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Recent evolution in Baltic *Fucus vesiculosus*: reduced tolerance to emersion stresses compared to intertidal (North Sea) populations

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ABSTRACT: The Baltic is a young, brackish and non-tidal sea, supporting an impoverished marine flora compared with adjacent open coastal areas. Populations of the normally intertidal brown alga *Fucus vesiculosus* L. are permanently submerged in the Baltic. We tested the hypothesis that these populations have evolved a reduced ability to withstand water-stresses caused by aerial exposure (desiccation and freezing), relative to adjacent intertidal populations in the North Sea. Desiccation and freezing tolerance were compared using chlorophyll fluorescence to monitor photosynthetic status during stress and recovery. To control for the influence of growth salinity on stress tolerance, the experimental material consisted of either adult algae cross-acclimated at Baltic and North Sea salinities (6.5 and 20 to 24 practical salinity units [psu], respectively), or juveniles from both populations grown in the Baltic from embryos (submersed, 6.5 psu). Baltic algae were less able to recover maximum photochemical yield (F_v/F_m) after freezing at –15°C than North Sea algae, and neither acclimation (adults) or growth salinity (juveniles) accounted for between-population differences. During desiccation at 5°C, differences in the response of variable fluorescence (F_v), as well as in initial fluorescence (F_0) and F_v during recovery, indicated that impaired photoprotective processes may contribute to the inability of Baltic algae to fully recover F_v/F_m after stress, in contrast to North Sea algae which displayed dynamic and rapidly recoverable reductions of F_v/F_m. Subsequent desiccation experiments during the summer (at 25°C) showed that, relative to North Sea algae, the effective photochemical yield ($\Delta F/F_m'$) of Baltic algae started to decline at lower tissue-water content (TWC) and recovered less completely after a return to seawater. A critical TWC of ca 10% for Baltic populations was identified, below which $\Delta F/F_m'$ did not fully recover. In addition, Baltic algae were less able to regain initial TWC during recovery. These results indicate that, in ca 7500 yr since the recruitment of the present marine flora to the Baltic, *F. vesiculosus* has evolved reduced tolerance to emersion stresses compared to adjacent intertidal populations.

KEY WORDS: Baltic Sea · Desiccation · Emersion stress tolerance · Evolution · Freezing · Fucoid algae · *Fucus vesiculosus* · Local adaptation · Water stress

INTRODUCTION

The Baltic is a semi-enclosed sea, with a gradient of decreasing salinity, and with only narrow connections to the North Sea. The brackish and essentially non-tidal nature of the Baltic is of great interest and potential importance for studies of recent evolutionary change in the quite limited numbers of marine species able to exist there. The marine flora of the Baltic was probably recruited at the beginning of the Littorina Sea period (ca 7500 before present, BP), when the connection between the Baltic and the Atlantic was wider, and has been experiencing a progressive decline in

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salinity since the gradual end of this period (ca 3000 BP; Ignatius et al. 1981). The conditions currently prevailing in the Baltic are, therefore, very recent in evolutionary terms, raising the question of how much evolutionary change has been possible in this short period. Algal studies of adaptations to Baltic conditions have largely focused on salinity tolerances for survival and/or growth (Russell 1985, 1988, Rietema 1991, 1995, Bäck et al. 1992a, Rueness & Kornfeldt 1992), ecophysiological responses to salinity (Bäck et al. 1992b), and reproductive adaptations to salinity (Serrão et al. 1996a, 1999b). These studies have provided evidence that several marine macroalgae species from the Baltic may have undergone ecotypic differentiation with regard to salinity tolerance.

The brown alga *Fucus vesiculosus* L. is the most conspicuous macrophyte throughout most of the Baltic Sea, penetrating into the Bothnian Bay to salinities as low as 4 practical salinity units (psu) (Kautsky et al. 1992, Serrão et al. 1996a, 1999b). Populations within the Baltic are permanently submerged, due to the combined effects of lack of tides, ice scour in winter, and extended periods of low water levels in spring. Thus, Baltic *F. vesiculosus* populations are likely to have been isolated in a habitat lacking emersion stresses for a period of a few thousand years. In general, intertidal fucoids are tolerant of a variety of emersion-related water stresses, e.g., desiccation (Dring & Brown 1982), and freezing (Davison et al. 1989, Pearson & Davison 1993, reviewed by Chapman 1995 and by Davison & Pearson 1996). Selection for emersion-stress tolerance is presumably strong in the harsh environmental gradient existing in the intertidal zone, where strong selection for molecular traits has indeed been shown to occur on the metre scale for an intertidal gastropod (Johannesson et al. 1995), and is one of the competing hypotheses to explain allozyme distributions in the intertidal fucoid *Silvetia compressa* (formerly *Pelvetia fastigiata*; see Serrão et al. 1999a) on a similar scale (Williams & Di Fiori 1996). Genetic differentiation between Baltic and North Sea populations of the blue mussel *Mytilus edulis* may also be maintained by selection (Johannesson et al. 1990). However, it is generally thought that the benefits of stress tolerance are associated with costs for the organism in terms of other fitness-related traits (Hoffmann & Parsons 1997); for example, competitive ability, growth rate, or reproductive output. In addition to gaining new stress-tolerance mechanisms under novel conditions, the rapid evolution of organisms invading, or exposed to, novel environments (see reviews by Orr & Smith 1998, and Thompson 1998), should also involve the loss of costly stress-related traits that are no longer required.

In this study, we tested the hypothesis that Baltic populations of *Fucus vesiculosus* are physiologically less able to tolerate emersion stresses (freezing and desiccation) than adjacent North Sea populations. Two populations of *F. vesiculosus* were studied, from the central Baltic Sea at Askö (constantly submerged at 6.5 psu) and from the North Sea at Tjärnö, on the Swedish west coast (intertidal, 20 to 30 psu). In order to account for the effects of differing growth salinities and emersion histories (i.e., hardening) on the responses of the 2 populations to emersion, we acclimated adult algae to the salinities prevailing at the 2 sites, under immersed conditions. In addition, *in vitro* fertilized zygotes of both Baltic and North Sea *F. vesiculosus*, both grown from embryos in the Baltic until the juvenile stage, were compared.

Our data show that there were intrinsic differences in the ability to recover from emersion stresses between the 2 populations, which remained significant after cross-acclimation. Furthermore, these differences were maintained in marine individuals grown from embryos in the Baltic, suggesting a genetic basis for the divergence in stress tolerance.

MATERIALS AND METHODS

Study sites and culture conditions. Adult *Fucus vesiculosus* L. plants (ca 20 individuals) were collected at Tjärnö, on the Swedish west coast, and at Askö in the central Baltic Sea (for map, see Serrão et al. 1996a), during January and again during June 1998, for the winter and summer experiments, respectively. Although spring tide amplitudes on the Swedish west coast are only around 30 cm, atmospheric pressure changes can cause much greater fluctuations (Johannesson 1989), often resulting in extended periods of exposure for intertidal organisms. Thus, high pressure during winter can cause extended periods (days) of freezing at or below the temperatures used in the experiments reported here. Similarly, summer exposures may occur at temperatures above 30°C (Beer & Kautsky 1992).

Salinity acclimations of apical tissue (average length ca 5 cm) were carried out in eight 10 l aquaria in constant temperature walk-in culture chambers at Askö (5°C winter; 12°C summer); photon-flux density (PFD) was ca 50 µmol photons m^{-2} s^{-1}, under a 16:8 h light:dark (L:D) photoperiod. Seawater (SW) was either natural Askö SW (6.5 psu), or Askö SW plus salts to give salinities of 20 psu (winter) or 24 psu (summer), which were the salinities measured at Tjärnö at the time of the experiments. Salt concentrations for each salinity were determined from the concentrations given in Serrão et al. (1996a). Algae were acclimated for a minimum of 10 d prior to use in the freezing and desiccation experiments described below.

