

Ecophysiological performance of the primitive red alga *Dixoniella grisea* (Rhodellophyceae) to irradiance, temperature and salinity stress: growth responses and the osmotic role of mannitol

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A culture strain of the benthic unicellular red alga *Dixoniella grisea* was investigated under different stress conditions. The effects of salinity, temperature and irradiance on growth rates were examined in two-factorial experiments and the accumulation of mannitol in response to increasing salinity investigated. The strain grows in a broad salinity range, from brackish water to twice seawater (60 psu). At optimal salinity (10 psu) and optimal temperature conditions (25–30°C), *D. grisea* grew best at moderate photon flux densities (PFDs; 50–100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). However, interactive effects between all factors were present. At suboptimal salinities and temperatures, maximal growth rates were shifted to lower PFD and growth was considerably reduced at 50 and 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The polyol mannitol was the main low molecular weight carbohydrate in *D. grisea*. This was verified by ¹³C-nuclear NMR spectroscopy and HPLC analysis. Mannitol levels increased from 2 to 52 $\mu\text{mol g}^{-1}$ dry weight (dw) with increasing salinities between 10 and 60 psu, indicating its role as an osmolyte for the first time in a unicellular red alga.

KEY WORDS: *Dixoniella grisea*, Growth, Low molecular weight carbohydrate, Mannitol, Osmolyte

INTRODUCTION

Dixoniella grisea is a benthic unicellular red alga assigned to the class Rhodellophyceae (Yoon *et al.* 2006), formerly classified in the monophyletic clade Porphyridiales 1 of the Bangiophyceae (Müller *et al.* 2001). It occurs in freshwater (Geitler 1970), brackish water (Deason *et al.* 1983), full seawater and salt marshes (Fresnel *et al.* 1989), indicating a broad salinity tolerance. Although there is data on the morphology, taxonomy and phylogeny, nothing is known on the ecophysiology of *D. grisea*. Most interestingly, this species does not contain the typical red algal heterosides floridoside or digeneaside (Kremer 1978), but contains the polyol mannitol as the only low molecular weight carbohydrate (LMWC) (Karsten *et al.* 1999).

Among the algae, mannitol represents the main photosynthetic product in the Phaeophyta (Reed *et al.* 1985). In contrast, the occurrence of mannitol within the red algae is rather uncommon. So far it has been only detected in the florideophycean genus *Caloglossa* (Karsten *et al.* 1992). All rhodellophycean species investigated so far (*Rhodella* species and *D. grisea*) exhibit mannitol as the main LMWC (Karsten *et al.*, 1999, 2003). The pattern of mannitol occurrences as well as other LMWCs fit the new phylogeny of the primitive red algal lineages. Their role as chemotaxonomic characters was already suggested by Karsten *et al.* (1999, 2003).

Mannitol has several physiological functions. Besides its role of being the major photoassimilatory product in Phaeophyta and the red alga *Caloglossa*, one of the most important functions is the control of cell turgor by acting as an osmolyte. Laboratory studies have shown that intracellular mannitol

concentrations vary as a direct function of external salinity in several marine brown algae including *Dictyota dichotoma*, *Scytosiphon lomentaria* (Kirst & Bisson 1979) and *Pilayella littoralis* (Reed & Barron 1983), indicating an osmotic role. This function was also shown for *Caloglossa* (Karsten *et al.* 1992; Mostaert *et al.* 1995). Mannitol as a compatible solute, even in high concentrations, does not disrupt enzyme function as was shown in *Caloglossa leprieurii* (Karsten *et al.* 1996a) and in lysozyme (Singh & Singh 2003). Mannitol also functions as an antioxidant owing to its ability to scavenge free oxygen radicals (Shen *et al.* 1997; Jennings *et al.* 1998).

The present study was designed to investigate the ecophysiological performance of *Dixoniella grisea* by measuring the growth response as a function of salinity and in relation to the interactive effects of temperature and photon flux density (PFD). For the first time in a primitive red alga, the physiological role of mannitol in a range of salinities was determined.

