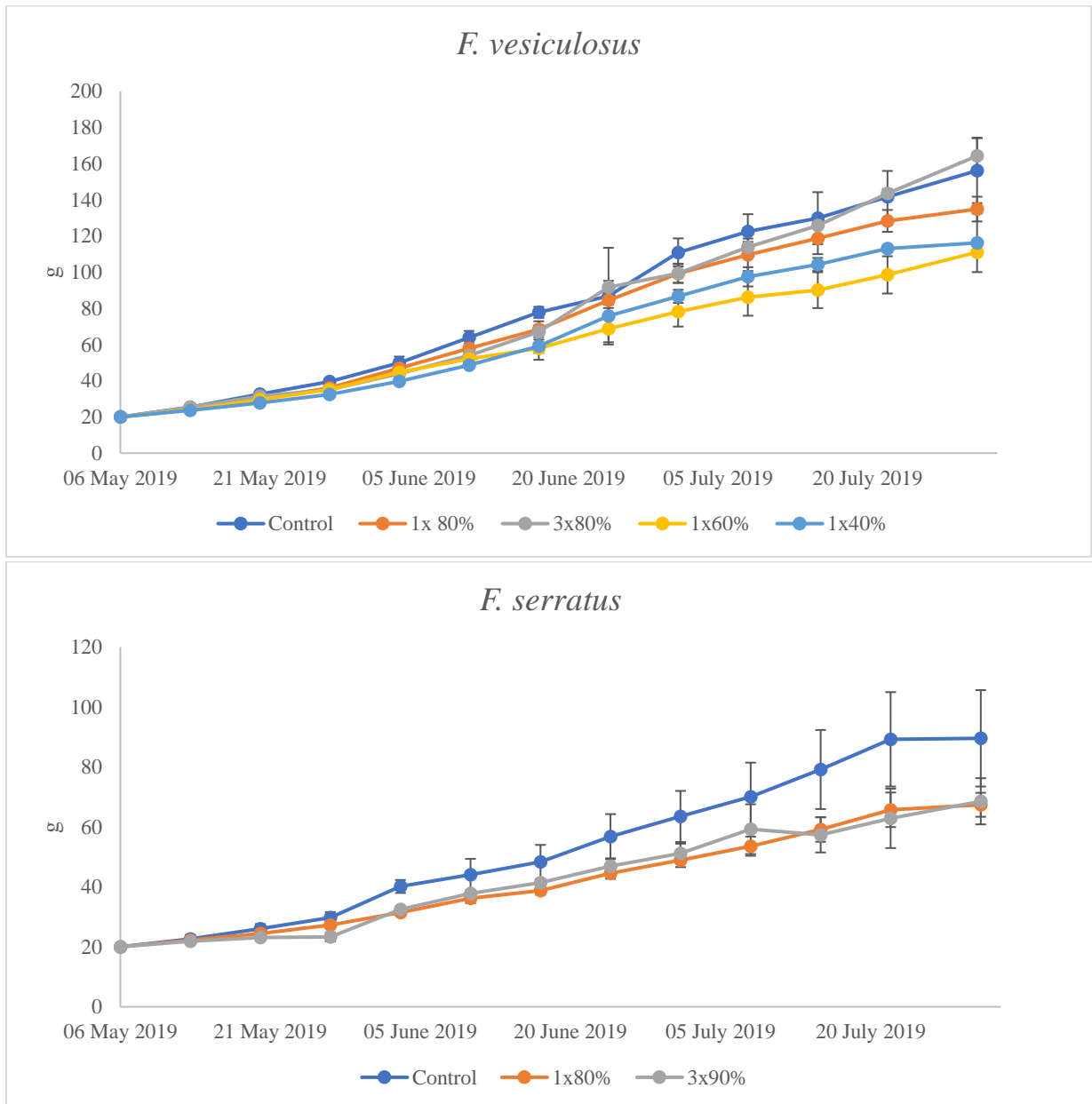
Growth of *Fucus* over time

Fig. S3: Weight of cultivated thalli over the entire experimental time calculated as epizoan-free, using the number of epizoans counted at the respective sampling day and the weights measured at 31 July 2019 (see “Material and methods”). Error bars indicate standard deviation, N=3.