For the growth of juvenile algae in the field at Askö, male and female gametes were released from reproductive receptacles of North Sea (Tjärnö) and Baltic (Askö) algae and fertilized *in vitro* essentially as described by Serrão et al. (1996a). Gamete release and fertilizations were carried out in SW of 6.5 psu for Baltic and 24 psu for North Sea populations. Approximately 20 individuals from each population were used in each case. Zygotes were seeded onto 10 × 10 cm plates of Seagoin' Poxy Putty™ (Permalite Plastics, Newport Beach, California, USA), cast as described in Serrão et al. (1996b). After attachment of zygotes, plates were attached to bricks and placed in the field close to the laboratory at a depth of ca 1.5 m in May (Tjärnö population) and June (Askö population) 1996, until used in experiments in January and June 1998.

Emersion stress experiments. ***Freezing:*** Freezing experiments were conducted in January 1998 with (1) adult tissue from both populations acclimated at 6.5 and 20 psu, and (2) juveniles which were at this time 19 to 20 mo old. Plates bearing juvenile thalli were brought into the laboratory and maintained in running Askö SW at 5°C under low light (ca 30 µmol photons m^{-2} s^{-1}) for 1 d before the experiments. For freezing treatments, adult apices or juvenile thalli were incubated for 3 h in darkness in freezers at –7 and –15°C, or at 5°C in darkness in boxes containing damp tissue paper (controls). After 1 and 3 h recovery in 6.5 psu (juveniles and low-salinity-acclimated adult tissue) or 20 psu (high-salinity-acclimated adult tissue), the maximum photochemical yield (F_v/F_m) was determined on dark-adapted tissue (10 min) using a pulse amplitude-modulated fluorometer (Diving PAM; Watlz, Effeltrich, Germany). Recovery from freezing was carried out under low PFD (ca 30 µmol photos m^{-2} s^{-1}) at 5°C .

Desiccation: Winter desiccation experiments (January 1998) were conducted at 5°C on cross-acclimated adults only. PFD in the culture chamber was 30 µmol photons m^{-2} s^{-1} during desiccation (on a mesh screen in order to minimize variations in drying rate caused by accumulation of water between the tissue and the surface) and recovery in SW (salinity 6.5 or 20 psu, depending on acclimation salinity). In studies to calculate drying rate, algae were weighed initially, allowed to desiccate for 11 h, under the conditions described above, and weighed at intervals during this period. The tissue was then dried overnight at 80°C to obtain dry weights. From these data, the tissue-water contents (TWC) were calculated as a percentage of the initial (100%) from the following relationship:

$$\text{TWC} = (\text{IW} - \text{DW})/(\text{FW} - \text{DW}) \times 100$$

where FW = fresh weight, IW = intermediate weight, and DW = dry weight.

In the initial experiment, apices were allowed to desiccate for 3 h and to recover in SW for 1 h. The F_v/F_m of dark-adapted tissue (10 min) was measured at the beginning of the experiment, and at hourly intervals during desiccation and recovery. In a second experiment under the same conditions, apices were allowed to desiccate for 10 h, and fluorescence was measured at the end of this period and after 3 and 12 h recovery in SW. In both experiments, controls were emersed at 5°C but remained fully hydrated in plastic boxes containing damp paper towels.

Summer desiccation experiments were carried out by drying adult apices or juvenile thalli on a mesh screen (to minimize variations in drying rate caused by accumulation of water between the tissue and the surface) at 25°C. Drying rates were initially determined for Askö (6.5 psu-acclimated) and Tjärnö (24 psu-acclimated) apical tissue, as described above for the winter desiccation experiments. Pulse amplitude-modulated fluorescence (PAM) was used to monitor effective photochemical yield ($\Delta F/F_m'$) under steady-state illumination (80 µmol photons m^{-2} s^{-1}) during desiccation and recovery in SW. Controls were emersed at 25°C and 80 µmol photons m^{-2} s^{-1}, and were kept hydrated in boxes containing wet paper towels. $\Delta F/F_m'$ was determined with the fiber optic probe clamped in position at an angle of approximately 60° to the tissue. At the beginning of each experiment, and following each yield determination, the tissue sample was briefly weighed. At the end of the experiment, the dry weight was determined to allow TWC calculations to be made as described above.

Statistics. The design of freezing and desiccation experiments involved repeated measurements of replicate adult apical tissue or whole juvenile algae. Such data lack independence in time (measurement at time_1 is not independent of measurement at time_0). In such cases we have chosen to compare only final recovery values (expressed as a percentage of the initial prestress value) between treatments, and since these are independent measurements, ANOVA is appropriate. Differences between means were detected with Tukey tests. The statistical analyses presented, including 2- and 3-factor ANOVAs and linear regressions, were performed using Systat Version 5.2.1. (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Winter desiccation experiments

Rates of desiccation of *Fucus vesiculosus* from the central Baltic and the North Sea at 5°C were similar, with 30 to 40% TWC remaining after 5 h desiccation,

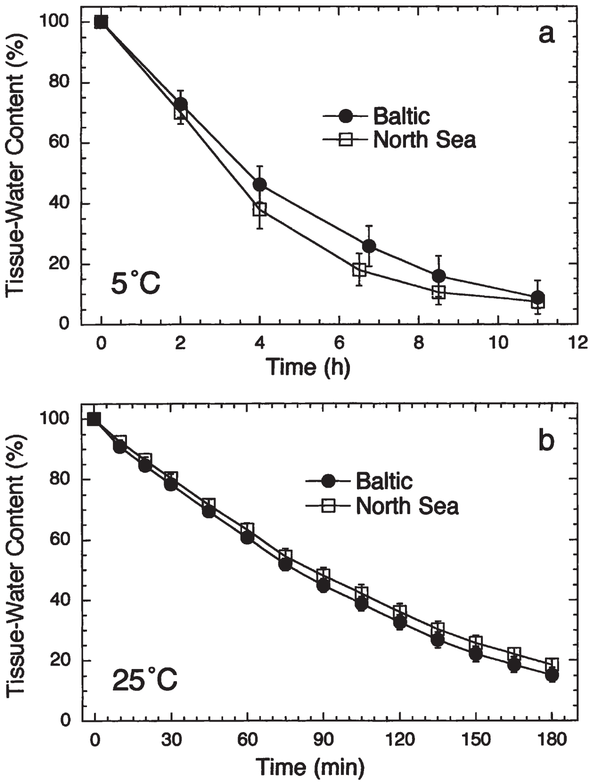

Fig. 1. *Fucus vesiculosus.* Desiccation rates of apical tissue of algae from the central Baltic Sea (Askö) and the North Sea (Tjärnö, Swedish west coast). Tissue-water content was determined (a) in the winter at 5°C (n = 15), and (b) in the summer at 25°C (n = 10). Algae were cultured in the laboratory for >10 d at salinities based on those measured *in situ* (6.5 psu for Baltic algae, 20 psu for North Sea algae in winter, and 24 psu for North Sea algae in summer). Values are means ± SE

and 10 to 15% TWC remaining after 10 h (Fig. 1a). The slightly lower rate of water loss from Askö algae can probably be explained by the higher starting weight of this material (Askö, 426.2 ± 20 mg; Tjärnö, 360.0 ± 26 mg).

Fluorescence-induction kinetics of cross-acclimated algae from Baltic and North Sea populations, determined on dark-adapted material during a 5 h desiccation period and subsequent recovery (at the same acclimation salinity), showed that, in all cases, recovery of F_v/F_m was complete within 1 h of re-immersion (Fig. 2a,b). However, it is clear from Fig. 2 that the relative changes in fluorescence components were different between the 2 populations; F_v/F_m was more depressed during desiccation in North Sea than in Baltic algae (Fig. 2a,b). This was mainly attributable to variable fluorescence, F_v (Fig. 2c,d), which increased after 5 h desiccation in Baltic algae (more evident at 6.5 psu), but decreased in North Sea algae at both acclimation salinities. Initial fluorescence (F_0) increased after 5 h desiccation relative to undesiccated (emersed) controls in algae from both populations. Following re-immersion, both F_v and F_0 returned to control levels following 1 h recovery in North Sea algae and Baltic algae at 20 psu, but remained elevated in Baltic algae at 6.5 psu. (Fig. 2c to f).