MATERIAL AND METHODS

Isolate and culture conditions

Unialgal stock cultures of *D. grisea* (Geitler) Scott *et al.* (UTEX strain 2320) were grown in 800 ml sterile filtrated Baltic Sea water (membrane filter, 0.2 μm , NL16, Schleicher & Schuell, Germany) adjusted to a salinity of 33 psu with artificial sea salt (hw Meersalz professional, Germany) and enriched with full-strength Provasoli's nutrients (Starr & Zeikus 1993). Cultures were kept at 20°C and a PFD of 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of photosynthetic active radiation (400–700 nm, Osram, Lumilux de Luxe, Daylight, Germany) at 16 : 8 h day length, and aerated with filtered air to keep the cells in

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suspension. NaHCO_3 was added as a carbon source with a final concentration of 3 mM.

Under these culture conditions, cells of *D. grisea* were solitary but formed loose aggregates due to an extremely thick mucilaginous sheath surrounding each *Dixoniella* cell. Cells are spherical and 100 photographed cells had a mean diameter (excluding a mucilaginous sheath) of 8.5 μm and a mean volume of 310 μm^3 . The abundance of logarithmically growing cells was approximately 40×10^3 cells ml^{-1} . As 1 ml logarithmically growing algal cultures amounted to approximately 0.05 mg dry weight (dw), 1 g dried culture corresponded to 248 mm^3 total cell volume.

Growth experiments

Relative growth rates (RGRs) were determined using an *in vivo* fluorimeter (Hansatech MFMS, Norfolk, UK) according to the technique of Karsten *et al.* (1996b). To investigate the effect of salinity on growth, *D. grisea* cultures were grown at 9 different salinities (5, 10, 15, 20, 25, 33, 45, 52, 60 psu) at a constant temperature (20°C) and a constant PFD (20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 16 : 8 h day length). To evaluate interactive effects of salinity and PFD, RGRs were determined at three salinities (10, 20, 33 psu) and four PFD levels (10, 20, 50, 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 16 : 8 h day length). Interactive effects between temperature and PFD were investigated at optimum salinity (10 psu) at six different temperatures (5, 15, 20, 25, 30, 33°C) and four different PFD levels (10, 20, 50, 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 16 : 8 h day length). Hypersaline media were adjusted with artificial sea salt (hw Meersalz professional, Germany) and hyposaline media were diluted with distilled water. Several layers of grey gauze were used to adjust different PFD levels. Temperature experiments took place in climate chambers. Algae were pre-acclimated to the different experimental conditions for 14 d by inoculating 30–50 ml stock culture into 800 ml medium.

A volume (0.05–0.5 ml) of acclimated and logarithmically grown algal suspensions was transferred to disposable Petri dishes (5 cm diameter, Kleinfeld, Hannover, Germany) containing 15 ml of the experimental medium. The volume was chosen to receive always a fluorescence signal 20–30 mV higher than the blank value of the medium. The growth of the samples, i.e. the exponential increase of the fluorescence signal, was followed at 1 d intervals for 6–16 d. The salinity response of the growth rate was fitted to the nonlinear model of Blanchard *et al.* (1996).

Low molecular weight carbohydrate quantification

Low molecular weight carbohydrates were separated and quantified by HPLC according to Karsten *et al.* (1991). Biomass was harvested by centrifugation, depending on growth rate, after 7–14 d of cultivation at the different salinities. Extracellular sea salt was removed by washing the samples in ice-cold $\text{Ca}(\text{NO}_3)_2$ solution (Mostaert *et al.* 1995). Samples were shock frozen in liquid nitrogen and freeze-dried (Lyovac GT2, Seris GmbH, Hürth, Germany). Ethanol extracts of algal samples (10–15 mg dw) were prepared for HPLC analysis according to Karsten *et al.* (1991).

NMR measurements

For NMR spectroscopy, an ethanol extract of 80 mg of algal dw of *Dixoniella grisea* was prepared as for the HPLC analysis, but was redissolved in 0.5 ml D_2O . The ^{13}C NMR spectra were recorded with a Bruker AVANCE 500 spectrometer (13C: 125.8 MHz). Typically, a sweep width of 30,000 Hz, 32,000 time domain points, and a 30° pulse of 3.0 μs were used for acquisition, with composite pulse decoupling (number of scans: 20,000). Samples were run at a temperature of 27°C and referenced from acetone ($\delta = 31.1$ ppm). For the purpose of comparison the spectrum of reference mannitol has been recorded under the same conditions and proved to be identical with the spectrum recorded for the sample of algal dw of *D. grisea*.