## Abundance of epizoans over time

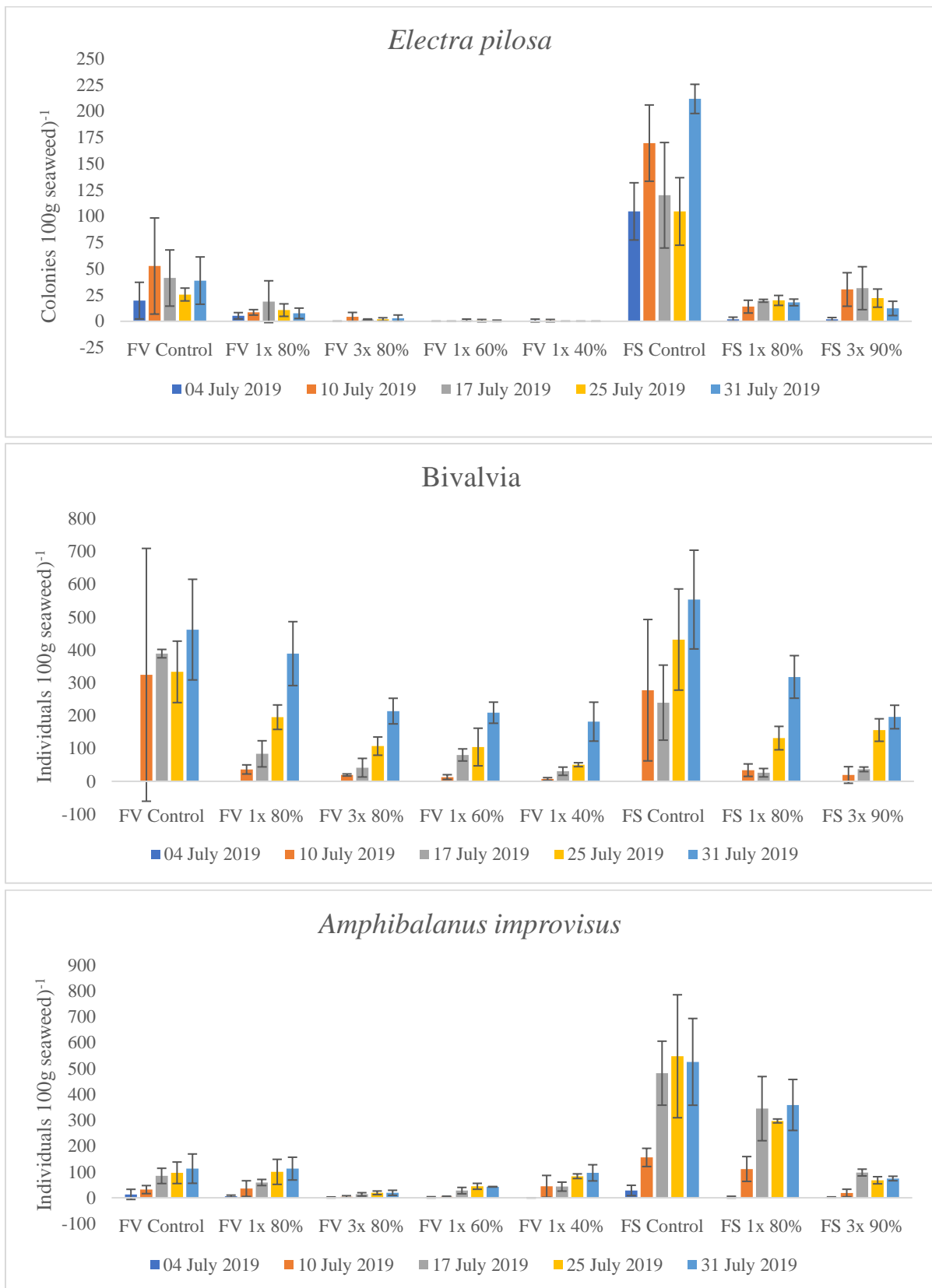


Fig. S4: Number of epizoans per 100g of *Fucus* biomass (calculated as epizoan free biomass) from 04 July 2019 (first observed occurrence of epizoans) to 31 July 2019 (end of experiment). FV: *F. vesiculosus*; FS: *F. serratus*. Error bars indicate standard deviation, N=3.

## **Chapter V – Synthesis and outlook**

## Synthesis

### Summary of the thesis results

The main objective of this thesis was to experimentally investigate core questions concerning the vegetative aquaculture of *Fucus* species (*F. vesiculosus*, *F. serratus*). In the following, the main results will be summarized briefly:

In **Chapter II**, the question was raised, if thalli survive long-term cultivation using the described cultivation method (for details on the cultivation method see Introduction). Further, the effect of fertility on growth in culture was studied and it was asked if low-fertility, which was found to be crucial, can be obtained through acclimatization of thalli from attached high-fertility populations towards low fertility and/or through use of thalli from unattached populations, which exhibit low-fertility in the field.

The experiments revealed that *Fucus* thalli do survive long-term unattached cultivation in baskets/net cages; the growth potential of the meristems was not affected by the cultivation method and cultivated thalli showed growth of vegetative apices for two consecutive years. Low fertility was found to be crucial for cultivation success since the high fertilities typical for attached field populations resulted in almost no growth or even weight loss due to degrading receptacles. Culture biomass with constant low fertility could only be obtained from an unattached field population from Glücksburg (Flensburg fjord), which retained its naturally low fertility in culture and did not suffer from fertility-related biomass losses. Thalli from attached populations did not acclimatize towards reduced fertility by long-term unattached cultivation and are therefore not useful for the envisaged culture method.

In **Chapter III**, the question was raised, how much annual yield can be expected from culture with the described cultivation method. In order to answer this question, growth rates at different initial stocking densities and during different seasons were measured and used to simulate growth during one year for different cultivation scenarios (different initial stocking densities and harvesting densities). Furthermore, it was asked if growth parameters determined in small-scale culture can be transferred to commercial-scale culture in 1.2m<sup>2</sup>-net cages.

The growth rates in experimental culture varied significantly depending on cultivation season (typical seasonal cycle with low growth rates during winter and high growth rates during summer) and initial stocking density (parabolic yield-density relationship for *F. vesiculosus* and asymptotic yield-density relationship for *F. serratus*). Based on the measured growth rates, maximal annual yields of 7.71 kg m<sup>-2</sup> were modelled for *F. vesiculosus* (requiring 6 harvests and a cultivation from 2 kg m<sup>-2</sup> to 3.25 kg m<sup>-2</sup>) and of 6.35 kg m<sup>-2</sup> for *F. serratus* (requiring 5 harvests and a cultivation from 3.75 kg m<sup>-2</sup> to 5 kg m<sup>-2</sup>), respectively. Slightly different cultivation scenarios resulted in only marginally lower yields with only 2-3 harvests required. Generally, higher yields should be possible when optimized culture conditions are used (outside a Jetfloat system, with higher light exposure and water exchange). Growth rates measured in small-scale culture were equal to growth rates measured in 1.2m<sup>2</sup>-net cages. However, technical improvements of the net cages are needed for successful cultivation of the comparatively small thalli of *F. vesiculosus*.