Since both Baltic and North Sea populations recovered F_v/F_m completely after 5 h desiccation, a second experiment investigated the recovery of algae exposed to 10 h desiccation (10 to 15% TWC; see Fig 1a). The results, shown in Fig. 3, indicate that there were differences in the extent of recovery between the populations, irrespective of acclimation salinity, which had no significant effects (3-factor ANOVA, population × treatment interaction, $F_{1,40} = 12.69$; $p = 0.001$). North Sea algae recovered F_v/F_m to control levels within 3 h of recovery in brackish (6.5 psu) or marine (20 psu) water, whereas between 3 and 12 h recovery F_v/F_m had not returned to control values in Baltic algae at either acclimation salinity (Tukey tests after 3 h recovery).

Winter freezing experiments

Freezing had significant effects on the recovery of F_v/F_m in adult acclimated tissue (3-factor ANOVA, population × salinity × temperature interaction, $F_{2,60} = 3.96$; $p = 0.024$). The adult Baltic algae acclimated at 6.5 psu responded poorly to freezing for 3 h at –15°C , and F_v/F_m was only ca 50% that of unfrozen controls following 3 h recovery (Fig. 4a). Baltic algae at 20 psu were also significantly affected by freezing at –15°C, but recovered F_v/F_m more completely than at 6.5 psu (Fig. 4a). In contrast, adult algae from the North Sea population acclimated to either 6.5 or 20 psu were not significantly affected by the same freezing treatment (Fig. 4a, Tukey tests). Freezing at –7°C for 3 h had no significant effects on F_v/F_m in either population (Tukey tests), although a slight decline in F_v/F_m was observed in the Baltic algae (at 6.5 psu) following 3 h recovery (Fig. 4a).

We also compared the freezing tolerances of juvenile (19 to 20 mo) Baltic and North Sea algae which had been grown from embryos in the field at Askö (central Baltic; 6.5 psu). A 2-factor ANOVA indicated a significant interaction between population and temperature on subsequent recovery of F_v/F_m ($F_{2,30} = 48.62$; $p < 0.001$). While no effects of freezing on F_v/F_m at –7°C were observed for either population, Baltic juveniles were severely affected by freezing for 3 h at –15°C, and no recovery took place within 3 h of a return to brackish water (Fig. 4b). Athough juveniles from the North Sea population were also affected by

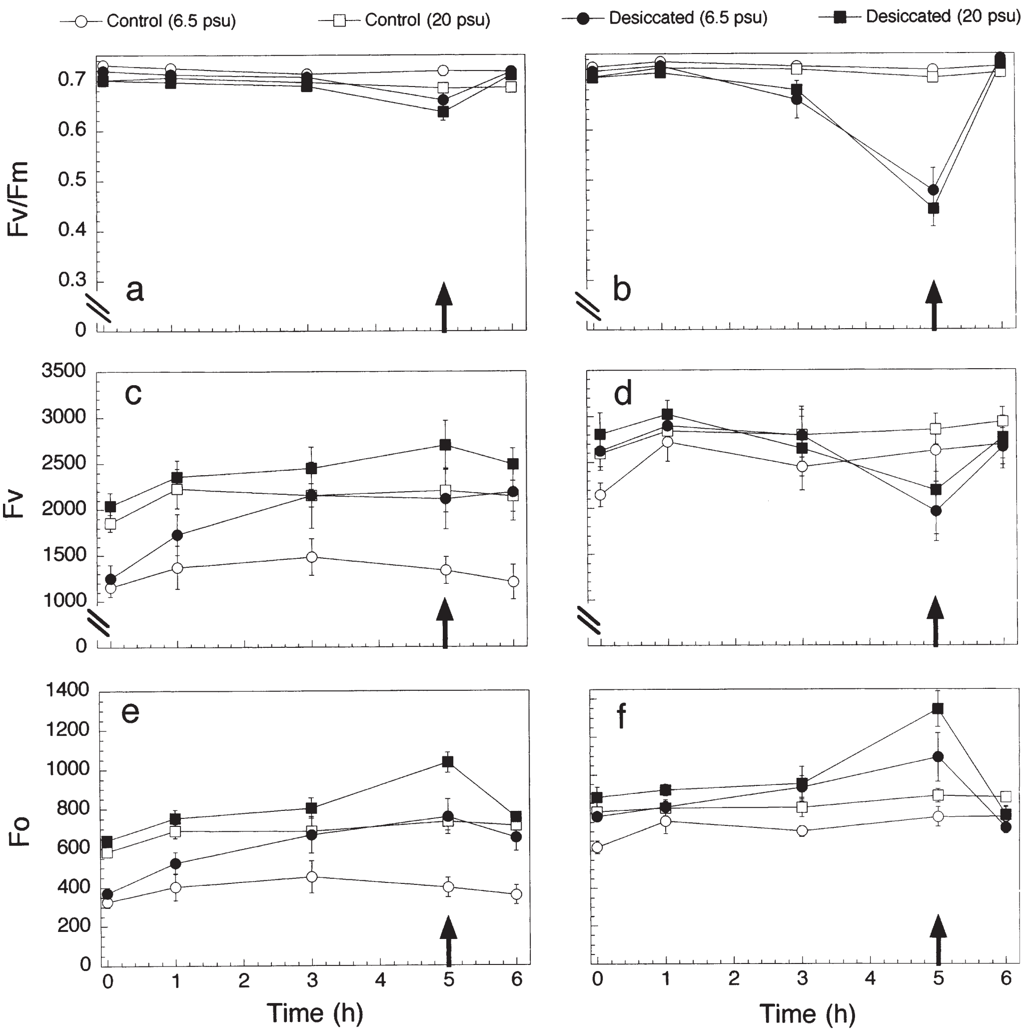

Fig. 2. *Fucus vesiculosus.* Effects of low-temperature desiccation (5 h) and recovery (1 h) at 5°C on fluorescence induction kinetics of dark-adapted adult algae from the central Baltic Sea (Askö: a, c, e) and the North Sea (Tjärnö: b, d, f). Maximum photochemical yield, F_v/F_m (a, b); variable fluorescence, F_v (c, d); initial fluorescence, F_0 (e, f) of algae acclimated at 6.5 and 20 psu. Arrows on *x*-axis: time of re-immersion in seawater of acclimation salinity; controls were kept fully hydrated in air. Values are means ± SE (n = 6)

freezing for 3 h at –15°C, recovered F_v/F_m was significantly greater than for their Baltic counterparts (cf. adult acclimated material in preceding paragraph).

Summer desiccation experiments

The desiccation rates of adult Askö (central Baltic; 6.5 psu) and Tjärnö (North Sea; 24 psu) *Fucus vesiculosus* at 25°C were not significantly different for apical tissue of similar initial weight (Askö, 407 ± 28.1 mg; Tjärnö, 412 ± 32.9 mg; Fig. 1b). Since, however, desiccation rate was dependent upon the initial size of the piece of tissue, in experiments to investigate $\Delta F/F_m'$ during desiccation and recovery, the weight of each replicate apical tip was recorded together with fluorescence measurements to allow subsequent analyses of $\Delta F/F_m'$ versus water content.

