



Double-edged sword of desalination: Decreased growth and increased grazing endanger range-margin *Fucus* populations

Leona J.M. Milec^{a,1,*}, Veijo Jormalainen^b, Luca Rugiu^a, Ricardo T. Pereyra^a, Eva Rothäusler^c, Jon Havenhand^a, Pierre R. De Wit^a

^a Department of Marine Sciences, University of Gothenburg, Hättebacksvägen 7, 452 96 Strömstad, Sweden

^b Department of Biology, University of Turku, 20014 Turun yliopisto, Finland

^c Centro de Investigaciones Costeras, Universidad de Atacama (CIC – UDA), Avenida Copayapu 485, Copiapó, Atacama, Chile

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ABSTRACT

Coastal ecosystems worldwide are facing intense and diverse pressures caused by anthropogenic climate change, which compromises physiological tolerance of organisms, as well as causes shifts in their biotic interactions. Within-species genetic variation plays an important role in persistence of populations under such changes by providing building blocks for adaptation. The brackish-water Baltic Sea is predicted to experience a significant desalination by the end of this century. The Baltic Sea is dominated, in terms of biomass, by a few species with locally adapted populations, making it a suitable model for studying shifting biotic interactions under changing abiotic conditions. We exposed two foundation species of brown algae, *Fucus vesiculosus* and *Fucus radicans*, to end-of-the-century projected salinity together with grazing pressure in experimental tanks. We measured growth, grazing and phlorotannin content and compared these traits between the current and projected future salinity conditions, between *Fucus* species, and between high and low genotypic diversity groups. Grazing, phlorotannin content and growth of both *F. radicans* and *F. vesiculosus* all showed genotypic variation. Future decreased salinity hampered growth of *F. vesiculosus* irrespective of genotypic diversity of the experimental population. Furthermore, the growth response to desalination showed variation among genotypes. *F. radicans* was more susceptible to grazing than *F. vesiculosus*, and, in the high genetic diversity group of the latter, grazing was higher in the future than in current salinity. Climate change induced hyposalinity will thus challenge *Fucus* populations at their range margins in the Baltic Sea both because of the growth deterioration and changes in grazing. Differences between the species in these responses indicate a better ability of *F. radicans* to cope with the changing environment. Our results emphasize the complexity of biotic interactions in mediating the climate change influences as well as the importance of genetic diversity in coping with climate change.

1. Introduction

Coastal ecosystems worldwide are facing intense and diverse pressures caused by anthropogenic climate change (Harley et al., 2006). Responses to these pressures are seldom straightforward (Berg et al., 2010; Walther, 2010); individual species are directly affected by abiotic stress, such as warming, acidification or desalination (Poloczanska et al., 2013), but also experience indirect effects through shifts in their biotic interactions (Blois et al., 2014; Van der Putten et al., 2010). For instance, organisms may experience changes in frequency or strength of their

positive interactions (e.g. mutualisms), or become more vulnerable to negative impacts (e.g. competition, predation or herbivory) (Gilman et al., 2010). These manifest throughout the food web, especially when ecologically dominant species are affected (Coleman and Williams, 2002; Jones et al., 1994; Zarnetske et al., 2012), as for example in mussel beds (Gutiérrez et al., 2003), coral reefs (Hoegh-Guldberg et al., 2007) or large seaweed stands (Harley et al., 2012).

Within-species genetic variation plays an important role in the tolerance of populations towards both biotic and abiotic stress (reviewed in (Laikre et al., 2016)). In the absence of strong gene flow, low genetic

* Corresponding author.

E-mail addresses: leona.milec@nord.no (L.J.M. Milec), veijor@utu.fi (V. Jormalainen), luca.rugiu@gu.se (L. Rugiu), ricardo.pereyra@marine.gu.se (R.T. Pereyra), eva.rothausler@uda.cl (E. Rothäusler), jon.havenhand@marine.gu.se (J. Havenhand), pierre.de_wit@marine.gu.se (P.R. De Wit).

¹ Present address: Nord University, Universitetsalléen 11, 8026 Bodø, Norway; Hasselt University, Agoralaan building D, 3590 Diepenbeek, Belgium.

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diversity may reflect adaptation to local conditions following selection, with only tolerant genotypes remaining (Johannesson and André, 2006). On the other hand, a population with a higher diversity of genotypes harbours a greater variety of phenotypic responses, increasing the chance of having more beneficial genotypes in the population and, thus, persistence at the population level. For instance, in brown algae, low genetic diversity has been linked to a narrower range of physiological responses, greater damage and slower recovery after a perturbation (Pearson et al., 2009; Wernberg et al., 2018). Higher diversity in tolerance-related traits can facilitate rapid adaptation to moving fitness optima (Reusch and Wood, 2007). A lack of genetic diversity may thus hinder evolutionary rescue effects in the face of anthropogenic threats e. g. through population bottlenecks, founder effects, and reduced gene flow due to isolation (Chakraborty and Nei, 1977; Johannesson and André, 2006). This can be particularly problematic in marginal seas, with limited connectivity and strong environmental gradients (Johannesson and André, 2006).

The Baltic Sea, as such a marginal sea, has a relatively low species diversity (Ojaveer et al., 2010; Zettler et al., 2014) and many local ecosystems are dominated a few key species (Furman et al., 2014), making it a suitable model for studies of shifting biotic interactions under changing abiotic conditions and genotypic variation in the underlying traits. Hence, communities can be more easily reconstructed under experimental conditions than in more complex ecological systems. Especially in marginal regions, such as the Gulfs of Bothnia and Finland, communities are comparatively low in complexity. In the Baltic Sea, the pressures imposed by climate change and other anthropogenic stressors are accelerated and magnified compared to other coastal sea regions (Reusch et al., 2018). Climate models predict a summer sea surface temperature increase of about 4 °C as well as a salinity decrease of up to 3 Practical Salinity Units (= PSU) throughout the Baltic Sea during the next century (Meier, 2006; Meier et al., 2012), which can have profound consequences for species distributions throughout the basin. Predicted desalination patterns strongly vary across the Baltic. The Baltic Proper, Archipelago Sea, Gulf of Riga, and Bothnian Sea will experience moderate decreases in the range of 1.5–2 PSU. The most extreme desalination is predicted in the Danish Straits and small localized coastal areas around the Baltic Proper and the Gulfs, while smaller absolute decreases are expected for the Gulfs of Bothnia and Finland (Jonsson et al., 2018; Meier, 2006; Meier et al., 2012). However, the velocity of desalination (in salinity change × km/decade) is predicted to be highest in precisely these latter two areas, as well as the southern Baltic (Jonsson et al., 2018).

Many marine Baltic Sea organisms currently live at the margin of their salinity tolerance range, in conditions which are predicted to change considerably within the next century. This might jeopardize future distributions of the brown algae *Fucus vesiculosus* and its endemic sister species *Fucus radicans*, which are dominant habitat-forming species in the northern and easternmost areas of the Baltic Sea (HELCOM, 2009; HELCOM, 2013). Baltic Sea *Fucus* have adapted to cope with and thrive in low salinity (Johannesson and André, 2006; Johansson et al., 2017; Russell, 1985). However, recent studies modelling the distribution of *Fucus* under future climate scenarios predict a dramatic contraction of its range due to desalination despite local adaptation, especially in the marginal low salinity areas of the Baltic (Jonsson et al., 2018; Kotta et al., 2019). In addition, the genus currently lives at its reproductive limit in the north-eastern Baltic Sea, due to reduced fertilization success (Rothäusler et al., 2018b; Serrão et al., 1999; Serrão et al., 1996) and sporophyte growth in response to low salinity (Bäck et al., 1992; Nygård and Dring, 2008). The forecasted desalination in these areas is thus expected to negatively impact their fitness (Graham and Wilcox, 2000; Lüning, 1990; Rothäusler et al., 2018a; Rugiu et al., 2018; Takolander et al., 2017b). The sister species *F. vesiculosus* and *F. radicans* differ from each other in many respects (Bergström et al., 2005; Pereyra et al., 2009), including tolerance to low salinity (Pereyra et al., 2009) and susceptibility to grazing (Gunnarsson and Berglund, 2012), implying

that climate change may affect the two Baltic Sea fucoids differently. While there are no studies to date explicitly testing the physiological salinity tolerance limits of *F. vesiculosus* and *F. radicans*, their distributions suggest that *F. radicans* is more tolerant to low salinity. *Fucus vesiculosus* has occasionally been observed at salinities down to 2 PSU (Ardehed et al., 2016), but occurs most commonly at 4 PSU or higher, and the size of the thalli and frequency of sexual reproduction decrease as a function of salinity (Ruuskanen and Bäck, 1999; Takolander et al., 2017a). On the other hand, *F. radicans* only lives in low salinities (3–6 PSU), and reaches more northern parts of the Gulf of Bothnia than *F. vesiculosus* (Schagerström and Kautsky, 2016; Takolander et al., 2017a).

Abiotic factors such as salinity can also cause indirect effects on biotic interactions, which can act as structuring factors as well (Korpinen et al., 2007; Kotta et al., 2019). In the Baltic Sea, grazing by the isopod *Idotea balthica* has been observed to decimate *Fucus* stands considerably (Engkvist et al., 2000; Gunnarsson and Berglund, 2012). Fucoids have evolved a set of secondary metabolites such as phlorotannins, which can play a central role in anti-herbivore defence, UV protection, and antioxidant activity. The antiherbivore defence production strategy can be constitutive or inducible (Haavisto et al., 2017; Jormalainen and Ramsay, 2009; Toth and Pavia, 2007), and show phenotypic plasticity with respect to several environmental variables (Amsler and Fairhead, 2005; Jormalainen and Honkanen, 2008). Thus, environmental impacts on the production of phlorotannins might have side-effects on biotic interactions. Furthermore, salinity, through its impact on photosynthetic capacity and osmoregulation, is a strong predictor of biochemical attributes determining the palatability of *Fucus* for its herbivores. Storage of carbon (e.g. mannitol) and nitrogen (e.g. proteins) compounds is compromised in low salinity environments, reducing the nutritional value of the algae (Barboza et al., 2019).

