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Light and temperature acclimation of *Rhodomonas salina* (Cryptophyceae): photosynthetic performance

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ABSTRACT: Blooms of phototrophic cryptophytes have been observed in the highly eutrophic estuarine Darss-Zingst Bodden Chain (DZBC), Germany, during prolonged periods of light limitation due to ice and snow covering. The present study analyses possible mechanisms by which *Rhodomonas salina*, as a surrogate for bloom forming DZBC cryptophytes, maintains large densities during these low light/low temperature conditions. Growth, photosynthetic activity and pigment content were examined under 16 combinations of temperature (5 to 20°C) and irradiance (10 to 150 µmol photons m^{-2} s^{-1}) under nutrient-saturated conditions in a seawater-based medium. *R. salina* was tested for its capacity to photoacclimate to different light intensities in relation to temperature by calculating the photoadaptive index E_k (light saturation point of photosynthesis, P_{max}/α). P_{max}, the maximum photosynthesis rate and α, the efficiency of light utilisation at limited light intensities remained unchanged with respect to irradiance for every temperature tested. Consequently E_k, the irradiance at which photosynthesis rate ceased to be light-limited was constant (mean 49 µmol photons m^{-2} s^{-1}) within the chosen range of irradiances. This indicated that *R. salina* failed to adapt to down-shift changes in the light regime, at least in terms of photosynthetic parameters. Pigmentation analyses supported these results showing no acclimation of pigment ratios with regard to growth irradiance for a particular temperature. The calculated irradiance needed for 0 net photosynthesis (E_c) was about 26 µmol photons m^{-2} s^{-1} and did not show any significant variation in light or temperature. The failure of *R. salina* to respond to down-shift changes in the light regime did not result, however, in a reduction in growth at low irradiances (10 µmol photons m^{-2} s^{-1}). Judging from these results, *R. salina* seems to pursue an alternative strategy to capture energy under low light conditions which we hypothesise to be uptake of dissolved organic carbon from the seawater-based medium. Follow-up research will concentrate on the relative contribution of heterotrophy to the overall nutrition of *R. salina* under white ice covering.

KEY WORDS: Bloom · Cryptophytes · *Rhodomonas salina* · Ice · Photosynthesis · Photoacclimation

INTRODUCTION

Several occurrences of cryptophyte blooms have been reported for a broad spectrum of lake types and nutrient regimes, suggesting that these organisms contribute substantially to planktonic metabolic processes (reviewed in Stewart & Wetzel 1986, Klaveness 1989, Gervais 1997). Cryptophytes often arise during winter in ice-covered lakes (Stewart & Wetzel 1986). Most winter studies are performed in lakes located in polar and subpolar regions (e.g. Vincent 1981, Roberts & Laybourn-Parry 1999, McKnight et al. 2000) or in high mountain lakes (e.g. Rott 1988, Felip et al. 1999) of oligo- to mesotrophic character. Less information exists about blooms in shallow eutrophic to hypertrophic lakes of the temperate climate zone (reviewed in Nebaeus 1984, Wiedner & Nixdorf 1998) or eutrophic coastal areas (Bothnian Bay in the Baltic Sea, Ikävalko 1997). The Darss-Zingst Bodden Chain (DZBC), situated at the southern coastline of the Baltic Sea, is one such eutrophic to hypertrophic shallow water estuarine system. Small cryptophytes can reach extremely high abundances during periods of persistent ice cover

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(Wasmund 1994, Schumann unpubl. data). Whereas in arctic regions, low-light stress is caused by low annual doses of photosynthetic active radiation (e.g. Laybourn-Parry et al. 1996), in the DZBC high eutrophication results in light limitation of primary production (Schiewer 1997). High amounts of particulate and dissolved organic carbon (DOC) have a significant effect of underwater irradiance distribution in the DZBC (Schubert et al. 2001). Regular periods of ice and snow covering are a further stressful event. Ice covering lasts for up to 3 mo and opaque ice with snow covering plus sea ice crystals and particulate material result in almost total darkness below ice (Schubert et al. 2001).

Following Rodhe's (1955) question: 'Can plankton production proceed during winter darkness in subarctic lakes?' various hypotheses have been suggested to explain the winter survival of cryptophytes. The most commonly cited reasons are: (1) decreased predation pressure on cryptophytes (review in Stewart & Wetzel 1986); (2) extreme low-light adaptation, obtained by large and effective light harvesting complexes (phycobiliproteins) (Palmisano et al. 1985, Arrigo et al. 1993, Robinson et al. 1995); and (3) low metabolic rates (e.g. *Cryptomonas erosa*, Morgan & Kalff 1975) enabling long survival periods of these organisms. A controversial point in dark survival of cryptophytes is the importance of mixotrophy (Salonen & Jokinen 1988, Sanders et al. 1989, Tranvik et al. 1989, Gasol et al. 1993, Gervais 1997).