In all the acclimation treatments $\Delta F/F_m'$ during desiccation was similar to hydrated (emersed) controls for the first 90 min, followed by steep declines, becoming zero after ca 3 h desiccation (Fig. 5). In contrast, changes in $\Delta F/F_m'$ during a 24 h recovery period differed both between the populations at each acclimation salinity (cf. Fig. 5a with b, and c with d), and within populations at each acclimation salinity (cf. Fig. 5a with c, and b with d). A 3-factor ANOVA on the 24 h $\Delta F/F_m'$ data (as a percentage of the initial $\Delta F/F_m'$) revealed that the interaction of population (Baltic, North Sea), acclimation salinity (6.5, 24 psu) and desic-

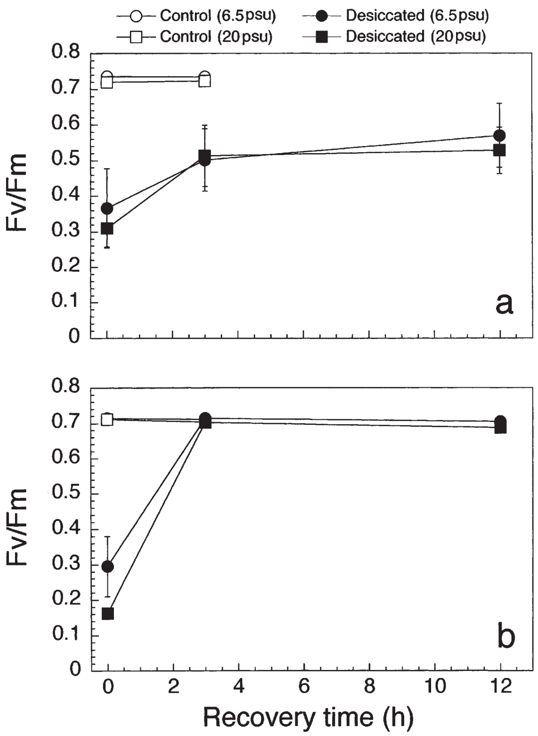

Fig. 3. *Fucus vesiculosus.* Recovery of maximum photochemical yield (F_v/F_m) in adults from (a) the central Baltic and (b) the North Sea acclimated at 6.5 or 20 psu, following low-temperature desiccation for 10 h at 5°C. Controls were kept fully hydrated in air and recovered in seawater. Values are means ± SE (n = 6)

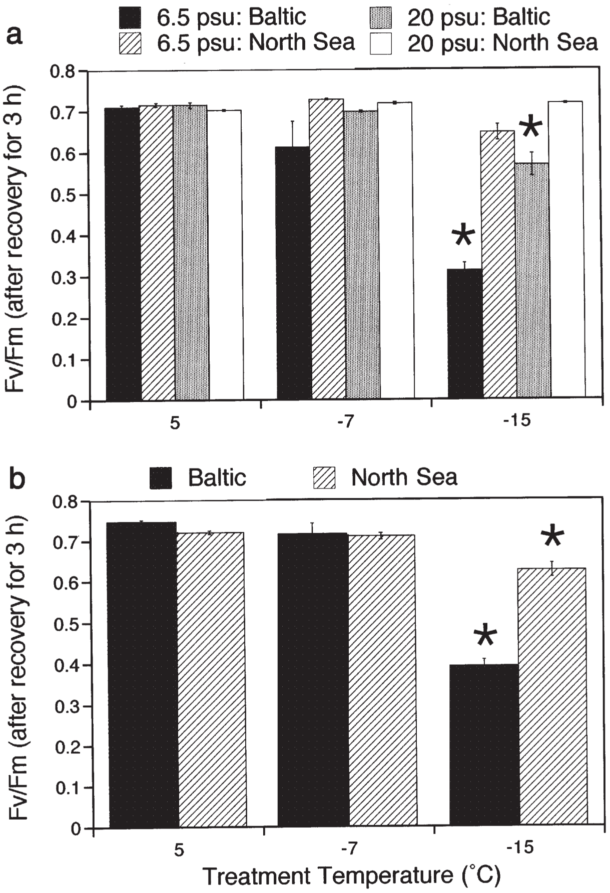

Fig. 4. *Fucus vesiculosus.* Recovery of maximum photochemical yield (F_v/F_m) after freezing in algae from the central Baltic and the North Sea. (a) Adult tissue acclimated at 6.5 or 20 psu. (b) Juvenile algae from *in vitro* fertilized zygotes after 1.5 yr in the field at Askö. Values are means ± SE (n = 6). Asterisks: means significantly different (p < 0.05) from the respective controls at 5°C

cation time (2, 4, 6 h) was significant ($F_{3,96} = 3.215$, p = 0.026). North Sea algae acclimated at 24 psu were the most desiccation-tolerant, recovering completely from 6 h drying (>90% tissue-water loss), while Baltic algae acclimated at 6.5 psu were the least tolerant, and no recovery of $\Delta F/F_m'$ occurred after 4 or 6 h desiccation (Fig. 5a,d). While cross-acclimation of North Sea algae at Baltic salinity (6.5 psu) led to a reduction in $\Delta F/F_m'$ during the early stages of recovery, the final recovery values after 24 h were not statistically different from the same population at 24 psu (Tukey tests). Moreover, North Sea algae acclimated at 6.5 psu remained much more able to recover from 4 and 6 h desiccation than Baltic algae at the same salinity (Tukey tests; Fig. 5a,b). Acclimation at 24 psu improved the desiccation tolerance of Baltic algae relative to the same population at 6.5 psu following 4 h desiccation (Tukey tests). However, recovery after 6 h desiccation remained lower than in North Sea algae at either 24 or 6.5 psu (Tukey tests; Fig. 5).

The relationship between $\Delta F/F_m'$ and TWC for acclimated adult tissue and juvenile algae is shown in Fig. 6 for each population and acclimation/growth salinity. The data for adults (Fig. 6a to d) are pooled from the initial experiment (data shown in Fig. 5) and 2 further experiments, and are repeated $\Delta F/F_m'$ measurements made during desiccation (n = 43 apical tips). Biphasic curves were found; $\Delta F/F_m'$ was constant until ca 40% TWC, followed by a rapid and linear decline in $\Delta F/F_m'$ at higher tissue-water losses. For each replicate, we determined the regression equation of the relationship over the linear part of the decline in $\Delta F/F_m'$. Together with the maximum $\Delta F/F_m'$, Y_{max}, the following parameters were derived: the slope of the decline in $\Delta F/F_m'$ (β); and the TWC at which $\Delta F/F_m'$ was half-maximum, $^1/_2 Y_{max}$. A 2-way ANOVA indicated that β was significantly different between populations ($F_{1,168} = 11.55$; p = 0.001; $MS_{error} = 122.54$) and between acclimation salinities ($F_{1,168} = 34.13$; p < 0.001; $MS_{error} = 122.54$). The interaction between the 2 factors was not significant. Thus, the decline in $\Delta F/F_m'$ was more steep for Baltic algae than for North Sea algae, and more steep at Baltic compared to