Projected future hyposaline conditions for the end of this century (Meier, 2006; Meier et al., 2012) may compromise growth and survival of Baltic *Fucus* in several non-exclusive ways. First, abiotic stress may induce a shift in allocation of energy from growth to maintenance (Perrin and Sibly, 1993; Rugiu et al., 2020) and to reduction in primary production in favour of hyposaline stress physiology. Second, increased maintenance needs may be covered further at the cost of resistance to herbivory. A reduction in allocation to production of secondary defensive compounds, such as phlorotannins, could result in increased susceptibility to grazing, as predicted by plant defence allocation models (Pavia and Toth, 2008). Knowledge of the genetic diversity in such tolerance-related traits and their response to hyposalinity is crucial to understand the future distribution of these ecologically important species.

The interplay between the two important factors determining the distribution of *Fucus* populations, salinity and herbivory, remains poorly understood. Here, we exposed *F. vesiculosus* and *F. radicans* to end-of-the-century projected salinity and grazing pressure in experimental tanks. We measured growth, grazing and phlorotannin content and compared these traits between the current and projected future salinity conditions, between the species, and between high and low diversity groups of *Fucus* genotypes. We tested the following hypotheses: (1) Future projected low salinity impairs growth in range margin populations of *Fucus* (*F. vesiculosus* and *F. radicans*); (2) Future projected low salinity reduces phlorotannin concentration and increases grazing in *Fucus*; (3) The responses to salinity show genotypic variation within species, (4) High genotypic diversity can mitigate the negative effects of low salinity on growth and grazing.

2. Materials and methods

2.1. Study organisms

In summer 2016, we collected 75 *F. vesiculosus* individuals from the shallow subtidal area in Rauma (61.07, °N, 21.31°E) and 30 *F. radicans*

in Närpes (62.47°N, 21.13°E) via snorkelling. All individuals were cleaned individuals from epiphytes and grazers under running freshwater. Microsatellite genotyping (see Section 2.2) was used to verify species identity and identify distinct genotypes (haplotypes) (see Section 2.2). We only sampled one stem from each holdfast (=individual) to avoid sampling of replicated genotypes. All individuals were genotyped at five microsatellite loci and cut into roughly similar-sized ramets to create replicates of the same genotype. The criteria for their size were 1) that each ramet contained a few apical meristems (on average 18.8 ± 11.5 meristems), on which the further growths is based, and 2) that there was some thallus biomass (on average 1258 ± 699 mg) to support them. Each ramet was coded for identification with a plastic tag. For the grazing treatment, we collected 480 *I. balthica* from the same *F. vesiculosus* populations. All organisms were kept in aquarium racks at a constant temperature of 17 °C before being transported from Finland (University of Turku) to the Sven Lovén Centrum, Tjärnö, Sweden (58.88°N, 11.15°E) in aerated coolers. Upon arrival, each ramet was individually attached to one ceramic tile and placed on the bottom of the experimental tanks, held upright by a piece of foam attached to them with a clothespin. Isopods were kept indoors in aerated plastic aquaria until the experiment started.

2.2. DNA extraction and microsatellite genotyping

DNA was extracted from lyophilized fresh algal tissue using a CTAB modified protocol for genomic DNA (Panova et al., 2016). Following this, samples were genotyped at five microsatellite loci shown to be diagnostic in previous studies of furoid species (Ardehed et al., 2016; Engel et al., 2003). We identified 71 genotypes of *F. vesiculosus* and 31 of *F. radicans* (Supplementary Table A.1). PCR products were pool-plexed and sized on a Beckman-Coulter CEQ 8000 capillary sequencer, and fragments were analyzed using the Fragment Analysis Software (Beckman-Coulter Inc., Fullerton, CA, USA). Genotypes were checked for null alleles, stuttering and allelic drop-out, using MICRO-CHECKER v. 2.2.3 (Van Oosterhout et al., 2004). GENCLONE 2.0 (Arnaud-Haond et al., 2007) was used for species identification, and to distinguish between unique and repeated genotypes produced by vegetative reproduction

through reattachment of adventitious branches.

2.3. Experimental setup

The outdoor experimental tank setup consisted of six 1500 l tanks, each equipped with an independent recirculating water system (Fig. 1). Translucent plastic sheets were mounted on wooden beams above the tanks, covering them from the top to protect against heavy rainfall and predation, while the sides were left open to allow airflow and avoid overheating. The water of the tanks was replaced weekly and enriched with Guillard's F/2 medium to ensure nutrient availability (Guillard, 1975; Guillard and Ryther, 1962). We diluted the medium in the tanks to 0.2 μM PO_4 to match the low summer nutrient concentration at the sampling sites (Fleming-Lehtinen et al., 2008). Handcrafted wave-generators were installed in every tank to provide the algae with the water movement necessary to prevent overgrowth by periphyton and filamentous algae (Kersen et al., 2011).

Tanks were set up in pairs, and each of them represented one block (2 tanks \times 3 blocks). The paired tanks within a block received the same set of 25 *F. vesiculosus* genotypes and of 10 *F. radicans* genotypes. Each tank was divided into a "high" and "low diversity treatment. They were physically separated from each other by an elevated section in the middle of the tank, preventing movement of grazers between the treatments. Since high and low diversity groups within one tank were sharing the same water, water-borne cues could have travelled between them, this was accounted for during statistical analyses (see Section 2.7). To realize the high diversity treatment, we added 20 *F. vesiculosus* genotypes to each tank with two replicated ramets and for the low diversity treatment five genotypes with 8 replicated ramets were added. In addition, to realize the high and low diversity treatment for *F. radicans*, each tank received 8 genotypes with 1 replicated ramet, and 2 genotypes with four replicated ramets, respectively. Hence, each tank contained a total of 96 ramets (see Supplementary Table A.1).

After one week of algal acclimation at 5.7 PSU, one of the paired tanks was kept at the "current" salinity, which represented the mean summer salinity (June–August 1995–2004) of Rauma (62.47°N, 21.14°E) and Storskäret, Finland (61.14°N, 21.30°E), while the other

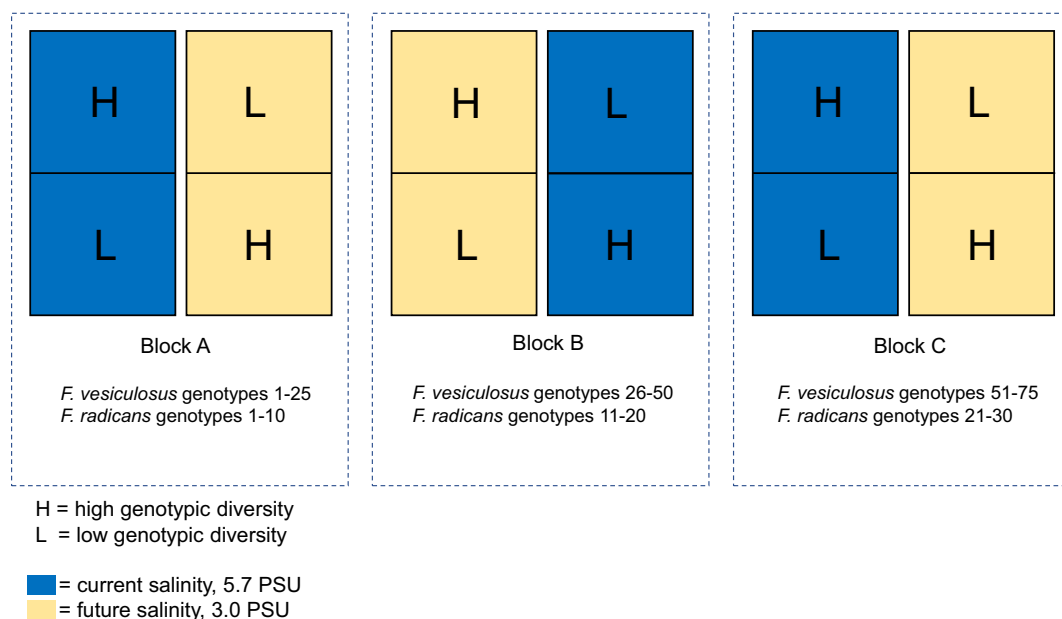


Fig. 1. Schematic overview of the experimental setup. Each block (A-C) contains two tanks, one for each salinity treatment. Two tanks within one block contain the same set of 25 *Fucus vesiculosus* genotypes and 10 *Fucus radicans* genotypes. Each tank contains two diversity treatments. The high genotypic diversity treatment (H) consists of 20 genotypes replicated twice for *F. vesiculosus* and 8 genotypes represented only once for *F. radicans*. The low genotypic diversity treatment (L) consists of five genotypes replicated eight times for *F. vesiculosus* and two genotypes represented four times for *F. radicans*. Each tank has an independent recirculating system and water within each tank is shared between diversity treatments.

tank was set to "future" salinity based on predictions for the next century (Meier, 2006). Therefore, the salinity in the future treatment was progressively lowered from 5.7 PSU (current) to 3.0 PSU over a period of three weeks (Supplementary Fig. B.1). Salinity treatments were created by mixing 35 PSU local seawater with freshwater in a large tank outside the system. At the start of the experiment, we added $N = 80$ isopods to each of the tanks in order to simulate grazing pressure. Isopods were not separately acclimated, since *I. balthica* is able to acclimate rapidly to changes in salinity (Hørlyck, 1973; Wood et al., 2014). The experiment lasted a total of 70 days (August – October 2016).

2.4. Abiotic measurements

Tank salinities were checked daily using a multimeter (WTW Multi 3430). Freshening by rain or condensation was corrected by adding sea water and evaporation by adding fresh water (Supplementary Fig. B.1). Temperature and light intensity were recorded every 10 min using HOBO pendant loggers (Onset computer Corporation). Average temperatures and midday (12:00–14:00) light intensities were not different across treatments. The mean (\pm SD) temperature was 12.7 ± 4.12 °C in current salinity and 12.6 ± 4.22 °C in future salinity. The mean (\pm SD) light intensity was 1388 ± 2130 Lux in current salinity and 1989 ± 3783 Lux in future salinity (Supplementary Figs. B.2 and B.3).

2.5. Growth and grazing measurements

All *Fucus* ramets were dried with paper towels and weighed (Sartorius BP221S analytical balance) to the nearest 0.1 mg, then held against a fixed light source to count the apical meristems and the number of grazing marks. Each ramet was photographed against the light source for subsequent measurement of branch length using ImageJ (Version 1.50i, (Schneider et al., 2012)). Length was averaged over all intact (non-grazed) branches from base to tip in each ramet. Two ramets were too fragmented to weigh, 23 were too fragmented or too intensely grazed to measure branch length and were excluded from these measurements. No receptacles were present on the thalli during the experiment.

