The meaning of genetic diversity
on the example of the bladder wrack
Fucus vesiculosus L.

Part II:
'Development of different fucoid genotypes in different water depths'

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Summary

In the Western Baltic the bladder wrack *Fucus vesiculosus* has shown a massive retreat from the deeper zones of its former distribution and in some regions it seems to disappear also in shallow waters. *Fucus vesiculosus* is assumed to provide habitat for many organisms and is the most important macroalga in the German Baltic Sea for evaluation according to the Water Framework Directive (WFD). A re-establishment of *Fucus vesiculosus* where it is locally vanished would be an indicator for improved water quality. Thus within the context of the RADOST project (Regional Adaption Strategies for the German Baltic Sea Coast), the Agency for Agriculture, Environment and Rural Areas of Schleswig-Holstein (LLUR) is thinking about opportunities to resettle *F. vesiculosus*. In shallow waters *F. vesiculosus* individuals are exposed to environmental stressors like high temperatures in summer. In greater depths development of *F. vesiculosus* might be impaired by low light availability. Because of Climate Change *Fucus* algae will be exposed to a drift of different environmental variables and thus to an increasing strengths of different stressors, such as rising temperatures. On behalf of RADOST project, the tolerance of early fucoid life stages towards thermal stress was analysed and after four days of thermal treatment a high percentage of the treated fucoid offspring survived. If inside species genotypes exist which are less sensitive against future environmental changes, the receipt of a strong ecosystem could be possible because of punctual dispersal of these individuals. For a resettlement of *F. vesiculosus* several essential questions have to be answered. One question which has to be explained is if the sensitivity towards possible stressors, such as lower light intensity in deeper zones of the Baltic Sea, varies among fucoid genotypes. To elucidate the development of specific sibling groups at different depths (1, 3, 5 m), fucoid offspring from specific sets of parents were exposed to the location Bülk (Kiel Fjord; German Baltic Sea). At lower depths mean values for maximum light intensity were about 1-10% of maximum values, occurred at a depth of 1 m. In contrast low variation of temperature between depths was found. Development of fucoid siblings was at a depth of 1 m higher than at 3 and 5 m. Further an inter-individual variability between fucoid sibling groups was found. For example, survival of *F. vesiculosus* individuals from specific sibling groups was higher than at other groups, also at greater depths. Thus it might be that inside

species genotypes exist which are less sensitive against future environmental changes.

Backround

Global climate change will affect marine ecosystems in several ways. The semi-enclosed Baltic Sea is characterized by its glacial development including species adapted to cold water conditions. Therefore strong ecological impacts are expected due to future global warming. The RADOST project (Regional Adaption Strategies for the German Baltic Sea Coast) aims to develop adaptation strategies for the Baltic coastline of Mecklenburg-Western Pomerania and Schleswig-Holstein. The Agency for Agriculture, Environment and Rural Areas of Schleswig-Holstein (LLUR) is one among 17 partners within the project. According to the Water Framework Directive (WFD), key aspects of their project activities include the development of concepts to protect the remaining populations of the bladder wrack (*Fucus vesiculosus*) and to re-establish the bladder wrack where it is locally vanished. Detailed management strategies of coastal ecosystems require scientific knowledge about the impact of climate change on marine macrophytes in the Baltic Sea. For example, if sensitivity towards possible stressors, like for example high temperatures in shallow waters or lower light intensities in greater depths, may differ among fucoid genotypes. To support the aims of the WFD, the RADOST project financed the investigation in 2011/2012 which has been focused on the question if sensitivity towards possible stressors, such as high temperatures in shallow waters and low light availability in greater depths, may differ among *F. vesiculosus* genotypes. Thus in the present study the development of fucoid offspring from specific sets of parents in different depths (1, 3, 5 m) was determined.

Introduction

Despite its general adaptation to fully marine and intertidal environments the bladder wrack *Fucus vesiculosus* has recruited since the last ice age into the Baltic Sea, where it is the most common canopy-forming and widespread species (Torn et al. 2006). But during the second half of the 20th century the *F. vesiculosus* has

experienced a massive retreat from the deeper zones of its former distribution and – in the Western Baltic - is now limited to the uppermost meters of the subtidal (0-3m) (Vogt & Schramm 1991, Torn et al. 2006), where it also seem to disappear in some regions. This decline is thought to be caused by effects of eutrophication such as decreased light penetration (Vogt & Schramm 1991). In the German Baltic Sea *F. vesiculosus* is the most important macroalga for evaluation according to the Water Framework Directive. A re-establishment of *F. vesiculosus* where it is locally vanished would be an indicator for improved water quality. Thus within the context of the RADOST project (Regional Adaption Strategies for the German Baltic Sea Coast), the Agency for Agriculture, Environment and Rural Areas of Schleswig-Holstein (LLUR) is thinking about opportunities to resettle the *F. vesiculosus*. Environmental conditions in greater depths can be different and may be imply stress for the resettled algae. Furthermore in the coming decades, beside scores of organisms in the Baltic Sea, *Fucus* individuals will be exposed to a drift of different environmental variables (temperature, salinity, pH, eutrophication, etc.) and therefore to an increasing strengths of different stressors. How strong the effect of this stressors is, is not only dependent from the tolerance range of different species but also from the genetically selected sensitivity within species. In a previous study the tolerance of early fucoid life stages towards thermal stress was analysed and an inter-individual variability between fucoid sibling groups was found (Maczassek 2009). Further, on behalf of the RADOST project, the mortality of early post-settlement stages of *F. vesiculosus* under thermal stress was determined and after four days of thermal treatment at 25°C, 74% of the treated fucoid offspring survived (Maczassek & Wahl 2011). But several essential questions have to be answered. One question which has to be explained is if the sensitivity towards possible stressors, like for example lower light intensity, varies among fucoid genotypes. Thus, on behalf of the Regional Adaptation Strategies for the German Baltic Sea Coast (RADOST project), we compared light and temperature conditions between different depths in the German Baltic Sea and analysed the development of different sibling groups (*F. vesiculosus*) under these conditions.

Material and methods

1. Sampling of mature individuals

In Juli 2011 mature specimens of *F. vesiculosus* were collected at a depth of 0.2-0.5 m at Bülk (Kiel Fjord, 54°27.327 N, 10°11.977; Fig. 1). Bülk is an exposed location with mainly hard substrate where *F. vesiculosus* predominantly occurs within the range of moles
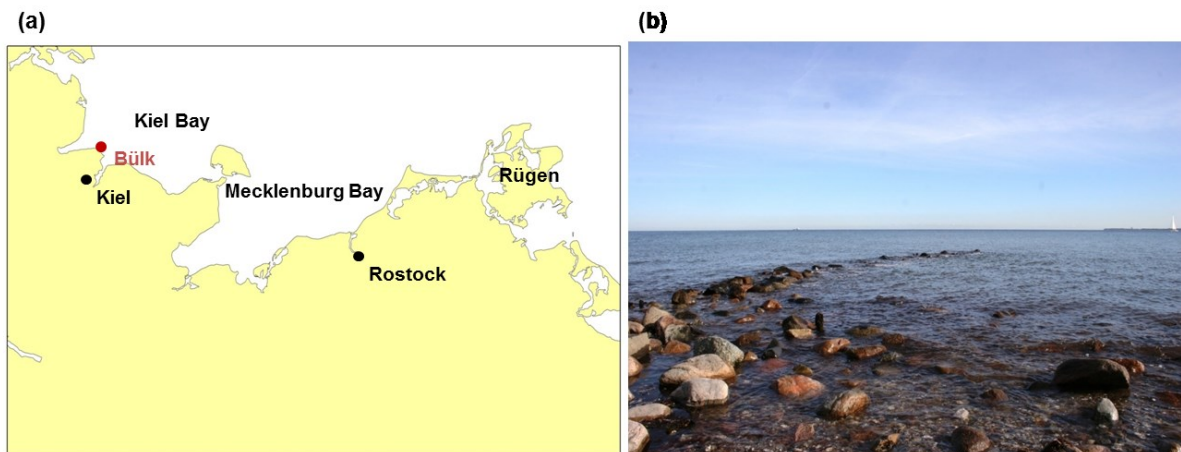

Fig. 1. Location Bülk of the German Baltic Coast where mature individuals of *Fucus vesiculosus* were collected (a), an exposed area with mainly hard substrate (b).