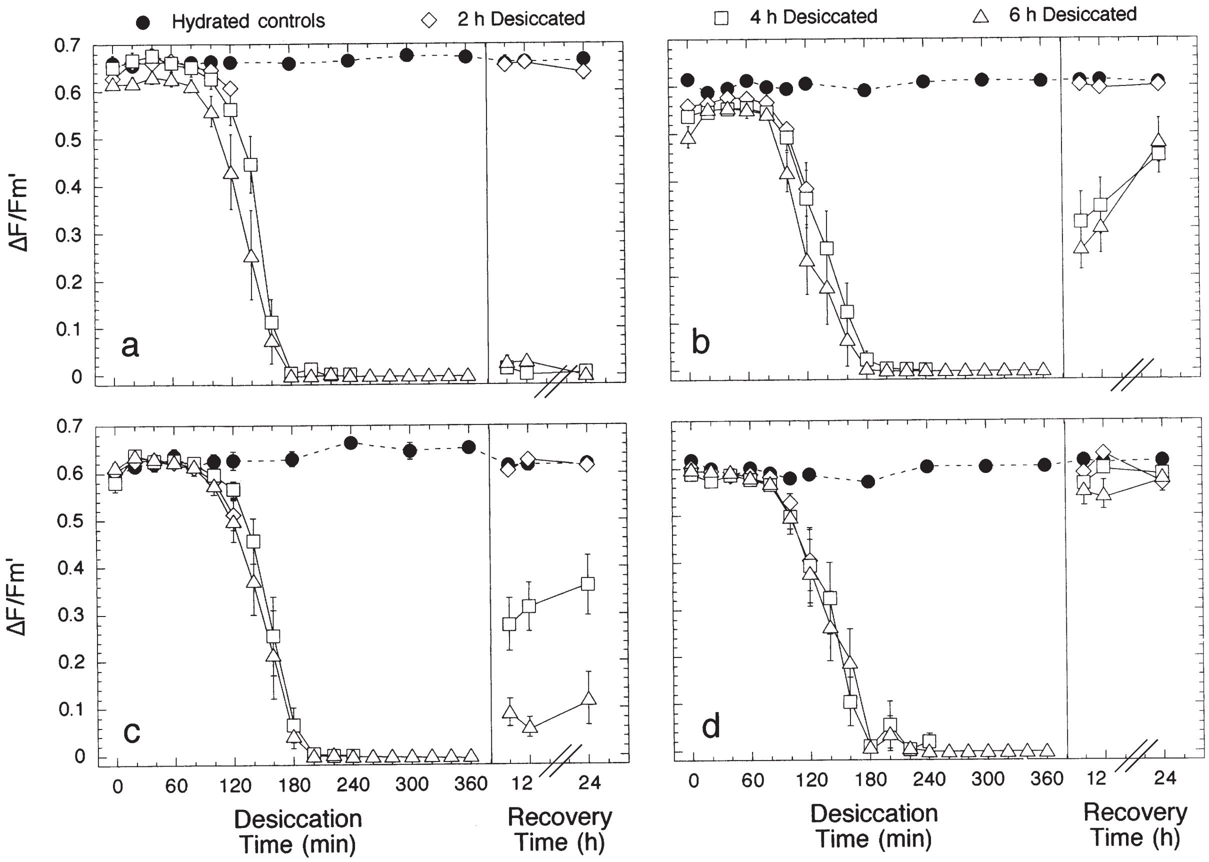

Fig. 5. *Fucus vesiculosus.* Effective photochemical yield ($\Delta F/F_{m}'$) during desiccation and recovery at 25°C of algae from (a, c) the central Baltic Sea and (b, d) the North Sea. (a) Baltic algae acclimated at 6.5 psu; (b) North Sea algae acclimated at 6.5 psu; (c) Baltic algae acclimated at 24 psu; and (d) North Sea algae acclimated at 24 psu. Controls were kept hydrated in air, and recovering algae were kept in seawater of the appropriate salinity, both at 25°C. Values are means ± SE (n = 7)

North Sea salinities. Differences in percentage water content at $^{1}/_{2}Y_{\max}$ occurred due to acclimation salinity rather than to intrinsic differences between populations (Table 1). Water contents at $^{1}/_{2}Y_{\max}$ were lower at 6.5 than at 24 psu ($F_{1,168} = 81.99$; $p < 0.001$; $MS_{error} = 25.03$), but were not significantly different between populations. Finally, Baltic populations at both acclimation salinities had higher $\Delta F/F_{m}'$ than North Sea populations (population × salinity interaction: $F_{1,168} = 10.56$; $p = 0.001$; $MS_{error} = 690.32$) (Table 1).

When the recovery of $\Delta F/F_{m}'$ (after 24 h re-immersion) was plotted against the TWC, a clear pattern emerged. Regardless of acclimation salinity, Baltic algae had a critical TWC of ca 10%, below which recovery of $\Delta F/F_{m}'$ to pre-stress levels did not occur (Fig. 7). In contrast, North Sea algae acclimated to 24 psu recovered $\Delta F/F_{m}'$ even after air-drying to <5% TWC; even following acclimation to 6.5 psu, only 3 replicates (of 43) showed reduced recovery after >90% tissue-water loss (Fig. 7). Using data from the

Table 1. *Fucus vesiculosus.* Parameters describing changes in effective photochemical yield ($\Delta F/F_{m}'$) of Baltic and Atlantic populations during desiccation in air at 25°C and low light intensity (80 µmol photons m^{-2} s^{-1}). Adult apical tissue was pre-acclimated in the laboratory at salinities of 6.5 or 24 psu. Slope (β) indicates the linear rate of decline of $\Delta F/F_{m}'$ during desiccation. Also shown are the tissue-water contents at which $\Delta F/F_{m}'$ was half-maximum (TWC at $^{1}/_{2}Y_{\max}$), and the maximum $\Delta F/F_{m}'$ attained in air ($Y_{\max}$). Values are means ± SE of 43 replicate apices; means with the same letter are not significantly different at p = 0.05 (Tukey tests)

Acclimation salinity (psu)	β	TWC at $^{1}/_{2}Y_{\max}$ (%)	$Y_{\max}$
Baltic Sea			
6.5	−44.37 ± 2.29	16.21^{a} ± 0.72	0.666^{a} ± 0.006
24	−31.56 ± 1.65	22.13^{b} ± 0.79	0.649^{b} ± 0.004
North Sea			
6.5	−35.69 ± 2.02	14.94^{a} ± 0.67	0.598^{c} ± 0.005
24	−28.77 ± 1.83	22.83^{b} ± 1.15	0.607^{c} ± 0.003