Biomass gain (g) and elongation (mm) were expressed as growth rate and calculated as follows:

$$(size_f - size_i) \times 70 \text{ days}^{-1}$$

Where $size_f$ and $size_i$ represents the biomass and length of the ramets at the end and at the beginning of the experiment. The change in number of apical meristems and grazing marks was calculated as the final count minus the initial count.

Since three ramets did not survive the experiment, they were excluded from the analyses, leaving a total sample size of $N = 573$.

2.6. Phlorotannin content

To assess chemical defences of the two *Fucus* species, we quantified the total phlorotannin content (% of dry weight) using a standard Folin-Ciocalteu analysis (Singleton et al., 1999) on freshly freeze-dried apical tissue at the end of the experiment. Briefly, the (poly)phenols in the sample react with the Folin-Ciocalteu reagent to form a spectrophotometrically quantifiable complex. We performed the analysis of phlorotannins on a paired subsample, for which 21 *F. vesiculosus* and 9 *F. radicans* genotypes were randomly chosen and one replicate was taken out of each salinity treatment ($N = 60$ ramets). 18 of the *F. vesiculosus* genotypes belonged to the high and 3 to the low diversity treatment. 6 of the *F. radicans* genotypes belonged to the high and 3 to the low diversity treatment. All phlorotannin measurements were determined using a PerkinElmer Lambda XLS + spectrophotometer.

2.7. Statistical analyses

2.7.1. Growth and grazing

Generalized mixed model analyses for growth (biomass gain $N = 573$, elongation $N = 552$, increase in number of apical meristems $N = 573$) and grazing (increase in number of grazing marks $N = 573$) were performed using the GLIMMIX procedure (Kiernan et al., 2012) in SAS 9.4 (SAS Institute Inc., 2015). We analyzed responses as a function of salinity treatment (2 levels), genotypic diversity treatment (2 levels) and their interaction as fixed effects. Random effects in the models were based on the experimental design and included block, genotype, genotype-by-salinity interaction and block-by-salinity interaction. We also tested the effect of tank nested in block, and its interaction with diversity, to account for dependence of diversity treatments sharing the same water within one tank. These latter factors did not improve the models or affect the results, so they were omitted for simplicity. For all models, we tested the significance of fixed effects using Type III tests with Kenward-Roger approximation of degrees of freedom (Kenward and Roger, 1997) and computed least squares means for each treatment with Tukey-Kramer adjustment for multiple testing. We analyzed the random effects by calculating the percentage of variance explained by each random variable from the covariance parameter estimates. We tested the significance of the random effects using the *covtest* option, which performs a log-likelihood test between the full (with random effect) and reduced (without random effect) model. Both covariance parameter estimates and test statistics of random factors were derived from the normally distributed, homoscedastic models. We tested for heteroscedasticity between salinity and diversity treatments using the *covtest* 'common variance' homogeneity option. When heteroscedasticity was supported by a significant result of this test and by a lower AIC of the heteroscedastic compared to the homoscedastic model, we allowed for unequal variances between treatments in the models. However, when the difference in AIC was small (≤ 4), we chose the simpler model (Burnham and Anderson, 2002). We verified assumptions of error distribution verified by visual inspection of model residuals. To visualize genotype effects and genotype-by-salinity interactions, we derived reaction norms of BLUPs (best linear unbiased prediction, (Robinson, 2008)) from the GLMMs in SAS 9.4, representing the deviation from the average response of each genotype in current versus future salinity.

We used normally distributed error variation in the models for biomass gain and elongation, Poisson error distribution in the model for the number of apical meristems and negative binomial error distribution in the model for number of grazing marks.

Effects on biomass gain and meristem formation, but not elongation, are expected to be mediated by the initial number of apical meristems (Moss, 1967). To control for the initial differences in the numbers of apical meristems, the initial number of apical meristems was included as a covariate in the models for biomass gain and apical meristems. Elongation was assumed to occur independently for each meristem, so it was not included as a covariate. For the grazing models, initial size in terms of biomass was included as a covariate.

Since *F. vesiculosus* and *F. radicans* inherently differ in growth characteristics (Bergström et al., 2005), the growth models were fitted separately for each species. Such inherent differences have not been reported for grazing by *I. balthica* or phlorotannins. We thus first fitted grazing and phlorotannin models using pooled data for both species, including species as a fixed factor and subsequently, if there was a significant species effect, separately for each species. The species-by-salinity interaction was tested, but was not significant ($P > 0.1$) and thus excluded from the pooled models. Model results were plotted using the *ggplot2* package in R (Wickham, 2016).

2.7.2. Phlorotannins

We constructed a linear mixed model to assess the effect of salinity, diversity, and species on phlorotannin content ($N = 60$). To test the effect of phlorotannin concentration on the probability of being grazed

while accounting for salinity, diversity, and species effects, we used a mixed logistic regression. The salinity-by-diversity interaction was not tested due to low sample size for the low diversity groups. For both models, the random effects of block, genotype and genotype-by-block were included. We used normally distributed error variation in the model for phlorotannins.

3. Results

3.1. The effect of salinity and genotypic diversity on growth

In *F. vesiculosus*, the salinity effect on biomass gain was mediated by the initial number of apical meristems (main effect of salinity: $F_{1, 6.2} = 0.23$, $P = 0.646$; salinity-by-covariate interaction: $F_{1, 383} = 145$, $P < 0.001$). While algae with more meristems gained biomass faster, the increase of growth rate per meristem was on average 50.4% smaller in future (= 3.0 PSU) than current (= 5.7 PSU) salinity (Fig. 2a, $F_{1, 218} = 36.7$, $P < 0.001$). We used a statistical model accounting for heteroscedastic variances because the variation in growth rate was 15.7% larger in the high diversity than in the low diversity treatment, although this effect was marginally non-significant (homogeneity test: $\chi^2 = 8.59$, $P = 0.072$). During experimentation, future salinity treated *F. vesiculosus*

formed significantly fewer apical meristems (-14.9% , main effect of salinity: $F_{1, 2.6} = 17.1$, $P < 0.05$) than those kept in the current salinity treatment and, as for biomass gain, the effect of salinity increased with the increasing initial size of thalli (salinity-by-covariate interaction: $F_{1, 471} = 23.8$, $P < 0.001$). There was no statistically significant effect of salinity on elongation ($F_{1, 3.8} = 1.90$, $P = 0.244$) (Supplementary Tables C.1a, C.3a and C.4a).

For *F. radicans*, growth in terms of biomass (Fig. 2b) and number of apical meristems only depended on the initial number of apical meristems (main effect of covariate, $F_{1, 54.2} = 11.0$, $P = 0.002$ and $F_{1, 90} = 9.44$, $P = 0.01$), and not on the salinity treatment (Supplementary Table C.1b). However, variation in biomass gain among ramets was 10 times lower in the future salinity compared to the current salinity treatment (homogeneity test: $\chi^2 = 18.6$, $P = 0.001$), which was again accounted for using a heteroscedastic model (Supplementary Tables C.1b, C.3b and C.4b).

There were no statistically significant main effects of genotypic diversity or interaction effects of salinity-by-diversity on growth in either of the two species in any of the three growth responses measured (Supplementary Table C.1).

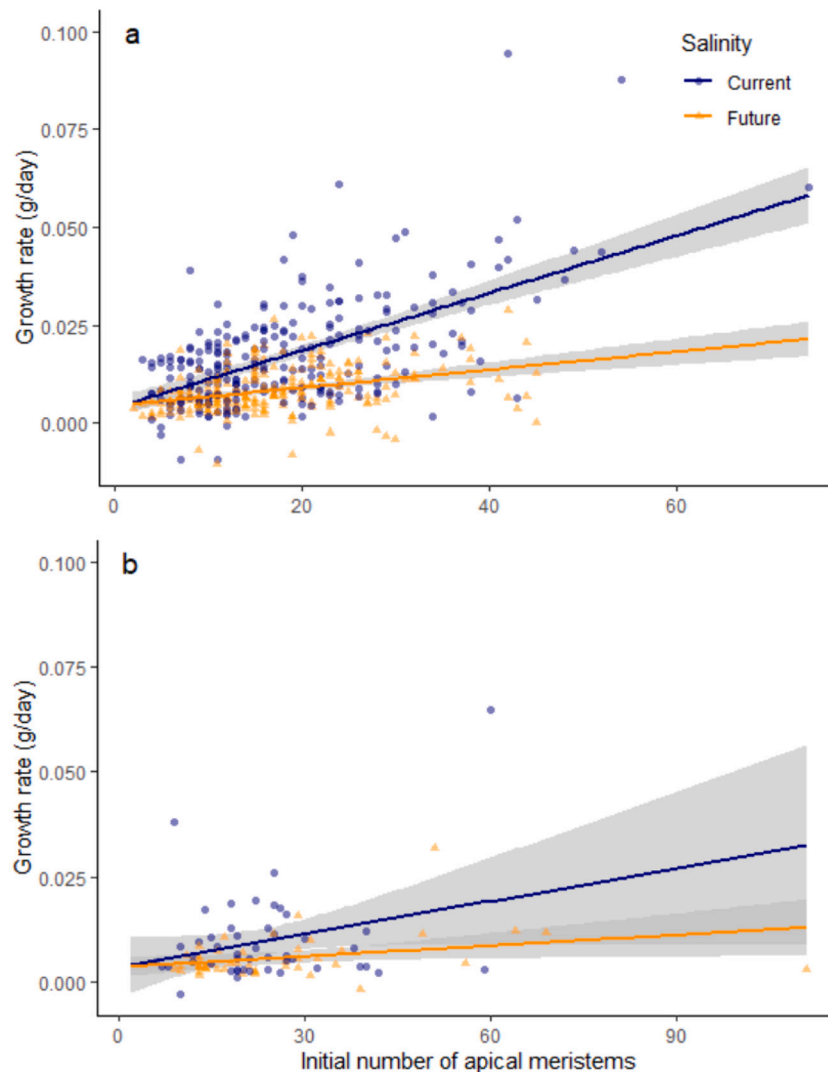


Fig. 2. Growth rate in terms of biomass (g/day) as a function of the initial number of apical meristems for a) *Fucus vesiculosus* and b) *Fucus radicans* in current (= 5.7 PSU) and future (= 3.0 PSU) salinity. Points represent raw data points, lines and grey shaded areas represent linear mixed model predictions and 95% confidence intervals, respectively.

