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Growth, photosynthesis and fertility of *Chara aspera* under different light and salinity conditions

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Abstract

The main aim of this study was to investigate if the wide salinity range of *Chara aspera* C.L. Willdenow is caused by acclimation capabilities alone, or if freshwater and brackish water populations have different salinity optima. In an outdoor experiment, plants collected from one freshwater and one brackish water site (about 8 PSU) in southern Sweden were incubated under different conditions of salinity and light. Elongation rate, final biomass, photosynthesis parameters, pigment composition as well as development of gametangia were determined.

Plants collected from freshwater all died at the highest salinity condition tested (20 PSU) possibly due to salinity stress. For most parameters tested, these plants performed best at low salinity. Thus, maximum photosynthetic rate in the absence of photoinhibition (P_{max}) and onset of light saturation and index of light acclimation (E_k)were higher at 0 PSU compared to 5 and 10 PSU. The number of gametangia tended to decrease with increasing salinity. In contrast, plants collected from brackish water performed best at intermediate salinities. Thus, final ash-free dry weight (AFDW) was lower at 20 PSU compared to all other salinity conditions and tended to be lower at 0 PSU compared to 5 and 10 PSU. P_{max} and E_k as well as the number of gametangia had maximum values at intermediate salinities (5 and 10 PSU). Also pigment composition was influenced by salinity. In both groups, chlorophyll a/chlorophyll b quotients increased with increasing salinity. Chlorophyll a/carotenoid quotients decreased with increasing salinity in the freshwater population, but had maximum values at intermediate salinities in the brackish water population.

Apart from salinity, light influenced the physiological performance of both populations. As expected, P_{max}, E_k and chlorophyll a/chlorophyll b quotients increased with increasing irradiance during the incubation in both populations, while chlorophyll a/carotenoid quotients decreased. Shoot elongation increased with decreasing irradiance in both populations. This effect was less pronounced at higher salinities, indicating that shoot elongation may be hampered in brackish water.

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Our results indicate that *C. aspera* growing in freshwater has optimum conditions at 0 PSU, while plants collected from brackish water have their optimum at 5–10 PSU. As all parameters were recorded after several weeks of incubation, we suggest that genetic differences between the populations rather than physiological acclimation to the field conditions are responsible for these different salinity optima.

Keywords: Charophyta; Salinity; Light; Photosynthesis; Growth

1. Introduction

Plants adapted to brackish water are able to regulate their turgor according to the salinity conditions. Among characeans, this ability is widespread; most ancestors of modern characeans are suggested to have been adapted to conditions of higher salinity (Schudack, 1993). This is supported by observations that the same mechanism of turgor pressure regulation (K^+ accumulation) occurs in different taxa of euryhaline characeans (*Lamprothamnium*, *Chara*, *Tolypella*), suggesting that this mechanism already existed before the main lineages of modern charophytes separated (Winter et al., 1996). Charophytes thus belong to the group of K^+ algae, while Na^+ accumulation is an alternative mechanism of turgor pressure regulation among algae (Kirst and Bisson, 1979).

Possibly, recent brackish water species among charophytes originate from euryhaline species but lost their ability for complete turgor regulation (Winter et al., 1996). Plants belonging to this group of mesohaline species regulate their turgor by accumulation of both ions (mainly Na^+, K^+, Cl^-) and sucrose, but are not able to keep pace with Na^+ increase at higher salinities. Thus, these species are restricted to about 15–20 PSU (Winter and Kirst, 1992; Winter et al., 1996). *Chara aspera*, together with *Chara canescens* Lois, apply this "incomplete" turgor regulation (Winter and Kirst, 1991, 1992). In the laboratory, *C. aspera* regulated its turgor perfectly at salinities up to 8 PSU. At salinities between 8 and 18 PSU, turgor pressure as well as K^+:Na^+ ratio decreased slightly (Winter and Kirst, 1992). In the Baltic Sea, this species can be found at salinities of up to 20 PSU. In Denmark, *C. aspera* has been found in localities where the salinity at least temporarily reaches levels of 11,000 mg l^{-1} Cl^- (about 20 PSU; Olsen, 1944). Along the west coast of Sweden, *C. aspera* has regularly been found at salinities of up to 15 PSU and occasionally at localities where the salinity probably exceeds 20 PSU at least temporarily (Blindow, 2000). However, the species can also be found in low-salinity conditions in the innermost parts of the Bothnian and Finnish Bays. Furthermore, *C. aspera* is common in freshwater where it mainly occurs in calcium-rich water, but also in oligotrophic soft-water lakes (Hasslow, 1931; Stålberg, 1939; Blindow, 2000). In the Baltic Sea region, *C. aspera* covers the widest salinity range among charophytes (Blindow, 2000).

Generally, the taxonomy of characeans has been subject to considerable debate and changes (Wood, 1962, 1965; Proctor, 1975). Until today, very little is known about the number of "biological species" behind the single taxonomic entities described. Reproductive isolation among different populations has been found within several "species".

Consequently, these taxonomic entities have been defined as "species complexes" (Proctor, 1971; Grant and Proctor, 1972). There are some indications that *C. aspera* may represent such a "species complex". Thus, reproductive isolation has been found between geographically distant populations of *C. aspera* (Croy, 1982). Furthermore, fresh- and brackish-water forms of *C. aspera* are morphologically different from each other and have been distinguished as "f. stagnales" and "f. marinae", respectively (Hasslow, 1931). The main aim of our study was thus to investigate if the wide salinity range of *C. aspera* is caused by acclimation capabilities alone, or if freshwater and brackish water populations have different salinity optima which would indicate genetic differences between these populations.

Charophytes can form dense vegetation both in freshwater lakes (Blindow, 1992a; van den Berg et al., 1998) and sheltered brackish water bays (Munsterhjelm, 1997), but disappear during eutrophication (Kohler et al., 1971; Lindner, 1978; Lang, 1981). Charophytes are more sensitive to increased nutrient loading than most other submerged macrophytes and have thus been used as bio-indicators for oligo- to mesotrophic conditions (Krause, 1981). Light limitation by microalgae is the most probable factor responsible for the decline of charophytes in nutrient-enriched water (Blindow, 1992b). In shallow water ecosystems, this decline is not gradual, but occurs rapidly at a certain critical turbidity (Scheffer et al., 1993). As turgor regulation in *C. aspera* is partly reached by increased intracellular sucrose concentrations (Winter and Kirst, 1992), increasing salinity may be connected to increased energy demand. This should theoretically cause a higher light compensation point for growth and thus a lower critical turbidity in brackish water compared to freshwater ecosystems. According to our knowledge, however, this relationship has not been studied yet for submerged macrophytes.

In order to compare the physiological performances of freshwater and brackish water populations of *C. aspera* and to investigate the combined effect of light and salinity on these plants, growth and photosynthesis as well as development of gametangia of plants collected from one freshwater and one brackish water site were measured under different conditions of light and salinity in an outdoor experiment.

2. Material and methods

2.1. Site description

C. aspera was collected in mesotrophic, calcium-rich Lake Krankesjön, south Sweden (55°42′N, 13°29′E) in a monospecific, dense mat at about 35 cm depth during 13 May 2001. The plants were about 5 cm long and still sterile. For information on morphometry and water chemistry of Lake Krankesjön, see Blindow et al. (2000).