In **Chapter IV**, the topic of epizoan fouling on cultivated biomass was addressed. It was asked, if the abundance and individual weight of the most frequently encountered epizoans (blue mussels, barnacles, bryozoans) can be reduced by regular desiccation of the cultivated biomass and how this affects the growth rate of the biomass. Different desiccation regimes (combinations of desiccation intensity and frequency) were compared.

The experiment revealed that regular desiccation reduces the abundance and weight of colonizing epizoans significantly, while the growth rates of both *Fucus* species were not or only moderately reduced

by desiccation treatments. Individual epizoan species as well as the two *Fucus* species showed different responses to different desiccation regimes. Overall, the most effective regimes were frequent mild desiccations (*F. vesiculosus*: 3x week<sup>-1</sup> to 80% of wet weight, *F. serratus*: 3x week<sup>-1</sup> to 90% of wet weight) as they reduced the percentage of epizoan biomass of the total harvested biomass significantly (*F. vesiculosus*: from 13.0 ± 4.8 % in undesiccated controls to 1.8 ± 0.2 %; *F. serratus*: from 19.1 ± 2.7% in undesiccated controls to 1.0 ± 0.1 %) and had at the same time no significant effect on the *Fucus* growth rates. However, it was not possible to produce completely epizoan-free biomass by applying desiccation treatments.

### Summary of the cultivation process based on the thesis results

Including the results of this thesis, the cultivation process can be depicted as follows (Fig. 1): thalli from a wild unattached population with low fertility are collected as seedling biomass. The collection is accompanied by regular monitoring of the population to ensure its continued integrity. The collected thalli are either used directly or smaller meristem-containing pieces are cut off for usage and non-growing stipe parts are removed. The thalli are then reared either in baskets/ net cages deployed in the sea or in land-based tanks. In both cases, maximal possible light exposure and water exchange should be provided in order to achieve maximum growth. Initial stocking densities and harvest densities should be chosen according to the optimal regimes presented in Chapter III. If the used population is affiliated to *F. vesiculosus* but contains no vesicles, it is more likely that regimes similar to those modelled for *F. serratus* apply (see Chapter III). When the desired harvest density is reached, new seedlings are cut from the harvested biomass and used for the next cultivation period.

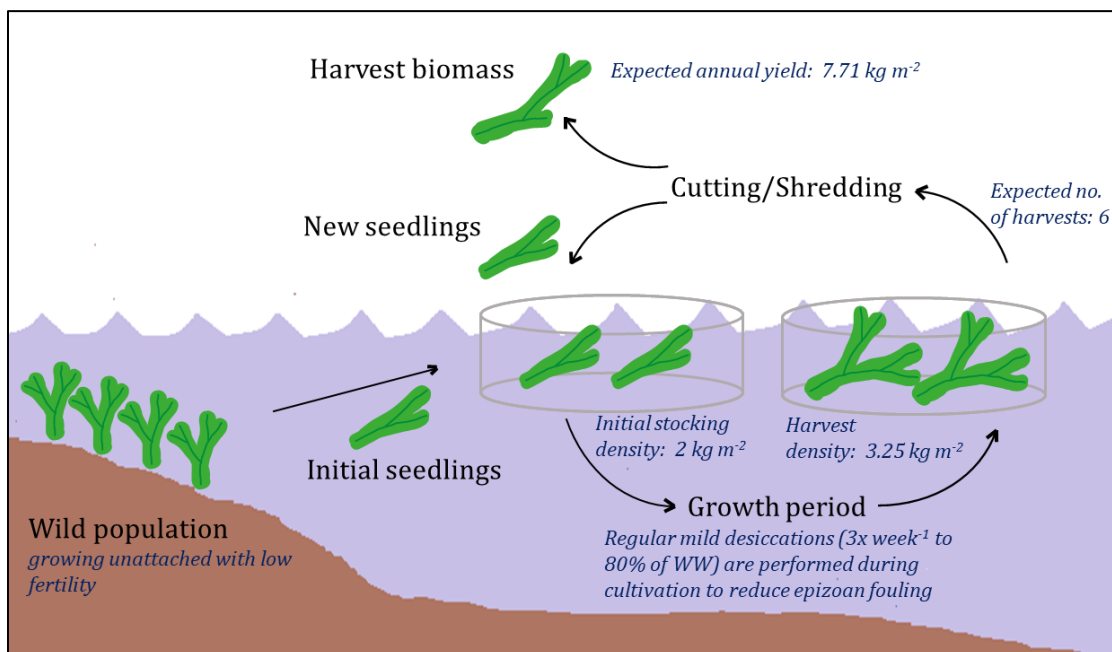


Fig. 1: Sketch of the envisaged cultivation process with information that has been obtained in the scope of this thesis (blue), exemplified for *F. vesiculosus*.

