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Effects of temperature and salinity on germling development and fitness in the brown macroalga *Fucus serratus* L.

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Abstract: Fucoid brown algae form extensive populations that dominate the intertidal and subtidal vegetation on temperate rocky shores. The persistence of populations of fucoid brown algae depends on their reproductive ability and the survival and growth of early life history stages (germlings) that are generally more susceptible to stressors than adults. In this study, the effects of temperature and salinity on germling development and fitness in the brown macroalgae *Fucus serratus* is assessed by studying rhizoid elongation, photosynthetic performance and survival in a matrix of four different temperatures and three salinities. It was hypothesized that both lower and higher than normal temperatures (12°C - 17°C) would constitute environmental stress and affect germling fitness, and that these effects would be exacerbated by sub-oceanic salinities below 32 psu. The results show that temperatures lower than the normal summer temperatures (6°C) have significant negative effects on both growth and survival as well as on photosynthesis, while there are no effects of higher than normal temperatures (22°C) alone. Sub-oceanic salinities affect growth and survival negatively, but only at the lowest salinity (18 psu), and have no effect on photosynthetic parameters. We conclude that higher water temperatures will not affect germling fitness, and thus *F. serratus* persistence, negatively, but low salinities and lower water temperatures will.

Key Words: *Fucus serratus*; photosynthesis; growth; survival

Introduction

Fucoid brown algae form extensive populations that dominate the intertidal and subtidal vegetation on temperate rocky shores. These important primary producers support marine ecosystems by providing food and shelter for various marine invertebrates and fish as well as substratum for epiphytic algae and fauna. While brown algae may resist some degree of

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environmental stress, their persistence depends on their reproductive ability and the survival and growth of early life history stages (germlings) that are generally more susceptible to stressors than adults (Fredersdorf *et al.*, 2009, Steen, 2004).

Germling fitness is often assessed by measuring germination success and rhizoid elongation. However, previous research (Nielsen *et al.*, 2003, Nielsen & Nielsen, 2005) has indicated that there are at least two distinctive end points for environmental stress in germlings: photosynthesis on one hand and rhizoid germination and elongation on the other. Photosynthesis that is targeted directly by stressors, or indirectly as a result of the production of reactive oxygen species (ROS), is physiologically separated from the cellular processes that underlie rhizoid germination and elongation in germlings, since processes of cell division and expansion constitute separate targets for environmental stress (Nielsen *et al.*, 2003, Nielsen & Nielsen, 2005).

While there is no concrete information available on the specific effects of temperature stress on photosynthesis in germlings, the response may be similar to that of flowering plants (Suzuki & Mittler, 2006) and related to inactivation of enzymes and induction of reactive oxygen species (ROS). ROS that results in cellular damage may also be induced by imbalanced osmotic potential (Coelho *et al.*, 2002) in brown algae. Particularly the D1 protein of photosystem II (PSII) is very sensitive to ROS that may induce photoinhibition. Photoinhibition occurs when the excitation energy that is harvested by chlorophyll *a* exceeds that required to excitate electrons at PSII to support steady state photosynthesis. Excess excitation energy may react with O_2 derived from reduction of H_2O at PSII and produce ROS which may lead to photodamage. Photoinhibition may also occur during inactivation of photosynthetic enzymes as a result of temperature stress (Verhoeven *et al.*, 1996) and may contribute to reduced growth due to reduced energy fixation.

While reduced salinity is known to target developmental events in germlings selectively (Andersson & Kautsky, 1996), information on targets for temperature stress on developmental events is limited. The enzymatic processes that support rhizoid germination and elongation may be slowed down as a result of temperature stress although direct inhibitory effects on rhizoid development are unknown. In contrast, hypo-osmotic conditions may induce direct inhibition of germling development. Germination and rhizoid elongation occur by tip growth where apical expansion of the rhizoid is directed by a Ca^{2+} gradient at the rhizoid apex (Roberts *et al.*, 1993). Similarly, germlings resist hypo-osmotic swelling by an osmoregulatory process that is initiated by entry into the cell of external Ca^{2+} through stretch activated ion channels and formation of a Ca^{2+} wave that is initiated at the apex of the swollen rhizoid (Taylor *et al.*, 1997).

This response may overrule the weaker Ca^{2+} signal that conducts rhizoid germination and elongation with the result that these processes are inhibited.