2. Gamete release and production of zygotes

The following method to obtain fucoid gametes was slightly modified from the method described by Karez (1997):

Gamete release by specific male and female algae (*F. vesiculosus*) was induced by cutting off mature tips (receptacles) (Fig. 2), rinsing them with fresh water and placing them in darkness in a climate chamber at a constant temperature of 15 °C.

Fig. 2. Mature tips (receptacles) of *F. vesiculosus*.

After 5 days, the tips were immersed in seawater (salinity 15, temperature 15°C) and exposed to light (200 µmol $m^{-2}s^{-1}$) for 2-5 hours. The time of exposure to light required for gamete release decreases with the maturity of the receptacles, and gametes only survive a few hours after release (Serrão et al. 1999). Consequently, receptacles were monitored continuously not to miss their gamete release, and fertilization was initiated as soon as possible after gamete release. A suspension of gametes from both male and female individual algae was placed into beakers and stirred to obtain a homogenous suspension. After fertilization, sibling zygotes from 11 sets of parents were achieved (11 beakers, each with a specific sibling group). 40 ml of the homogenous fucoid zygote suspension from every beaker was dispersed on 24 sand stone cube areas (2 cm^2) using a 10 ml glass pipette. The cubes then were fixed on PVC plates.

3. Development of sibling groups and transplantation into different water depths

Fucoid zygotes were placed first for 7 days at 100 µmol $m^{-2}s^{-1}$ 16:8 hours) in the climate chamber (15°C) and secondly from August-December 2011 into Kiel benthocosms, 4000 L experimental units at the Kiel Fjord (Fig. 3 b) (http://www.geomar.de/de/news/article/die-ostsee-der-zukunft-im-miniformat/). In the Kiel benthocosms, fucoid offspring was treated with natural seawater from the Kiel Fjord (salinity depending on the occurring salinity in the Kiel Fjord, which is mainly

around 16), daylight and environmental temperature. There, a high and uniform density of 7 sibling groups developed from an overall of 11 sibling groups (*F. vesiculosus*) (Fig. 3 a).
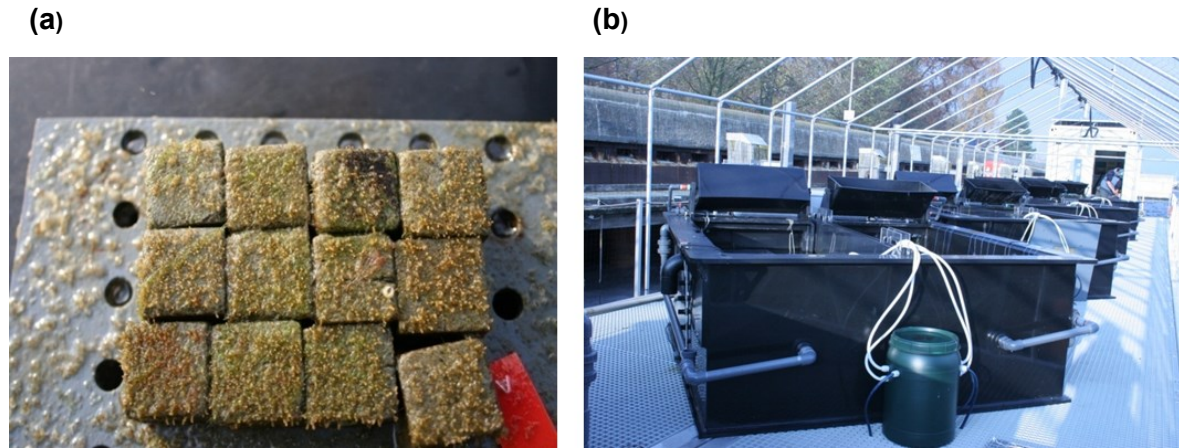

Fig. 3. Young development stages (5 months old; 1-10 mm) of *F. vesiculosus*, attached on sandstone cubes (a), placed into Kiel benthocosms, 4000 L experimental units (b).

Before well-developed sibling groups (SG 1-SG 7) were exposed to the location Bülk (Fig. 1) in 1, 3, and 5 m water depth, per sibling group total number of *F. vesiculosus* individuals on 2 cm^2 sand stone cube area were counted and length of *F. vesiculosus* individuals on 0.5 cm^2 sand stone cube area were measured to the nearest 1 mm, using a stereo microscope (6.4 x). For counting and measuring algae, cubes were detached from PVC plates. Subsequently cubes were distributed again on PVC plates, in such a way that on one PVC plate 7 different sibling groups were fixed, every sibling group marked with a cable clip of different colour. To avoid drifting of PVC plates by stream and swell, before plates were exposed to the filed, they were fixed with a thread bar, 30 cm in length, on sidewalk flagstones. To protect sibling groups from sedimentation its distance from the sea floor was 20 cm (Fig. 4).
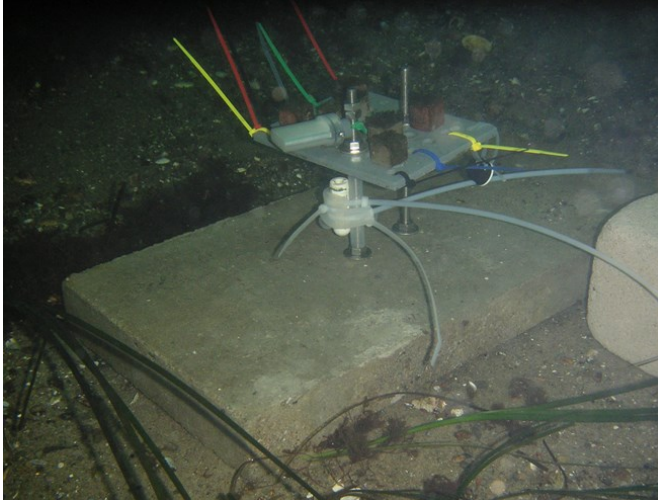
Fig.4. PVC-plate with 7 specific siblling groups (*F. vesiculosus*) attached on sand stone cubes and marked by cable clips with different coloures. PVC plates were fixed 20 cm over the sea floor with a thread bar on sidewalk flagstones. For temperature- and light intensity measurements a data logger (HOBO®, Onset Computer Corporation) was fixed at the thread bar.

PVC plates were fixed with a thread bar, 30 cm long, on sidewalk flagstones (Fig. 4); so that sibling groups. Afterwards sibling groups were exposed to the location Bülk (Fig. 1) in 1, 3, and 5 m water depth. Per depth (1, 3 and 5 m) every sibling group, was replicated four times (Fig. 5), resulting in 84 sand stone cubes with fucoid offspring.
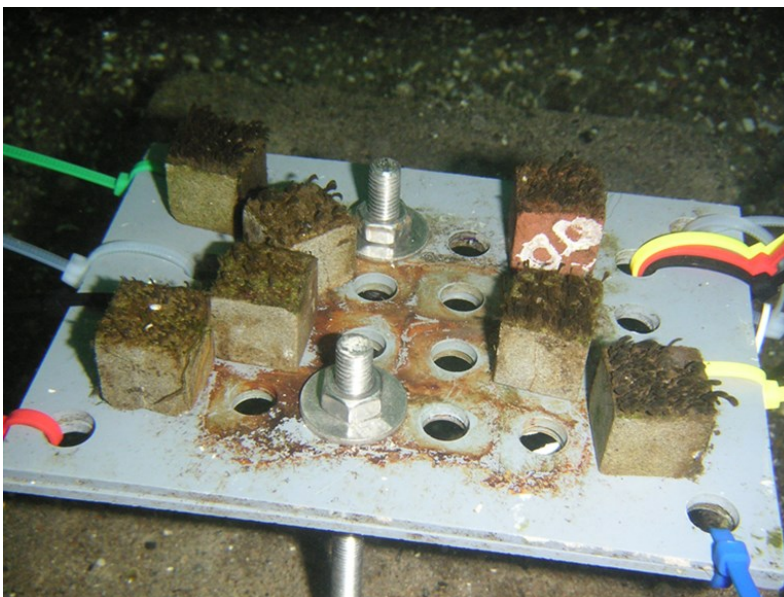

Fig. 5. One replicate of the present study. PVC-plate with 7 specific siblling groups (*F. vesiculosus*) attached on sand stone cubes and marked by cable clips with different coloures.