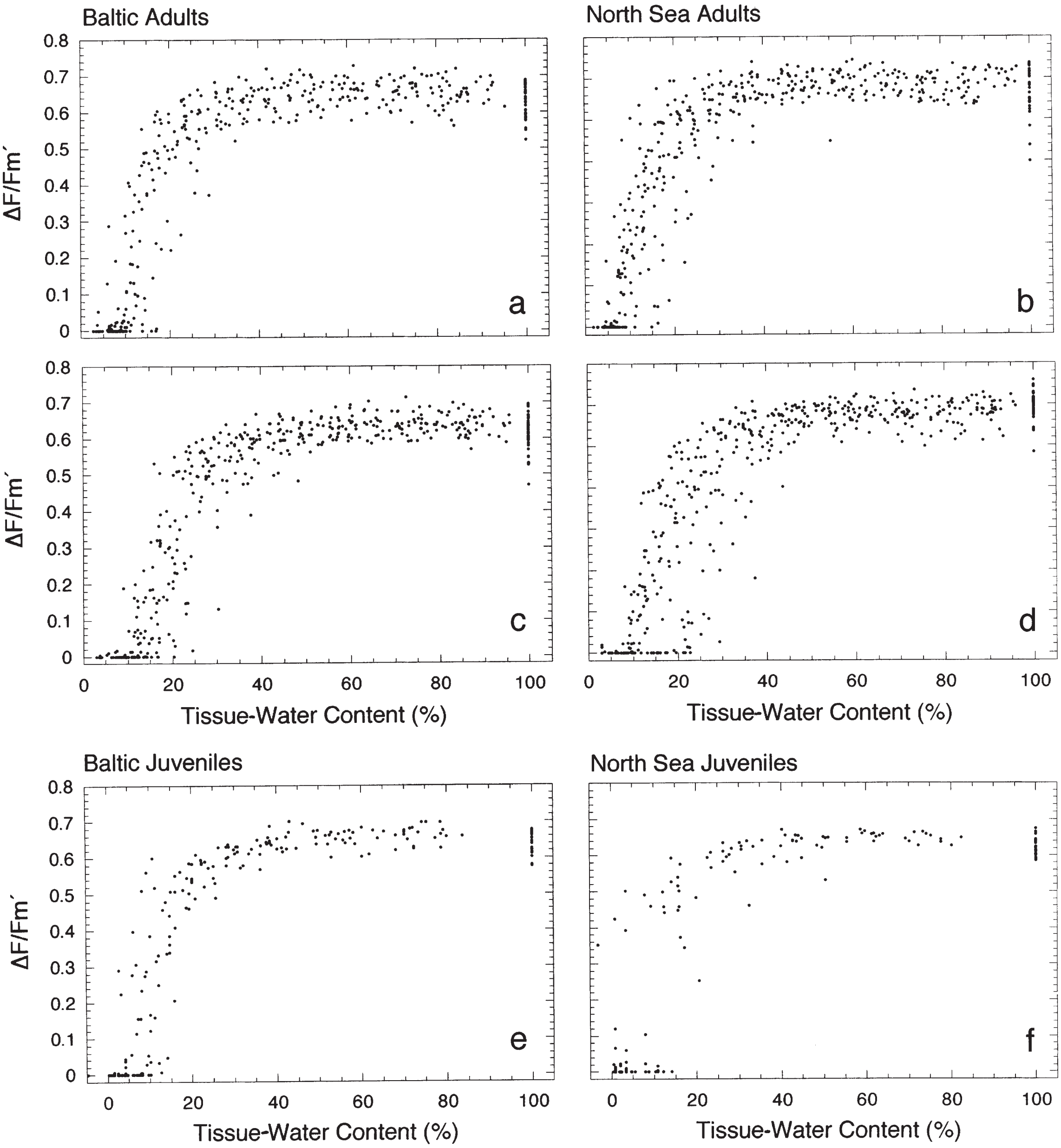

Fig. 6. *Fucus vesiculosus.* Relationship between $\Delta F/F_m'$ and tissue-water content during desiccation at 25°C for adult algae from the Baltic and the North Sea acclimated in the laboratory at either 6.5 or 24 psu (a to d), or juveniles grown in the field in the central Baltic (6.5 psu: e, f). (a) Baltic, 6.5 psu; (b) North Sea, 6.5 psu; (c) Baltic, 24 psu; (d) North Sea, 24 psu; (e) Baltic juveniles; (f) North Sea juveniles. Data are repeated measures on 43 replicates

same experiments, the ability to regain tissue water following desiccation was plotted against recovery of $\Delta F/F_m'$ (Fig. 8). At both 6.5 and 24 psu, Baltic algae regained less tissue-water than North Sea algae, presumably reflecting impairment of osmoregulatory ability and/or physical disruption of the plasma membrane in some cells or tissues. Inability to regain TWC close to initial values (i.e., pre-desiccation) was strongly associated with a failure to recover $\Delta F/F_m'$ in Baltic algae, and, to a much lesser extent, in North Sea algae at 6.5 psu (Fig. 8). North Sea *Fucus vesiculosus* at 24 psu always regained at least 70% of the original TWC, while at 6.5 psu only 2 replicates failed to do so. These data suggest that the failure of the photosynthetic machinery to recover from desiccation stress may at least in part result from more general cellular damage at the level of the cell wall and/or plasma membrane.

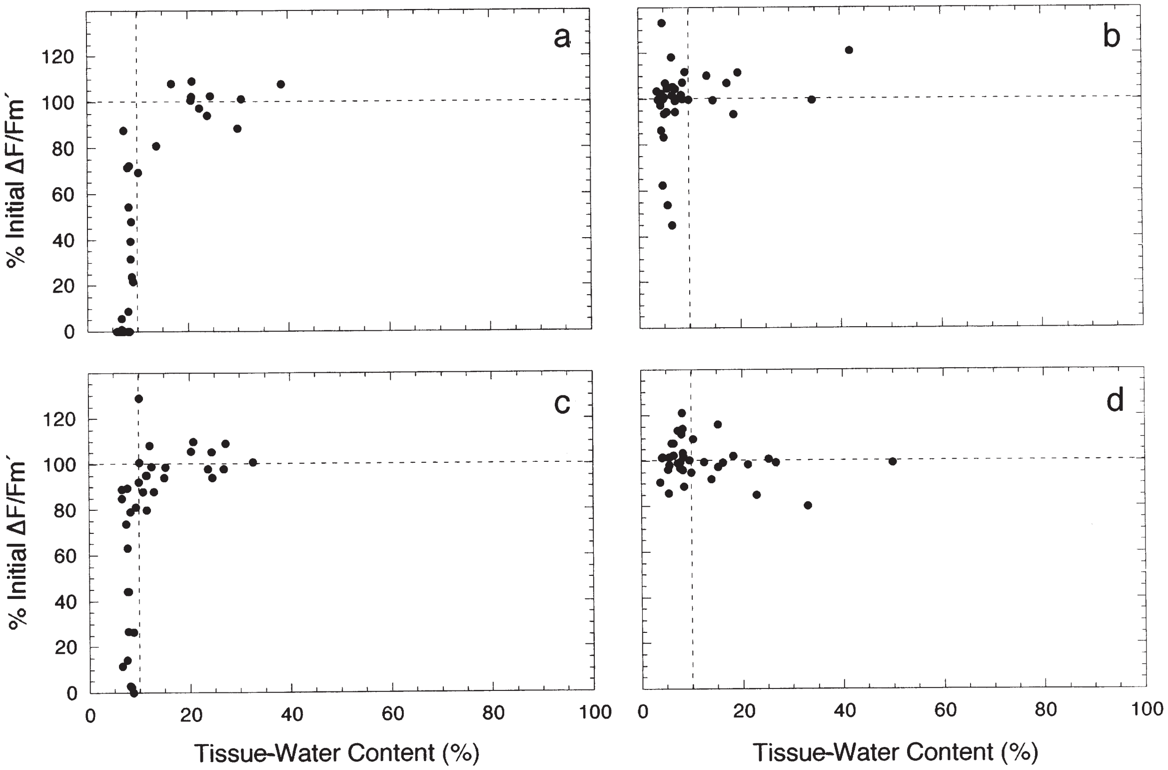

Fig. 7. Relationship between tissue-water content during desiccation and recovered $\Delta F/F_m'$ following 24 h in seawater (6.5 or 24 psu). (a) Baltic, 6.5 psu; (b) North Sea, 6.5 psu; (c) Baltic, 24 psu; (d) North Sea, 24 psu. Each point represents replicate apical tip

These data show that differences in desiccation tolerance between the 2 populations remained following acclimation at either population's growth salinity. Differences in the responses to desiccation were detected arising from salinity acclimation, but were of less overall importance in desiccation responses than the origin of the population.

The response of juveniles (25 to 26 mo) (Fig. 9) during desiccation followed a similar pattern to that of adult plants. There was a rapid decline in $\Delta F/F_m'$ to zero after a slight increase during initial emersion, probably a response of the photosynthetic apparatus to increased CO_2 diffusion rate in air (Johnston & Raven 1986). The decline in $\Delta F/F_m'$ occurred earlier in time in juvenile than in adult tissue. However, $\Delta F/F_m'$ versus TWC plots showed that this was due to more rapid desiccation, since $\Delta F/F_m'$ did not begin to decrease until <40% TWC remained, as for adult tissue (cf. Fig 6a,b with e,f). The ability to recover from desiccation was lower in Baltic than in North Sea juveniles, despite the fact that the former had been grown post-germination under the same field conditions in the Baltic Sea. A 2-way ANOVA on 24 h recovery data indicated significant differences between treatments related to population and desiccation time ($F_{3,48} = 10.73$; $p < 0.0001$; $MS_{error} = 397.05$). For each desiccation treatment (1, 2, 3 h), North Sea juveniles recovered more completely than Baltic juveniles (Tukey tests; Fig. 9).