3.2. The effect of salinity and genotypic diversity on grazing pressure and phlorotannins

In a model pooling together grazing of both *Fucus* species ($N = 573$), the interaction of salinity and diversity was significant ($F_{1, 50.2} = 10.8$, $P < 0.05$). This was due to increased grazing in the future salinity in the high diversity treatment: in the high diversity treatment, algae in future salinity received 50% more grazing marks than those in current salinity. There was no significant difference in grazing marks between salinity conditions in the low diversity treatment (Fig. 3a, Supplementary Tables C.5a and C.6a). The number of grazing marks also increased with the initial weight of the algae ($F_{1,287} = 17.3$, $P < 0.001$) and differed strongly between species ($F_{1,91.8} = 18.5$, $P < 0.001$), with *F. radicans* receiving 37.8% more grazing marks than *F. vesiculosus* (Supplementary Tables C.2b, C.5a and C.6a). Because of the difference in grazing

between species we re-ran the model for each species separately (Fig. 3b, c). Results showed that the initial weight effect remained consistent across both species (Supplementary Table C.2b, c), but the salinity-by-diversity interaction in the pooled data was driven by *F. vesiculosus* (Fig. 3b, $F_{1, 330} = 8.67$, $P = 0.004$) while in *F. radicans* variation within treatments was high and the interaction effect was marginally non-significant (Fig. 3c, $F_{1, 35} = 3.48$, $P = 0.071$).

No significant effects of salinity ($F_{1,2.81} = 0.84$, $P = 0.430$) or diversity ($F_{1,25} = 0.51$, $P = 0.481$) or species ($F_{1,25} = 1.07$, $P = 0.311$) were found on algal phlorotannin content (mean \pm SD = 10.1 ± 1.86). Finally, the probability of being grazed was not influenced by phlorotannin concentration (logistic regression, effect of phlorotannins: $F_{1,46.9} = 2.20$, $P = 0.145$) nor were there any significant salinity ($F_{1,3.65} = 0.34$, $P = 0.592$), diversity ($F_{1,27.4} = 0$, $P = 0.982$) or species effects ($F_{1,26.7} = 0.02$, $P = 0.886$).

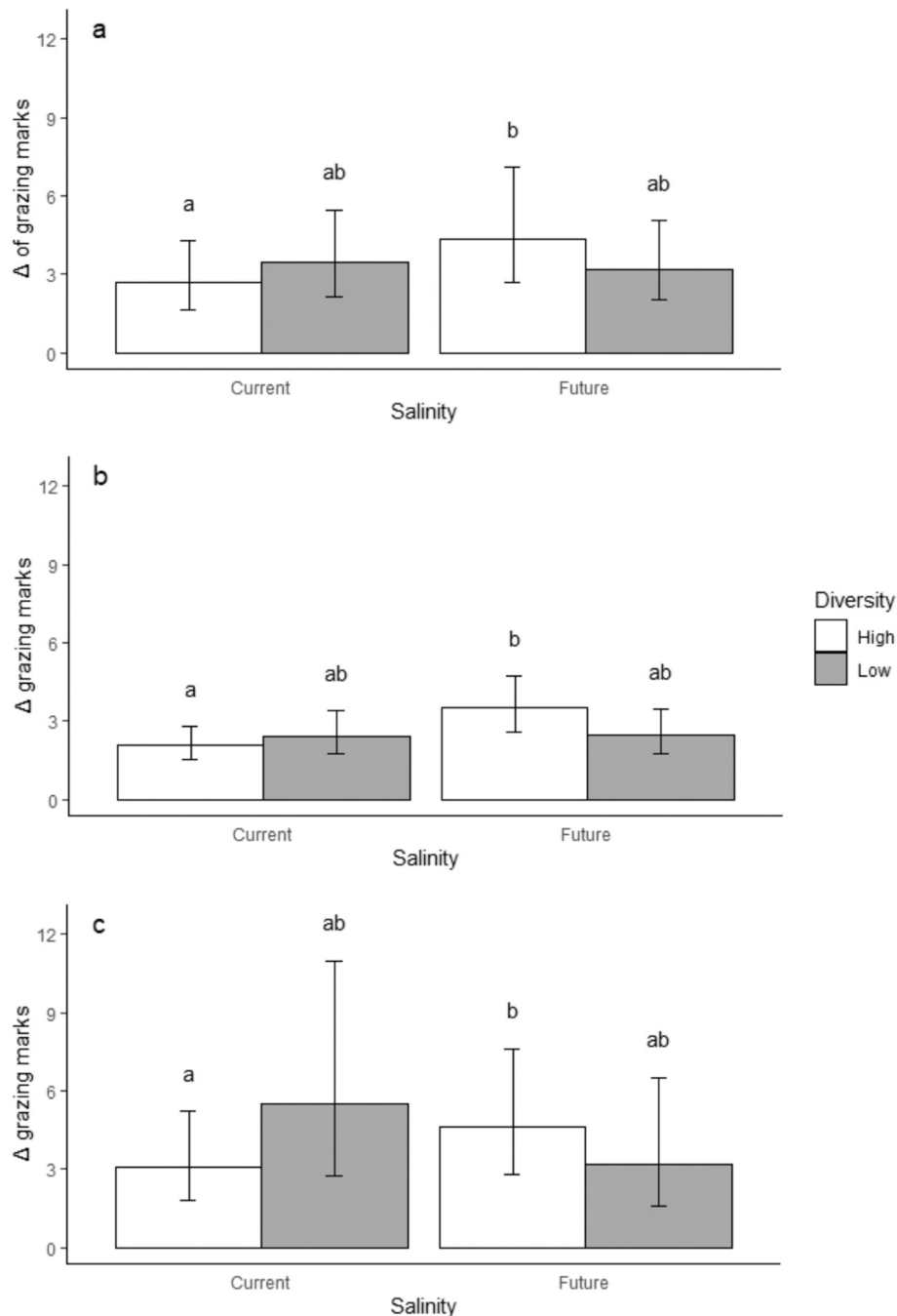


Fig. 3. Number of grazing marks (least squares means \pm 95% confidence intervals) gained during the experiment for a) *Fucus vesiculosus* and *Fucus radicans* pooled, b) *F. vesiculosus* only and c) *F. radicans* only. Comparisons are between current (= 5.7 PSU) and future (= 3.0 PSU) salinity, and high and low genotypic diversity treatments. Significant effects were found for species as a factor. Different letters between treatments indicate significant Tukey-Kramer adjusted post-hoc comparisons (see Supp. Table 6).

3.3. Genotypic variation of growth and grazing responses

For *F. vesiculosus*, but not *F. radicans*, we found a salinity-by-genotype interaction in terms of the increase in number of apical meristems, suggesting genotypic variation in the growth response to decreased salinity (Fig. 4). The interaction explained 6.8% of the variance in *F. vesiculosus* ($\chi^2 = 5.53$, $P < 0.01$, Supplementary Table C.1a, Fig. 4a). We found no such salinity-by-genotype interaction effects on other growth responses (Supplementary Table C.1).

However, in *F. vesiculosus*, genotype explained a significant proportion of variance in growth rate in terms of biomass gain (22.6%, $\chi^2 = 23.2$, $P < 0.001$), elongation (28.9%, $\chi^2 = 17.9$, $P < 0.001$), and apical meristems (8.5%, $\chi^2 = 10.1$, $P < 0.001$, Supplementary Table C.1a). We found no significant genotypic variation in *F. radicans* (Supplementary Table C.1b).

When both species were pooled, the genotype-by-salinity interaction explained 24.2% of the total variation in the number of grazing marks ($\chi^2 = 14.5$, $P = 0.0001$, Supplementary Table C.2a). When the two species were modelled separately, the proportion of variance in the amount of grazing marks explained by genotype was 12.7% in *F. vesiculosus* ($\chi^2 = 7.22$, $P < 0.01$, Supplementary Table C.2b), and the genotype-by-salinity interaction explained 55.3% of the variance in *F. radicans* ($\chi^2 = 12.7$, $P < 0.001$, Supplementary Table C.2c).

Phlorotannin content showed considerable genotypic variation, with genotype explaining 37.6% of the variance ($\chi^2 = 7.11$, $P < 0.01$, Supplementary Table C.2a).

4. Discussion

Here we showed that two *Fucus* species vary in their growth response to the projected future desalination as well as in grazing by the isopod *I. balthica*. Furthermore, there was genotypic variation in the amount algae were grazed and salinity-by-genotype-interactions, suggesting existence of genotypic variation in tolerance to future desalination. Grazing was higher in future projected than in current conditions, though this increase only occurred when the genotypic diversity of the grazed algae was high.

It is known that Baltic *Fucus* populations tolerate low-salinity conditions better than their North Sea counterparts (Nygård and Dring, 2008), probably as a result of natural selection and adaptation during colonization of the Baltic Sea (Rothäusler et al., 2016). However, we observed a diminished growth of *F. vesiculosus* when salinity was reduced compared to their native habitat. Our result is similar to a recent study with other marginal populations of *F. vesiculosus* (Rugiu et al., 2018), as well to another brown alga from the Danish coast (Kristiansen et al., 1994). The biomass gain of *F. vesiculosus* was related to the number of apical meristems in our experiment. Indeed, algae with more initial apical meristems grew faster in current than in future salinities, both in terms of biomass and as an increase in the number of apical meristems. However, there was no difference in the elongation rate between the salinity treatments, suggesting that the biomass increase is controlled by the formation of new meristems and not by the cellular division rates within individual meristems. The salinity outside the optimum of an alga can affect its growth through multiple mechanisms, such as changes in osmotic pressure and cellular organic solute