In Höllviken, a bay of the Baltic Sea in south Sweden (55°25′N, 12°56′E), *C. aspera* was collected on 11 and 12 June 2001. The plants grew scattered in a mixed vegetation together with *C. baltica* Bruz., *C. canescens*, *Tolypella nidifica* v. Leonh and *Zannichellia major* Boenn on 50–100 cm depth. During the sampling occasion, *C. aspera* plants were about 5 cm long. Most plants were sterile, but single fertile plants, both males and females, were found. The salinity was 8.3 PSU. The distance between the two sampling sites is about 50 km.

2.2. Description of the experiment

In the laboratory, apices of *C. aspera* were cut 1–2 days after the sampling some millimetre below an internode to a length of about 3 cm. Each apex was planted in a 400 ml beaker containing 1 cm of sediment from Lake Krankesjön (mixed and sieved, 2.5 mm mesh size) and water. For freshwater treatments, filtered (63 μm mesh size) water of Lake Krankesjön was used. For the salinity treatments, NaCl, Na_2SO_4, $MgCl_2$, $MgSO_4$, $CaCl_2$ and KCl were added to water from Lake Krankesjön corresponding to the composition of macroconstituents in natural seawater (containing per l: 19.8 g Cl^-, 11.0 g Na^+, 2.8 g SO_4^{2-}, 1.3 g Mg^{2+}, 0.43 g Ca^{2+}, 0.41 g K^+) and to the salinity condition in question. In total, 96 apices were planted (6 replicates for each of the 4 salinity and the 4 light conditions). To avoid damage of the plants which were used for the experiments, an additional 30 apices were cut for the determination of fresh weight, dried externally by means of household tissue.

After 3–6 days of precultivation in the laboratory (room temperature, low light), the start length of each plant was measured, and the beakers were transferred to an outdoor experimental set-up. For each light condition, all 24 beakers were placed in one compartment. Three of the four compartments were covered with "neutral density" foils which reduced the incoming light equally for the whole PAR and UV spectrum to 7, 14 and 56% of incident solar irradiance, respectively. The compartment for the 100% light treatment was left uncovered or covered with a transparent plastic foil during rainy conditions, respectively. All four compartments were connected with each other and filled with water which was mixed by pumps during sunny conditions to avoid temperature differences among the treatments.

Every day during the experiment, the length of each plant was measured, and all beakers in each compartment were rotated to avoid different shading conditions related to the position in the compartments. Once per day, the water level in each beaker was filled up with distilled water to replace losses due to evaporation.

The experiments were terminated 30–33 days (plants collected from freshwater) and 20–23 days (plants from brackish water), respectively, after the start of the incubation. For all fertile plants, the number of gametangia was counted under a stereomicroscope. The plants were divided into two groups. For each salinity and light condition, three plants were used for measurement of photosynthesis main parameters and pigment concentrations. The other three plants were used for the determination of dry and ash-free dry weight. Fresh weight of all plants was determined after the termination of the photosynthesis measurements as described above. Dry weight was determined after 4 h at 105 °C, ash weight after 1 h ashing at 525 °C. Elongation rate was calculated as daily length increase for the period of linear growth (4–14 days) during the experiment. For plants originating from freshwater, ash-free dry weight (AFDW) data of the 7 and 14% light treatments were combined ("low light") as well as values of the 56 and 100% light treatments ("high light") because of the low sample sizes due to some missing values.

2.3. Photosynthesis measurements

Net oxygen exchange rates at nine increasing light intensities from 0 to approximately 2000 μmol photons m^{-2} s^{-1} for each of the 16 irradiance-salt treatments were determined by using a computer-controlled Light Dispensation System (MK2, ILLUMINOVA, Sweden,

described in Wolfstein and Hartig, 1998). Temperature was set according to the respective temperature in the beaker. Photosynthetic parameters maximum photosynthetic rate in the absence of photoinhibition (P_{max}), α (light dependency of photosynthesis at limiting irradiances) and β (slope of decay of photosynthesis rates at oversaturating intensities) were derived by a fitting procedure using an iterative exponential regression with the equation given in Walsby (1997). E_k, the onset of light saturation and index of light adaptation, was calculated by dividing P_{max} by α (Talling, 1957). Several investigations have shown that E_k can be used to describe the photoacclimation status of algae (Talling, 1957; Henley, 1993; Falkowski and Raven, 1997).

2.4. Determination of pigment concentrations

The chlorophyll and carotenoid contents of the algae were determined spectrophotometrically after extraction in 3 ml of DMF (*N*,*N*-dimethylformamide) in darkness at 4 °C for ca. 12 h. Absorption was measured with an UV-Vis spectrophotometer (Specord M42, Carl Zeiss Jena, Germany). Pigment contents ($\mu g\,ml^{-1}$) of the extracts were calculated from absorbance spectra according to the equations derived by Porra et al. (1989).

2.5. Statistical analysis

All data sets were transformed to achieve non-significant heteroscedasticity (*F*-test). Transformations used were $\ln(Y)$ for AFDW, P_{max} and E_k, $\ln(Y + 1)$ for chlorophyll a/chlorophyll b, chlorophyll a/carotenoid and number of gametangia, $\ln(Y + 0.01)$ for elongation rate and arcsin (root *Y*) for proportion of ash weight in total dry weight. Apart from the occasions when homoscedasticity could not be achieved even after a transformation, a two-way analysis of variance (ANOVA) was performed on each transformed data set. Factors analysed were salinity and light. Scheffe's post hoc tests were used to determine differences between groups. Confidence intervals given in Fig. 5 and Figs. 7–10 are calculated by the TC3D software (Jandel).

3. Results

3.1. Growth

All plants from the brackish water population survived until the end of the experiment. However, all plants originating from freshwater that were exposed to 20 PSU died within the first days of the experiment, possibly due to salinity shock. Furthermore, one plant exposed to 5 PSU and one plant exposed to 10 PSU did not survive. Overall weight gain (fresh weight) during the cultivation period was about 56 and 110% for plants collected from freshwater and brackish water, respectively.

End-AFDW of plants collected from freshwater did not differ significantly among the individual salinity and light treatments. Plants collected from brackish water had their highest end-AFDW at 5 and 10 PSU, while AFDW was significantly lower at 20 PSU and had a tendency to be lower at 0 PSU (Fig. 1, Tables 1 and 2).