There is so far no detailed knowledge on temperature responses and interactions between temperature and other stressors in the physiology of brown algae germlings. Climate change may not only affect temperature regimes in coastal marine areas but also salinity through changes in precipitation and subsequent changes in freshwater discharges to these areas (Parry *et al.*, 2007, Royer & Grosch, 2006, Sanchez-Gomez *et al.*, 2009). Studies of the effects of temperature and salinity and their interactions – if any – on brown algae germlings are therefore timely. In this paper, studies of the stress response of the photosynthetic apparatus are carried out in parallel with an assessment of rhizoid development to provide more complete information on germling responses to environmental stress. The effects of temperature and salinity on germling development and fitness in the brown macroalgae *Fucus serratus* L. are assessed by studying rhizoid elongation, photosynthetic performance and survival in a matrix of four different temperatures (6°, 12°, 17° and 22° C) and oceanic salinity (32 psu), as well as two lower salinities (24 psu and 18 psu, respectively). We have chosen to test temperatures that are both higher and lower than present day summer means in the North Sea region. As *F. serratus* releases its gametes from July to September (Knight & Parke, 1950), the two middle temperatures were chosen to bracket the diurnal mean temperatures during the warmest month of the year in Northern Europe (around 15° C). We hypothesized that both lower and higher temperatures (here represented by 6° and 22° C, respectively) would constitute environmental stress and affect germling fitness, and that these effects would be exacerbated by sub-oceanic salinities.

Materials and methods

Gamete acquisition and culture of zygotes

Adult *Fucus serratus* were collected at low tide from Belhaven Bay on the SE of the Firth of Forth, Scotland, UK. Samples were transported to the laboratory on ice within 1.5 hours of collection. Mature receptacles were cut from male and female algae, blotted dry and stored in the dark at 3-5°C for up to two weeks. Gamete release was stimulated by rinsing receptacles in tap water and exposing them to natural day light for approximately 45 minutes. Transfer of receptacles to artificial seawater (ASW) induced gamete release. ASW was made with Milli-Q water containing 33.2 g l^{-1} of Instant Ocean (Aquarium Systems, Sarrebough, France). The concentration of gametes was adjusted by measuring the absorbance of gametes in ASW in a 1 ml cuvette on a spectrophotometer. The gamete concentration was kept constant at approximately 4.25 x 10^6 cells ml^{-1} which was equivalent to an absorbance of 0.2 at a

wavelength of 480 nm, the primary absorption wavelength of carotenoids. The egg concentration was kept constant at approximately 200 ml^{-1} which was equivalent to an absorbance of 0.1 at a wavelength of 450 nm, the primary absorption wavelength of chlorophyll. Mixing of male and female gametes in ASW induced fertilization. Fertilization was terminated after 30 min. by filtering zygotes through a 100 µm mesh into fresh ASW and the zygote concentration adjusted to the desired density.

To determine survival, rhizoid length and photosynthesis, germlings were plated in small Petri dishes fitted with coverslip bases in ASW at an approximate density of 100 cm^{-2}. After settlement and initial attachment of zygotes, the dishes were filled with 5 ml ASW. Incubation dishes and beakers were placed in an incubator at 17° C at an irradiance of 100 µmol m^{-2} s^{-1} in a 16/8 hrs. light/dark cycle. At 24 hrs. after fertilization (AF) germlings were transferred to the following salinities: 18, 24 and 32 psu (60, 80 and 100% of that of oceanic seawater). Simultaneously the incubation dishes and beakers were transferred to incubators at 6°, 12°, 17° and 22° C at an irradiance of 100 µmol m^{-2} s^{-1} in a 16/8 hrs. light/dark cycle. The temperatures were selected so that the two middle temperatures bracket the summer mean temperature of the region (around 15°C) and the low and high temperature would then represent low and high temperature stress respectively. Germlings were cultured for a total of 7 days (until 7 days AF) and the incubation medium was changed at 2, 4 and 6 days AF. At the end of the incubation the following endpoints were measured: germling survival, rhizoid length, and photosynthesis. The whole experiment was repeated three times, each time with a different batch of germlings, making the number of replicates, N = 3 for all further data analysis.

Germling survival

Germling survival was determined as the number of live germinated germlings out of a total of 80 germinated germlings for each treatment in each of the three batches, evaluated under a microscope at 10 x magnification.

Rhizoid length

Digital images were recorded of 40 germlings for each treatment in each of the three batches at 10 x magnification (Sony Cyber-Shot, 3.3 megapixels, Sony Corporation, Tokyo, Japan). The rhizoid length was defined as the distance from the wall dividing the thallus from the rhizoid to the rhizoid tip, and was measured using the image analysis software SIGMA SCAN PRO v. 5.