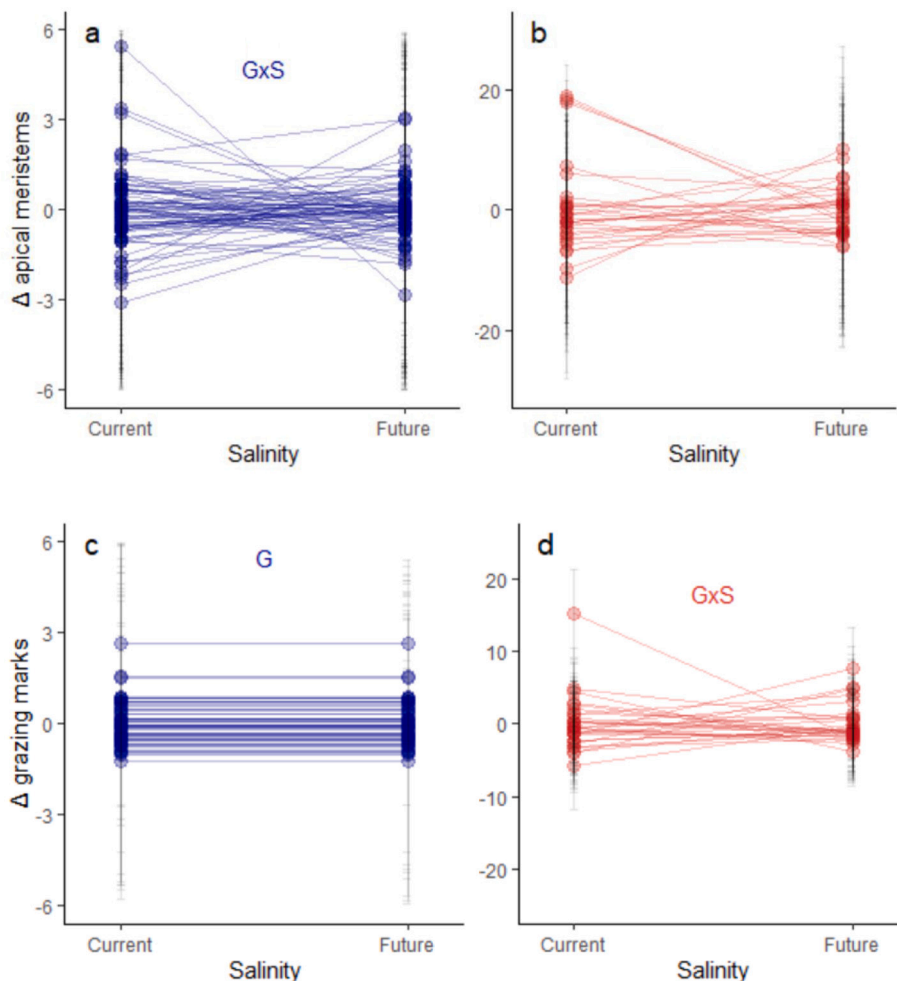


Fig. 4. Genotypic variation in growth, grazing and their response to current (= 5.7 PSU) and future (= 3.0 PSU) salinity. The Y-axis shows GLMM-derived BLUPs (deviation from the average response of genotypes in each salinity treatment level) of increase in number of apical meristems in a) *Fucus vesiculosus* and b) *Fucus radicans* and increase in number of grazing marks in c) *F. vesiculosus* and d) *F. radicans* during the experimental period (70 days). Each point represents the mean and 95% confidence interval for a genotype in the corresponding salinity treatment. Lines connecting the same genotype in different conditions represent the change of these estimates from the current to the future salinity treatment. Note the difference in scale of the y-axis. Significant effects of genotype and genotype-by-salinity are indicated by G and GxS, respectively (see Tables C.1 and C.2).

composition, ionic distributions, oxidative stress and ROS formation (Bisson and Kirst, 1995; Hurd et al., 2014; Rugiu et al., 2020). All these regulatory mechanisms require energy, thus generating a trade-off between cellular homeostasis and the growth of *F. vesiculosus*.

The genotype-by-salinity interaction explained 6.8% of the variation in apical meristem formation in *F. vesiculosus*. Since apical meristem proliferation is the main trigger for growth, this indicates a genotypic variation in the ability of *F. vesiculosus* to cope with future salinity conditions in the Baltic Sea. The number of apical meristems can also be considered a proxy for fitness, as they are responsible for the production of receptacles (Knight and Parke, 1950). Thus, a reduced formation of new meristems may result in a lower reproductive output such as found by Rothäusler et al. (Rothäusler et al., 2018a). Similar to our study, they were able to distinguish between meristems differentiating into receptacles and vegetative apices in their experiment. In parallel with our findings, they showed variation in the response to climate change among genotypes. Populations with high genetic diversity would be more likely to be able to evolve to track future environmental change in the Baltic Sea, as the high-tolerance genotypes would persist and increase in frequency.

Low salinity led to increased grazing of *F. vesiculosus* by the generalist herbivore *I. balthica*, but this increase only took place in the experimental population with high genotypic diversity, while in the low-diversity treatment, the amount of grazing did not change. Since we did not measure isopod mortality in this study, we cannot formally distinguish between increased grazing intensity by the isopods and inherent grazing susceptibility of the algae. Either mechanism is plausible, and they are not mutually exclusive. *Idotea balthica* has a wide salinity tolerance range reaching down to 2.7 PSU (Leidenberger et al., 2012) and has demonstrated a high degree of plasticity under different experimental salinities without any significant changes in food consumption or survival (Wood et al., 2014), supporting increased grazing intensity. On the other hand, *I. balthica* has been shown to suffer higher mortality under future hyposaline conditions in combination with warming (Rugiu et al., 2021), supporting increased grazing susceptibility. Finally, osmoregulatory adjustments of euryhaline mesograzers to desalination can induce an increase or decrease in metabolic rate, efficiency of nutrient absorption and ultimately food consumption. The outcome depends on the species, population of origin, and the magnitude of the salinity transition (Bulnheim, 1974; Łapucki and Normant, 2008; Normant and Lamprecht, 2006).

Increased grazing susceptibility in the high-diversity-future-salinity group can possibly be explained by direct effects of hyposalinity on algae. Given a trade-off between growth, cellular homeostasis and anti-herbivore defences (Herms and Mattson, 1992; Sunda and Hardison, 2010) in a stressful environment, energy allocation to maintain growth and homeostasis may lead to low grazing resistance. Changes in algal traits, such as thallus morphology (Van Alstyne, 1989), tissue toughness (Rothäusler et al., 2017; Taylor et al., 2002) and production of secondary metabolites (Amsler and Fairhead, 2005; Dethier et al., 2005) have all been shown to play an important role in grazer deterrence. Increased consumption may also indicate changes in the quality of algae as food for grazers. Although we did not find any change in the content of total phlorotannins, there could have been changes in the proportions of separate phlorotannin polymers (Jormalainen et al., 2011) or in the contents of other defensive compounds (Deal et al., 2003), both of which remained unquantified here.

Palatability and food quality of brown algae are complex traits and can be altered by salinity in numerous ways. The thallus of *F. vesiculosus* has been found to become softer in hyposaline conditions (Rothäusler et al., 2017) which may allow faster consumption. Furthermore, its nutritional content may have been reduced in future conditions (Barboza et al., 2019; McArt and Thaler, 2013), forcing isopods to compensatory feeding to meet their energy requirements (Lee et al., 2004; Simpson and Raubenheimer, 1993). *Fucus vesiculosus* reared under hyposaline conditions has been shown to accumulate lower amounts of

mannitol (major carbon storage compound of brown algae) and nitrogen (proxy for protein content), both indicators of the quality of *F. vesiculosus* as a food source (Barboza et al., 2019). Conversely, high C:N ratios and mannitol content are feeding cues for *I. balthica* (Weinberger et al., 2011), the reduction of which could have counteracted compensatory consumption effects to some extent. Algae in hyposaline conditions can also retain more water (Rothäusler et al., 2017), thereby diluting nutritious substances, which results in higher biomass consumption by herbivores (Cruz-Rivera and Hay, 2000; Jormalainen et al., 2011). In this study, we found significant genotypic variation in grazing of *F. vesiculosus* and in phlorotannin production of both the species, indicating genetic variation in the palatability of the algae. In addition, there was a salinity-by-genotype interaction in the grazing of *F. radicans*, implying that distinct genotypes responded differently to hyposalinity in their susceptibility to grazing.

Despite genotypic variation in growth, grazing and defence, the combined pressure of reduced growth and intensification of herbivory on *F. vesiculosus* may still form a serious future threat in the Baltic Sea. Even though *F. vesiculosus* can disperse long distances by floating, at least occasionally (Rothäusler et al., 2015; Rothäusler et al., 2020), a recent study suggests that the dispersal rate of *F. vesiculosus* may not be able to match the rate of climate change (Jonsson et al., 2018). *Fucus radicans* could be even more at risk, due to a combination of its low standing genetic variation, mostly asexual reproduction and limited rafting dispersal due to a lack of buoyancy (Tatarenkov et al., 2005). In addition, the southern distribution range edge of *F. radicans* is partly determined by grazing pressure, due to food preference behaviour of *I. balthica*, and also by competition with the faster-growing *F. vesiculosus* (Forslund et al., 2012; Gunnarsson and Berglund, 2012; Schagerström and Kautsky, 2016). Thus, there is concern that *F. radicans* might end up “squeezed” between their physiological and biotic limit, a hypothesis which is supported by our finding of higher grazing susceptibility of *F. radicans* than *F. vesiculosus*. This future scenario is particularly concerning, as *F. radicans* is endemic to the Baltic Sea, and would thus go extinct if it is not able to withstand future changes. However, there are some glimmers of hope: *F. radicans* has evolved a high tolerance to low salinity during the short time since the Baltic Sea opening (more so than *F. vesiculosus*), and according to our results there is genotypic variability in both salinity tolerance and grazing resistance, indicating that further evolution is possible.