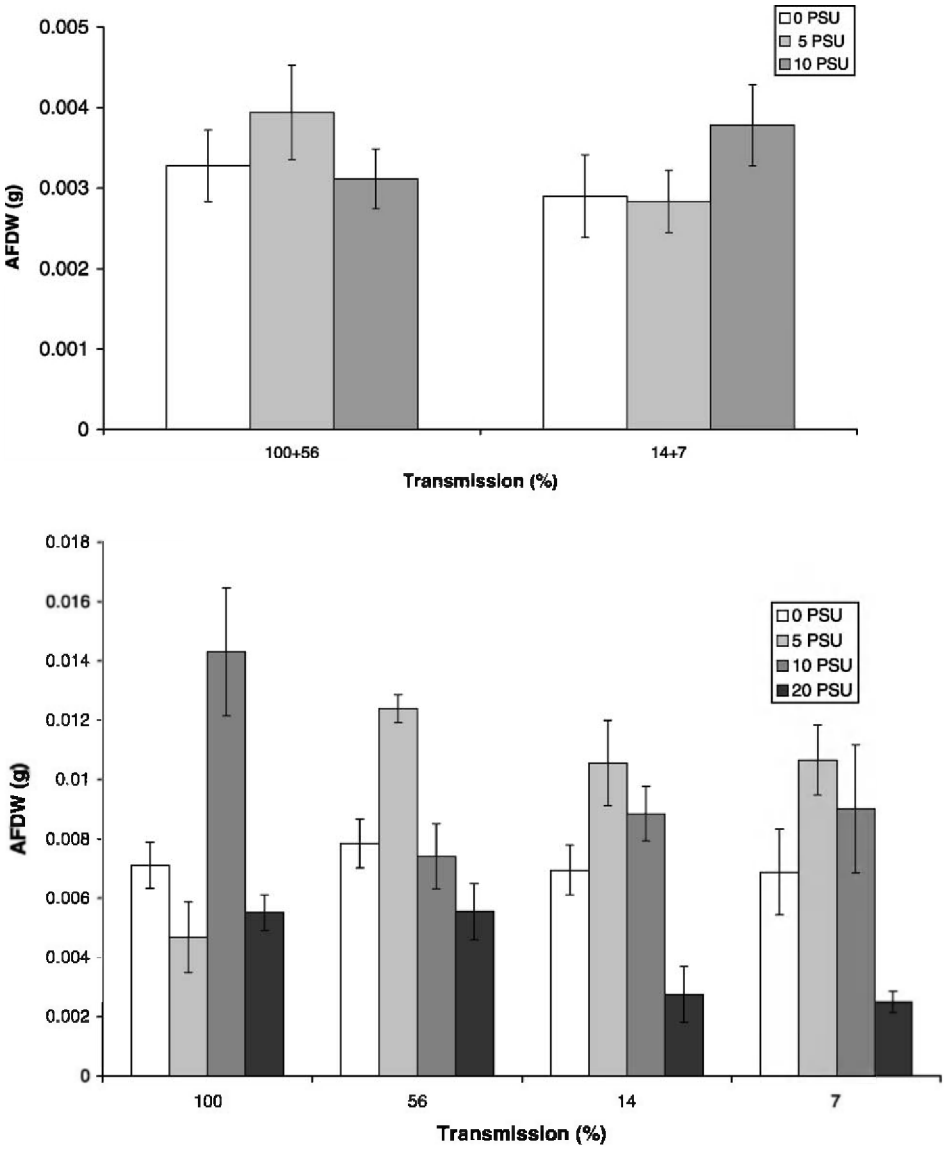

Fig. 1. End-AFDW of *Chara aspera* collected from freshwater (above) and brackish water (below) for the different salinities and light treatments. Mean values ± S.E. are given.

Plants collected from freshwater had a higher ash content (about 75–90% of dry weight) than plants collected from brackish water (about 50–75% of dry weight). For both populations, values for ash content at the end of the experiment were higher at 0 PSU compared to 5 and 10 PSU. Plants collected from brackish water had higher ash content at 20 PSU compared to 5 and 10 PSU (Tables 1 and 2, Fig. 2).

Table 1
Main ANOVA results: significances (P-values) of the effects of salinity, light and their interaction, respectively, on different parameters

Parameter	Origin of plants	Salinity	Light	Salinity × light
AFDW	Freshwater	n.s.	n.s.	n.s.
	Brackish water	<0.0001	n.s.	0.0004
Elongation rate	Freshwater	n.s.	0.0023	0.0014
	Brackish water	<0.0001	<0.0001	n.s.
Ash weight (% d.w.)	Freshwater	0.0044	n.s.	n.s.
	Brackish water	0.0002	n.s.	0.0046
P_{max}	Freshwater	<0.0001	<0.0001	<0.0001
	Brackish water	<0.0001	<0.0001	<0.0001
E_k	Freshwater: no homoscedasticity !			
	Brackish water	0.041	<0.0001	n.s.
α	Freshwater and brackish water: no homoscedasticity!			
Chlorophyll a/chlorophyll b	Freshwater	<0.0001	<0.0001	n.s.
	Brackish water: no homoscedasticity !			
Chlorophyll a/carotenoid	Freshwater	0.0005	<0.0001	n.s.
	Brackish water	<0.0001	<0.0001	<0.0001
Number of gametangia	Freshwater	n.s.	n.s.	n.s.
	Brackish water	<0.0001	0.0086	0.0132

In both groups, shoot elongation increased with decreasing irradiances, but was also influenced by salinity (Tables 1 and 2, Fig. 3). At high salinities, the increase of elongation rate at low irradiances was much lower or not obvious at all (Fig. 3). Thus, the interaction effect from light × salinity was significant for plants collected from freshwater (Table 1). For plants originating from brackish water, elongation rates were significantly lower at 20 PSU compared to all other salinities and lower at 10 PSU than at 0 PSU (Tables 1 and 2, Fig. 3). For both populations, ratios of final length and end-AFDW were significantly higher at 0 and 5 PSU, and low light compared to all other treatments (Fig. 4).

3.2. Photosynthesis

In both populations, salinity and light had a distinct effect on P_{max} (Tables 1–3). For the freshwater population, P_{max} increased with increasing irradiance, but decreased with increasing salinity. Plants originating from brackish water had higher P_{max}-values when grown at 100% transmission compared to all other irradiances, and higher P_{max}-values at 5 and 10 PSU compared to 0 and 20 PSU (Table 2). P_{max} was also strongly affected by the salinity × light interaction in both populations (Table 1). Thus, increase of P_{max} with

Table 2
Results of Scheffe's post hoc tests

Ash content	AFDW		Ash weight		Shoot elongation		P_{max}		E_k		Chlorophyll a/ chlorophyll b		Chlorophyll a/ carotenoid		Number of gametangia	
	Fresh	Brackish	Fresh	Brackish	Fresh	Brackish	Fresh	Brackish	Fresh	Brackish	Fresh	Brackish	Fresh	Brackish	Fresh	Brackish
Light																
7–14%	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.	–	***	n.s.	–	***	***	n.s.	n.s.
7–56%	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	n.s.	–	***	***	–	***	***	n.s.	n.s.
7–100%	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	***	–	***	***	–	***	***	n.s.	n.s.
14–56%	n.s.	n.s.	n.s.	n.s.	*	***	***	n.s.	–	***	***	–	***	***	n.s.	n.s.
14–100%	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	***	–	***	***	–	***	***	n.s.	n.s.
56–100%	n.s.	n.s.	n.s.	n.s.	n.s.	**	***	***	–	***	*	–	***	***	n.s.	**
Salinity																
0–5 PSU	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	–	*	n.s.	–	***	***	n.s.	n.s.
0–10 PSU	n.s.	n.s.	**	***	n.s.	***	***	**	–	***	***	–	***	***	n.s.	n.s.
0–20 PSU	–	***	–	n.s.	–	***	–	n.s.	–	***	–	–	–	n.s.	–	***
5–10 PSU	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.	–	*	***	–	n.s.	n.s.	n.s.	n.s.
5–20 PSU	–	***	–	n.s.	–	***	–	***	–	n.s.	–	–	–	***	–	***
10–20 PSU	–	***	–	*	–	***	–	***	–	n.s.	–	–	–	***	–	***

The tests were performed for all possible combinations of light and salinity conditions, respectively, when ANOVA showed a significant effect of this factor. n.s.: test not performed or not significant ($P > 0.05$); *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; –: missing value, or ANOVA not performed.