In the beginning of the cultivation process, it is probably necessary to use the entire harvest as seedling biomass in order to produce a larger stock of biomass since most unattached populations are small and the amount that can be obtained from the wild is low. Once sufficient biomass is available, larger (commercial) cultivation volumes can be realized and only a small portion of the harvest has to be used for seedling production while the rest can be processed. High epizoan and epiphyte loads on the cultured biomass are likely, but regular desiccation can help to reduce the colonization. For effective desiccation, the desiccation resistance of the used *Fucus* population should be experimentally determined before. In

open-water culture, desiccations should be performed during the night or at cloudy but not rainy days to ensure a predictable air temperature regime during the desiccation process (no strong micro-climatic gradients). However, the production of epizoan-free biomass will be impossible in open-water culture. In tank culture, epizoan fouling might be prevented by pre-filtering or sterilizing the used sea water. The mentioned tank culture options are so far only theoretical and have not been tested in experiment but appear to be promising and will therefore be included in the following discussion.

### **Limitations of the developed cultivation method**

The developed cultivation method is not suited for the fast production of large biomass volumes. First, because unattached wild populations are needed for initial seedling generation, these populations are mostly small and will provide only limited initial seedling biomasses. Second, because the general growth potential of *Fucus* species is low compared to other vegetatively reproduced seaweeds like *Kappaphycus alvarezii* and *Euchema denticulatum* (Kasim and Mustafa 2017, Kimathi et al. 2018). Therefore, some of the large-volume applications of seaweed aquaculture typically mentioned in the related literature (Kraan et al. 2010) are rather unrealistic, for example the production of biofuel or feed products. A realistic application of the developed cultivation method is the production of low-volume products like cosmetic extracts and food supplements and in this context it might serve as an alternative to the currently used harvesting of wild stocks. Further research is needed in order to decide if open-water culture or tank culture should be preferred for the corresponding applications, especially with respect to the required cleanness of the produced biomass (i.e., the presence and amount of epiphytes and epizoans, Chapter IV). Significant nutrient extraction from large eutrophic water bodies (Fei et al. 2004) also appears to be beyond the possibilities of the developed cultivation method due to the abovementioned low growth potential. However, the method might be suitable for the treatment of smaller water bodies or small waste water volumes in integrated multitrophic aquaculture systems.

### **Comparison to culture based on sexual reproduction**

An alternative to the developed vegetative cultivation method would be aquaculture based on sexual reproduction. In this case, the cultivation process would include the following steps: first, the production of gametes and their fusion to zygotes, second, the seeding of zygotes on cultivation substrate and the subsequent rearing of germlings, third, the out-planting of seeded substrates to the sea and the cultivation of *Fucus* plants to harvest size. The production of zygotes and germlings is well-established for lab experiments (e.g., Maczassek 2014, Bogaert et al. 2015), but it is unclear if and under which conditions germlings survive and grow on commercial cultivation substrates (e.g., ropes). It is also unknown if and when an out-planting to the field should be performed. In cultivation trials performed so far, zygotes were seeded on ropes in indoor tanks but the germlings arrested in growth some weeks after seeding (F. Weinberger pers. comm., own observation) and no cultivation to harvest size could be achieved. Hence, this method is at the very beginning of development and it is unclear if successful culture is possible at all. The use of sexual reproduction would have two main advantages, first, the more common attached populations of *Fucus* species with usual fertilities could be used, second, large numbers of plants could be generated in a short amount of time based in a small number of reproductive parental plants. The main disadvantage of this method, which led to the focus of this thesis on vegetative aquaculture, is the need for a land-based hatchery process for the cultivation of the germling stages (Kerrison et al. 2018). If seeding could successfully be performed *in situ*, i.e., directly at the farm without a hatchery phase, sexual aquaculture would be a very effective alternative to the method developed here.

Fouling would probably become a problem in sexual aquaculture as well (Walls et al. 2017). In this case, the desiccation method for fouling reduction described in Chapter IV could potentially be applied in a similar manner, e.g., by air-exposing cultivation ropes.



### Comparison to harvesting of wild stocks

The intention of this thesis was to develop an aquaculture method as alternative to the harvesting of wild *Fucus* stocks, which have declined or are expected to decline in several regions including the Baltic Sea (Kautsky et al. 1986, Vogt and Schramm 1991, Jueterbock et al. 2013, Nicastró et al. 2013). The environmental impact of harvesting wild seaweed stocks in general is a matter of ongoing research: *Laminaria hyperborea* and *Ascophyllum nodosum* are harvested at large scales along North Atlantic coasts (Vea and Ask 2011, Guiry and Morrison 2013, Araújo et al. 2021). For both species, harvesting has been performed for several decades and the impact of harvesting on the stocks as well as the associated ecosystems has been studied repeatedly with complex results (Keser et al. 1981, Ugarte and Sharp 2001, Ugarte et al. 2006, Lauzon-Guay et al. 2021). The applied harvesting scheme (frequency, intensity) was found to be critical for the effect on the standing stock biomass; under optimal conditions the stock recovers completely before the next harvest is performed and thus the standing stock biomass does not change in the long term (Ugarte et al. 2006, Gendron et al. 2017, Lauzon-Guay et al. 2021). However, the age structure of the stocks can change due to harvesting activities (Steen et al. 2016). Further, negative effects on associated epiphyte species have been observed (Garbary 2017) and effects on the associated fish fauna are debated (Black and Miller 1991, Rangeley 1994). Therefore, it is generally recommended to employ a whole-ecosystem approach when evaluating the effect of harvesting wild seaweeds and not focus on the standing stock alone (Lotze et al. 2019). In contrast to the abovementioned species, studies on the effects of *Fucus* harvesting are scarce: it has been shown that the cutting height (0cm, 15cm, 25cm) as well as the seasonal timing of the harvest influence the impact on the standing stock biomass (Keser et al. 1981, Ulaski et al. 2020). However, published studies on the effects on the entire ecosystem are lacking to the knowledge of the author and therefore no general conclusion about the sustainability of harvesting wild *Fucus* stocks can be drawn. In comparison to wild harvesting, *Fucus* aquaculture has the definite advantage that wild stocks are spared, which is especially important in regions where *Fucus* stocks are at risk. Yet, in the particular case of vegetative aquaculture, initial seedling biomass has to be generated from unattached vegetatively reproducing populations (Chapter II), which are *per se* vulnerable due to their low regenerative potential. Therefore, the generation of initial seedlings from these populations should be accompanied by even more rigorous monitoring than the commercial harvesting of attached populations. Once a sufficient culture stock is developed, cultivation is potentially independent of biomass from wild stocks and at this point the advantage of sparing wild stocks does apply. Other advantages of seaweed aquaculture mentioned in the literature (e.g., provision of additional habitat for invertebrates) are less convincing and might be offset by disadvantages like the addition of artificial materials (Campbell et al. 2019). The environmental benefits associated to biomass removal (nutrient extraction, carbon extraction) apply to both, aquaculture and wild harvesting. In conclusion, based on the paramount incentive of protecting wild stocks, it is definitely a future-oriented effort to develop aquaculture solutions for *Fucus*. Even though the solution presented in this thesis is not suited for the production of large volumes, it represents a step on the way to efficient *Fucus* production independent of wild stocks and is therefore in line with the general notion of Araújo et al. (2021), who state that “it is widely recognized that a transition from wild stock seaweed harvesting to aquaculture is needed to meet the growing demand while avoiding the overexploitation of wild seaweed resources”.