DISCUSSION

The results of this study indicate that a considerable reduction in the ability to tolerate emersion stresses has taken place in populations of *Fucus vesiculosus* (L.) in the brackish, non-tidal Baltic Sea compared to neighbouring populations in the North Sea. Tolerance of *F. vesiculosus* to freezing and desiccation was higher in a population from Tjärnö on the Swedish west coast than in a central Baltic population from Askö. The Baltic Sea can be considered a novel or unusual habitat for intertidal fucoid algae in 2 major respects: Firstly, it is atidal and therefore normally intertidal algae like *F. vesiculosus* are present as permanently submerged populations. Secondly, the brackish conditions, particularly of the central and northern Baltic (ca 7 to 3 psu, respectively), impose unique osmotic and ionic conditions upon marine macroalgal populations (Serrão et al. 1996a, 1999b). In order to account for possible effects of growth salinity on emersion-

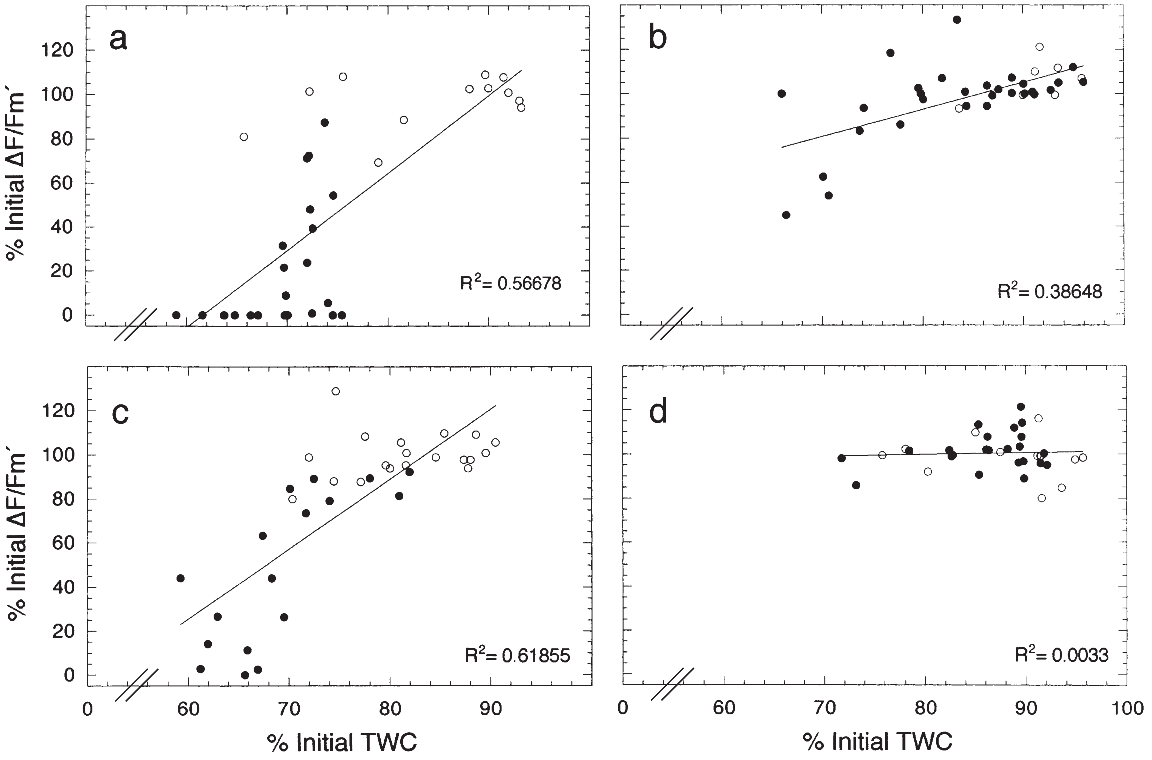

Fig. 8. *Fucus vesiculosus.* Relationship between recovered $\Delta F/F_m'$ and recovered tissue-water content (TWC), 24 h following desiccation in algae from (a, c) the Baltic and (b, d) the North Sea. Algae were acclimated at salinities of (a, b) 6.5 psu or (c, d) 24 psu ●: replicates which had desiccated TWC <10%; ○: replicates which had desiccated TWC >10%

stress tolerance, we cross-acclimated both populations to salinities typical of the central Baltic and North Sea (Skagerrak) in the laboratory prior to stress-tolerance experiments. In addition, we grew juveniles of both populations from *in vitro*-fertilized zygotes for a period of up to 2 yr in the central Baltic. These individuals therefore had an identical salinity history (6.5 psu), and had never experienced emersion prior to the experiments reported here.

We made several observations suggesting that the differences between the responses of the 2 populations to freezing stress are not solely due to growth salinity: (1) Although freezing tolerance in Baltic algae was increased after acclimation at higher salinity, it remained lower than that of the North Sea population at 20 psu. (2) Algae from the North Sea population, following acclimation at 6.5 psu, were only slightly less freezing-tolerant than those remaining at 20 psu, and remained considerably more freezing-tolerant than Baltic algae at 6.5 psu. The effects of salinity acclimation on freezing tolerance reported here are similar to those found by Pearson & Davison (1994) for *Fucus distichus*, a less freezing-resistant fucoid (see Davison et al. 1989). (3) Juvenile algae grown in the Baltic for ca 1.5 yr displayed physiological responses similar to those of adult algae after short-term salinity acclimation. Juveniles from the North Sea population were significantly more freezing-tolerant than those originating from the Baltic, despite having developed post-fertilization at the same salinity in the absence of emersion stress. The latter result suggests that genetic differences underly the differences in freezing responses of the 2 populations. There are 2 further points of interest with regard to the freezing tolerance of juvenile algae. Firstly, our experiments indicated that, by the second winter in the field, the freezing tolerance of juveniles was very similar to that of adults (cf. Fig. 4a with b). This is in marked contrast to desiccation tolerance (discussed below). Secondly, the levels of freezing tolerance observed in juveniles developed in the absence of emersion-dependent hardening processes (Schonbeck & Norton 1979), although the possibility that exposure to seasonal low water temperatures in the field may play a role in hardening cannot be discounted (Bird & McLachlan 1974, MacDonald et al. 1974).

Desiccation at low temperatures (5°C) resulting in a TWC of 30 to 40% had no effect on the recovery of F_v/F_m after re-immersion, but the responses of F_v and F_v/F_m during desiccation suggest different physiological responses to desiccation between Baltic and North

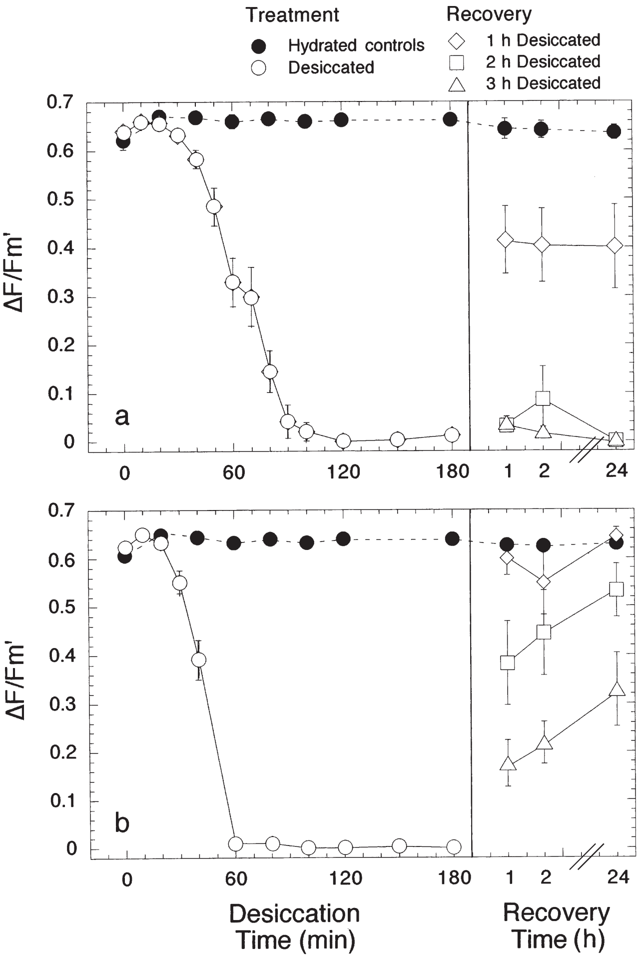

Fig. 9. *Fucus vesiculosus.* Effective photochemical yield ($\Delta F/F_m'$) during desiccation and recovery at 25°C of juveniles grown in the central Baltic Sea. (a) Baltic algae, (b) North Sea algae. Controls were kept hydrated in air, and recovering algae were kept in Baltic seawater, both at 25°C. Values are means ± SE (n = 7)

Sea *Fucus vesiculosus.* Although further physiological data are required in order to more fully understand the particular ways in which these 2 populations differ (e.g., gas-exchange measurements, fluorescence-quenching analysis, changes in photoprotective xanthophyll cycle components), our data do provide some indications. The decline in F_v/F_m in North Sea *F. vesiculosus*, which occurred to a much lesser extent in Baltic algae, was due to a concomitant increase in F_0 (also observed in Baltic algae and usually interpreted as an indicator of accumulated inactive PSII reaction centers) together with a decline in F_v. In contrast, F_v increased in Baltic algae as TWC decreased. Declining F_v reflects increased nonradiative energy loss (heat dissipation), and an increase in photoprotective processes (Krause & Somersalo 1989, Krause & Weis 1991). Thus, these data suggest that protection of the photosynthetic apparatus may occur to a greater extent in the North Sea than in the Baltic population; an interpretation which is supported by results showing impaired recovery in Baltic algae after more prolonged and severe desiccation.