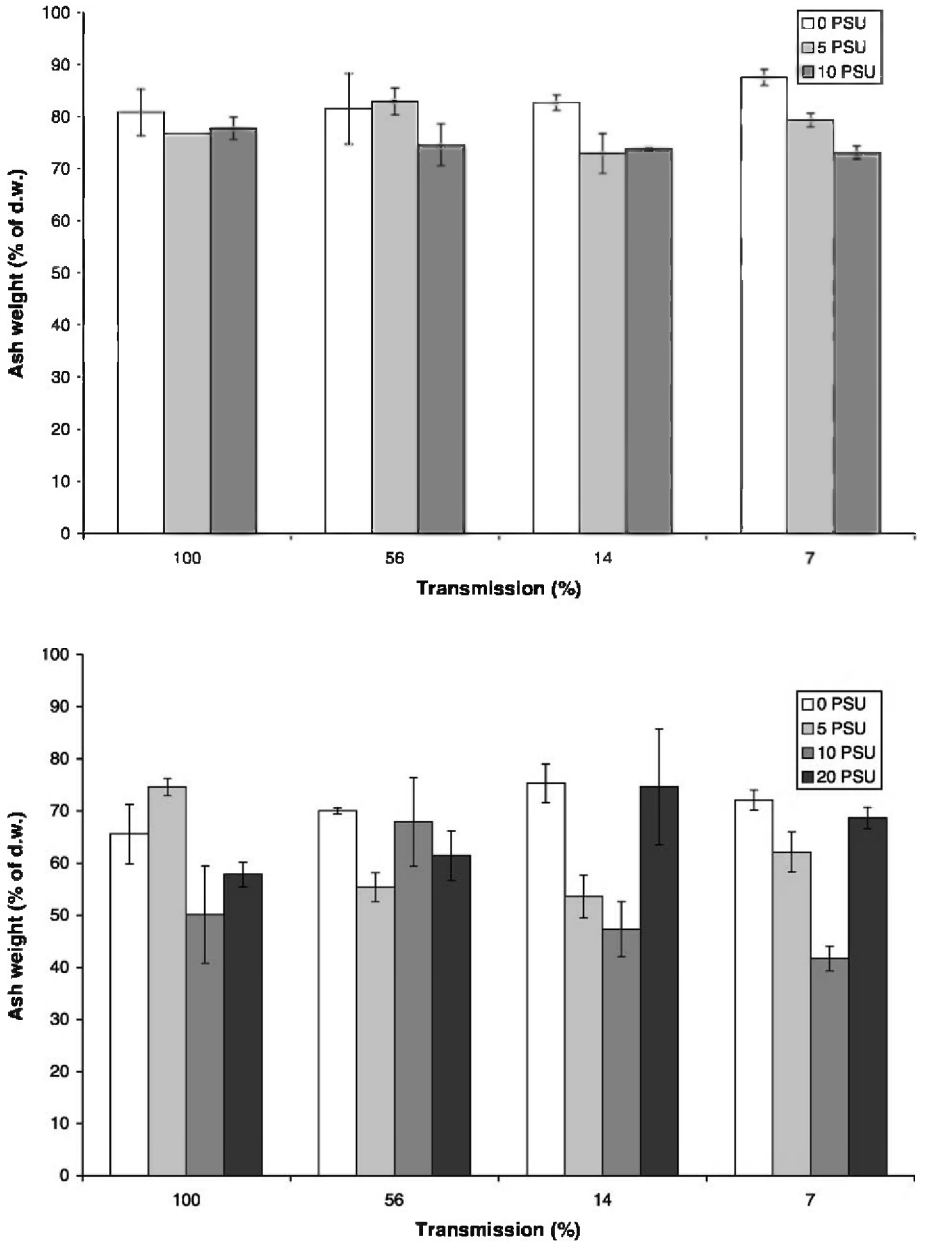

Fig. 2. Ash weight of *Chara aspera* collected from freshwater (above) and brackish water (below) for the different salinities and light treatments. Mean values ± S.E. are given.

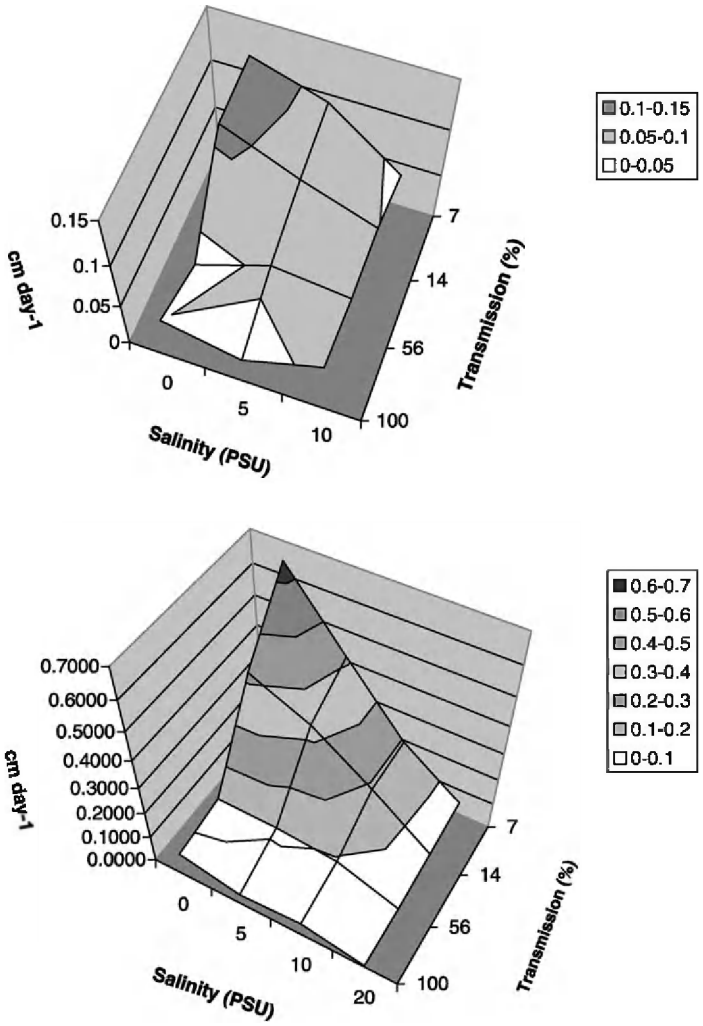

Fig. 3. Shoot elongation (centimetre per day) for *Chara aspera* collected from freshwater (above) and brackish water (below) for the different salinities and light treatments.

increasing irradiance was most distinct at higher salinities (10 PSU for plants originating from freshwater, 20 PSU for plants collected from brackish water) (Table 3).

In all treatments except for the 10 PSU treatment of plants originating from freshwater, the increase of irradiance was accompanied by a decreasing value of α (Table 3). In contrast to P_{max} and α-values, the dependency of values for E_k (the light saturation point of photosynthesis) on treatment conditions differed considerably between plants originating from freshwater and brackish water, respectively. For plants originating from freshwater, E_k-values were influenced by both irradiance and salinity. While E_k-values increased with