When light availability is the major factor controlling growth of ice algae (Marra & Boarman 1984, Sakshaug & Holm-Hansen 1986), phototrophic organisms should respond to variations in the light climate by photoadaptive processes. Several studies (e.g. Haxo & Fork 1959, Wright 1964, Morgan & Kalff 1975) have shown that cryptophytes are able to adapt to low mean irradiance by increasing the amount of light harvesting pigments. In contrast, Post et al. (1984) argued that these flagellates may depend more on motility than on photoadaptional capacity to obtain optimal light intensity. Whereas morphology (e.g. Santore 1977, Klaveness 1981), pigmentation (e.g. MacColl et al. 1976, Thinh 1983), as well as systematics and phylogeny (Klaveness 1985) have been extensively studied, a photosynthetic characterisation of Cryptophyceae exists only for very few species (*Cryptomonas lis*, Thinh 1983, 1988; *C. obavata*, Giroldo & Vieira 1999). To our knowledge E_k, a useful index of acclimation to different light conditions (Talling 1957, Henley & Ramus 1989), has been calculated only for *C. lis* (Thinh 1983, 1988). Therefore, information on cryptophyte photoacclimation kinetics is needed to clarify whether or not special features in light utilisation capabilities are responsible for the observed winter dominance of cryptophytes. Moreover, ecophysiological studies (*in situ* and *in vivo*) of growth kinetics are still scarce for Cryptophyceae (Cloern 1977, Ojala 1993a).

In this article, we assess the complex effect of various combinations of light and temperature on photosynthesis and growth rates of *Rhodomonas salina*, used as a surrogate for DZBC bloom cryptophytes, under nutrient-saturated conditions. Of particular interest were the effects of irradiance and temperature on modulation of photosynthetic efficiency, which is thought to be the most important acclimation mechanism of autotrophic individuals (Henley & Ramus 1989).

MATERIALS AND METHODS

Experimental design. We followed changes in growth, pigment content and photosynthetic activity of the cryptophyte alga *Rhodomonas salina* at varying light and temperature conditions. Cultures acclimated to the appropriate conditions were grown under combinations of 4 quantum irradiances from 10 to 150 µmol photons m^{-2} s^{-1} and 4 temperatures from 5 to 20°C. Samples were taken daily for growth rate determination. Biovolume, pigmentation and photosynthetic parameters were measured in the mid-exponential growth phase, which, depending on the light/temperature regime, occurred between Days 6 and 15. For each treatment, we determined the exact time of the mid-exponential growth phase in pre-experiments, adjusting our main experiments accordingly. Experiments were replicated by using 2 to 3 concurrently grown batches of cultures.

Organism and culture conditions. The cryptophyte *Rhodomonas salina* (Wislouch) Hill and Wetherbee (Cryptophyceae) (978/24) was obtained from the Culture Collection of Algae and Protozoa (CCAP) and originates from a brackish, eutrophic and lower latitude water body comparable to DZBC.

Cultures were maintained in f/2 medium, a seawater-based medium enriched with nitrate (742 µM), phosphate (36.2 µM), vitamins and trace metals to maintain nutrient-saturated growth. Medium salinity was 6.8 psu and the pH was 8.0, with both values lying well within the ranges of 2 to 13.2 psu and pH 7.4 to 9.5, for which Wasmund (1994) established cryptophyte occurrence in the DZBC (maximum occurrence at 8.3 psu and pH 8.4).

Cultures used for inocula were allowed to acclimate to the appropriate conditions by 2 repeated transfers of cells in the mid-exponential growth phase (Days 6 to 15 depending on treatment, see above) into fresh sterile medium. At the beginning of the experiments, these pre-acclimated cultures were diluted with sterile medium to a cell density of approximately 2×10^5 ml^{-1}.