In summer desiccation experiments, there was more evidence that salinity acclimation affected desiccation tolerance than in the winter desiccation experiments (possibly a consequence of higher desiccation rates at 25°C). This could have been due to (1) differences in the extent and/or rate of desiccation between winter and summer experiments, (2) interactive effects between temperature and desiccation, (3) the fact that $\Delta F/F_m'$ rather than F_v/F_m was measured, or (4) because of seasonal or temperature influences on acclimation processes. The fact that salinity-induced acclimation to freezing did occur in winter experiments (particularly in Baltic populations), shows that acclimation to this stress was effective, and that its effects could be detected by measurement of F_v/F_m. In view of the evidence for common physiological components in the response of fucoids to water stresses in general (Pearson & Davison 1994), together with the narrow range of TWC separating full and partial recovery (Fig. 8), explanations (1) and/or (2) appear the most likely.

Despite the large differences in recovery of $\Delta F/F_m'$ between Baltic and North Sea *Fucus vesiculosus* populations, and to a lesser extent between acclimation salinities, the behaviour of $\Delta F/F_m'$ during desiccation was similar across treatments. This has been noted before in interspecific responses of photosynthetic gas exchange during desiccation in fucoids (Dring & Brown 1982); recovery ability rather than responses during stress largely define overall differences in tolerance. However, one of the advantages of chlorophyll fluorescence measurements is the ease and rapidity with which they can be made, allowing a greater number of treatments and replicate measurements to be made than would be possible using, e.g., standard gas-exchange techniques. By making measurements on a large number of replicates we were able to show that the declining slope of $\Delta F/F_m'$ was steeper in Baltic than in North Sea populations during desiccation. Together with data showing that TWC for $^1/_2 Y_{max}$ did not differ, this indicates that the photochemical yield begins to decline at lower TWC (i.e., more severe desiccation) in Baltic *F. vesiculosus* than in intertidal populations. Without more detailed physiological characterization it is not possible to draw conclusions, although this phenomenon may reflect differences in non-photochemical quenching processes (i.e., photoprotection) that may significantly affect subsequent recovery, and deserves further study.

The differences in recovery ability that we observed between Baltic and North Sea populations persisted after salinity cross-acclimation. Moreover, recovery ability following short-term acclimations at 6.5 psu were consistent with the behaviour of juveniles grown in the field over longer periods. Acclimation salinity, while not accounting for the physiological differences between populations, did have considerable effects on desiccation tolerance, and thus appears to play a general role in modifying water-stress responses in fucoids (freezing tolerance; Pearson & Davison 1994, this study). The reason why acclimation at increasing salinity increases desiccation tolerance remains unknown, but compensatory increases in the concentration of internal osmotica is likely to play a role. The major organic osmoticum in brown algae is mannitol (Reed et al. 1985), which increased in response to salinity in long-term cultures of Baltic and Atlantic *F. vesiculosus* (Bäck et al. 1992b). As well as acting as compatible solutes, polyols are thought to have other protective roles within cells undergoing water stress, e.g., redox regulation (Ansell et al. 1997).

Our experiments suggest that there is a critical TWC of ca 10% below which Baltic algae cannot recover. Acclimation to higher salinity improved the extent of recovery, but total recovery still did not occur below this threshold TWC. In contrast, for North Sea algae at their growth salinity we were unable to find a TWC (even <5%) below which they were unable to fully recover $\Delta F/F_m'$. Acclimation of North Sea algae at Baltic salinity reduced desiccation tolerance in some replicates, but recovery was <50% in only 1 replicate. The recovery of $\Delta F/F_m'$ following desiccation to a water content of 5.9 ± 1.1% TWC of North Sea *Fucus vesiculosus* in this study was essentially complete within 1 h of re-immersion (see Fig. 5d). In a study of the same North Sea population, Beer & Kautsky (1992) found positive rates of net photosynthesis after rehydration from a similar extent of tissue-water loss, but at considerably lower rates than those of algae having withstood TWC down to 20%, which recovered fully. In their experiments, however, desiccation was carried out in full sunlight, which may have resulted in greater photoxidative stress than would be expected under the lower PFD used in this study, which was not saturating for photosynthesis. In addition, gross photosynthesis data are not available in the study by Beer & Kautsky (1992); these would be necessary in order to make more meaningful comparisons of the techniques used for photosynthetic measurements.

Measurement of $\Delta F/F_m'$ is a powerful tool to investigate photosynthetic status (Genty et al. 1989); obviously however, caution should be used in making inferences about overall physiological status from one kind of measurement. The ability of Baltic *Fucus vesiculosus* in particular to regain TWC was impaired after severe desiccation (to <10% TWC; Fig. 8). Furthermore, there was a correlation between water-uptake ability and recovery of $\Delta F/F_m'$ in salinity-acclimated algae, with the exception of the North Sea population at 24 psu. Although the causal relationship between water uptake and photosynthetic status is not known, these results suggest the possibility that the cell wall and/or plasma membrane is a major site of desiccation-induced injury, and that photosynthetic impairment following recovery is, therefore, a consequence of osmoregulatory impairment.

The effects of desiccation on juveniles appear more severe given the shorter drying times required to disrupt recovery. However, this was largely a factor of the relative drying rates for different ages of tissue: juveniles from the North Sea had TWC of only 4.20 ± 0.69% after 1 h desiccation, compared with 56.85 ± 1.58% for adults after 1 h desiccation. Juveniles from the Baltic (which were on average larger than those from the North Sea) had TWC of 13.93 ± 1.45% after 1 h desiccation, while adults had 58.13 ± 1.04% after the same period. Higher rates of tissue-water loss for juveniles should have ecological consequences for growth and survival, even if physiologically the responses of juvenile and adult tissue is similar.

It is interesting that the extent of recovery of North Sea juveniles declined between 1 and 3 h drying, although the TWC did not decrease further, remaining at ca 4%. This suggests that the duration of drying at stable TWC can affect subsequent recovery in addition to the final TWC reached.

It has been observed by Russell (1985) that the present flora of the Baltic is largely composed of species which are intertidal throughout the rest of their distributions, and he has suggested that intertidal species that commonly experience wide fluctuations in salinity might be ‘preadapted’ to a brackish-water existence because of their genetic plasticity. In highly (osmotically) variable environments like the intertidal zone, selection may simultaneously favour mechanisms to tolerate both water stress (freezing, desiccation) and hypoosmotic stress (precipitation, freshwater runoff). However, selection would strongly favour low salinity tolerance in submerged populations in the comparatively stable salinity environment of the Baltic. Thus, if water-stress tolerance carries costs in terms of fitness components, it would be selectively disadvantageous. Evidence from several studies now supports both parts of this hypothetical framework. The best evidence for low salinity adaptation comes from studies of growth and ecophysiological comparisons between Atlantic and Baltic populations (Bäck et al. 1992a,b), and from assessment of the osmotic capabilities of Baltic versus Atlantic gametes (Serrão et al. 1996a). Our study shows that over the past several thousand years of relative isolation in the Baltic, *Fucus vesiculosus* has lost a significant proportion of its ability to tolerate water stresses such as freezing and desiccation. Much recent interest has focused on the role of ecological processes in driving rapid evolution and divergence or speciation (Orr & Smith 1998, Thompson 1998). The challenge now is to investigate the nature of the genetic changes in Baltic *F. vesiculosus* which are responsible for its physiological divergence from Atlantic populations.

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