## Outlook

The results of this thesis leave a series of open questions concerning the vegetative aquaculture of *Fucus*, which should be focal points of future research. The open questions can be assigned to three main categories, i) the general biology of *Fucus* species, ii) abiotic interactions relevant for the cultivation process, iii) biotic interactions relevant for the cultivation process. These categories will be discussed separately in the following. As technical aspects of the cultivation process were not specifically treated in the scope of this thesis, corresponding questions will be discussed only marginally where appropriate.

### Open questions concerning the general biology of *Fucus*

#### Long-term low fertility/ asexuality in culture

Based on the results of this thesis, unattached low-fertility *Fucus* populations are needed as seedling source for vegetative *Fucus* aquaculture: in Chapter II, the used naturally unattached population from Glücksburg retained a very low fertility over 1 year in culture (maximally  $2.0 \pm 3.1\%$  fertile apices). However, it remains unclear if the low fertility of this and other unattached populations is genetically determined or if it is the result of acclimatization to environmental conditions, or both (Norton and Mathieson 1983, Mathieson et al. 2006). Thus, a long-term guarantee of low-fertility in culture cannot be given at the current state of knowledge (Chapter II). Future research should focus on the identification of genes involved in receptacle formation of furoid brown algae and on the influence of hybridization and polyploidy, which have been observed in unattached low-fertility populations (Wallace et al. 2004, Coyer et al. 2006), on receptacle formation/ fertility. If any genetic determinant for low fertility or even total asexuality can be found (e.g., hybridization of certain parental species/populations), this might be used for the generation of asexual culture strains or culture strains with a guaranteed low level of fertility. On the other hand, the influence of cultivation conditions (salinity, nutrient concentrations, water movement etc.) on long-term fertility of the cultivated biomass should be further investigated (Maczassek 2014).

#### Induction of low fertility in culture

Unfortunately, wild unattached *Fucus* populations are rare compared to attached populations, which are found all along temperate rocky coasts of the northern hemisphere (Lüning 1985). Yet, they are not useful for aquaculture due to their high fertility (Chapter II). A potential option to generate low-fertility seedling biomass from attached populations might be an intervention in the (potential) photoperiodic control of receptacle formation of attached populations: it has been shown that *Fucus distichus* subsp. *distichus* from Nova Scotia, which is reproductive from late autumn to spring, does not produce receptacles during long-day (i.e., short-night) light regimes but during short-day (i.e., long-night) light regimes under controlled laboratory conditions (Bird and McLachlan 1976). Similar patterns have been observed for *Ascophyllum nodosum*, a close relative of the genus *Fucus* (Terry and Moss 1980), indicating that both species have a photoperiodic control of receptacle formation, more specifically, that receptacle formation is initiated under short-day conditions. Other abiotic factors (water temperature, light intensity during light period) did hardly influence the photoperiodic response (Bird and MacLachlan 1976, Terry and Moss 1980). For *Ascophyllum nodosum*, night breaks reduced the number of formed receptacles drastically during short day conditions, which indicates that the algae measure night length and not day length in order to regulate receptacle formation (Terry and Moss 1980). The mentioned night breaks might be an option to enable the cultivation of *Fucus* biomass from attached populations without fertility-related biomass losses as they are supposed to strongly reduce the number of initiated receptacles. Yet, it can only be speculated that Baltic Sea *Fucus* populations employ the same photoperiodic control as *F. distichus* subsp. *distichus* and *Ascophyllum nodosum*. For *F. vesiculosus*, two genotypes have been described from the Kiel Bay (Chapter II, see also Berger et al.