Data analysis

Salinity effects on RGR and mannitol accumulation were analysed using one-way ANOVA. The Tukey-test was used to find *a posteriori* homogeneous subgroups of means that differed significantly. The salinity response of RGRs was additionally fitted to the nonlinear model of Blanchard *et al.* (1996) using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). The increase of mannitol content as a function of salinity was additionally described using the linear regression model. The 95% confidence band of the curve was calculated, i.e. the true best-fit curve lies within that band with a probability of 95%. The coefficient of determination (r^2) and the parameters of both regression models with the respective standard errors are given.

Salinity and PFD effects were analysed using two-way ANOVA. The significance of the main effects of the two factors, i.e. the effects of salinity levels averaged across all PFD levels and *vice versa*, as well as of the interaction effect of salinity and PFD were calculated. Differences between PFD levels at one salinity level were analysed using simple main effects analysis. Significant differences among PFD levels were further analysed by pairwise comparisons using the Šidak adjustment for multiple comparisons. Analysis of temperature and PFD effects was analogously carried out.

RESULTS

Testing the interactive effect of temperature and PFD on growth in *D. grisea*, both factors separately and significantly affected this process ($P < 0.001$, Table 1, Fig. 1). With respect to temperature, RGRs averaged over all PFD levels were optimal at 25°C, i.e. RGRs decreased with decreasing temperatures between 5 and 20°C as well as $\geq 30^\circ\text{C}$. With respect to PFD, RGRs averaged over all temperature levels were highest at 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, intermediate at 100 and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and lowest at the lowest PFD of 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 1). The interactive effects of temperature \times PFD were also highly significant ($P < 0.001$, Table 1), indicating that PFD had different effects on growth in *D. grisea* cultivated at 5, 15, 20, 25 and 30°C. Therefore, simple main effects of PFD at the different temperature levels were analysed, and found to be highly significant ($P < 0.001$). *Dixoniella grisea* cultivated at 5°C grew only at 10 and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, i.e. did not grow at all at higher PFDs tested

Table 1. Statistical results of two-way and one-way ANOVAs for effects of temperature and photon flux density (PFD) treatments, salinity and PFD treatments and salinity on growth and effects of salinity on mannitol content.

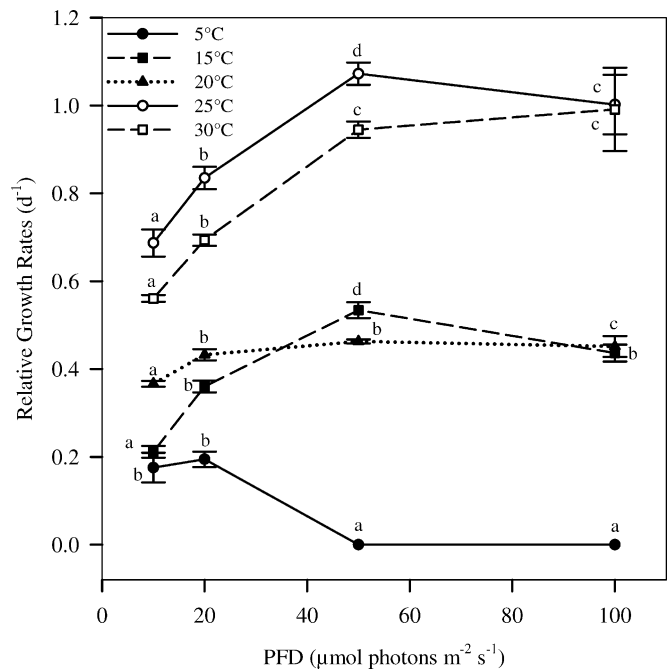
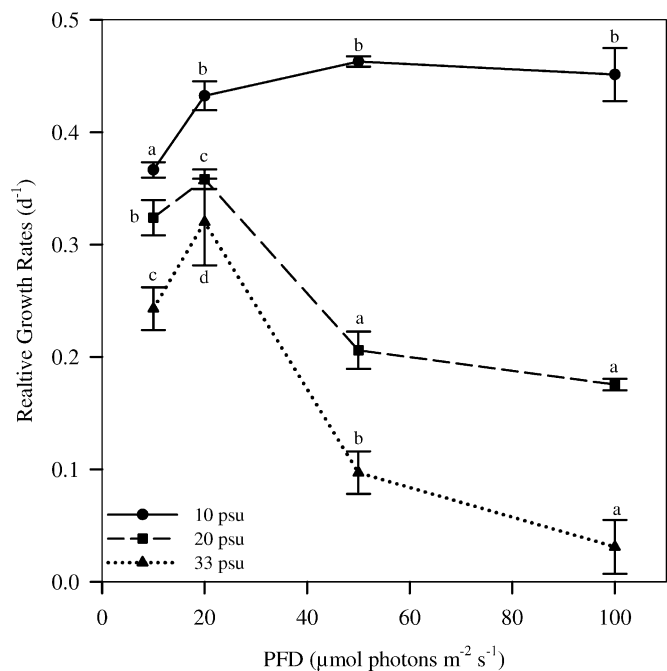
Source of variation	MS	df	F	P
Temperature and PFD on growth				
Temperature	2.151	4	2183.9	<0.001
PFD	0.205	3	208.5	<0.001
Temperature × PFD	0.078	12	79.7	<0.001
Residual	0.001	80		
Salinity and PFD on growth				
Salinity	0.334	2	932.0	<0.001
PFD	0.063	3	176.0	<0.001
Salinity × PFD	0.035	6	98.0	<0.001
Residual	<0.001	46		
Salinity on growth				
Between groups	0.256	9	464.2	<0.001
Within groups	0.001	40		
Salinity on mannitol content				
Between groups	790.888	9	217.7	<0.001
Within groups	3.632	20		