Experimental batch cultures were grown under aeration in 150 ml carboys (Duran glass), thermostated at 5,

10, 15 and 20°C under a 12:12 h light:dark cycle. These incubation temperatures were within the range of 0.1 to 21.4°C, for which Wasmund (1994) established cryptophyte occurrence in the DZBC. The daily courses of underwater light in the DZBC for the time of cryptophyte blooms (November to February) were calculated from simulated surface light courses after correction for reflection according to Walsby (1997). In ice-free winters, daily light doses were approximately 6.54 mol m^{-2} between November and February, corresponding to the maximum value of 150 µmol photons m^{-2} s^{-1} (= 6.48 mol m^{-2}) that we chose for the experimental 12:12 h cycle. In the DZBC, a maximum of 0.43 mol m^{-2} d^{-1} reaches the cryptophytes under white ice covering, which corresponds to the experimental 10 µmol photons m^{-2} s^{-1}. Irradiation at 10, 30, 60 and 150 µmol photons m^{-2} s^{-1} (growth irradiation, E_g) was obtained by combining 5 cool white fluorescent tubes (Radium NL 36W/25) with neutral density filters. Irradiance was measured inside the culture glass vessels using a spectroradiometer (Macam SR9901, Macam) equipped with a 4 π-spherical underwater sensor.

Biovolume, growth rates, pigments and primary production. Daily cell counts of living flagellates and volume determinations of 1% ice-cooled glutaraldehyde preserved samples were made by microscopic examination within 3 d after fixation, using a Bürker counting chamber. Average cell volume was determined from the size of at least 50 cells, according to the equation for spheroids given in Tranvik et al. (1989).

Growth rates were calculated from the equation $\mu = (\ln_{N2} - \ln_{N1})/(t_2 - t_1)$, where N1 and N2 are the average values of cell numbers at times t_1 and t_2, corresponding to the beginning and the end, respectively, of exponential growth. We used GraphPad Prism 2.0 (GraphPad Software) to determine the beginning and end of the exponential growth.

For chlorophyll *a* (chl *a*) determination, duplicate subsamples of 5 to 10 ml of the algal suspension, filtered onto a Whatman GF/F filter, were extracted in 3 ml of DMF (*N*,*N*-dimethylformamide) and stored in darkness at 4°C for ca. 5 h. Absorption was measured with an UV/VIS spectrophotometer (Lambda 2, Perkin Elmer). Pigment contents (µg ml^{-1}) were calculated from the absorbance spectra of the extracts according to Porra et al. (1989). Phycoerythrin (PE) content was determined spectrophotometrically as described in Sciandra et al. (2000).

Measurements of the photosynthesis-irradiance (*P-E*) curves were performed for 14 out of the 16 light-temperature treatments as well as for their replicates. Chl *a* contents were insufficient to allow oxygen electrode measurements of the irradiance-temperature regimes 60/150 µmol photons m^{-2} s^{-1} and 5°C. To avoid variability due to possible fluctuation of photosynthesis, experiments were always carried out 2 h after the start of a light phase. Net oxygen exchange rates, at 9 increasing light intensities from 0 to approximately 1100 µmol photons m^{-2} s^{-1}, were measured with a Clarke type oxygen electrode using a computer controlled Light Dispensation System (MK2, ILLUMINOVA, described in Wolfstein & Hartig 1998). One measurement of a *P-E* curve took about 40 min. After an equilibration time of 10 min darkness, measurement started with 10 min darkness, followed by a stepwise, successive increase of irradiance. Each irradiance step lasted for 4 min. Temperature was set according to the respective acclimation temperature. Photosynthetic parameters were derived by a fitting procedure applied to each individual sample. Because photoinhibition was not evident, we used an iterative exponential regression with the 2-parameter equation of Webb et al. (1974):

$$P = P_{max}(1 - e^{\alpha E/P_{max}}) + R$$

where P is the photosynthesis rate at irradiance E, P_{max} is the maximum photosynthesis rate obtained at saturating irradiance in the absence of photoinhibition, R is the rate of respiration and α the initial slope of the *P-E* curve (light dependency of photosynthesis at limited irradiances). P_i, the photosynthesis rate at growth irradiance E_g, was calculated according to the respective fitted equation. E_k (light saturation point and index of light adaptation, P_{max}/α, Talling 1957) and E_c (compensation irradiance at which respiratory oxygen demand is balanced by photosynthesis) were determined according to Platt et al. (1980).

Statistics. All analyses were conducted using GraphPad Prism 2.0 (GraphPad Software). After $\log_{10}$-transformation in order to stabilise the variances, 2-way ANOVAs were performed to test for differences between light and temperature exposure history with respect to growth rate, pigments and photosynthetic parameters. Post tests were applied to test for linear trends and to identify groups differing from the others if there was a significant difference ($p < 0.05$) for the respective parameters (Tukey's test). 3D graphics were derived by a surface fitting procedure using simple polynomial equations (TableCurve 3D, AISN software). Equations are given in the legends with r^2, standard error and *F*-value.