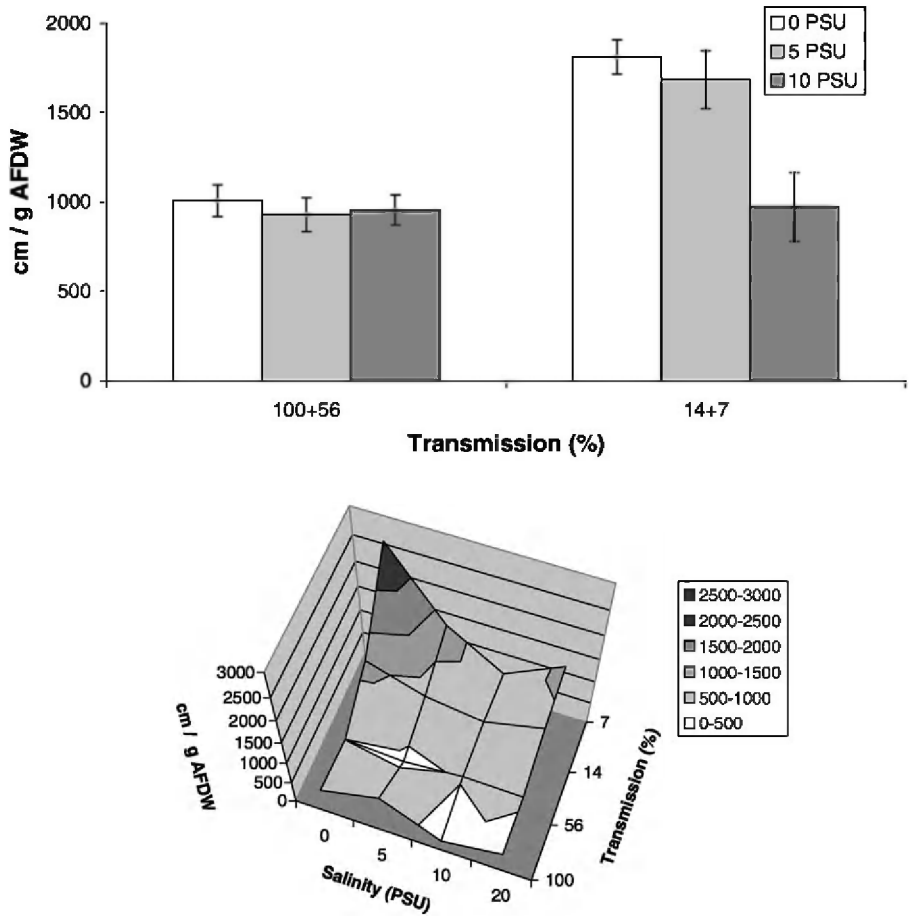

Fig. 4. Final length/AFDW quotient of *Chara aspera* collected from freshwater (above: mean values ± S.E. are given) and brackish water (below) for the different salinities and light treatments.

increasing irradiance in the 0 and 5 PSU treatments, they were low in all 10 PSU treatments (Fig. 5). For the brackish water group irradiance was the main determinant for E_k, which increased with increasing irradiance of the treatments (Tables 1 and 2, Fig. 5). Also salinity had a significant effect on E_k-values of the brackish water population with the highest values recorded at 5 and 10 PSU and the lowest at 0 PSU.

When all light treatments were combined, plants collected from freshwater had significantly ($P = 0.0190$, Mann–Whitney U-test) higher P_{max} at 0 PSU than plants collected from freshwater. For the other salinities tested, P_{max} did not differ significantly between populations. For determinations of E_k, there was a non-significant tendency for higher values at 0 PSU among the plants collected from freshwater. At 10 PSU, plants collected from freshwater had significantly ($P = 0.0022$, Mann–Whitney U-test) lower E_k than plants collected from brackish water (Fig. 6).

Table 3
Photosynthetic main parameters of *Chara aspera* originating from freshwater and brackish water after about 20 and 30 days of incubation, respectively, at different salinity and light conditions

Salinity	Irradiance	Brackish water group		Freshwater group	
		P_{max}	α	P_{max}	α
20	7	140 (4)	1.00 (0.130)	n.d.	n.d.
	14	159 (16)	0.87 (0.026)	n.d.	n.d.
	56	178 (7)	0.81 (0.044)	n.d.	n.d.
	100	262 (18)	0.72 (0.056)	n.d.	n.d.
10	7	192	1.55	110 (7)	1.00 (0.028)
	14	178 (15)	1.01 (0.063)	179 (4)	1.42 (0.037)
	56	209 (18)	0.86 (0.082)	275 (20)	1.99 (0.132)
	100	282 (18)	0.76 (0.019)	304 (10)	1.73 (0.084)
5	7	212 (9)	1.84 (0.287)	163 (11)	1.40 (0.088)
	14	206 (17)	1.21 (0.086)	199 (8)	1.59 (0.029)
	56	209 (9)	0.86 (0.054)	204 (6)	0.83 (0.067)
	100	289 (8)	0.74 (0.026)	299 (15)	0.74 (0.050)
0	7	245 (26)	1.99 (0.080)	185 (3)	1.57 (0.034)
	14	181 (34)	1.31 (0.283)	245 (13)	1.31 (0.044)
	56	131 (26)	0.86 (0.295)	217 (8)	0.94 (0.039)
	100	169 (10)	0.53 (0.047)	399 (15)	0.87 (0.057)

Mean values and standard deviations (in brackets) are given for three measurements of independent individuals (except for 10 PSU, 4% irradiance brackish water group with $n = 1$). Units and abbreviations: salinity (PSU), irradiance (percentage of incident ambient light), P_{max} (mmol O_2 (g chlorophyll a)$^{-1}$ h^{-1}), α (mmol O_2 (g chlorophyll a)$^{-1}$ h^{-1} μmol photons^{-1} m^2 s); n.d.: not determined (Section 3).

Respiration increased considerably with increasing irradiance and salinity for both plants originating from freshwater and brackish water. Respiration rates at low salinities and low light conditions were lower for the freshwater group, but the slope of the increase with increasing irradiance and salinity was much steeper in the freshwater group compared to the brackish water group (Fig. 7).

The dependency of chlorophyll a/chlorophyll b quotients from salinity and irradiance conditions did not differ much between plants originating from brackish water and freshwater. In both groups the quotients were high at high irradiances, indicating small antennae sizes, and increased slightly with increasing salinity. However, the brackish water group generally had lower quotients, indicating larger antennae at all conditions (Fig. 8). For the freshwater group, both salinity and irradiance had a significant effect on chlorophyll a/chlorophyll b quotients (Tables 1 and 2).

In all cases the highest chlorophyll a/carotenoid quotients were found at low irradiances. Differences between the two populations exist with respect to the absolute values. When comparing similar conditions, the freshwater group always exhibited lower chlorophyll a/carotenoid quotients than the brackish water group. While chlorophyll a/carotenoid quotients in algae from brackish water peaked at 5–10 PSU, this quotient declined with increasing salinity for plants originating from freshwater (Fig. 9). For both populations, effects from both salinity and light on the chlorophyll a/carotenoid quotient were highly significant (Tables 1 and 2).

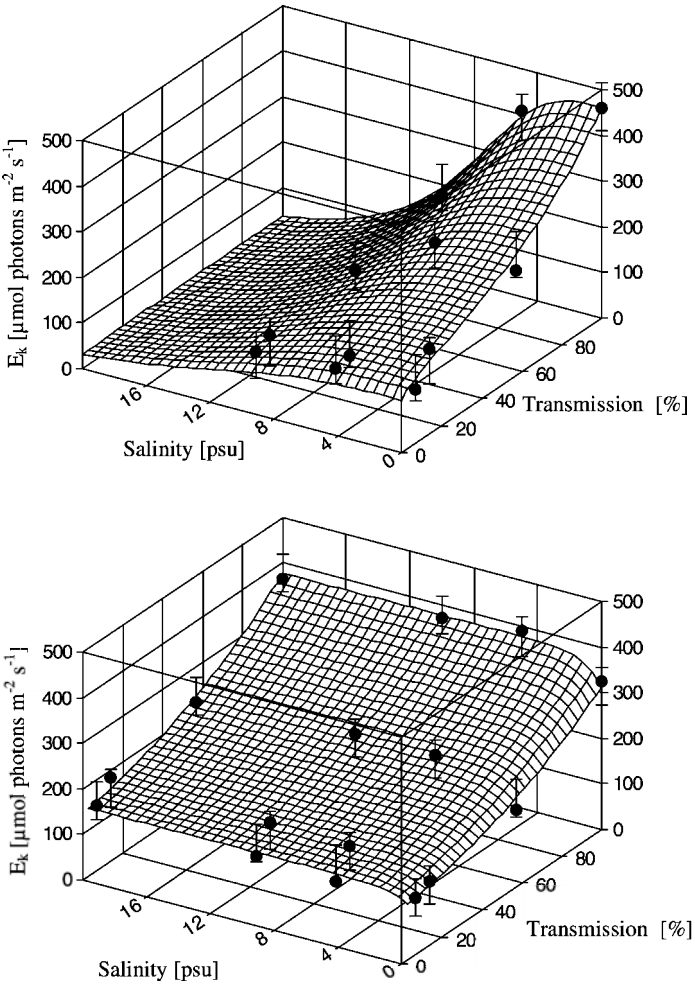

Fig. 5. E_k-values (µmol photons m^{-2} s^{-1}) of *Chara aspera* collected from freshwater (above) and brackish water (below) at different salinities and light treatments. Mean values and confidence intervals (95%) are given.