(Fig. 1). At the other temperatures, RGRs generally increased with PFD between 10 and 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Rates were similar or slightly lower at 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ as compared to 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Testing the interactive effect of salinity and PFD on growth in *D. grisea*, both factors separately exhibited significant main effects ($P < 0.001$, Table 1, Fig. 2). With respect to salinity, RGRs averaged over all PFD levels were highest at 10 psu, followed by 20 and 33 psu. With respect to PFD, RGRs averaged over all salinity levels were highest at 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, intermediate at 10 and 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and lowest at the highest PFD of 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 2). The interactive effects of salinity × PFD were also highly significant ($P < 0.001$, Table 1), indicating that PFDs differently influenced growth of algae cultivated at 10, 20 and 33 psu. Therefore, simple main effects of PFD at the different salinity levels were analysed. All were highly significant too ($P < 0.001$). RGRs of *D. grisea* at 10 psu increased with PFD, reaching highest values between 20 and 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. In contrast, RGRs of samples at 20 and 33 psu were highest at intermediate PFD of 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. RGRs decreased drastically in these samples at 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to 50 and 10% of the maximal value at 20 and 33 psu, respectively (Fig. 2).

Salinity had a highly significant effect on growth of *D. grisea* (one-way ANOVA: $P < 0.001$, Table 1, Fig. 3). Values were highest at brackish salinities and decreased significantly at salinities > 25 psu. The data followed the nonlinear model of Blanchard *et al.* (1996) (regression model: $P < 0.001$, $r^2 = 0.93$), i.e. salinity-growth response represented an optimum curve. Highest growth rates of $0.59 \pm 0.02 \text{ d}^{-1}$ were reached at a salinity of 9 ± 2 psu (Fig. 3). According to the regression model, good growth, i.e. more than 80% of the maximal RGR and sufficient growth, i.e. more than 20% of the maximal RGR, were achieved up to 23 psu and 46 psu, respectively.

The HPLC method guarantees separation and quantification of a number of red algal LMWCs, including heterosides and polyols. HPLC analysis revealed only one peak in all samples of

**Fig. 1.** Temperature × photon flux density (PFD)—growth response curves of *Dixoniella grisea*. Growth was followed, depending on growth rate, for 7–14 d at three salinities (10, 20, 33 psu) and four PFDs (10, 20, 50, 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Shown are mean values \pm standard deviation ($n = 5$). Different letters per salinity level represent significant differences at $P < 0.05$ among PFD levels according to the analysis of simple main effects.**Fig. 2.** Salinity × photon flux density (PFD)—growth response curves of *Dixoniella grisea*. Growth was followed, depending on growth rate, for 7–14 d at three salinities (10, 20, 33 psu) and four PFDs (10, 20, 50, 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Shown are mean values \pm standard deviation ($n = 5$). Different letters per salinity level represent significant differences at $P < 0.05$ among PFD levels according to the analysis of simple main effects.