The number of *F. vesiculosus* individuals per depth was partly different between but not within sibling groups (Table 1).

Table 1. Number of *F. vesiculosus* individuals (mean ± SD, n = 4) from different sibling groups (SG 1-SG 7), exposed to different depths at the location Bülk in December 2011.

	SG 1	SG 2	SG 3	SG 4	SG 5	SG 6	SG 7
1 m	202 ± 43	304 ± 98	295 ± 56	255 ± 72	40 ± 23	167 ± 12	16 ± 2
3 m	178 ± 49	251 ± 28	324 ± 37	165 ± 39	24 ± 5	223 ± 58	21 ± 9
5 m	128 ± 39	224 ± 14	297 ± 51	204 ± 15	31 ± 5	197 ± 67	16 ± 8

The percent coverage of sibling groups on sand stone cubes was estimated visually by one and the same person during monthly SCUBA dives from March-September 2012. Before coverage was estimated, filamentous algae growing on cubes and *F. vesiculosus* individuals were removed. Afterwards the rhizoid cover of each sibling group on 2 cm^2 sand stone cube area was documented. To observe also the thalli growth of *F. vesiculosus* algae during time, in addition the length of the largest thallus branch from the greatest individual per sibling group (maximal length) was measured to the nearest 1 mm from April-September.

In situ temperature and light intensity were monitored continuously in 1, 3 and 5 m depths over 9 months (January–September 2012) using data loggers (HOBO®, Onset Computer Corporation), taking one measurement per hour (Fig. 4).

In September 2012, mortality, length and physiological condition of *F. vesiculosus* individuals were quantified. For it sibling groups were sampled by SCUBA divers from 1, 3 and 5 m water depth to the surface and to the lab. To avoid exposure of *F. vesiculosus* individuals to a possible light stress at the water surface, sibling groups were transferred shaded (covered with a dark foil). The physiological condition of the algae was estimated by measuring the maximal photochemical yield (Fv/Fm) of photosynthesis with a Diving-PAM-fluorometer (Walz GmbH, Effeltrich, Germany). Fv/Fm is a chlorophyll fluorescence measuring parameter and tests if stress affects the photosystem II of plants. The greater the plant stress, the fewer open PS II reaction centers available, and the Fv/Fm ratio is lowered. Fv or variable fluorescence is the difference between maximum fluorescence (Fm), determined

under strong light, i.e. when all PS II centers are closed, and minimum fluorescence (Fo), the dark-adapted leaf pre-photosynthetic fluorescent state, i.e. when all reaction centres are active or open. A typical Fv/Fm ratio for a well-functioning photosynthetic apparatus is 0.8 (Björkman & Demmig 1987). For *F. vesiuclosus* from the Swedish west coast the maximal Fv/Fm value, measured under close to optimal conditions, was 0.63 ± 0.05 (mean ± SD) (Magnusson 1997). In the present study the Fv/Fm value of at least five *F. vesiculosus* individuals, depend from number of survived algae (Table 2), per sibling group was determined. For it a clip was attached to the upper section of one branch and a shutter plate was closed so 1 diameter area of the branch was dark adapted. After a dark adaptation of five minutes, Fv/Fm measurement was taken. Afterwards from every sibling group surviving *F. vesiculosus* individuals were counted and length of the largest thallus branch of survived algae (Table 2) was measured. Length data will be referred to as growth of *F. vesiculosus* throughout the text.

Table 2. Number of survived *F. vesiculosus* individuals (mean ± SD, n = 4) from different sibling groups (1-7) in different depths (1, 3, 5 m) at the location Bülk from Dec. 11-Sep. 12. Young development stages of *F. vesiculosus* (1-10 mm) were exposed to Bülk in Dec. 11; survival of *F. vesiculosus* individuals was analysed in September 2012.

	SG 1	SG 2	SG 3	SG 4	SG 5	SG 6	SG 7
1 m	4 ± 8	17 ± 10	33 ± 9	26 ± 5	14 ± 13	24 ± 9	7 ± 7
3 m	4 ± 5	3 ± 4	3 ± 4	8 ± 10	7 ± 3	16 ± 17	2 ± 4
5 m	8 ± 9	4 ± 3	5 ± 8	11 ± 8	2 ± 2	29 ± 19	3 ± 3

4. Statistical Analyses

4.1 Coverage of fucoid sibling groups

Effect of depth, sibling group and month on the coverage of *F. vesiculosus* sibling groups were analyzed using a mixed effects-model (Pinheiro & Bates 2000). It was fitted with the linear mixed model formula lme (implemented in the NLME library in the R environment (version 2.15.2; http: //www.R-project.org.). Depth, sibling group and month were specified as fixed factors. Graphical diagnostics in R were used to confirm normality of errors (normal-probability plots) and homogeneity of variances (fitted values vs. residuals plots). The response variable coverage (%) of fucoid sibling groups in different depths at different months was normally distributed. Since

no homoscedasticity was found, the α-level for ANOVA was reduced to p < 0.01, in order to avoid a type 1 error (Underwood 1997).

4.2 Survival, growth and maximal photochemical yield of fucoid sibling groups

Statistical data analysis was conducted with the Statistica 8.0 software package (Statsoft, Hamburg): The response variables growth, survival (%, transformed with arcsine transformation), and photochemical yield of fucoid sibling groups were compared among the treatment factors depth and sibling groups by a two-way ANOVA. Since sibling group did not affect the photochemical yield of *F. vesiculosus*, the analysis was reduced to one-way ANOVA with the factor depth. Response variables were not normally distributed (Shapiro-Wilks test, p < 0.05) and no homoscedasticity was found (Levine's test, p < 0.05). Therefore the α-level was reduced to p < 0.01 to avoid a type 1 error. Tukey´s multiple comparison tests (p < 0.01) were used for further analysis of growth, survival, and maximal photochemical yield of sibling groups.

Results

1. Environmental conditions at different depths

<u>Light and temperature</u>

Data loggers for temperature and light intensity measurements were lost at a depth of 3 and 5 m so that in the present study no temperature and light intensity data at 3 m from January–March 12 and no light intensity data at 5 m from April–June 12 are shown.

Light intensity increased from January until May (1, 3 m), respectively at a depth of 5 m until July, and dropped again until September (Fig. 6). Light intensity was at 3 and 5 m only 8% of the light intensity values measured at 1 m (January-September). At a depth of 1 m highest light intensity (mean value: 1222 µmol m^{-2} s^{-1}) was analyzed in May. At 3 m only 5% of the light intensity value, measured at 1 m (May), was found. Regrettably the data logger was lost. Thus no comparison between light intensity at 1