3.3. Development of gametangia

By the end of both experiments, most plants were fertile. For none of the populations, a significant difference in number of gametangia between females and males could be found (Mann–Whitney *U*-test, $P > 0.05$). Therefore, data for male and female plants were combined in the following analysis.

The number of gametangia per plant was highest at 0 PSU for plants collected from freshwater, but highest at 5–10 PSU for plants collected from brackish water. For both populations, gametangia developed best at 56% light transmission (Fig. 10). However, effects of salinity and light as well as the interaction effect salinity × light were not significant

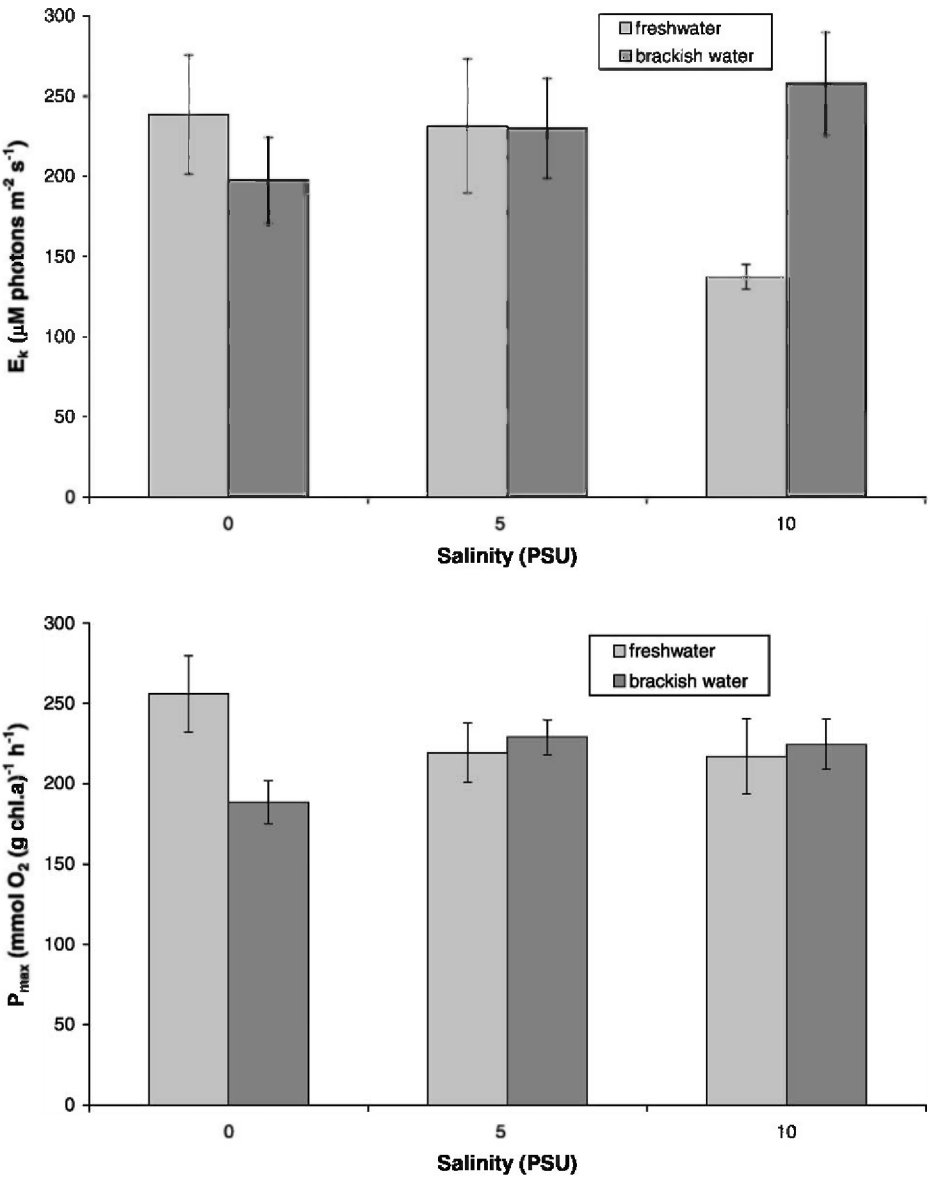

Fig. 6. E_k-values (mmol photons m^{-2} s^{-1}: above) and P_{max} (mmol O_2 (g chlorophyll a)$^{-1}$ h^{-1}: below) of *Chara aspera* collected from freshwater and brackish water for the different salinities combined for all light treatments. Mean values ± S.E. are given.

for plants collected from freshwater. For plants originating from brackish water, both salinity and light had a significant effect on gametangia numbers (Tables 1 and 2). Thus, plants grown at 20 PSU had significantly lower numbers of gametangia than plants from all other salinity treatments. Those grown at 100% light had significantly lower numbers of gametangia

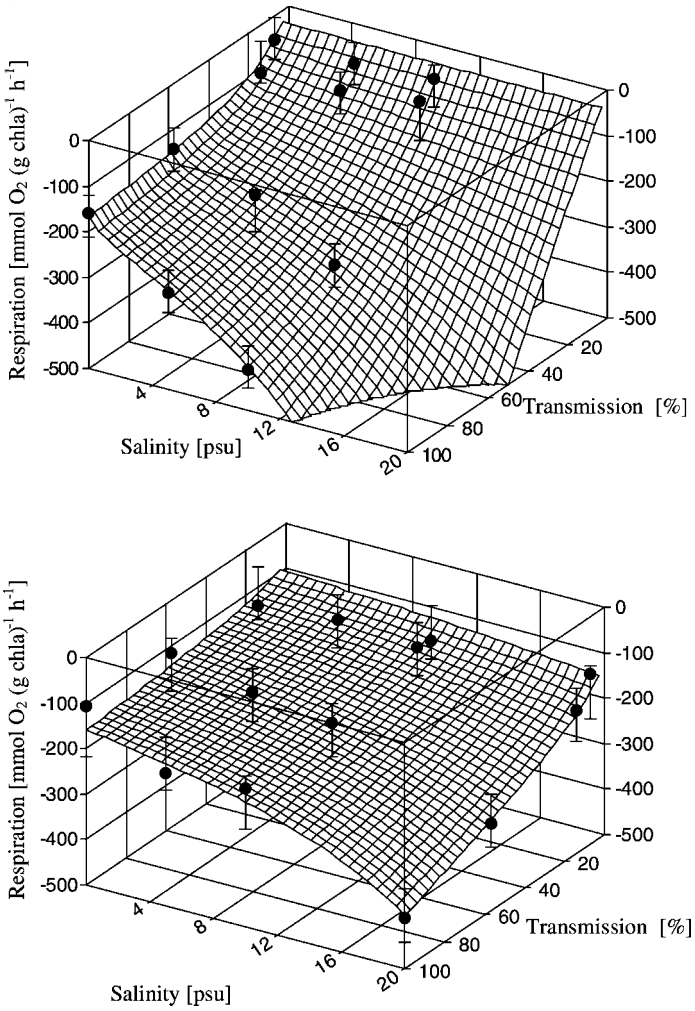

Fig. 7. Respiration (mmol O_2 (g chlorophyll a)$^{-1}$ h^{-1}) of *Chara aspera* collected from freshwater (above) and brackish water (below) at different salinities and light treatments. Mean values and confidence intervals (95%) are given.

than those grown at 56% transmission. Plants grown at 100% light and 20 PSU had no gametangia at all.