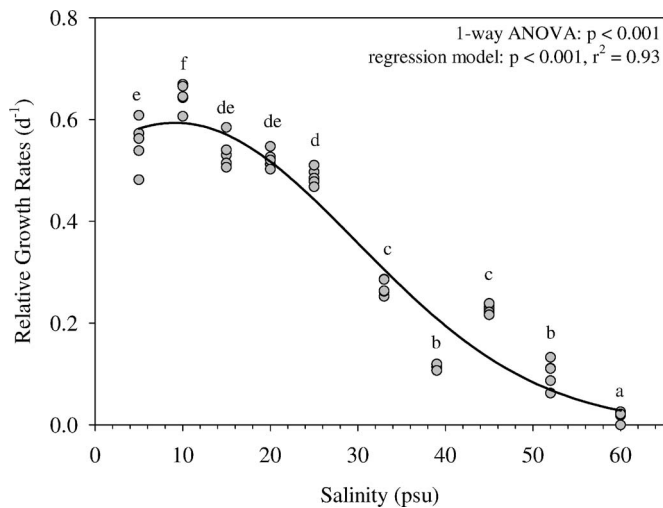


Fig. 3. Salinity—growth response curve of *Dixoniella grisea* at 20°C and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Growth was followed, depending on growth rate, for 7–14 d at salinities ranging from 5 to 60 psu. Shown are individual measurements ($n = 5$) and fitted optimum curve according to Blanchard *et al.* (1996). Given are P values of the statistical analyses. Different letters represent significant differences at $P < 0.05$ among salinities according to the Tukey *post hoc* test.

Dixoniella grisea at a retention time of 6.24 min (Fig. 4a). ^{13}C -NMR spectroscopy identified this peak as mannitol (Fig. 4b).

Salinity had a significant effect on the mannitol content in *D. grisea* (one-way ANOVA: $P < 0.001$, Table 1, Figs 4a, 5). The mannitol concentration increased linearly between 10 and 60 psu from 2 to 52 $\mu\text{mol g}^{-1} \text{dw}$ with a mean slope of $8.5 \pm 0.5 \mu\text{mol g}^{-1} \text{dw}$ per 10 psu (regression model: $P < 0.001$; $r^2 = 0.92$; Fig. 5). No mannitol was detected in cells growing at 5 psu. According to 95% confidence band of the linear regression model, *D. grisea* contains a significant amount of mannitol with a probability of 95% at minimal salinities of 5–11 psu (x -intercept when $y = 0.0$).

DISCUSSION

Salinity, temperature and irradiance are among the adverse environmental factors commonly encountered by marine algae. Their effects on growth in one strain of the unicellular benthic red alga *D. grisea* have been investigated in two-factorial experiments. The strain grows well in a broad salinity range between 5 and 50 psu, a typical feature for a euryhaline organism. This is consistent with the species distribution in freshwater (Geitler 1970), brackish water (Deason *et al.* 1983), full marine water and salt marshes (Fresnel *et al.* 1989). However, *D. grisea* had a growth optimum at 10 psu and showed good growth at salinities < 25 psu, indicating a clear preference for brackish waters. Estuarine algal populations were more resistant to hyposaline stress (Reed & Barron 1983; Young *et al.* 1987; Benjamin *et al.* 1999). However, true brackish water species or at least ecotypes are not very common within the algae and so far mainly described for macroalgae of the Baltic Sea (*Fucus radicans* sp. nov.: Bergström *et al.* 2005; *Ceramium strictum*: Rueness & Kornfeldt 1992; *Phycodrys rubens*: Rietema 1991; *Delesseria sanguinea*: Rietema 1993). *Dixoniella grisea* is a warm-temperate species since it grew best at 25–30°C. At optimal salinity (10 psu) and optimal temperature conditions (25–30°C), *D.*