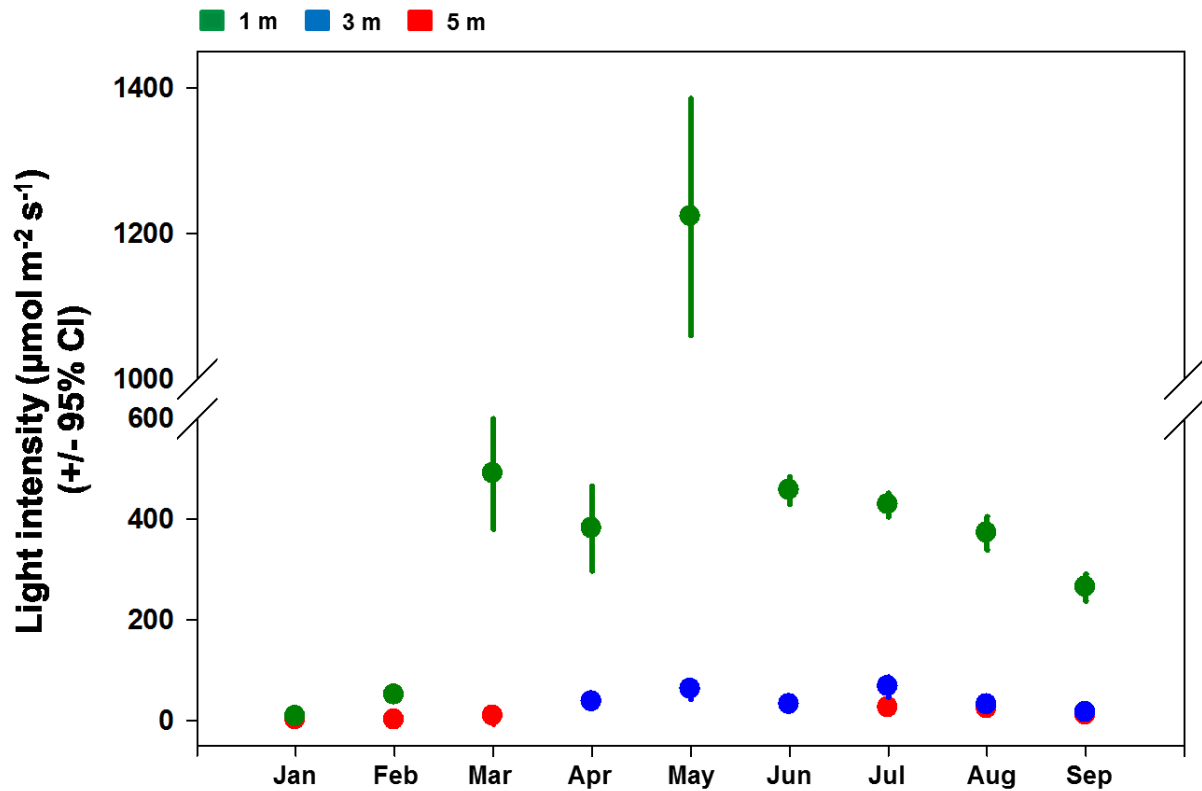and 5 m for May could be made. At 5 m highest light intensity was analyzed in July, 94% lower than at 1 m.

Fig. 6. Light intenstity (μmol m^{-2} s^{-1}) at a water depth of 1 m (Jan.–Sep.), 3 m (Apr.–Sep.) and 5 m (Jan.–Mar.; Jul.–Sep.); measured at the location Bülk (Kiel Fjord) by data loggers (HOBO®, Onset Computer Corporation) taking one measurement per hour (mean ± 95% CI). Mean (central symbol), 95% CI (thin lines).

Temperature (Fig. 7) varied not much between different depths. In January at a depth of 1 and 5 m mean value of temperature was around 5°C, decreased in February (1 m: 2°C, 5 m: 1°C) and increased from March–August (1 m: 19°C, 3 m: 18°C, 5 m: 17°C) with maximum values of 21°C (1 m) and 20°C (3 and 5 m). In September at all different depths mean value of temperature was around 16°C.
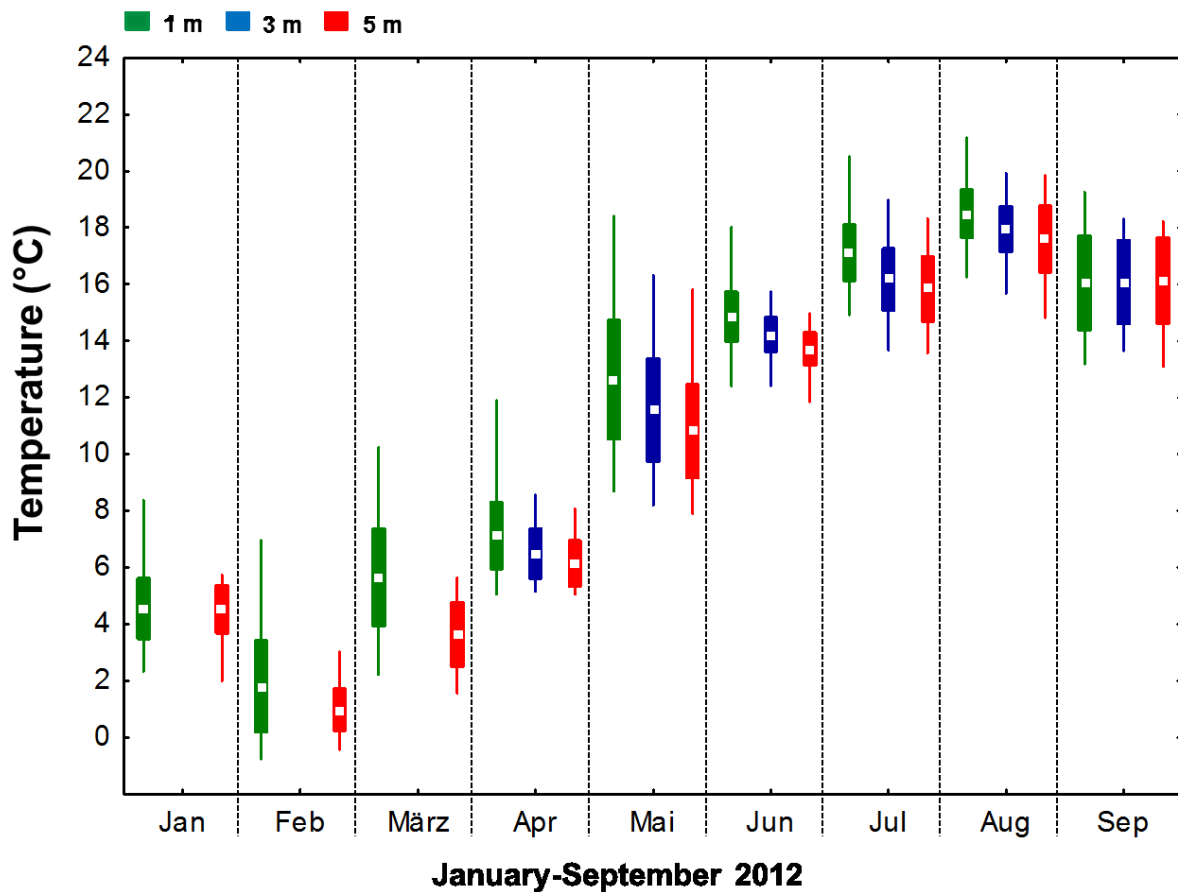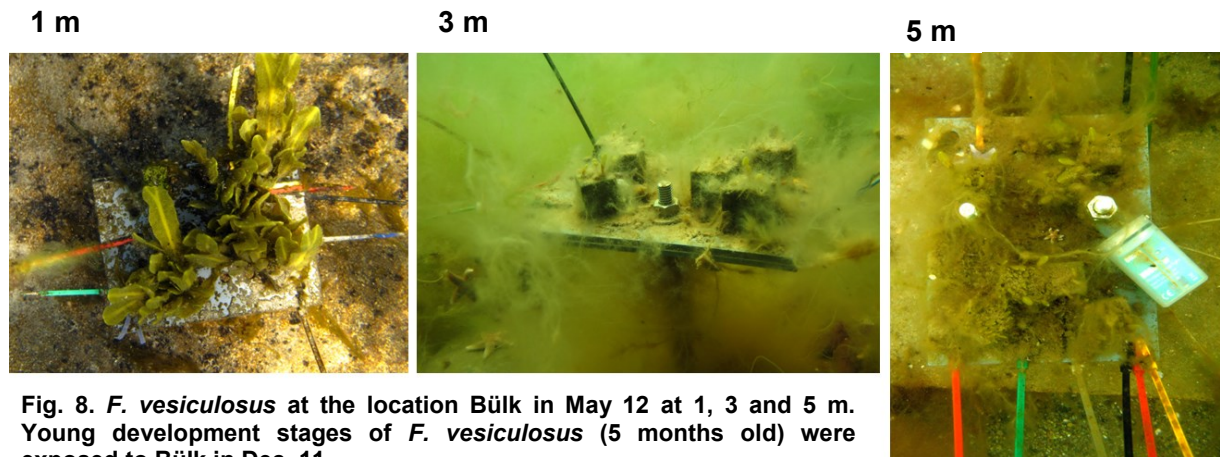
Fig.7. Water temperatures (°C) at a water depth of 1 m (Jan–Sep.), 3 m (Apr.–Sep.) and 5 m (Jan.-Sep.); measured at the location Bülk (Kiel Fjord) by data loggers (HOBO®, Onset Computer Corporation) taking one measurement per hour. Mean (central symbol), SD (columns), extremes (min.-max.: thin lines).