Photosynthesis

Photosynthesis was evaluated using chlorophyll fluorescence. The following parameters were recorded: Maximum quantum yield of PSII (F_v/F_m), non-photochemical quenching (NPQ) and electron transport rate (ETR) (Maxwell &

Johnson, 2000). All parameters were determined on a microscopy PAM (Walz, Effeltrich, Germany) using default factory settings in an air-conditioned room at 20°C. Germlings were dark adapted for 15 minutes and F_0 and F_m were determined prior to each series of measurements. Subsequently F'_m and F_t were determined at 8 different light intensities, gradually increasing from 0 to 520 µmol m^{-2} s^{-1}. Each step lasted 30 sec. An assumed light absorbance of 84% was used to calculate relative ETR. Photosynthetic parameters were measured for nine germlings at each treatment in each of the three batches.

Statistical analysis

Statistical analysis was carried out using SYSTAT 12 and according to Quinn & Keough (2002). Data were tested for homogeneity of variance (Cochran's test) and normal distribution before parametric testing. Data were subjected to two-factor analysis of variance (ANOVA) and differences between individual means were tested further by post hoc multiple range test (Tukey test). All tests were carried out at a level of significance of $p < 0.05$, and errors are displayed graphically as ± 1 SE.

Results

Rhizoid elongation

A statistically significant increase in rhizoid elongation with temperature from 6° C, over 12° C to 17° C can be observed (Fig. 1, ANOVA, $p < 0.0001$). There were no statistically significant differences in rhizoid elongation at 17° C and 22° C, respectively (Fig. 1). At 6° C there was no effect of decreased salinity (Fig. 1), while at the three other temperatures a statistically signify-cant decrease in rhizoid elongation can be seen at the lowest salinity (18 psu), compared to the two other salinities (Fig. 1, ANOVA, $0.05 > p > 0.01$). No statistically significant differences in rhizoid elongation were found between 32 psu and 24 psu salinity at any temperature (Fig. 1). No statistically significant interactions between temperature and salinity can be observed for rhizoid elongation at any treatment (Fig. 1).

Photosynthetic parameters

A statistically significant increase in F_v/F_m is seen from 6° C to 12° C (Fig. 2; ANOVA, $p < 0.0001$), however, no further increase in F_v/F_m is seen with increasing temperatures above 12° C (Fig. 2). No statistically significant effects of salinity are seen at any temperature (Fig. 2), and no statistically significant interactions between temperature and salinity are observed. Variations in ETR_{max} are parallel to variations in F_v/F_m. There is a statistically significant increase in ETR_{max} from 6° C to 12° C (Fig. 2, ANOVA, $p < 0.0001$), with no further increase with increasing temperature (Fig. 2). No statistically significant effects of salinity are seen at any temperature (Fig. 2) and there are no statistically significant interactions (Fig. 2). Variations in NPQ_{max} are in accordance with this

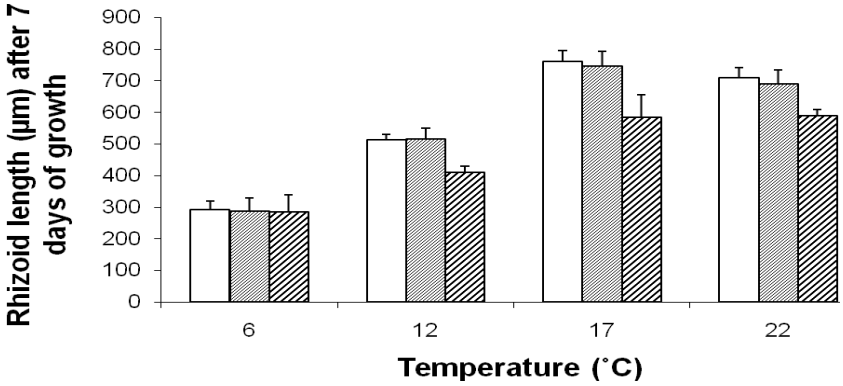

Fig. 1: Germling rhizoid elongation in µm measured after 7 days of cultivation at the various temperatures (° C) and salinities, employed in this work. Open bars (left): Salinity 32 psu (100% oceanic seawater), finely hatched bars (center): Salinity 24 psu (80% oceanic seawater), coarsely hatched bars (right): Salinity 18 psu (60% oceanic seawater). Mean values ± 1 SE (N = 3) are shown.

pattern, but naturally in the opposite direction. A statistically significant decrease in NPQ_{max} is seen from 6° C to 12° C (Fig. 2, ANOVA, $p < 0.0001$), with no further decrease in NPQ_{max} at higher temperatures (Fig. 2), no statistically significant effects of salinity (Fig. 2), and no statistically significant interactions are seen at any temperature (Fig. 2).