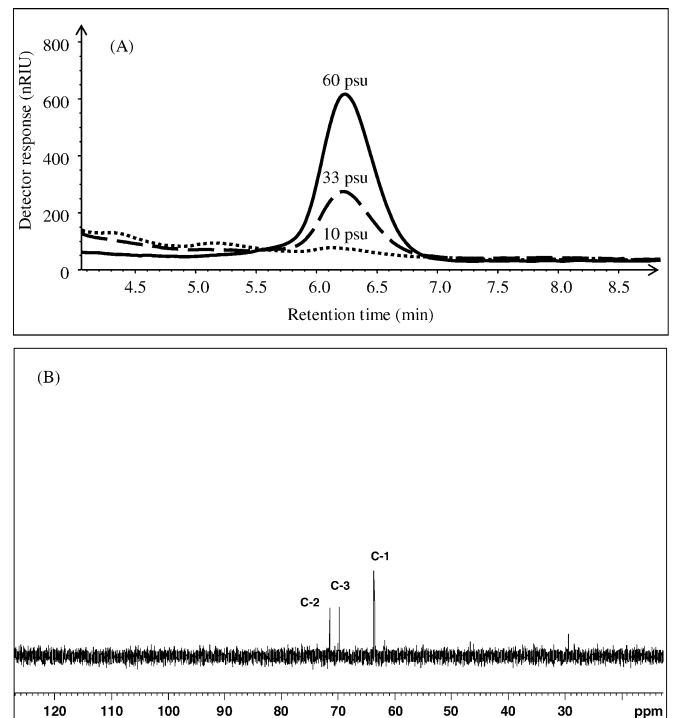


Fig. 4. (A) HPLC chromatograms obtained from ethanolic extracts of three cultures of *Dixoniella grisea* acclimated to 10 psu (dotted line), 33 psu (dashed line) and 60 psu (solid line). Each diagram shows one peak at a retention time of 6.22 min representing mannitol. Mannitol content of the samples is 36 nmol (10 psu), 437 nmol (33 psu) and 1126 nmol (60 psu). For all samples similar biomass of 20–22 mg dw was used. (B) ^{13}C NMR spectrum of the 70% ethanol extract of *D. grisea* in D_2O solution [$\delta_{\text{C-2}(\text{CHOH})} = 71.5$, $\delta_{\text{C-3}(\text{CHOH})} = 69.9$, $\delta_{\text{C-1}(\text{CH}_2\text{OH})} = 63.7$].

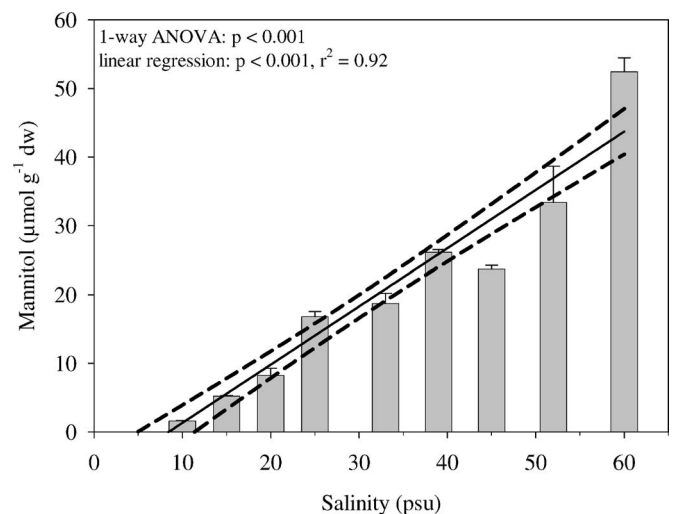


Fig. 5. The effect of salinity on the intracellular concentrations of mannitol in *Dixoniella grisea*. Algae were treated with salinities ranging from 5 to 60 psu. Data are expressed as mean value \pm standard deviation ($n = 3$). The best fit-line of the linear regression model and the 95% confidence band are shown. Given are the P values of the statistical analyses.

grisea exhibited highest growth rates at the highest tested photon flux densities (50–100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). A strain isolated in thermal springs at Piestany, Czechoslovakia, did not show light saturation of growth until 50–100 W m^{-2} , which approximates to 200–500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (conversion factor = 4.2 according to Morel & Smith 1974; Pekarkova *et al.* 1988).