Mesocosm experiments are necessarily imperfect simplifications of the natural habitat in which the study organisms are found. While we attempted to replicate the conditions of the Finnish sampling sites as closely as possible, certain physical and chemical attributes may have been different in our mesocosm setup in Sweden, as we created salinity treatment by diluting local sea water. The three main parameters of concern are temperature, pH and alkalinity. Mean sea surface temperature (SST) for the Bothnian Sea in August, September and October in 2018 was 18, 14, and 8 °C, respectively (Siegel and Gerth, 2018). The mean temperature in our experimental tanks varied accordingly, from 16.9 to 8.2 °C from the end of August until the end of October. The pH in the Bothnian Sea in August and September in 2016 was 7.8 (Swedish Ocean Archive (SHARK), The Swedish Agency for Marine and Water Management and the Swedish Meteorological and Hydrological Institute, retrieved 21-07-2021), which was equal to the pH of the local tapwater used for dilution (Strömstads kommun vattenverket, retrieved 14-07-2021). Finally, the alkalinity in the Baltic Sea does not scale 1:1 with salinity, and the relationship between the two differs across the subareas of the Baltic (see Fig. 2a in (Müller et al., 2016)). Alkalinity decreases more quickly as a function of salinity in Bothnian Sea waters than in the Kattegat/Skagerrak area. However, diluting Skagerrak sea water to 3–5.7 PSU with local tap water, which has an alkalinity of 770 µmol/kg (Strömstads kommun vattenverket, retrieved 14-07-2021), roughly equivalent to the alkalinity observed in Finnish waters, should achieve comparable final alkalinities. Alternative dilution water sources would be local river water, (Ö. Anräsälven, SLU environmental database

for soil-water-environment, retrieved 29-06-2021) at 390 $\mu\text{mol/kg}$, or distilled water, both of which would result in lower alkalinity compared to Finnish waters. While absolute alkalinity limits for growth of *Fucus* are not known, Fucaceae have been shown to acquire carbon from the surrounding water by extracting dissolved organic carbon without changing the buffering capacity of the surrounding water up to $\text{pH} > 9.5$ (Axelsson and Uusitalo, 1988). An experimental study testing the direct effects of pH on macroalgal growth showed a decrease in growth rates of *F. vesiculosus* at $\text{pH} > 8.5$ (Middelboe and Hansen, 2007). Theoretically, the $\sim 300 \mu\text{mol/kg}$ increase in alkalinity at 5.7 PSU in our experiment could thus allow *Fucus* in 5.7 PSU to take up more carbon before alkalinities drop too low and pH rises too high to grow. However, since we refreshed the water in the tanks weekly, we are confident that this 300 $\mu\text{mol/kg}$ increase did not affect the experiment. Regardless of the efforts and considerations mentioned above, an experimental setting at a non-native location cannot exactly replicate natural conditions. Nevertheless, given the small size of the deviations of these abiotic factors compared to the significant salinity reduction, we are confident that the main effect of desalination should still be valid.

5. Conclusion

Our results showed that future salinity conditions impaired the growth of rocky littoral fucoid algae and induced a shift in resistance to herbivory mediated by genotypic diversity. Genotypic variation was revealed for apical meristem proliferation and the amount of grazing, as well as their response to future salinity, emphasizing the importance of genetic diversity in coping with future predicted changes in abiotic factors. We conclude that concern about range margin *Fucus* populations is appropriate, firstly because of its complex responses and vulnerability to predicted changes, and secondly because of its crucial role as a foundation species. In general, abiotic stress can cause unexpected shifts in biotic interactions when traits with genotypic variation lie at the base of these interactions. In order to predict future distributions of ecologically important species, models should take into account the effects of these shifting biotic interactions and the genetic variability of responses to abiotic stress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jembe.2021.151666>.

References

Amsler, C.D., Fairhead, V.A., 2005. Defensive and sensory chemical ecology of Brown algae. *Adv. Bot. Res.* 43, 1–91. [https://doi.org/10.1016/S0065-2296\(05\)43001-3](https://doi.org/10.1016/S0065-2296(05)43001-3).
 Ardehed, A., Johansson, D., Sundqvist, L., Schagerström, E., Zagrodzka, Z., Kovaltchouk, N.A., Bergström, L., Kautsky, L., Rafajlovic, M., Pereyra, R.T., Johannesson, K., 2016. Divergence within and among seaweed siblings (*Fucus*

vesiculosus and *F. radicans*) in the Baltic Sea. *PLoS One* 11, 1–16. <https://doi.org/10.1371/journal.pone.0161266>.
 Arnaud-Haond, S., Duarte, C.M., Alberto, F., Serrão, E.A., 2007. Standardizing methods to address clonality in population studies. *Mol. Ecol.* 16, 5115–5139. <https://doi.org/10.1111/j.1365-294X.2007.03535.x>.
 Axelsson, L., Uusitalo, J., 1988. Carbon acquisition strategies for marine macroalgae. *Mar. Biol.* 97, 295–300. <https://doi.org/10.1007/bf00391315>.
 Bäck, S., Collins, J.C., Russell, G., 1992. Effects of salinity on growth of Baltic and Atlantic *Fucus vesiculosus*. *Br. Phycol. J.* 27, 39–47. <https://doi.org/10.1080/00071619200650061>.
 Barboza, F.R., Kotta, J., Weinberger, F., Jormalainen, V., Kraufvelin, P., Molis, M., Schubert, H., Pavia, H., Nylund, G.M., Kautsky, L., Schagerström, E., Rickert, E., Saha, M., Fredriksen, S., Martin, G., Torn, K., Ruuskanen, A., Wahl, M., 2019. Geographic variation in fitness-related traits of the bladderwrack *Fucus vesiculosus* along the Baltic Sea-North Sea salinity gradient. *Ecol. Evol.* 9, 9225–9238. <https://doi.org/10.1002/ece3.5470>.
 Berg, M.P., Toby Kiers, E., Driessen, G., van der Heijden, M., Kooi, B.W., Kuenen, F., Liefing, M., Verhoef, H.A., Ellers, J., 2010. Adapt or disperse: understanding species persistence in a changing world. *Glob. Chang. Biol.* 16, 587–598. <https://doi.org/10.1111/j.1365-2486.2009.02014.x>.
 Bergström, L., Tatarenkov, A., Johannesson, K., Jönsson, R.B., Kautsky, L., 2005. Genetic and morphological identification of *Fucus radicans* sp. nov. (Fucales, Phaeophyceae) in the brackish Baltic Sea. *J. Phycol.* 41, 1025–1038. <https://doi.org/10.1111/j.1529-8817.2005.00125.x>.
 Bisson, M.A., Kirst, O.K., 1995. Osmotic acclimation and turgor pressure regulation in algae. *Naturwissenschaften* 82, 461–471.
 Blois, J.L., Zarnetske, P.L., Matthew, C.F., Finnegan, S., 2014. Climate change and the past, present, and future of biotic interactions. *Science* (80-) 341, 499–504. <https://doi.org/10.1126/science.1237184>.
 Bulnheim, H.P., 1974. Respiratory metabolism of *Idotea balthica* (Crustacea, Isopoda) in relation to environmental variables, acclimation processes and moulting. *Helgoländer Meeresun.* 26, 464–480. <https://doi.org/10.1007/BF01627627>.
 Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference*. Springer, USA.
 Chakraborty, R., Nei, M., 1977. Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. *Evolution* 31, 347–356.
 Coleman, F.C., Williams, S.L., 2002. Overexploiting marine ecosystem engineers: potential consequences for biodiversity. *Trends Ecol. Evol.* 17, 40–44. [https://doi.org/10.1016/S0169-5347\(01\)02330-8](https://doi.org/10.1016/S0169-5347(01)02330-8).
 Cruz-Rivera, E., Hay, M.E., 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. *Ecology* 81, 201–219.
 Deal, M.S., Hay, M.E., Wilson, D., Fenical, W., 2003. Galactolipids rather than phlorotannins as herbivore deterrents in the brown seaweed *Fucus vesiculosus*. *Oecologia* 136, 107–114. <https://doi.org/10.1007/s00442-003-1242-3>.
 Dethier, M.N., Williams, S.L., Freeman, A., 2005. Seaweeds under stress: manipulated stress and herbivory affect critical life-history functions. *Ecol. Monogr.* 75, 403–418. <https://doi.org/10.1890/03-4108>.
 Engel, C.R., Brawley, S.H., Edwards, K.J., Serrão, E.A., 2003. Isolation and cross-species amplification of microsatellite loci from the fucoid seaweeds *Fucus vesiculosus*, *F. serratus* and *Ascophyllum nodosum* (Heterokontophyta, Fucaceae). *Mol. Ecol. Notes* 3, 180–182. <https://doi.org/10.1046/j.1471-8286.2003.00390.x>.
 Engkvist, R., Malm, T., Tobiasson, S., 2000. Density dependent grazing effects of the isopod *Idotea balthica* Pallas on *Fucus vesiculosus* L in the Baltic Sea. *Aquat. Ecol.* 34, 253–260. <https://doi.org/10.1023/A:1009919526259>.
 Fleming-Lehtinen, V., Laamanen, M., Kuosa, H., Haahti, H., Olsonen, R., 2008. Long-term development of inorganic nutrients and chlorophyll a in the open northern Baltic Sea. *Ambio* 37, 86–92. [https://doi.org/10.1579/0044-7447\(2008\)37\[86:LDOINA\]2.0.CO;2](https://doi.org/10.1579/0044-7447(2008)37[86:LDOINA]2.0.CO;2).
 Forslund, H., Eriksson, O., Kautsky, L., 2012. Grazing and geographic range of the Baltic seaweed *Fucus radicans* (Phaeophyceae). *Mar. Biol. Res.* 8, 322–330. <https://doi.org/10.1080/17451000.2011.637565>.
 Furman, E., Pihlajamäki, M., Valipakka, P., Myrberg, K., 2014. The Baltic Sea Environment and Ecology [WWW Document]. [https://doi.org/10.1016/0302-184X\(83\)90006-9](https://doi.org/10.1016/0302-184X(83)90006-9).
 Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W., Holt, R.D., 2010. A framework for community interactions under climate change. *Trends Ecol. Evol.* 25, 325–331. <https://doi.org/10.1016/j.tree.2010.03.002>.
 Graham, L.E., Wilcox, L.W., 2000. *Algae*. Prentice Hall, USA.
 Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), *Culture of Marine Invertebrate Animals*. Springer-Verlag, USA, pp. 29–60.
 Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. *Can. J. Microbiol.* 8, 229–239.
 Gunnarsson, K., Berglund, A., 2012. The brown alga *Fucus radicans* suffers heavy grazing by the isopod *Idotea balthica*. *Mar. Biol. Res.* 8, 87–89. <https://doi.org/10.1080/17451000.2011.594890>.
 Gutiérrez, J.L., Jones, C.G., Strayer, D.L., Iribarne, O.O., 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos* 101, 79–90. <https://doi.org/10.1034/j.1600-0706.2003.12322.x>.
 Haavisto, F., Koivikko, R., Jormalainen, V., 2017. Defensive role of macroalgal phlorotannins: benefits and trade-offs under natural herbivory. *Mar. Ecol. Prog. Ser.* 566, 79–90. <https://doi.org/10.3354/meps12004>.
 Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomaneck, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems. *Ecol. Lett.* 9, 228–241. <https://doi.org/10.1111/j.1461-0248.2005.00871.x>.