Germling survival

For germling survival, there are no statistically significant effects of temperature (Fig. 3), but there is a statistically significant decrease in germling survival at the lowest salinity (18 psu), compared to the two other salinities, at all temperatures (Fig. 3, ANOVA, $0.001 > p > 0.0001$). No statistically significant interaction effects are observed.

Discussion

Our results indicate that rhizoid elongation, or growth, increase with increasing temperatures from 6°C over 12°C to 17°C. At the same time, the results show that rhizoid elongation decrease under hypo-osmotic conditions. Photosynthesis shows the same response to temperature as rhizoid elongation, but no effect of salinities below full ocenanic levels (32 psu). Germling survival shows no temperature effects, but is affected negatively by salinities lower than full oceanic levels (18 and 24 psu). No interactions between temperature and salinity effects were

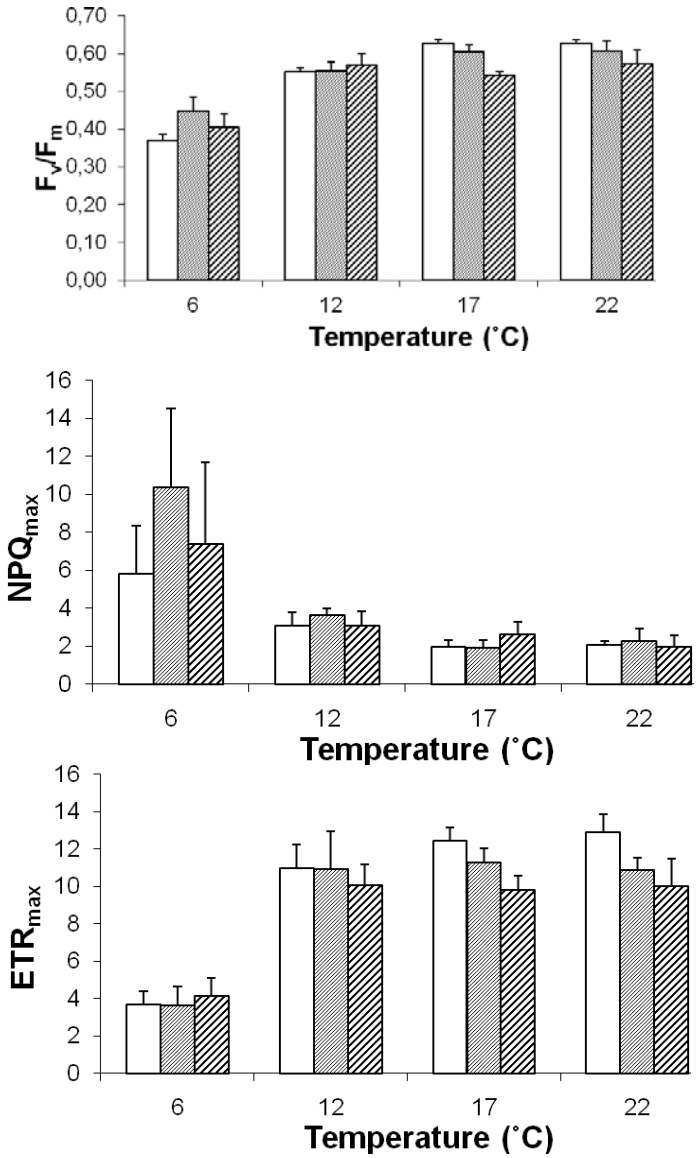

Fig. 2: Chlorophyll fluorescence parameters for *Fucus* germlings at the various temperatures (°C) and salinities, employed in this work. Top panel shows maximum photosynthetic efficiency, measured on dark adapted specimens (F_v/F_m). Mid panel shows maximum non-photosynthetic quenching (NPQ_{max}), bottom panel shows maximum electron transport rate (ETR_{max}). Open bars (left): Salinity 32 psu (100% oceanic seawater), finely hatched bars (center): Salinity 24 psu (80% oceanic seawater), coarsely hatched bars (right): Salinity 18 psu (60% oceanic seawater). Mean values ± 1 SE (N = 3) are shown.

found in the two-way ANOVAs, indicating that there are no synergistic effects between temperature and salinity stress. Our hypothesis that changing temperatures will exert environmental stress on *Fucus serratus* germlings was therefore only partially confirmed, only temperatures lower than present day summer water mean temperatures affect germling performance negatively. Sub-oceanic salinities do have a negative effect on germling performance, even at 18 psu, which is not uncommonly found in the habitat range of *F. serratus*. Salinity stress does not enhance temperature stress or vice versa within the ranges tested here. Different mechanisms must therefore be behind the effects observed on the different variables measured.