In most laboratory studies, a single environmental parameter is imposed and effects on algal growth are analysed. However, adverse abiotic factors are almost never present alone in natural habitats of benthic marine or estuarine algae. For example, high light stress caused by low tide is often accompanied by UVB radiation and temperature stress, and may also induce osmotic disturbance. Under conditions of multiple stresses, the negative effects on the alga's performance often interact in an additive or antagonistic manner, i.e. differ from those under a single adverse environmental condition. Interactive effects between temperature and PFD, and between salinity and PFD were clearly present in *D. grisea*. At suboptimal salinities and temperatures, maximal growth rates were shifted to lower PFD, and growth was considerably reduced at 50 and 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Thus, interactions between the respective two factors were additive. An increasing number of studies examined the interactive effects of multiple stressors on micro- and macroalgae during the last decade. However, only a few reports deal with growth as investigated in this paper. Additive effects of temperature and PFD on growth have been described for two marine diatoms (Harrison *et al.* 1993), of temperature and salinity in the red macroalga *Heterosiphonia japonica* (Bjaerke & Rueness 2004), of salinity and high nutrient load in the seagrass *Zostera marina* (van Katwijk *et al.* 1999), of UVB radiation and nutrient limitation in the chlorophyte microalga *Dunaliella tertiolecta* (Shelly *et al.* 2005), and of UVB radiation and temperature in the intertidal macroalga *Alaria marginata* (Hoffman *et al.* 2003). However, a lack of additive effects, i.e. no increased susceptibility to UVB stress under nutrient limitation has been reported for growth in natural populations of periphytic diatoms (Bothwell *et al.* 1993) and in the green alga *Selenastrum capricornutum* (Veen *et al.* 1997). As it has been emphasised by Davison & Pearson (1996) and Franklin & Forster (1997), the investigation of the response of algae to multiple stresses is an essential requirement for estimating effects of global climate change on marine plants.

The lack of growth of *Dixoniella grisea* cultivated at 5°C at 50–100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ is presumably based on photoinhibition or even damaged photosynthetic apparatus due to excessive light. Physiological studies showed that chilling temperatures increase high PAR stress by reducing photoprotective features in higher plants (Huner *et al.* 1993), *Chlorella vulgaris* (Maxwell *et al.* 1994) and *Ulva rotundata* (Henry & Ramus 1989; Franklin 1994). Chilling temperatures hamper photosynthesis due to reduced activities of key enzymes in the Calvin cycle (Holaday *et al.* 1992) and increased photoinhibition (Öquist *et al.* 1987). Low temperatures slow recovery from photoinhibitory damage, possibly by decreasing the rate of repair of the D1 protein in photosystem II (Greer *et al.* 1986; Franklin 1994). In addition, cool temperatures slow down formation of zeaxanthin by de-epoxidation of xanthophyll cycle components, a process linked to increased non-radiative dissipation of excess energy in photosystem II (Bilger & Björkman 1991).

Accordingly, decreased growth rates of *D. grisea* cultivated at 20 and 33 psu at 50–100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ are presumably based on lower photosynthetic rates due to enhanced photoinhibition. Photoinhibition caused by excessive light is substantially enhanced upon salinity stress. This is reported for the green alga *Chlamydomonas reinhardtii* (Neale & Melis 1989) or the seagrass *Halophila ovalis* (Ralph 1999). Enhanced susceptibility arises due to the lower light-saturated rates of photosynthesis under salinity stress and due to a salinity-induced inhibition of the repair of cells from the photodamage.

At optimal temperature and low PFD conditions, *D. grisea* is well adapted to a broad salinity range between 5 and 50 psu. Intracellular LMWC contents were analysed by HPLC in ethanol extracts of *D. grisea* acclimated to salinities between 5 and 60 psu. Using ^{13}C NMR analysis, it could be verified that mannitol is the main LMWC synthesised by this species supporting an earlier result of Karsten *et al.* (1999). The data shown, however, represent the first experimental proof for a linear increase of the intracellular mannitol concentration as a function of salinity in a primitive red alga. Mannitol concentrations increased from 2 to 52 $\mu\text{mol g}^{-1} \text{dw}$ between 10 and 60 psu. Therefore it is reasonable to assume that this polyol plays an important role in osmotic acclimation in *D. grisea* as it was demonstrated for the florideophycean genus *Caloglossa* (Karsten *et al.* 1992). It can be further assumed that mannitol has two functions in *D. grisea*, first as an osmolyte and second as a compatible solute, i.e. protecting enzymes under hyperosmotic stress (Karsten *et al.* 1996a). However, despite mannitol accumulation, growth rates decreased considerably in *D. grisea* at salinities > 25 psu. Synthesis of organic osmolytes represents an energy cost and causes a drain on metabolites needed for cell growth, i.e. strong accumulation of mannitol is accompanied with decreased growth.