4. Discussion

Distinct ecophysiological differences exist between freshwater and brackish water populations of *Chara aspera*. Ritzl (2000) observed that bulbils of *C. aspera* originating from brackish water were able to germinate at salinities between 0 and 7 PSU, while bulbils of

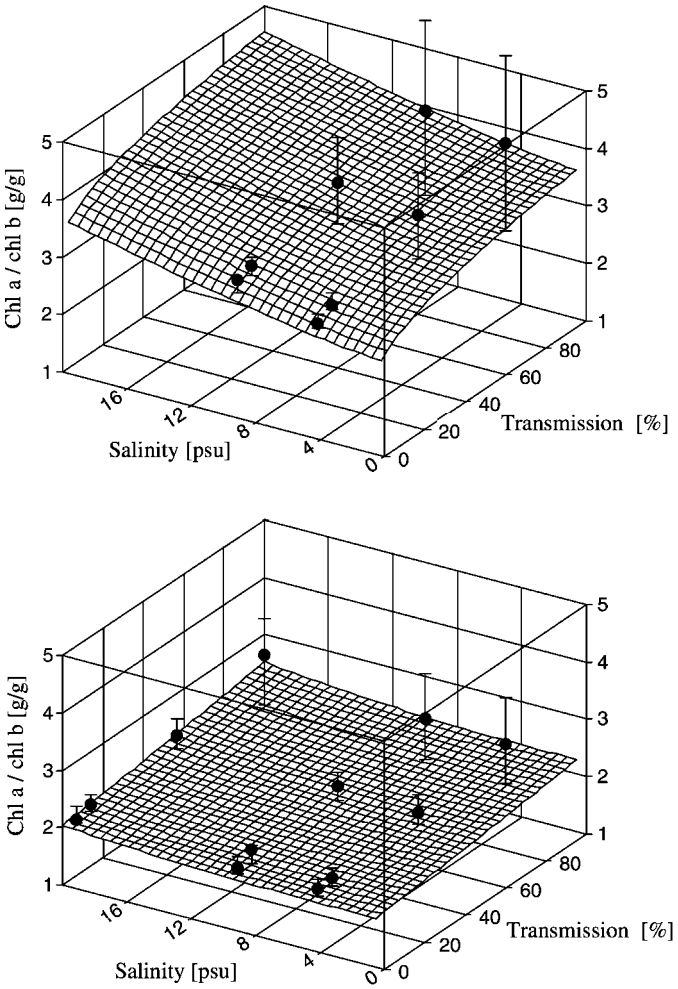

Fig. 8. Chlorophyll a/chlorophyll b quotients of *Chara aspera* collected from freshwater (above) and brackish water (below) at different salinities and light treatments. Mean values and confidence intervals (95%) are given.

freshwater plants failed to germinate at 7 PSU. In the experiments described above, salinity optima of light saturation point of photosynthesis and development of gametangia showed clear differences between the two populations of *C. aspera*. For all three parameters, plants collected from freshwater performed best at 0 PSU, whereas plants collected from brackish water had their optima at 5–10 PSU. A similar tendency was found for P_{max}. For plants collected from brackish water, there was also a tendency, though non-significant, for higher final AFDW at 5 and 10 PSU compared to 0 PSU.

As these comparisons were made after several weeks of incubation, we suggest that the ecophysiological differences between *C. aspera* originating from freshwater and brackish water are not caused by physiological acclimation to the conditions of their natural

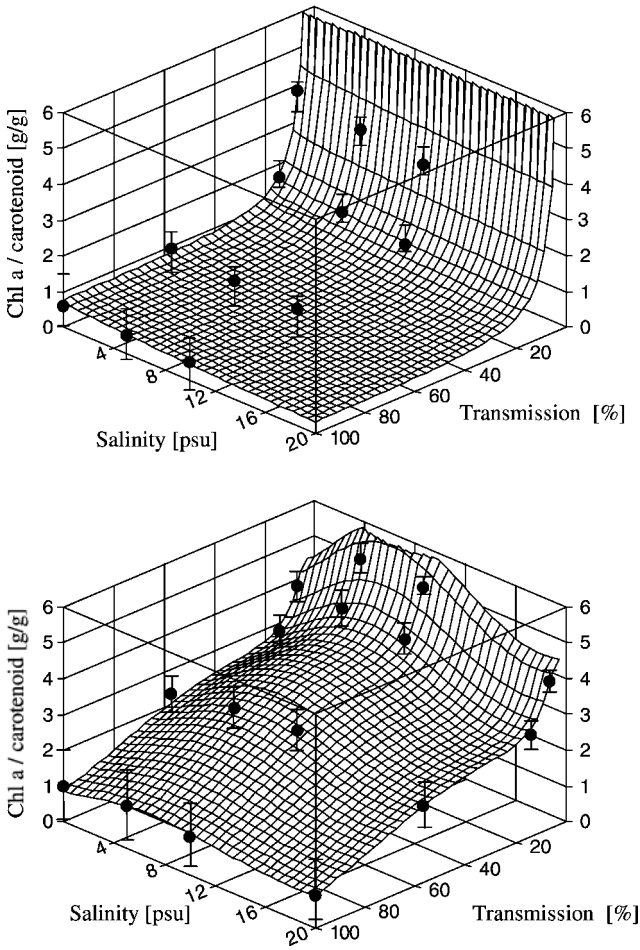

Fig. 9. Chlorophyll a/carotenoid quotients of *Chara aspera* collected from freshwater (above) and brackish water (below) at different salinities and light treatments. Mean values and confidence intervals (95%) are given.

locations alone. Instead, the existence of genetic differences between these populations is probable. Apart from distinct morphological differences between *C. aspera* originating from freshwater and brackish water (Hasslow, 1931, own observations), recent findings of genetic differences by means of AFLP-analysis between freshwater and brackish water populations of this plant (Mannschreck, personal communication) confirm this suggestion. Similarly, Ritzl (2000) suggested ecotypic differentiation between freshwater and brackish water populations for *C. tomentosa* L. Considering the recent history of the Baltic Sea, which has existed as a brackish water environment for no longer than about 7500 years, such a differentiation is striking, but has also been described for other plant groups. Baltic Sea populations can be separated from their suggested origins by both morphology and salt tolerance, a feature observed for plants which probably invaded the Baltic Sea from