- Harley, C.D.G., Anderson, K.M., Demes, K.W., Jorve, J.P., Kordas, R.L., Coyle, T.A., Graham, M.H., 2012. Effects of climate change on global seaweed communities. *J. Phycol.* 48, 1064–1078. <https://doi.org/10.1111/j.1529-8817.2012.01224.x>.
- HELCOM, 2009. Biodiversity in the Baltic Sea. *Balt. Sea Environ. Proc.* 116, 188.
- HELCOM Red List Macrophyte Expert Group, 2013. Species Information Sheet *Fucus vesiculosus*.
- Herns, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. *Q. Rev. Biol.* 67, 283–335. <https://doi.org/10.1086/417659>.
- Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., Steneck, R.S., Greenfield, P., Gomez, E., Harvell, C.D., Sale, P.F., Edwards, A.J., Caldeira, K., Knowlton, N., Eakin, E.M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R.H., Dubi, A., Hatzioles, M.E., 2007. Change and ocean acidification. *Science* (80-) 318, 1737–1743.
- Horlyck, V., 1973. The osmoregulatory ability in three species of the genus *Idotea* (Isopoda, Crustacea). *Ophelia* 12, 129–140. <https://doi.org/10.1080/00785326.1973.10430123>.
- Hurd, C.L., Harrison, P.J., Bischof, K., Lobban, C.S., 2014. *Seaweed Ecology and Physiology*, 2nd ed. Cambridge University Press.
- Inc., S.I., 2015. SAS/CONNECT 9.4 user's Guide. Third Edition, Third. ed. Cary, North Carolina, USA.
- Johannesson, K., André, C., 2006. Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. *Mol. Ecol.* 15, 2013–2029. <https://doi.org/10.1111/j.1365-294X.2006.02919.x>.
- Johansson, D., Pereyra, R.T., Rafajlović, M., Johannesson, K., 2017. Reciprocal transplants support a plasticity-first scenario during colonisation of a large hyposaline basin by a marine macro alga. *BMC Ecol.* 17, 1–9. <https://doi.org/10.1186/s12898-017-0124-1>.
- Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. *Oikos* 69, 373–386.
- Jonsson, P.R., Kotta, J., Andersson, H.C., Herkül, K., Virtanen, E., Sandman, A.N., Johannesson, K., 2018. High climate velocity and population fragmentation may constrain climate-driven range shift of the key habitat former *Fucus vesiculosus*. *Divers. Distrib.* 24, 892–905. <https://doi.org/10.1111/ddi.12733>.
- Jormalainen, V., Honkanen, T., 2008. Macroalgal chemical defenses and their roles in structuring temperate marine communities. In: Amsler, C.D. (Ed.), *Algal Chemical Ecology*. Springer, Berlin Heidelberg, pp. 57–89.
- Jormalainen, V., Ramsay, T., 2009. Resistance of the brown alga *Fucus vesiculosus* to herbivory. *Oikos* 118, 713–722. <https://doi.org/10.1111/j.1600-0706.2008.17178.x>.
- Jormalainen, V., Koivikko, R., Ossipov, V., Lindqvist, M., 2011. Quantifying variation and chemical correlates of bladderwrack quality - herbivore population makes a difference. *Funct. Ecol.* 25, 900–909. <https://doi.org/10.1111/j.1365-2435.2011.01841.x>.
- Kenward, M.G., Roger, J.H., 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53, 983–997. <https://doi.org/10.2307/2533558>.
- Kersen, P., Kotta, J., Bučas, M., Kolesova, N., Değere, Z., 2011. Epiphytes and associated fauna on the brown alga *Fucus vesiculosus* in the Baltic and the north seas in relation to different abiotic and biotic variables. *Mar. Ecol.* 32, 87–95. <https://doi.org/10.1111/j.1439-0485.2010.00418.x>.
- Kiernan, K., Tao, J., Gibbs, P., 2012. Tips and strategies for mixed modeling with SAS global forum 2012. *SAS Glob. Forum* 2 (332), 1–18.
- Knight, M., Parke, M., 1950. A biological study of *Fucus vesiculosus* L. and *F. serratus* L. *J. Mar. Biol. Assoc. United Kingdom* 29, 439–514.
- Korpinen, S., Honkanen, T., Vesakoski, O., Hemmi, A., Koivikko, R., Loponen, J., Jormalainen, V., 2007. Macroalgal communities face the challenge of changing biotic interactions: review with focus on the Baltic Sea. *Ambio* 36, 203–211. [https://doi.org/10.1579/0044-7447\(2007\)36\[203:MCFTCO\]2.0.CO;2](https://doi.org/10.1579/0044-7447(2007)36[203:MCFTCO]2.0.CO;2).
- Kotta, J., Vanhatalo, J., Jänes, H., Orav-Kotta, H., Rugiu, L., Jormalainen, V., Bobsien, I., Viitasalo, M., Virtanen, E., Sandman, A.N., Isaeus, M., Leidenberger, S., Jonsson, P. R., Johannesson, K., 2019. Integrating experimental and distribution data to predict future species patterns. *Sci. Rep.* 9, 1–14. <https://doi.org/10.1038/s41598-018-38416-3>.
- Kristiansen, A.A., Pedersen, P.M., Moseholm, L., 1994. Salinity-temperature effects on growth and reproduction of *Scytosiphon lomentaria* (Fucophyceae) along the salinity gradient in Danish waters. *Phycologia* 33, 444–454.
- Laikre, L., Lundmark, C., Jansson, E., Wennerström, L., Edman, M., Sandström, A., 2016. Lack of recognition of genetic biodiversity: international policy and its implementation in Baltic Sea marine protected areas. *Ambio* 45, 661–680. <https://doi.org/10.1007/s13280-016-0776-7>.
- Łapucki, T., Normant, M., 2008. Physiological responses to salinity changes of the isopod *Idotea chelipes* from the Baltic brackish waters. *Comp. Biochem. Physiol. Part A* 149, 299–305. <https://doi.org/10.1016/j.cbpa.2008.01.009>.
- Lee, K., Raubenheimer, D., Simpson, S.J., 2004. The effects of nutritional imbalance on compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar. *Physiol. Entomology* 29, 108–177. <https://doi.org/10.1111/j.0307-6962.2004.00371.x>.
- Leidenberger, S., Harding, K., Jonsson, P.R., 2012. Ecology and distribution of the isopod genus *Idotea* in the Baltic Sea: key species in a changing environment. *J. Crustac. Biol.* 32, 359–381. <https://doi.org/10.1163/193724012X626485>.
- Lüning, K., 1990. *Seaweeds: Their Environment, Biogeography, and Ecophysiology*. Wiley, USA.
- McArt, S.H., Thaler, J.S., 2013. Plant genotypic diversity reduces the rate of consumer resource utilization. *Proc. R. Soc. B* 280, 1–8.
- Meier, H.E.M., 2006. Baltic Sea climate in the late twenty-first century: a dynamical downscaling approach using two global models and two emission scenarios. *Clim. Dyn.* 27, 39–68. <https://doi.org/10.1007/s00382-006-0124-x>.
- Meier, H.E.M., Hordoir, R., Andersson, H.C., Dieterich, C., Eilola, K., Gustafsson, B.G., Höglund, A., Schimanke, S., 2012. Modeling the combined impact of changing climate and changing nutrient loads on the Baltic Sea environment in an ensemble of transient simulations for 1961–2099. *Clim. Dyn.* 39, 2421–2441. <https://doi.org/10.1007/s00382-012-1339-7>.
- Middelboe, A.L., Hansen, P.J., 2007. Direct effects of pH and inorganic carbon on macroalgal photosynthesis and growth. *Mar. Biol.* Res. 3, 134–144. <https://doi.org/10.1080/17451000701320556>.
- Moss, 1967. The apical meristem of *Fucus*. *New Phytol.* 66, 67–74.
- Müller, J.D., Schneider, B., Rehder, G., 2016. Long-term alkalinity trends in the Baltic Sea and their implications for CO₂-induced acidification. *Limnol. Oceanogr.* 61, 1984–2002. <https://doi.org/10.1002/lno.10349>.
- Normant, M., Lamprecht, I., 2006. Does scope for growth change as a result of salinity stress in the amphipod *Gammarus oceanicus*? *J. Exp. Mar. Biol. Ecol.* 334, 158–163. <https://doi.org/10.1016/j.jembe.2006.01.022>.
- Nygård, C.A., Dring, M.J., 2008. Influence of salinity, temperature, dissolved inorganic carbon and nutrient concentration on the photosynthesis and growth of *Fucus vesiculosus* from the Baltic and Irish seas. *Eur. J. Phycol.* 43, 253–262. <https://doi.org/10.1080/09670260802172627>.
- Ojaveer, H., Jaanus, A., Mackenzie, B.R., Martin, G., Olenin, S., Radziejewska, T., Telesh, I., Zettler, M.L., Zaiko, A., 2010. Status of biodiversity in the Baltic Sea. *PLoS One* 5, 1–19. <https://doi.org/10.1371/journal.pone.0012467>.
- Panova, M., Aronsson, H., Cameron, R.A., Dahl, P., Godhe, A., Lind, U., Ortega-martinez, O., Pereyra, R., Tesson, S.V.M., Wrangle, A., Blomberg, A., Johannesson, K., 2016. DNA extraction protocols for whole-genome sequencing in marine organisms. In: Bourlat, S.J. (Ed.), *Marine Genomics*. Humana Press, New York, USA, pp. 13–44. <https://doi.org/10.1007/978-1-4939-3774-5>.
- Pavia, H., Toth, G.B., 2008. Macroalgal models in testing and extending defense theories. In: Amsler, C.D. (Ed.), *Algal Chemical Ecology*. Springer, Berlin Heidelberg, pp. 147–172.
- Pearson, G.A., Lago-Leston, A., Mota, C., 2009. Frayed at the edges: selective pressure and adaptive response to abiotic stressors are mismatched in low diversity edge populations. *J. Ecol.* 97, 450–462. <https://doi.org/10.1111/j.1365-2745.2009.01481.x>.
- Pereyra, R.T., Bergström, L., Kautsky, L., Johannesson, K., 2009. Rapid speciation in a newly opened postglacial marine environment, the Baltic Sea. *BMC Evol. Biol.* 9, 1–9. <https://doi.org/10.1186/1471-2148-9-70>.
- Perrin, N., Sibly, R., 1993. Dynamic models of energy allocation and investment. *Annu. Rev. Ecol. Syst.* 24, 379–410. <https://doi.org/10.1146/annurev.es.24.110193.002115>.
- Poloczanska, E.S., Brown, C.J., Sydeman, W.J., Kiessling, W., Schoeman, D.S., Moore, P. J., Brander, K., Bruno, J.F., Buckley, L.B., Burrows, M.T., Duarte, C.M., Halpern, B.S., Holding, J., Kappel, C.V., O'Connor, M.I., Pandolfi, J.M., Parmesan, C., Schwing, F., Thompson, S.A., Richardson, A.J., 2013. Global imprint of climate change on marine life. *Nat. Clim. Chang.* 3, 919–925. <https://doi.org/10.1038/nclimate1958>.
- Reusch, T.B.H., Wood, T.E., 2007. Molecular ecology of global change. *Mol. Ecol.* 16, 3973–3992. <https://doi.org/10.1111/j.1365-294X.2007.03454.x>.
- Reusch, T.B.H., Dierking, J., Andersson, H., Bonsdorff, E., Carstensen, J., Casini, M., Czajkowski, M., Hasler, B., Hinsby, K., Hyytiäinen Johannesson, K., Jomaa, S., Jormalainen, V., Kuosa, H., Kurland, S., Laikre, L., MacKenzie, B.R., Margonski, P., Melzner, F., Oesterwind, D., Ojaveer, H., Refsgaard, J.C., Sandström, A., Schwarz, G., Tonderski, K., Winder, M., Zandersen, M., 2018. The Baltic Sea as a time machine. *Sci. Adv.* 4.
- Robinson, 2008. That BLUP is a good thing: the estimation of random effects. *Stat. Sci.* 6, 15–32.
- Rothäusler, E., Sjöroos, J., Heye, K., Jormalainen, V., 2016. Genetic variation in photosynthetic performance and tolerance to osmotic stress (desiccation, freezing, hyposalinity) in the rocky littoral foundation species *Fucus vesiculosus* (Fueales, Phaeophyceae). *J. Phycol.* 52, 877–887. <https://doi.org/10.1111/jpy.12455>.
- Rothäusler, E., Correll, H., Jormalainen, V., 2015. Abundance and dispersal trajectories of floating *Fucus vesiculosus* in the northern Baltic Sea. *Limnol. Oceanogr.* 60, 2173–2184. <https://doi.org/10.1002/lno.10195>.
- Rothäusler, E., Haavisto, F., Jormalainen, V., 2017. Is the future as tasty as the present? Elevated temperature and hyposalinity affect the quality of *Fucus* (Phaeophyceae, Fueales) as food for the isopod *Idotea balthica*. *Mar. Biol.* 164, 1–15. <https://doi.org/10.1007/s00227-017-3237-3>.
- Rothäusler, E., Rugiu, L., Jormalainen, V., 2018a. Forecast climate change conditions sustain growth and physiology but hamper reproduction in range-margin populations of a foundation rockweed species. *Mar. Environ. Res.* 141, 205–213. <https://doi.org/10.1016/j.marenvres.2018.09.014>.
- Rothäusler, E., Uebermuth, C., Jormalainen, V., Haavisto, F., 2018b. Living on the edge: gamete release and subsequent fertilisation in *Fucus vesiculosus* (Phaeophyceae) are weakened by climate change-forced hyposaline conditions. *Phycologia* 58, 111–114. <https://doi.org/10.1080/00318884.2018.1524246>.
- Rothäusler, E., Rugiu, L., Tiitonen, T., Jormalainen, V., 2020. It takes two to stay afloat: interplay of morphology and physiological acclimation ensures long-term floating dispersal of the bladderwrack *Fucus vesiculosus* (Phaeophyceae, Fueales). *Eur. J. Phycol.* 55, 242–252. <https://doi.org/10.1080/09670262.2019.1694706>.
- Rugiu, L., Manninen, I., Rothäusler, E., Jormalainen, V., 2018. Tolerance and potential for adaptation of a Baltic Sea rockweed under predicted climate change conditions. *Mar. Environ. Res.* 134, 76–84. <https://doi.org/10.1016/j.marenvres.2017.12.016>.
- Rugiu, L., Panova, M., Pereyra, R.T., Jormalainen, V., 2020. Gene regulatory response to hyposalinity in the brown seaweed *Fucus vesiculosus*. *BMC Genomics* 21, 1–17. <https://doi.org/10.1186/s12864-020-6470-y>.