2001), “only spring bloomers” reproduce exclusively in spring with the receptacles being initiated in late autumn/ winter. “Spring and autumn bloomers” reproduce in spring and additionally during late summer with receptacle initiation in July, i.e., during a phase of short nights. *F. serratus* reproduces in winter with receptacle initiation starting in August. All three cases might be under photoperiodic control but with different specific thresholds of night length that need to be exceeded for the initiation of receptacle formation. It is also possible that increasing night lengths after a time of short nights induce receptacle formation. In any case, night breaks might prevent receptacle initiation which would potentially lead to a continually low fertility of cultivated biomass. Many questions concerning this approach need to be answered for successful application in culture, for example: Do *Fucus* species from the Baltic Sea show reduced fertilities if night breaks are applied? How long do night breaks need to be? Which light quality and quantity is needed for the night breaks? Are other growth parameters affected by night breaks? Experiments on this topic are highly encouraged.

#### Genetic impoverishment

Propagation of a small initial seedling biomass by vegetative reproduction might lead to genetic impoverishment over time, especially as the unattached source populations, which reproduce vegetatively, might already be genetically less diverse than sexually reproducing attached populations (Tooby 1982, Norton and Mathieson 1983, Wallace et al. 2004, Coyer et al. 2006, Mathieson et al. 2006, Johannesson et al. 2011, Sjøtun et al. 2017). Genetic impoverishment of cultures might become problematic under changing environmental conditions and especially with respect to resistance against pathogens (Tooby et al. 1982, Manning and Dickson 1986, examples from red algal aquaculture in: Loureiro et al. 2015, Charrier et al. 2017). However, it has to be remembered that wild unattached *Fucus* populations survive for decades although they are assumed to reproduce mainly by vegetative reproduction. One example is the unattached population in List/ Sylt which was described by Nienburg in 1931 and exists to this day. Genetic impoverishment in culture could be avoided by regular addition of seedlings from genetically different wild populations. Alternatively, crossing (i.e., sexual reproduction) with fertile thalli from wild populations might be a solution (Akem et al. 2000). However, it is unclear, i) if the few receptacles developed by low-fertility culture material from unattached populations produce viable gametes, ii) if these gametes are able to fuse with gametes from wild population of unattached/attached origin and produce viable germlings, and iii) if the offspring retains the desired low fertility necessary for successful vegetative culture (Chapter II, Sjøtun et al. 2017). Besides, the successful rearing of artificially produced *Fucus* germlings to adult size is still a problem (see above) and hence this approach is very theoretical at the moment.

Another potential factor, which might mitigate genetic impoverishment, is inter-clonal variation, i.e. variation in the capacity of single thalli (“ramets”) originating from the same clone to respond to environmental conditions. This phenomenon has been observed in many clonal organisms, and somatic mutations, mitotic recombination, transposons and epigenetic differences have, among others, been suggested as possible causes (Eriksson 1988, Santelices and Varela 1993, Sanchez-Gomez et al. 2011). Inter-clonal variation has also been observed in the red alga *Gracilaria chilensis* (now *Agarophyton chilense*), which is also cultivated in vegetative aquaculture (Santelices and Varela 1993). If inter-clonal variation occurs in vegetative *Fucus* populations is unclear.

#### Efficient seedling production

During the cultivation process, seedlings, i.e., thallus pieces containing an apical meristem, have to be generated initially from wild thalli and later from the harvested biomass when the next cultivation season is started. During the cultivation experiments, seedling production was performed manually using scissors. However, in larger-scale culture, this process has to be automated for example by shredding the harvested biomass. For efficient seedling production by shredding, the process should result in a

shredding product with the following qualities: i) a predictable size distribution of the resulting thallus pieces with the majority of thalli in the desired size range and only small portions being too small or too large, ii) a predictable and low share of thallus pieces which contain no apical meristems and are thus probably useless as seedlings (but see below). Due to the amount of labor, a sorting of the shredding product is probably not feasible if no automated sorting solutions are developed.

#### Formation of meristems at cut faces of seedlings

In the scope of this thesis it was repeatedly observed that small fronds (“adventive branches”) grow from the cut surfaces of cultivated thalli. The same can be observed in the field when adventive branches grow from wound surfaces of damaged thallus parts (Fulcher and McCully 1969, own observation). Adventive branches can grow from cut surfaces of thallus pieces with apical meristem as well as from cut surfaces of thallus pieces with removed apical meristem (Moss 1964). In a climate chamber experiment, even comparatively small thallus pieces without apical meristem (12 x 4mm) produced adventive branches after 4 weeks (own observation). In the open-water cultivation experiments of this thesis, cultivated thalli often developed adventive branches from cut surfaces. However, the adventive branches never grew to considerable sizes and the biomass produced from adventive branches was negligible compared to the biomass produced from normal thallus apices. When seedling biomass is produced by automated shredding, a portion of the shredded biomass is expected to contain no apical meristems (see above). The formation of adventive branches might be a possibility for these thalli to continue growth and thus add to the overall yield of the culture. However, it is unclear if thallus pieces without apical meristems survive the time of wound healing at the cut surfaces in open-water culture and if the biomass produced from adventive branches adds a significant share to the overall yield of the culture.