Fouling

F. vesiculosus individuals at a depth of 1 m were less fouled by filamentous algae than at 3 and 5 m at which higher densities of filamentous algae were observed at 3 m than at a depth of 5 (Fig.8).

1 m   **3 m**   **5 m**

Fig. 8. *F. vesiculosus* at the location Bülk in May 12 at 1, 3 and 5 m. Young development stages of *F. vesiculosus* (5 months old) were exposed to Bülk in Dec. 11.

2. Coverage and maximal length of fucoid siblings at different depths

2.1 Coverage

Depth, sibling group and time affected the coverage of *F. vesiculosus* significantly (Table 3). Compared to 3 and 5 m, a continuous higher coverage at 1 m was analyzed, except for sibling group 1, which coverage was from March-July higher at greater depths (Fig. 9). At Sibling group 1 (May-July), 2, 4 (March-August), 3 (May-August) and 6 (March-September) coverage was at a depth of 5 m higher than at 3 m. Furthermore coverage of sibling group 5 differed not between water depths from March-April and was at a depth of 1 m higher than at lower depths from May-September. At all different depths coverage of sibling group 1, 5 and 7 was lower than at the other sibling groups. Since at the beginning of our field experiment number of *F. vesiculosus* individuals from sibling group 5 and 7 was much less than from sibling group 1-4 and 6 (Table 1) the significant effect of sibling group and the low coverage of sibling group 5 and 7 must be interpreted with care.
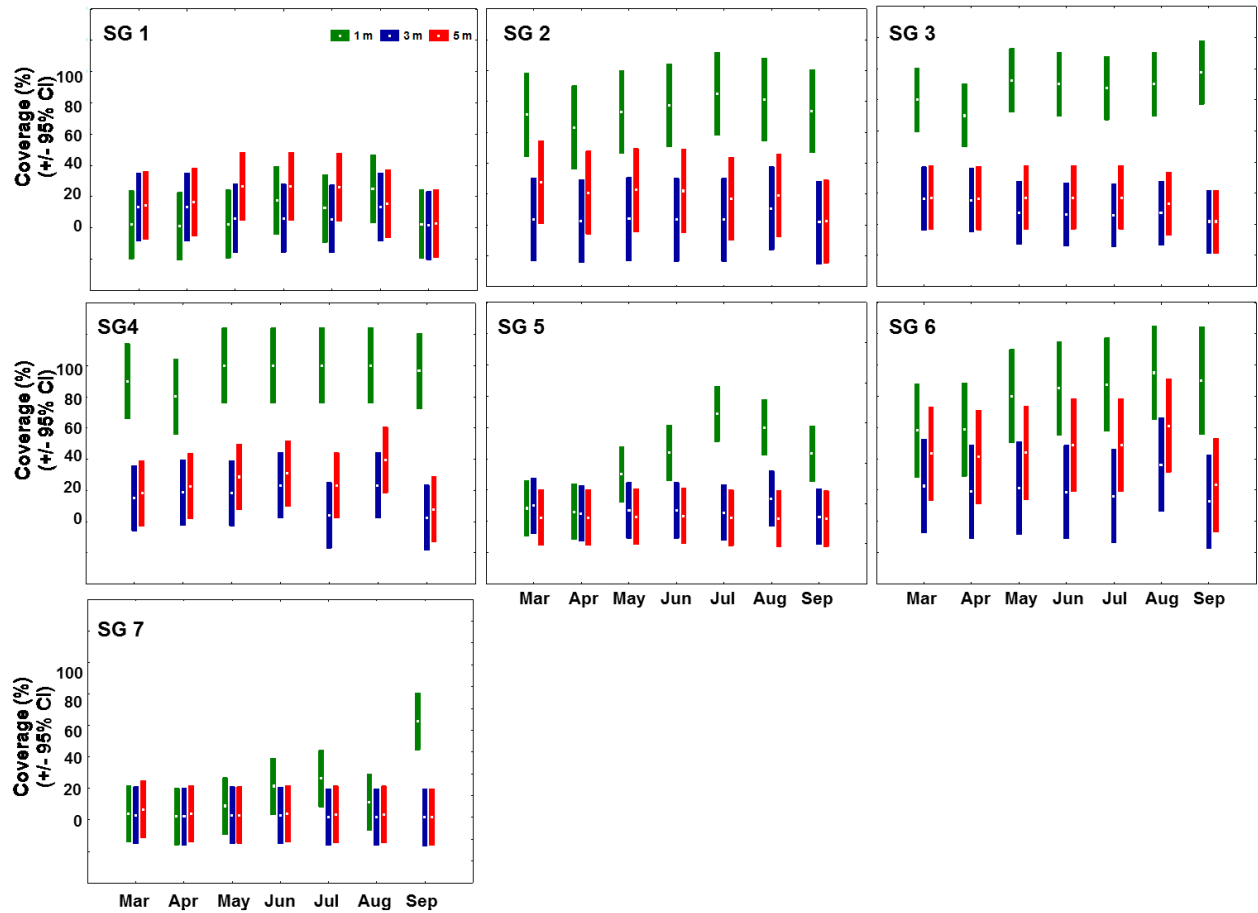
Fig. 9. Coverage of *F. vesiculosus* (%, mean +/- 95% CI; n = 4) from different sibling groups (SG 1-SG 7) in different depths (1, 3, 5 m) at the location Bülk. Young development stages of *F. vesiculosus* (1-10 mm) were exposed to Bülk in Dec. 11; the coverage was estimated monthly from March-September 2012.

Table 3. Mixed effect model for the effect of the factors sibling group (1-7), month (Mar. 11-Sep. 12) and depth (1, 3, 5 m) on the coverage of sibling groups (*F. vesiculosus*). Significant factors (p < 0.01) are shown in bold.

	df	df (denominator)	F-value	p-value
Depth	**2**	**62**	**40.39737**	**0.0001**
Sibling group	**6**	**62**	**8.49068**	**0.0001**
Month	**6**	**372**	**12.55708**	**0.0001**
Depth x Sibling group	**12**	**62**	**3.96233**	**0.0001**
Depth x Month	**12**	**372**	**13.13131**	**0.0001**
Sibling group x Month	36	372	1.37855	0.0772
Depth x Sibling group x Month	72	372	0.82892	0.8330

2.2 Maximal length

At the beginning of our field experiment in December 11 maximal length (Fig. 10) of sibling groups was smaller than 1 cm. *F. vesiculosus* algae grew not before April. At a depth of 1 m, highest values for maximal length of *F. vesiculosus* individuals (n = 7) were 89% greater than at 3 m and 93% greater than at 5 m. No differences between 3 and 5 m are shown, except for individuals from sibling group 5 which maximal length tended to be higher at a depth of 3 m than at 5 m (May-September).