Intracellular mannitol concentration differs considerably between red algae and even between the related rhodellophycean genera *Rhodella* and *Dixoniella* (Table 2). The mannitol content of different *Rhodella* species is 40-fold higher than that of *D. grisea*. A reason for this difference is the extremely thick mucilaginous sheath (15–20 μm thickness) surrounding each *Dixoniella* cell, which has a typical diameter (excluding sheath) of 8.5–17.0 μm (unpublished data; Scott *et al.* 1992). Thus, the volume of the sheath contributes up to > 80% of the total cell volume (including the sheath). Depending on cell age and physiological condition, up to 75% of total dry biomass could be fixed in the mucilaginous sheath (Pekarkova *et al.* 1988). Given data therefore underestimate mannitol concentration of the cells (excluding the sheath) with the factor 4. Hence, dry weight represents an insufficient reference parameter to calculate the amount of LMWC compounds in *D. grisea*. Alternatively, cell volume or intracellular protein content could be used. *Dixoniella* cultures growing at 33 psu had a mannitol concentration of 19 $\mu\text{mol g}^{-1} \text{dw}$. As 1 g dried culture of logarithmically growing algae corresponded to 248 mm^3 total cell volume, this mannitol concentration is equivalent to 77 mol m^{-3} cell volume. However, mannitol is presumably not evenly distributed throughout the cell but localised in the cytoplasm as it has been proposed for marine brown algae (Reed *et al.* 1985) and the red alga *Caloglossa leprieurii* (Mostaert *et al.* 1996). Therefore, mannitol may account for an even larger fraction of the total osmotic pressure

Table 2. Intracellular mannitol concentrations in different red algal species under culture conditions (c) and from field collections (f) at the respective extracellular salinity.

Organism	Source	Salinity (psu)	Mannitol ($\mu\text{mol g}^{-1}$ dw)
<i>Dixoniella grisea</i> (c)	This study	10–60	2–52
		33	19
<i>D. grisea</i> (c)	Karsten et al. 1999	30	12
<i>Rhodella violacea</i> (c)	Karsten et al. 1999	30	848
<i>R. reticulata</i> (c)	Karsten et al. 1999	30	158
<i>R. maculate</i> (c)	Karsten et al. 1999	30	206
<i>Caloglossa lepriurii</i> (c)	Karsten et al. 1992	35	202
<i>C. lepriurii</i> (c)	West et al. 1992	35	360–396
<i>C. adnata</i> (c)	Karsten & West 1993	5–60	200–800
		30	550
<i>C. ogasawaraensis</i> (c)	Karsten & West 1993	5–60	100–1000
		30	400
<i>C. lepriurii</i> (c)	Karsten & West 1993	5–60	200–650
		30	350
<i>C. ogasawaraensis</i> (c)	Karsten & West 1993	5–60	50–750
		30	250
<i>C. stipitata</i> (c)	Karsten & West 1993	5–60	25–1000
		30	300
<i>C. apomeiotica</i> (c)	Karsten & West 1993	5–70	150–750
		32	300
<i>C. lepriurii</i> (c) ¹	Mostaert et al. 1995	0.2–70	100–408
		35	141
<i>C. lepriurii</i> (f)	Pedroche et al. 1995	32	235
<i>C. lepriurii</i> (f)	Pedroche et al. 1995	32	308
<i>C. ogasawaraensis</i> (f)	Pedroche et al. 1995	2	61
<i>C. lepriurii</i> (f)	Pedroche et al. 1995	2	171
<i>C. lepriurii</i> (f)	Pedroche et al. 1995	20	431
<i>C. stipitata</i> (f)	Pedroche et al. 1995	20	233
<i>C. lepriurii</i> (f)	Pedroche et al. 1995	28	179

¹ Values are recalculated based on 70–80% cell water content.

in the cell compartment. Thus, mannitol makes a significant contribution to the water balance of the cells.

In conclusion, *D. grisea* is a warm-temperate, euryhaline organism. The synthesis of the polyol mannitol presumably supports this broad salinity range in its function as an osmolyte and a compatible solute. However, *D. grisea* grows best at brackish salinities < 25 psu. At otherwise optimal conditions, maximal growth is achieved at moderate PFD levels (50–100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). However, interactive effects between salinity and PFD and temperature and PFD are present. The results are consistent with the species distribution and the conditions of the natural habitat where *D. grisea* strain 2320 was isolated (Mississippi Sound, USA).

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