### Open questions concerning interactions with the abiotic environment during cultivation

#### Light

*Fucus* species are generally considered as high-light-adapted when compared to subtidal seaweed species (Bäck and Ruuskanen 2000, Fortes and Lüning 1980). They show fairly high saturation irradiances for photosynthesis (e.g., between 200 and 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  for *Fucus vesiculosus* (Bäck and Ruuskanen 2000, Nygård and Dring 2008)) and can prevent photodamage at irradiances above the saturation level by dynamic photoinhibition (Ekelund et al. 2007, own observation). Accordingly, no signs of growth reduction by light stress have been observed during the experiments of this thesis and highest growth rates were observed when the cultivation baskets were deployed outside the Jetfloat system, i.e., at optimal light exposure (Chapter III, IV). Therefore, commercial cultivation devices should be designed to allow for maximal incoming irradiance, especially since not all thalli within a cultivation device are equally well light-exposed (Chapter III). In tank culture, additional artificial illumination might be an option to increase growth. This could be achieved for example by increasing the available irradiance during times of low natural light levels (e.g., during winter) or by extending the natural day length. The latter might be an option even during summer as *Fucus* species show growth saturation only at day lengths of 18h and more (Strömberg 1977). How efficient the suggested uses of additional artificial illumination are, is unclear at the current state of knowledge. It is also unclear if long-term cultivation is successfully possible in the complete absence of natural sun light, e.g. in indoor integrated multitrophic aquaculture facilities.

#### Temperature

The studied *Fucus* species show temperature optima for growth between 10°C and 15°C depending on geographical origin (Fortes and Lüning 1980, Nygård and Dring 2008). A permanent cultivation at the temperature optimum would be desirable but is of course impossible in open-water culture. In the case

of tank culture, economic considerations will decide if and to which degree heating/ cooling should be used to control water temperatures. The following remarks only concern open-water culture without artificial temperature control: During winter, freezing poses threat to cultures even though short-time freezing is tolerated by *Fucus* species to some degree (Bird and McLachlan 1974, Davison et al. 1989). Lowering the culture devices and air bubbling might be options to avoid freezing damages. During summer, it is critical to avoid water temperatures above the tolerated maximum (e.g., 27°C for western Baltic Sea *F. vesiculosus* (Graiff et al. 2015b, Pansch et al. 2018)). Heat-related degradation of culture material might be avoided by temporal lowering of the culture devices or by pumping up cold water from greater depths. The latter might additionally serve as a non-invasive method of fertilization during potential nutrient limitation in late summer (Pedersen and Borum 1996, see below). The efficiency and potential adverse effects of the suggested measures should be tested in future experiments.

#### Nutrients and water movement

As discussed in Chapter III, nutrient limitation may occur in late summer and may be amplified by reduced water movement in enclosed cultivation devices. Therefore, open-water cultivation devices should allow for sufficient water flow through and movement. In tank culture, the water should be exchanged frequently and sufficient water movement should be guaranteed, for example by a wave generator (similar to wave generators described in Wahl et al. 2015), pumping or air bubbling. Even full tumble culture might be a possibility as it is used for instance in the culture of some red seaweeds (Pang and Lüning 2004, da Mata 2008). Artificial fertilization is discouraged as it is expected to foster mainly the growth of ephemeral epiphytes and microalgae in the cultivation system (Pedersen and Borum 1996, Worm and Sommer 2000). In open-water culture, artificial fertilization should generally be avoided as most coastal waters suffer from eutrophication already (Selman et al. 2008). Pumping up nutrient-rich water from greater depths might be an ecologically acceptable alternative (see above).

#### Salinity, dissolved inorganic carbon concentration and pH

The chemical water parameters salinity, dissolved inorganic carbon concentration (DIC) and pH affect the growth of *Fucus* species to different degrees. Salinity is a crucial factor influencing physiology and growth of *Fucus* species significantly as for instance Bäck et al. (1992a, b) and Nygård and Dring (2008) could show. The ambient salinity found at the natural habitat of *Fucus* populations intended for culture is not necessarily the optimal salinity for growth (Bäck et al. 1992b, Nygård and Dring 2008). Therefore, adjusting the salinity to the optimum, if known, might be a means to increase growth in tank culture. However, habitat-specific salinity conditions have been suggested to be a potential cause for the low fertility of some unattached populations (implying that low fertility is the result of an acclimatization process, which is not clear (Norton and Mathieson 1983, Mathieson et al. 2006)). Therefore, changing salinity in culture might not only influence growth but also fertility, and an increase of the fertility of the culture material should be avoided (Chapter II).

DIC is the only source of carbon for most seaweed species including Fucales (Hurd et al. 2014), which are able to utilize CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> but not CO<sub>3</sub><sup>2-</sup> for photosynthetic carbon fixation (Surif and Raven 1989). DIC concentrations co-vary with salinity and are also influenced by temperature and other factors (Hurd et al. 2014). Generally, it is believed that seaweeds are not carbon limited in full saline habitats (Surif and Raven 1989, Hurd et al. 2014). However, in brackish conditions, a growth-stimulating effect of CO<sub>2</sub> fertilization on *F. vesiculosus* has been observed (Nygård and Dring 2008, Graiff et al. 2015a). Therefore, further experiments should be performed to evaluate the potential of CO<sub>2</sub> fertilization for *Fucus* aquaculture including testing for interactions with other factors like salinity and light availability.

Although the pH of surface seawater is fairly constant (~8-8.25, Jiang et al. 2019), significantly higher pH levels have been observed in macroalgal beds as well as in rock pools (Middelboe and Hansen 2007).

At high pH, the inorganic carbon equilibrium shifts to the side of  $\text{CO}_3^-$  and thus pH can exert a direct effect on the plants as well as an indirect effect via the alteration of the DIC composition and thus the availability of carbon species for carbon fixation. Middelboe and Hansen (2007) detected a direct negative pH effect on growth of *F. vesiculosus* at pH levels above 8.5 and also found signs for an indirect growth inhibition via DIC alteration at pH 9.3. In open-water culture with sufficient water flow through, pH levels are expected to remain at non-problematic levels. However, in tank culture, it might be necessary to control pH.