Maximal length varied between sibling groups, especially at a depth of 1 m. So grew algae from sibling group 1 and 7 not before May whereas individuals from sibling group 2-6 started growing already in April. In addition highest maximal length was mainly analyzed in September (sibling group 1, 4, 6 and 7: 13.8-19.5 cm), except at sibling group 3 and 5 with highest maximal length in July (17-17.9 cm) and sibling group 2 with highest maximal length in August (18.6 cm).
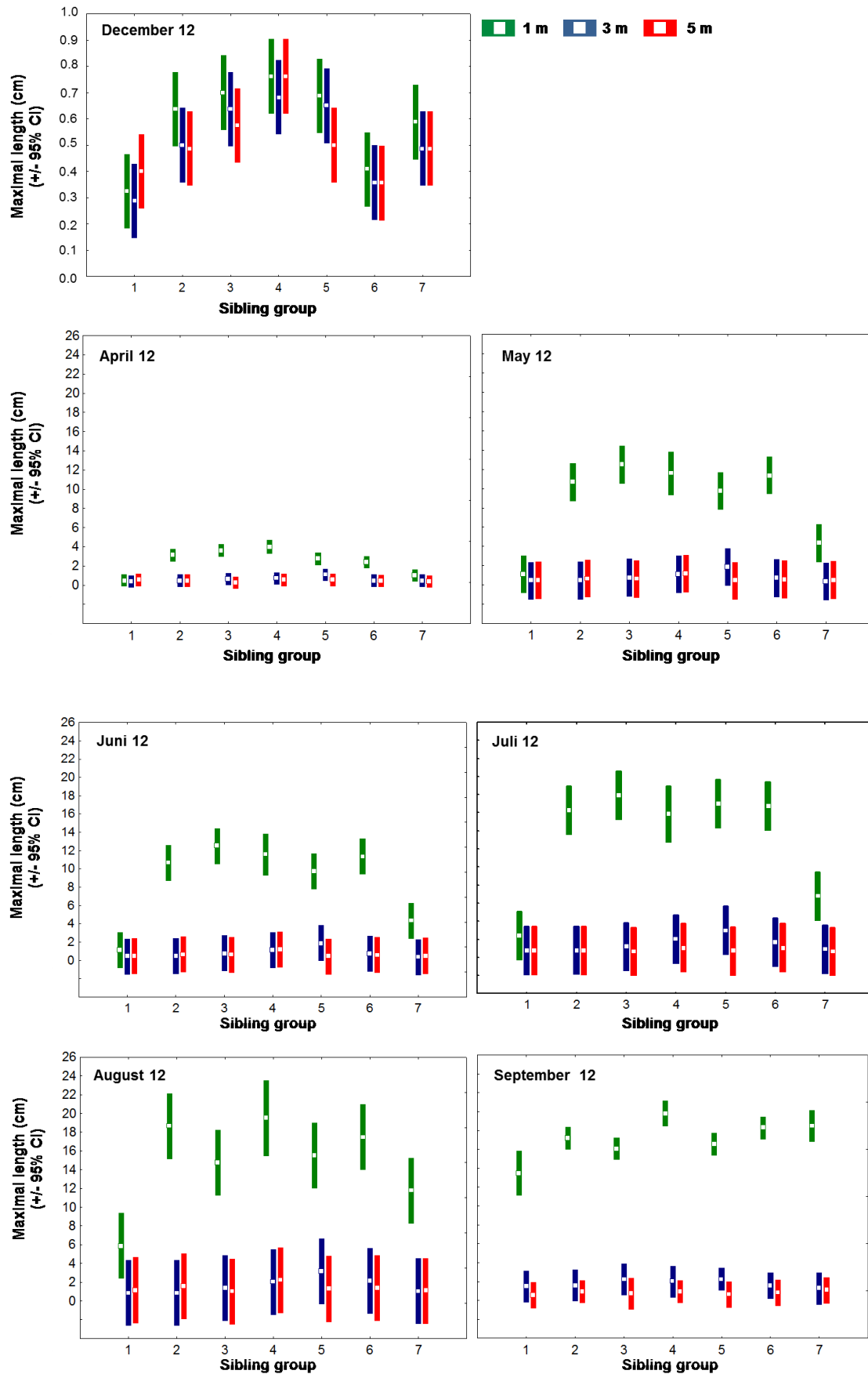
Fig. 10. Maximal length of *F. vesiculosus* (cm, mean +/- 95% CI; n = 4) from different sibling groups (SG 1-SG 7) in different depths (1, 3, 5 m) at the location Bülk in December 11 and from April-September 2012. The length of the largest *F. vesiculosus* individual per sibling group (maximal length) was measured monthly.

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3. Survival rate, grwoth and maximal photochemical yield of fucoid siblings at different depths

3.1 Survival rate

Depth and sibling group significantly affected the survival of *F. vesiculosus* (Table 4). At a depth of 1 m, fucoid algae survived with a significantly higher rate of individuals from sibling group 5 and 7 than was observed in individuals from sibling groups 2 and 3 at a depth of 3 m (Fig. 11). At a depth of 1 m more individuals of sibling group 5 and 7 than of sibling group 1-4 and 6 survived. Further, at a depth of 3 m more individuals of sibling group 5 than of sibling group 1-4 and 6-7 survived.
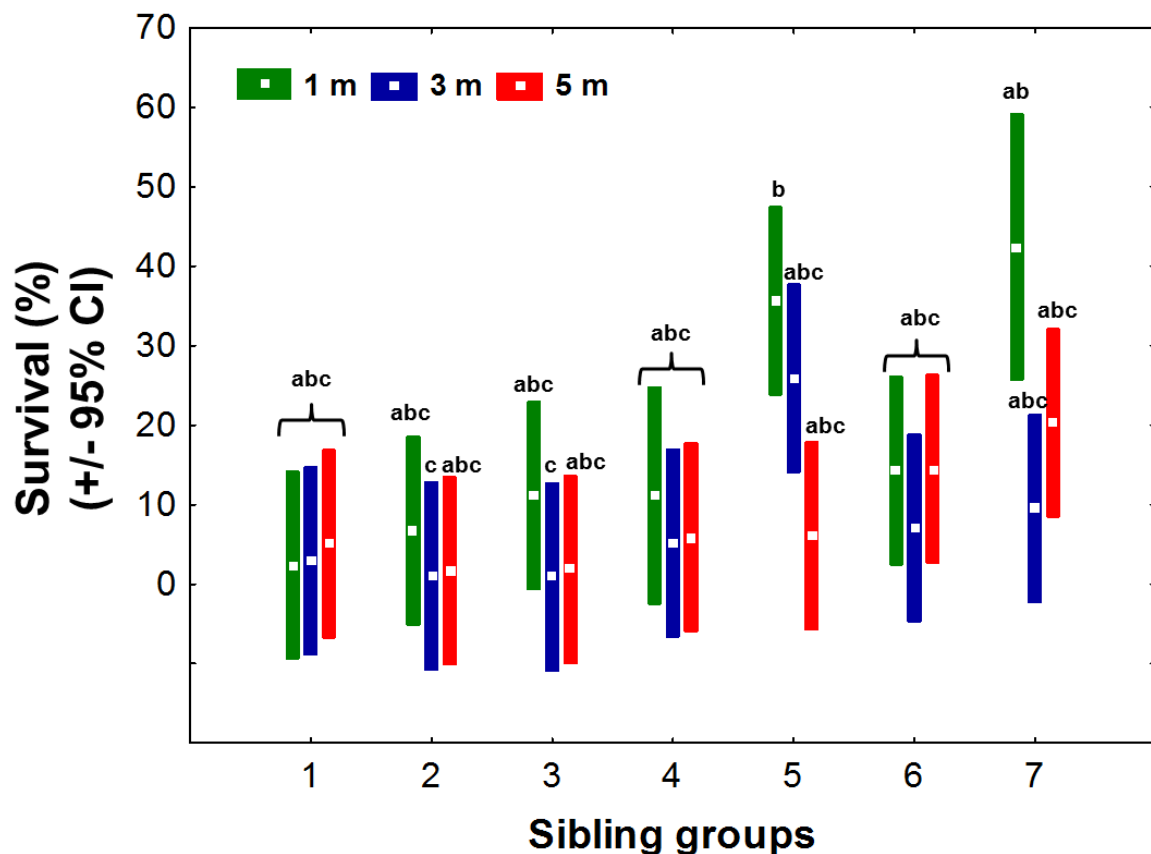

Fig. 11 Survival of *F. vesiculosus* (%, mean ± 95% CI, n = 4) from different sibling groups (1-7) in different depths (1, 3, 5 m) at the location Bülk from Dec. 11 until Sep. 12. Young development stages of *F. vesiculosus* (1-10 mm) were exposed to Bülk in Dec. 11; survival of *F. vesiculosus* individuls was analysed in September 2012 and compared separately between different treatment combinations (depth x sibling group by Tukey´s test. Values represented by vertical bars with different letters differ significantly (p < 0.01 by Tukey´s test).