- Rugiu, L., De Wit, P., Kostian, I., Jormalainen, V., 2021. Climate change driven hypersalinity as a selective agent in the littoral mesoherbivore *Idotea balthica*. Mar. Environ. Res. 163, 105216. <https://doi.org/10.1016/j.marenvres.2020.105216>.
- Russell, G., 1985. Recent evolutionary changes in the algae of the Baltic Sea. Br. Phycol. J. 20, 87–104. <https://doi.org/10.1080/00071618500650111>.
- Ruuskanen, A., Bäck, S., 1999. Morphological variation of northern Baltic Sea *Fucus vesiculosus* L. Ophelia 50, 43–59. <https://doi.org/10.1080/00785326.1999.10409388>.
- Schagerström, E., Kautsky, L., 2016. Despite marine traits, the endemic *Fucus radicans* (Phaeophyceae) is restricted to the brackish Baltic Sea. Eur. J. Phycol. 51, 378–386. <https://doi.org/10.1080/09670262.2016.1183234>.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1007/978-1-84882-087-6_9.
- Serrão, E.A., Kautsky, L., Brawley, S.H., 1996. Distributional success of the marine seaweed *Fucus vesiculosus* L. in the brackish Baltic Sea correlates with osmotic capabilities of Baltic gametes. Oecologia 107, 1–12. <https://doi.org/10.1007/BF00582229>.
- Serrão, E.A., Brawley, S.H., Hedman, J., Kautsky, L., Samuelsson, G., 1999. Reproductive success of *Fucus vesiculosus* (Phaeophyceae) in the Baltic Sea. J. Phycol. 35, 254–269. <https://doi.org/10.1046/j.1529-8817.1999.3520254.x>.
- Siegel, H., Gerth, M., 2018. Sea Surface Temperature in the Baltic Sea 2018. HELCOM.
- Simpson, S.J., Raubenheimer, D., 1993. A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. Philos. Trans. R. Soc. B 342, 381–402. <https://doi.org/10.1098/rstb.1993.0166>.
- Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1).
- Sunda, W.G., Hardison, D.R., 2010. Evolutionary tradeoffs among nutrient acquisition, cell size, and grazing defense in marine phytoplankton promote ecosystem stability. Mar. Ecol. Prog. Ser. 401, 63–76. <https://doi.org/10.3354/meps08390>.
- Takolander, A., Cabeza, M., Leskinen, E., 2017a. Climate change can cause complex responses in Baltic Sea macroalgae: a systematic review. J. Sea Res. 123, 16–29. <https://doi.org/10.1016/j.seares.2017.03.007>.
- Takolander, A., Leskinen, E., Cabeza, M., 2017b. Synergistic effects of extreme temperature and low salinity on foundational macroalga *Fucus vesiculosus* in the northern Baltic Sea. J. Exp. Mar. Bio. Ecol. 495, 110–118. <https://doi.org/10.1016/j.jembe.2017.07.001>.
- Tatarenkov, A., Bergström, L., Jönsson, R.B., Serrão, E.A., Kautsky, L., Johannesson, K., 2005. Intriguing asexual life in marginal populations of the brown seaweed *Fucus vesiculosus*. Mol. Ecol. 14, 647–651. <https://doi.org/10.1111/j.1365-294X.2005.02425.x>.
- Taylor, R.B., Sofka, E., Hay, M.E., 2002. Tissue-specific induction of herbivore resistance : seaweed response to amphipod grazing. Oecologia 132, 68–76. <https://doi.org/10.1007/s00442-002-0944-2>.
- Toth, G.B., Pavia, H., 2007. Induced herbivore resistance in seaweeds: a meta-analysis. J. Ecol. 95, 425–434. <https://doi.org/10.1111/j.1365-2745.2007.01224.x>.
- Van Alstyne, K., 1989. Adventitious branching as a herbivore-induced defense in the intertidal brown alga *Fucus distichus*. Mar. Ecol. Prog. Ser. 56, 169–176. <https://doi.org/10.3354/meps056169>.
- Van der Putten, W.H., Macel, M., Visser, M.E., 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. Philos. Trans. R. Soc. B Biol. Sci. 365, 2025–2034. <https://doi.org/10.1098/rstb.2010.0037>.
- Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>.
- Walther, G.R., 2010. Community and ecosystem responses to recent climate change. Philos. Trans. R. Soc. B Biol. Sci. 365, 2019–2024. <https://doi.org/10.1098/rstb.2010.0021>.
- Weinberger, F., Rohde, S., Oschmann, Y., Shahnaz, L., Dobretsov, S., Wahl, M., 2011. Effects of limitation stress and of disruptive stress on induced antigrazing defense in the bladder wrack *Fucus vesiculosus*. Mar. Ecol. Prog. Ser. 427, 83–94. <https://doi.org/10.3354/meps09044>.
- Wernberg, T., Coleman, M.A., Bennett, S., Thomsen, M.S., Tuya, F., Kelaher, B.P., 2018. Genetic diversity and kelp forest vulnerability to climatic stress. Sci. Rep. 8, 1–8. <https://doi.org/10.1038/s41598-018-20009-9>.
- Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
- Wood, H.L., Nylund, G., Eriksson, S.P., 2014. Physiological plasticity is key to the presence of the isopod *Idotea baltica* (Pallas) in the Baltic Sea. J. Sea Res. 85, 255–262. <https://doi.org/10.1016/j.seares.2013.05.009>.
- Zarnetske, P.L., Skelly, D.K., Urban, M.C., 2012. Biotic multipliers of climate change. Science 336 (6088), 1516–1518. <https://doi.org/10.1126/science.1222732>.
- Zettler, M.L., Karlsson, A., Kontula, T., Gruszka, P., Laine, A.O., Herkül, K., Schiele, K.S., Maximov, A., Haldin, J., 2014. Biodiversity gradient in the Baltic Sea: a comprehensive inventory of macrozoobenthos data. Helgol. Mar. Res. 68, 49–57. <https://doi.org/10.1007/s10152-013-0368-x>.