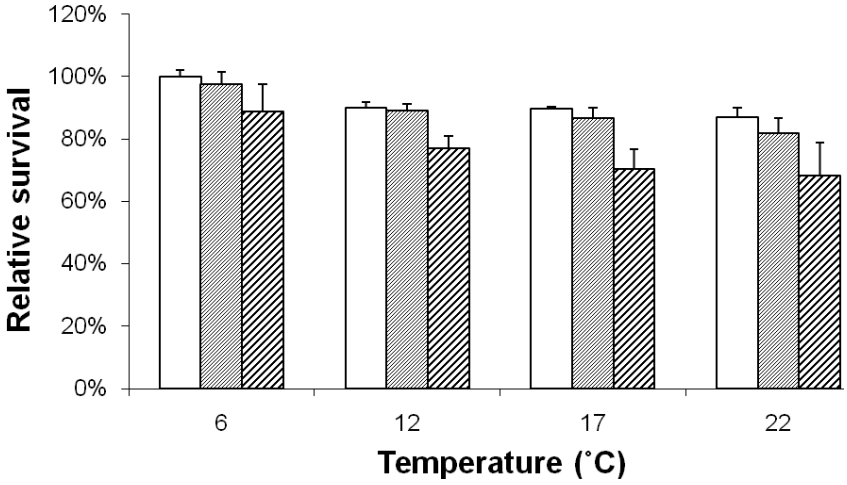

Fig. 3: Relative germling survival in % (out of 80 individuals) as a function of the various temperatures (° C) and salinities, employed in this work. Open bars (left): Salinity 32 psu (100% oceanic seawater), finely hatched bars (center): Salinity 24 psu (80% oceanic seawater), coarsely hatched bars (right): Salinity 18 psu (60% oceanic seawater). Mean values ± 1 SE (N = 3) are shown.

Rhizoid elongation in *Fucus* germlings shows a maximum response at 17° - 22° C and as such is not affected by increased temperatures, relative to present day summer temperatures, represented by the temperature range 12° -17° C in this experiment. The inhibition of rhizoid elongation in hypo-osmotic conditions may be related to modification of Ca^{2+} uptake mechanisms and weakening of Ca^{2+} gradient formation. Two different inhibitory mechanisms are probably

interacting here. Rhizoid elongation is directed by a Ca^{2+} gradient at the rhizoid apex (Roberts *et al.*, 1993), but at the same time it is known that the germlings resist hypo-osmotic swelling by an osmoregulatory process through formation of a Ca^{2+} wave, also initiated at the rhizoid apex (Taylor *et al.*, 1997). This response may overrule the weaker Ca^{2+} signal accompanying rhizoid elongation so that rhizoid elongation and growth is inhibited when germlings are required by environmental conditions to increase osmoregulatory activity. Low temperatures may inhibit growth and rhizoid elongation because enzymetic processes are slowed down. The lack of salinity effects at the lowest temperature could then be ascribed to low temperature inhibiting the Ca^{2+} gradient-formation involved in both rhizoid elongation and in osmoregulatory mechanisms equally.

Photosynthetic parameters show the same overall response to temperature as rhizoid elongation, although different mechanisms must be involved. Generally, a temperature optimum of 12° - 22° C is found, indicating no effect of increased temperatures on photosynthetic parameters, but a negative effect of low temperatures. The effects of low temperatures on photosynthesis are probably similar to the effects of low temperatures on flowering plants, with inactivation of photosynthetic enzymes leading to less light utilized in photosynthesis and eventually to ROS induction (Suzuki & Mittler, 2006, Verhoeven *et al.*, 1996). The decreased survival rate of germlings under hypo-osmotic conditions is consistent with observations of other algal taxa (Fredersdorf *et al.*, 2009, Nygard & Dring, 2008), and is probably related to the increased metabolic costs related to osmotic compensation (Kamer & Fong, 2000). Since all photosynthetic parameters are directly affected by low temperatures, but not by low salinities, it is therefore important not only to measure photosynthetic parameters, e.g. by PAM techniques as done here, but also to measure growth and survival, that are the integrated products of physiological processes, to fully evaluate the effect of stress on algal germlings. To the temperature and salinity effects indicated here can be added the effects of anthropogenic pollution, not included in this study, but previously shown to enhance negative effects of temperature and hypo-osmotic conditions on other species of brown algae (Burridge & Bidwell, 2002, Burridge *et al.*, 1999).

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