Table 4. Two-way ANOVA for the effect of the factors depth and sibling groups (1-7) on the survival of _F. vesiculosus_ individuals. Significant factors (p < 0.01) are shown in bold.

	df	MS	F	p
Depth	**2**	**0.255456**	**7.9655**	**0.000855**
Sibling group	**6**	**0.209041**	**6.5182**	**0.000025**
Depth x Sibling group	12	0.051315	1.6001	0.115996
Error	60	0.032070		

3.2 Growth

Depth affected growth of _F. vesiculosus_ individuals significantly and interacted with sibling group (Table 5). From December 11-September 12, young developmental stages of fucoid siblings (1-10 mm) grew around 12.2-21.0 cm at a depth of 1 m, whereas siblings at 3 and 5 m only grew between 0.3-2.5 cm in length. Variation in length among sibling groups was larger at a depth of 1 m where sibling group 4 and 6 grew significantly more than sibling group 5 (Fig. 12). Since the factor 'sibling group' had no significant effect on length the higher growth of sibling group 4 and 6, compared to sibling group 5, is due to the interaction between the two factors and interpretations of variation in length among sibling groups must be taken with care.
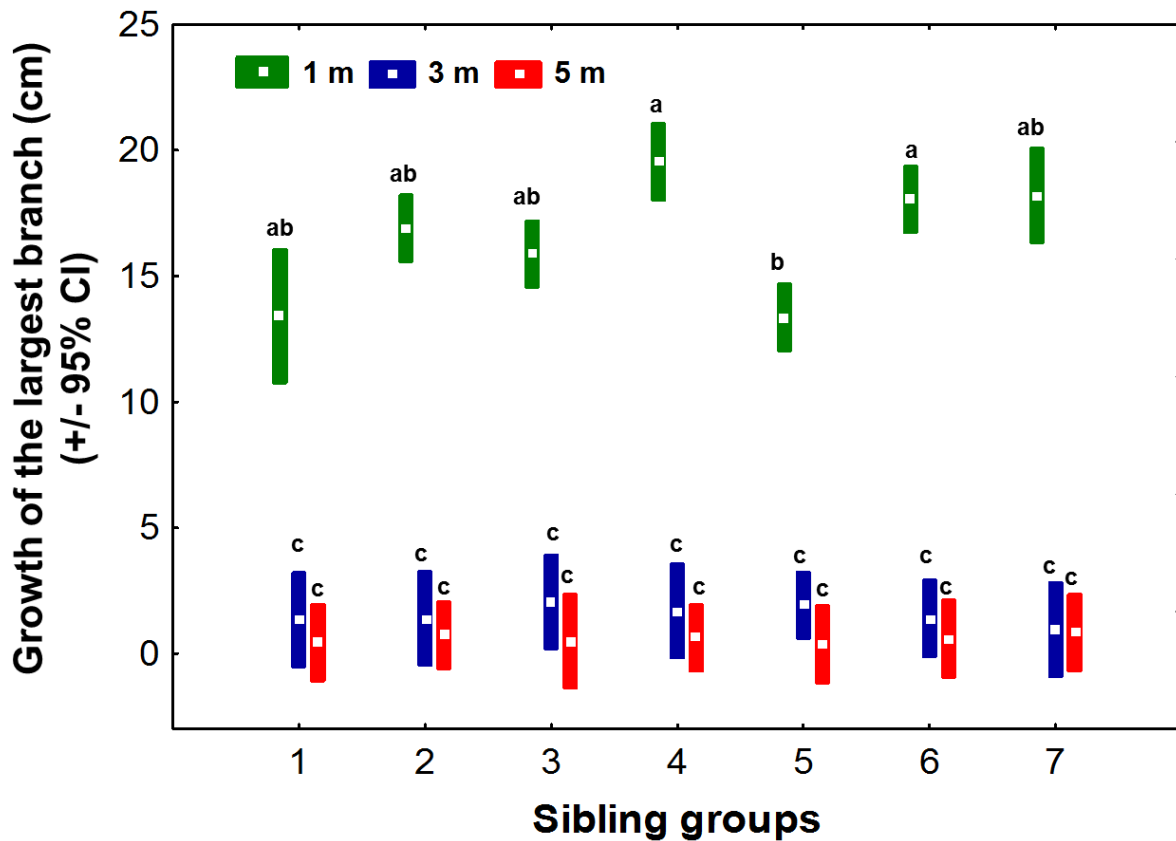
Fig. 12. Growth of *F. vesiculosus* from different sibling groups (1-7) in different depths (1, 3, 5 m) at the location Bülk from Dec. 11 until Sep. 11 (cm, mean ± 95% CI, n = 4). Young development stages of *F. vesiculosus* (1-10 mm) were exposed to Bülk in Dec. 11; Length of the largest thallus branch of survived *F. vesiculosus* individuls (number of individuals are shown in Table 2) was analysed in Sep. 12 and compared separately between different treatment combinations (depth x sibling group) by Tukey´s test. Values represented by vertical bars with different letters differ significantly (p < 0.01 by Tukev´s test).

Table 5. Two-way ANOVA for the effect of the factors depth and sibling groups (1-7) on the growth of *F. vesiculosus* individuals. Significant factors (p < 0.01) are shown in bold.

	df	MS	F	p
Depth	**2**	**1414.439**	**820.064**	**0.000000**
Sibling group	6	5.094	2.953	0.017575
Depth x Sibling group	**12**	**5.503**	**3.190**	**0.002849**
Error	40	1.725		

3.3 Maximal photochemical yield (Fv/Fm)

Depth significantly affected the maximal photochemical yield (Fv/Fm) of *F. vesiculosus* individuals (Table 6). Since no significant effect of sibling group was found, neither within nor between different depths, the mean maximal photochemical data for every depth are combined for the different sibling groups (Fig. 13). At a depth

of 1 m, the maximal photochemical yield of fucoid algae was significantly higher than at 3 and 5 m, between 3 and 5 m the Fv/Fm was not significantly different.
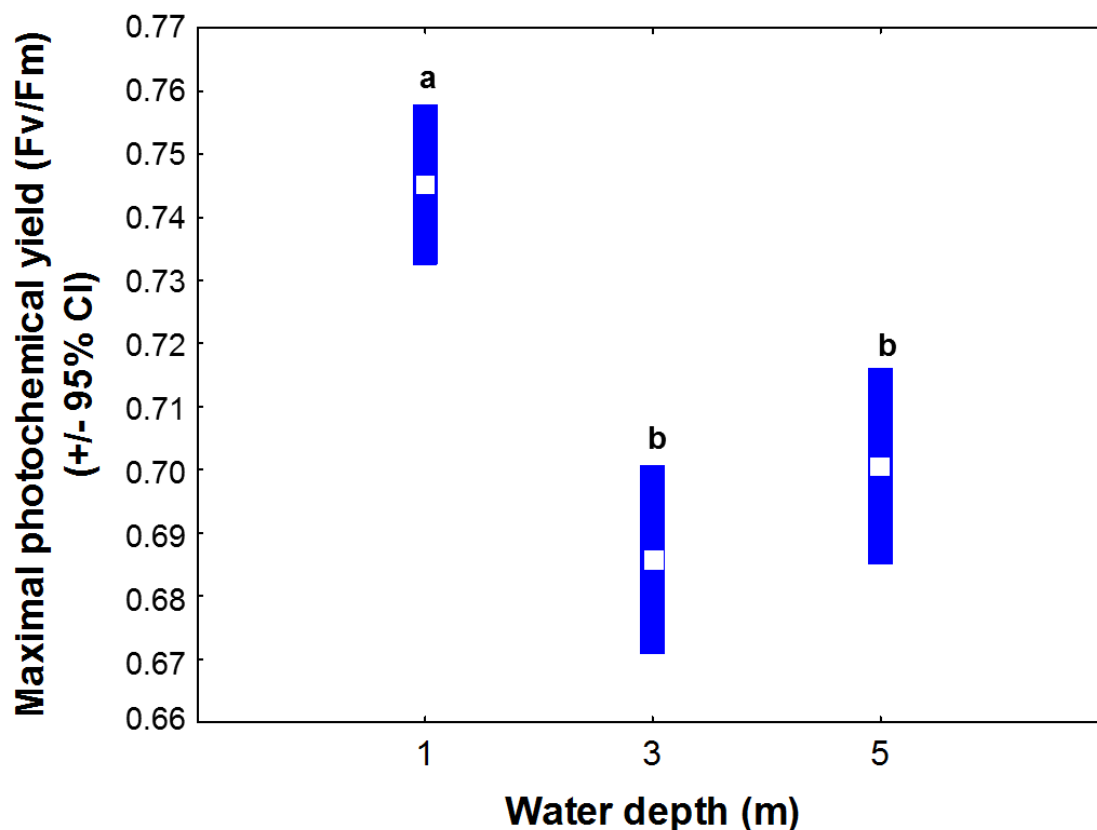

Fig. 13. Maximal photochemical yield of *F. vesiculosus* individuals in different depths (Fv/Fm, mean ± 95% CI; n** = 140) at the location Bülk. Young development stages of *F. vesiculosus* (1-10 mm) were exposed to Bülk in Dec. 11. Maximal photochemical yield was analysed in September 2012 and compared between different depth by Tukey´s test. Values represented by vertical bars with different letters differ significantly (p < 0.01 by Tukey´s test).
**Number of measurements, dependend from number of survived algae (see Table 2).