#### Depth

The depth of open-water baskets/ net cages or land-based tanks is a sum parameter that integrates other parameters (mainly light, potentially also temperature and other parameters). According to the requirements mentioned before, depth should be chosen minimal to ensure maximum light exposure, while care should be taken to prevent temperatures to rise above the tolerated maximum which is more likely in shallow culture devices.

#### **Open questions concerning interactions with the biotic environment during cultivation**

In the scope of this thesis, epizoan fouling is the only biotic interaction that has been paid attention to. Other biotic interactions that might be important for future cultivation efforts will be discussed briefly in the following.

#### Epiphytism

In Chapter IV, regular desiccation has been shown to be an effective method to reduce epizoan fouling. Fouling by epiphytic algae has not been dealt with in the scope of this thesis as it occurs only seasonally (Colina 1981, own observations), while epizoans remain on the thalli once settled. Therefore, epiphyte-free biomass can potentially be produced by harvesting cultures only during seasons with no/few epiphytes present. However, epiphytes are generally not desired in a commercial cultivation process as they lower the quality of the biomass and reduce light and nutrients available for the cultivated *Fucus* thalli (Worm and Sommer 2000). Desiccation (Chapter IV) might be effective against epiphytes but further experiments are needed to test this hypothesis. Another option could be periodic addition of periwinkles (*Littorina* sp.) during times of epiphyte growth as these grazers prefer epiphytes over *Fucus* and can be removed easily compared to crustacean grazers once the epiphyte season is over (Lubchenco 1983).

#### Grazing

Next to epizoan colonization, grazing was the most prevalent biotic interaction observed in the scope of this thesis (Chapter II). It has been shown that grazing by isopods (*Idotea balthica*) and amphipods (*Gammarus* sp.) can drastically reduce the number of meristematic apices and thus limit the growth potential of the cultivated biomass (Chapter II). If cultivation is performed in land-based tanks, grazing can be prevented by pre-filtering the used seawater, which is not possible in open-water culture. In open-water culture, regular air exposure, which is effective against epizoan foulers, might reduce grazer abundance as well, since desiccation is lethal for crustacean grazers (own observation). A regular treatment of the cultivated biomass with fresh water (either by spraying or submergence) could also be an effective method to keep the grazer abundance low (own observation). However, the effect of this treatment on growth of the cultivated *Fucus* species requires further studies. A highly speculative alternative method for the reduction of grazer abundance in cultures is co-cultivation with crustaceivorous fishes, similar to the use of wrasses (Labraeidae) for the control of salmon lice in salmon farming (Skiftesvik et al. 2013). Periwinkles (*Littorina* sp.), which colonize cultivated biomass through



planktonic larvae (own observation), are expected to be resistant against all suggested treatments and have to be removed manually.

### Diseases

Various diseases are known from commercially cultivated seaweeds, which can result from infections by viruses, bacteria, protists, fungi or algal endophytes (Bolton et al. 2009, Gachon et al. 2010, Hurd et al. 2014, Wahl et al. 2014). Diseases of *Fucus* field populations are, to the knowledge of the author, not described so far. Nevertheless, it is reasonable to expect disease outbreaks in culture; during an experiment of this thesis, few cultivated *F. serratus* thalli showed symptoms of a disease infection. The thalli stopped growth and lost their natural texture within a few days and decayed shortly after. The degradation was clearly distinct from degradation caused by over-desiccation (described in Chapter IV): the apparent brown coloration of the thallus margins observed in over-desiccated thalli did not occur, the loss of texture concerned the entire thallus simultaneously (and not the margins first as in over-desiccated thalli) and the degradation was more rapid in the case of the suspected infection. If *Fucus* culture will be extended to a commercial level, disease prevention should be addressed in future research, especially since vegetatively reproduced stocks have only a limited genetic repertoire to resist potential pathogens (see above).

### Conclusion

In this thesis, a feasible vegetative cultivation method for *Fucus* species has been developed which allows for reasonable biomass production if the culture biomass can be kept at low fertility. The identification of suitable unattached source populations and sustainable seedling generation from these populations is crucial for the implementation of the method. For the generation of high-value products, land-based culture solutions are probably superior and should be subject of future developments as epizoans and grazers can potentially be excluded. Open-water culture in combination with desiccation treatments might be preferred if a certain degree of epizoan fouling is tolerated or if other specific purposes (e.g., nutrient extraction) are pursued.

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## **Erklärung**

Ich versichere an Eides statt, dass diese Abhandlung - abgesehen von der Beratung durch meine Betreuer - nach Inhalt und Form eine eigenständige und nur mit den angegebenen Hilfsmitteln verfasste Arbeit ist. Weiterhin, dass diese Arbeit oder Teile dieser Arbeit bisher nicht im Rahmen eines Prüfungsverfahrens vorgelegen haben, veröffentlicht worden ist oder zur Veröffentlichung eingereicht wurde.

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I declare, under penalty of perjury, that this work - apart from guidance by my supervisors - is my own work in content and form and has been authored only by help of the cited resources. Further, that this work or parts of this work have so far not been subject of an examination process, and have not been published or submitted to publication in the context of an examination process. I also declare under penalty of perjury, that this work has been performed under the rules of good scientific practice of the German Research Foundation and that I was not withdrawn an academic title so far.

Kiel, the

*Rafael Meichßner*