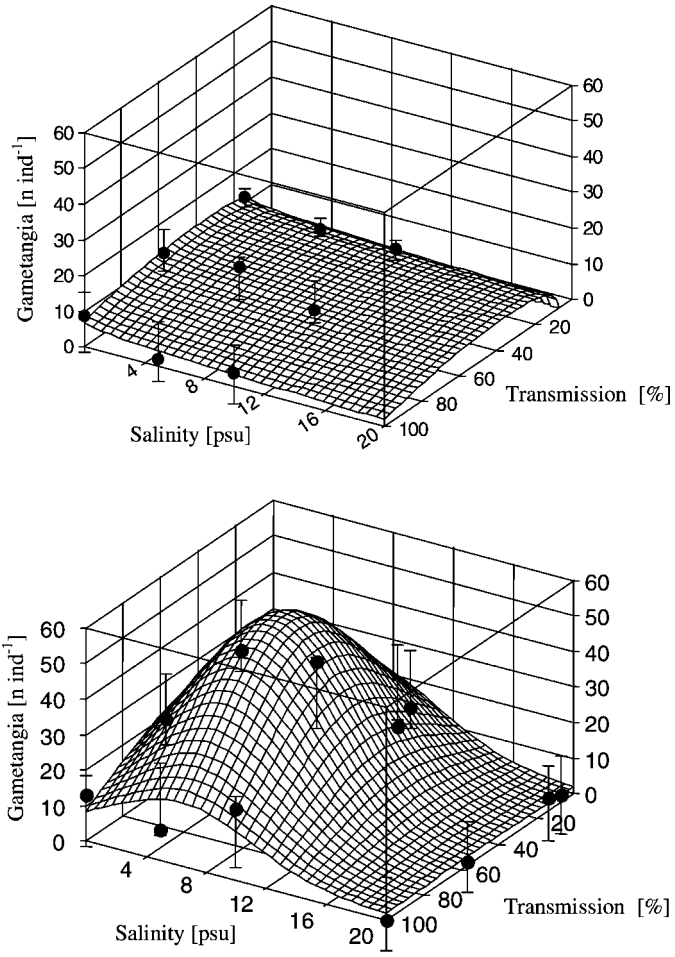

Fig. 10. Number of gametangia of *Chara aspera* collected from freshwater (above) and brackish water (below) at the end of the experiment at different salinities and light treatments. Mean values and confidence intervals (95%) are given.

marine environments like *Chorda filum* and *Fucus vesiculosus* as well as plants probably originating from freshwater like *Cladophora glomerata* and *Cladophora rupestris* (Russell and Thomas, 1988; Thomas et al., 1990). The origin of *C. aspera* in the Baltic Sea region is not known, but can theoretically be from either freshwater or other brackish water regions. In freshwater, *C. aspera* occurs in a wide geographical range including major parts of Europe, Asia and North America as well as northern Africa, but it has also been collected from other brackish habitats, for example, in Norway and southern France (Corillion, 1957; Langangen, 1974; Croy, 1982; Krause, 1997).

Our results do not give information about the upper salinity limit for the freshwater population of *Chara aspera*. All plants collected from this site died when exposed to 20 PSU,

but as the plants were not gradually adapted to increasing salinities, this mortality may have been caused by salinity shock. Plants collected from brackish water survived at 20 PSU, but reduced weight gain, photosynthesis and gametangia production, all indicate that such high salinities are a considerable stress factor for the plants. As the plants were not grown axenically, bacteria may have contributed considerably to the respiration values measured by us. Nevertheless, the distinct increase of the respiration rate at 20 PSU is another indicator for considerable salinity stress of the plants. Twenty PSU coincides with the upper salinity limit at which *C. aspera* can be found in the field (Olsen, 1944; Langangen, 1974; Blindow, 2000), and with the upper end of the physiological tolerance range of this species (Winter and Kirst, 1992). Additionally, charophytes can be restricted from high salinities by a reduction of fertility. In the laboratory, the euryhaline *T. nidifica* formed gametangia at salinities as high as 12 PSU, but no ripe oospores (Winter et al., 1996). For *C. aspera*, salinity dependence of oospore formation has not been studied yet, but the lower numbers of gametangia formed at 20 PSU indicate that fertility indeed may be reduced at high salinity.

For plants originating from both freshwater and brackish water, photosynthetic characteristics as well as pigment concentrations varied among light conditions as expected. The dependencies of the main photosynthesis parameters on irradiance indicate a "classical" irradiance acclimation. Thus, P_{max} increased with increasing irradiance, whereas α decreased. Comparable absolute values of P_{max}, α and E_k indicate similar overall photosynthetic capacities between freshwater and brackish water plants.

Interestingly, the highest P_{max} values were measured for the freshwater plants at 0 PSU. At this salinity, plants collected from brackish water had significantly lower P_{max}. The same tendency was observed for E_k. Our results thus support the CSR-model proposed by Grime (1979). According to this model, stress-tolerant plants are poor competitors under stress-free conditions as the achievement of stress-tolerance is connected with additional physiological costs.

Pigmentation reflects photosynthetic properties of phototrophic organisms. In particular, the chlorophyll a/chlorophyll b quotient indicates the relative size of light-harvesting complexes, and the chlorophyll a/carotenoid quotient is correlated with the capacity of light-protecting mechanisms. These quotients are useful parameters for comparisons within one species on a relative base. In both populations, lower chlorophyll a/chlorophyll b quotients and higher chlorophyll a/carotenoid quotients were observed at low irradiances indicating larger antennae and lower capacity for light protection under these conditions. Quotients of pigment concentration varied considerably between populations. Plants originating from freshwater generally had higher chlorophyll a/chlorophyll b quotients and lower chlorophyll a/carotenoid quotients than plants originating from brackish water indicating smaller antennae and higher demand or capacity for light protection.

Apart from higher ash content at 20 PSU in the brackish water population, which most probably reflected higher intracellular salt concentrations, the relative ash content generally decreased with increasing salinities. Higher calcium carbonate precipitation under lower salinities is the most probable explanation for this difference. Indeed, ash contents of charophytes originating from freshwater and brackish water seem to differ considerably. While *Chara tomentosa* collected from three calcium-rich lakes in Sweden had ash contents of about 60–70% of d.w., only 15–20% of ash content were found in a brackish water population (unpublished data). Low marl incrustation in brackish water compared to freshwater

forms of both *C. tomentosa* and *C. aspera* was mentioned also by Hasslow (1931) and may be explained by high of brackish water in combination with high concentrations of Mg^{2+} buffer capacity compared to Ca^{2+}. By the end of our experiments, plants collected from freshwater generally had higher ash content than plants collected from brackish water. This may be explained by the fact that freshwater plants already were incrusted with calcium carbonate at the start of the experiments. This assumption is supported by differences in colour between the populations, which was greyish in the freshwater plants and fresh green in the brackish water plants. However, this incrustation seems not to have influenced the photosynthetic capacity of the freshwater plants, nor was the demand for light protection reduced by this cover.

The decrease of both E_k and P_{max} with increasing salinity in plants collected from freshwater was especially pronounced at low light intensities. This supports our assumption that enhanced energy demand due to turgor regulation may cause a higher light compensation point. As a consequence, these plants can be expected to decline at a lower critical turbidity when exposed to higher salinities. Considering the optimum values for both E_k and P_{max} at 5 and 10 PSU, plants collected from brackish water behaved like facultative halophytes (Larcher, 1994) which are stimulated by salt at low concentrations. However, shoot elongation as a response to low light conditions occurred in both populations only at low salinities. Decreased turgor at higher salinities could have caused this suppression of shoot elongation. Similarly, shoot elongation was reduced in *Chara vulgaris* L. when salinity was increased to about 6 PSU in a laboratory experiment (Winter and Kirst, 1990). It is possible that shoot elongation generally is suppressed under high salinity conditions in *C. aspera* and possibly other species of charophytes. Such a behaviour could theoretically cause an exclusion of these plants from turbid water under higher salinities.

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