Table 6. One-way ANOVA for the effect of the factor depth on the maximal photochemical yield of *F. vesiculosus* individuals.

	df	MS	F	p
Depth	2	0.01743	21.28	0.000000
Error	47	0.00082		

Discussion

Development of fucoid sibling groups (coverage, maximal length, survival rate, growth and maximal photochemical yield) was at a depth of 1 m higher than at lower depths (Fig. 9-13). Low light availability in greater depth as a result of eutrophication is assumed to be one reason for the decline of Western Baltic *F. vesiculosus* populations (Vogt & Schramm 1991). In the present study at 1 m water depth light intensity was much higher than at 3 and 5 m (Fig. 6) which was certainly one reason for a better development of fucoid siblings at 1 m. So was rate of electron transport (rETR) and growth of *F. vesiculosus* in the Kiel Bay (Western Baltic) impaired by low light conditions in greater depths (Rohde et al. 2008). In the Gulf of Finland at 5 m light quantity only was about 4 to 6% of the surface light and the maximum growth depth of *F. vesiculosus* correlated with light intensity (Back & Ruuskanen 2000). However, mean Fv/Fm values of *F. vesiculosus* individuals from 3 and 5 m water depths (Fig. 13) indicate a good physiological condition of the algae since values were higher than 0.63 ± 0.05 (mean ± SD), a Fv/Fm value measured under close to optimal conditions (Magnusson 1997). Maximal photochemical yield as well as coverage of sibling groups 2-4 and sibling group 6 tended to be higher at a depth of 5 m than at 3 m. Compared to 1 and 5 m, during our field monitoring we observed a high density of filamentous algae at 3 m water depth (Fig. 8) which could have led to lower coverage and Fv/Fm of algae at 3 m. At a depth of 1 m *F. vesiculosus* individuals were less fouled than at 3 and 5 m which might be caused by the higher wave exposure in shallow waters, compared to greater depths. Better growth of filamentous algae at 3 m, compared to 5 m is certainly caused by the higher light intensity at 3 m water depth. The longer fucoid algae take to grow the longer they are endangered by stressors, such as grazing, sedimentation and competition by filamentous alga (Wahl et al. 2011). In the present study, individuals which grew faster than the others were maybe large enough to prevail against filamentous algae at 3 m which might be the cause for higher growth of sibling groups (Fig. 12) and maximal length of sibling group 5 (Fig. 10) at 3 m, compared to 5 m.

But why was coverage and growth high and in contrast survival of fucoid siblings low? For example, coverage of sibling groups 2-4 was at 5 m higher than at 3 m (Fig. 9) but at 3 m only a few algae survived until September (Fig. 11). Since also

coverage decreased from August until September, one explanation could be that most of the algae died in the end of our field experiment. This would mean that these fucoid siblings were able to survive at low light availability in greater depths, but only for a specific time.

Effect of time is especially shown by the increase of maximal length at 1 m water depth (Fig. 10), certainly caused by rising light intensity and temperature from January until August/September (Fig. 6, 7). Maximal length was from December to March very low and started to grow in May when highest light intensity occurred. Highest peak of maximal length at 1 m was achieved in August and September, when values for temperature were between 13-19°C, around the temperature (15°C) for growth of adult *F. vesiculosus* (Lüning 1985). Another reason could be that nitrogen reserves of fucoid tips (*F. vesiculosus)* were used for rapid growths in summer season (Lehvo et al. 2001).

In the present study at a depth of 1 m extreme values for temperature about 21°C occurred (Fig. 6). During the coming decades in the Baltic Sea temperature will increase as a result of global warming (Meier 2006, Neumann 2010) and especially in shallow waters *F. vesiculosus* individuals will be exposed to higher temperatures. Germination of *F. vesiculosus* zygotes from the German Baltic is strongly impaired by high temperature (25°C) (Maczassek 2009). Further in-situ surface temperatures in *F. vesiculosus* habitats showed that 25°C and higher values already occur today (Maczassek et al., in prep.). The predicted global and regional warming and more frequent heat waves in the Baltic Sea might reduce recruitment success of *F. vesiculosus* stronger. This thermal stress may further be enhanced by UV radiation which interferes with the germination of fucoid zygotes (Wiencke et al. 2000, Schoenwaelder et al. 2003) and can accompany high temperatures in shallow waters. The eutrophication and shading driven retreat of *F. vesiculosus* from its deeper range to shallow habitats, now exposes the populations to enhanced temperature stress (more pronounced in the uppermost meters of the water column). The persistence of *Fucus* in the western Baltic will depend on its potential for adaptation, also to low light availability in greater depths. In the present study development of *F. vesiculosus* individuals differed between sibling groups. Furthermore in previous studies (Maczassek 2009, Maczassek & Wahl 2011) fucoid germlings were conspicuously less sensitive against high temperature. Thus it might

be that genotypes may exist which are less sensitive against environmental stressors. One explanation may be that single genotypes of the intertidal North Sea gene pool of *F. vesiculosus* may have persisted, similar to what Johannesson et al. (2012) found for *F. radicans*: *F. radicans* is a dominant species in the northern Baltic Sea and has evolved from *F. vesiculosus* (Pereyra et al. 2009). Only single multilocus genotypes were able to fully recover from freezing (-15°C) or desiccation (3 h) and it was concluded that their high tolerance is what is left of the ancestral genotype of *F. vesiculosus* (Johannesson et al. 2012). Therefore, some Baltic *F. vesiculosus* populations might prevail the increasing temperatures during global warming because of their harbouring ancestral heat resistant genotypes in the genepool. Future molecular studies exploring candidate genes playing a key role in germling resistance to abiotic stressors may further elucidate the potential for Baltic *F. vesiculosus* to adapt to climate change. We consider a combination of new molecular tools for *F. vesiculosus* and selection experiments at the earliest life stages of this alga as a promising path for exploring its adaptive potential under the conditions of climate change. However, it should be noted that the initial density of germlings from sibling group 5 and 7 was lower than initial density of other sibling groups (Table 1). Since mortality of algae is higher in *Fucus* stands with higher initial densities (Karez 2003) high survival rate (Fig. 11) of sibling group 5 and 7 may not be caused by less sensitivity of these two groups against environmental stress but rather by their low initial density. Further was the lower coverage (Fig. 9) of sibling group 5 and 7, compared to other fucoid siblings certainly also caused by their low initial density.

Global warming may affect *Fucus* species also indirect by an increase of grazing pressure due to rising temperatures (Wahl et al. 2011). According to the RADOST project (Regional Adaption Strategies for the German Baltic Sea Coast) we determined how defence strength of *F. vesiculosus* is affected by high temperatures and if fucoid siblings exist which are less sensitive against high temperature (25°C) and proximate feeding stress by the isopod *Idotea baltica*. Regrettably, in our first study (Maczassek & Wahl 2011) the ensuing assessment of feeding impact by *Idotea baltica* on thermal stressed surviving fucoid germlings could not be analysed since the consumer unexpectedly avoided feeding on the young stages of *F. vesiculosus.* Thus in a second study sensitivity of thermal stressed fucoid recruits, with and

without anti-herbivore defence, against feeding pressure was determined by a pellet assay. Results will be shown in a final report for the Agency for Agriculture, Environment and Rural Areas of Schleswig-Holstein (LLUR) in April 2013.

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