RESULTS

Growth

Thirteen out of the 16 light-temperature combinations resulted in significant growth. Three combinations, T = 5°C, E_g = 30, 60 and 150 µmol photons m^{-2} s^{-1}, did not

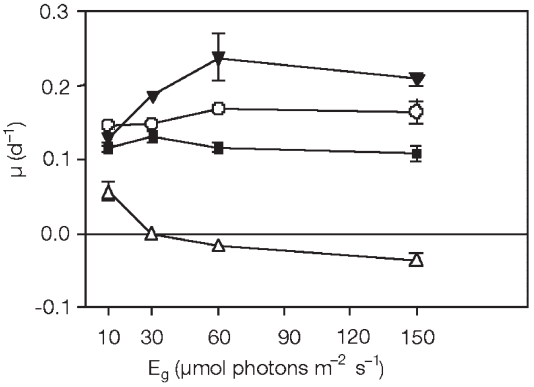

Fig. 1. *Rhodomonas salina*. Growth rates (μ) at different incubation irradiances (E_g) and temperatures. Error bars = ±SD. (△) 5°C, (■) 10°C, (○) 15°C, (▼) 20°C

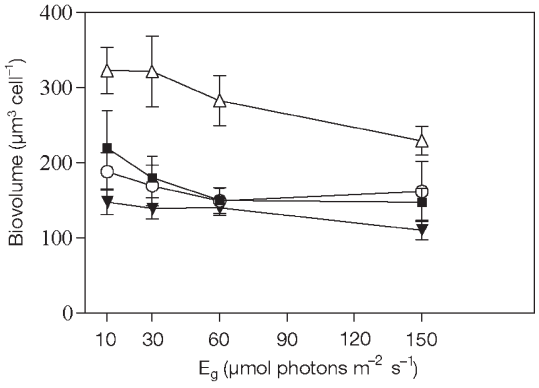

Fig. 2. *Rhodomonas salina*. Biovolume per cell at different incubation irradiances (E_g) and temperatures. Error bars = ±SD. (△) 5°C, (■) 10°C, (○) 15°C, (▼) 20°C

support growth (Fig. 1). The growth rate of *Rhodomonas salina* showed only small irradiance-dependent changes (10% of variances explained by irradiance; $p > 0.05$, 2-way ANOVA). This is most marked at 10 and 15°C with almost constant growth rates of 0.11 to 0.13 d^{-1} and 0.15 to 0.17 d^{-1}, respectively, within the range of growth irradiances. The irradiance for maximum growth slightly decreased ($p > 0.05$) with decreasing temperature; at 20°C, it was 60 μmol m^{-2} s^{-1} and at 5°C, it dropped to 10 μmol m^{-2} s^{-1}. Photoinhibition of growth could be observed in the low temperature cultures above 10 μmol photons m^{-2} s^{-1}. Temperature explained >70% of the variances in growth rate ($p < 0.001$, 2-way ANOVA), where growth increased significantly with increasing temperature ($r^2 = 0.62$, $p < 0.001$, post test for linear trend) and reached its maximum at 20°C, the highest temperature examined (0.24 d^{-1} at 60 μmol m^{-2} s^{-1}).

Biovolume

Similar to the growth rate, the cell size of *Rhodomonas salina* (mean 182 μm^3) was only slightly affected by irradiance (17% of variance explained; $p < 0.01$, 2-way ANOVA), showing an insignificant decrease with increasing irradiance ($p > 0.05$; Fig. 2), whereas temperature accounted for 78% of the variances ($p < 0.001$). The mean cell volume decreased significantly with increasing temperature ($r^2 = 0.73$, $p < 0.01$) at each level of irradiance. The smallest cells had an average volume of 110 μm^3 ($T = 20$°C, $E_g = 150$ μmol m^{-2} s^{-1}) and the largest of 341 μm^3 ($T = 5$°C, $E_g =$ 10 μmol photons m^{-2} s^{-1}) implying an up to 3-fold size difference within this strain.

Pigmentation

Cellular chl *a* content increased for all temperature regimes when irradiance dropped below 30 μmol photons m^{-2} s^{-1} (Fig. 3). Chl *a* contents at 10 μmol photons m^{-2} s^{-1} were significantly different from all other irradiance treatments for all temperature regimes ($p < 0.01$, Tukey's test). This increase was approximately 4-fold in the high temperature cultures and 2-fold in the low temperature cultures. A significant positive effect of temperature on chl *a* content per cell ($r^2 = 0.99$, $p < 0.01$) was observed for the low-light culture (10 μmol photons m^{-2} s^{-1}), whereas cultures grown at higher irradiances exhibited insignificant changes in cellular chl *a* with temperature ($p > 0.05$).

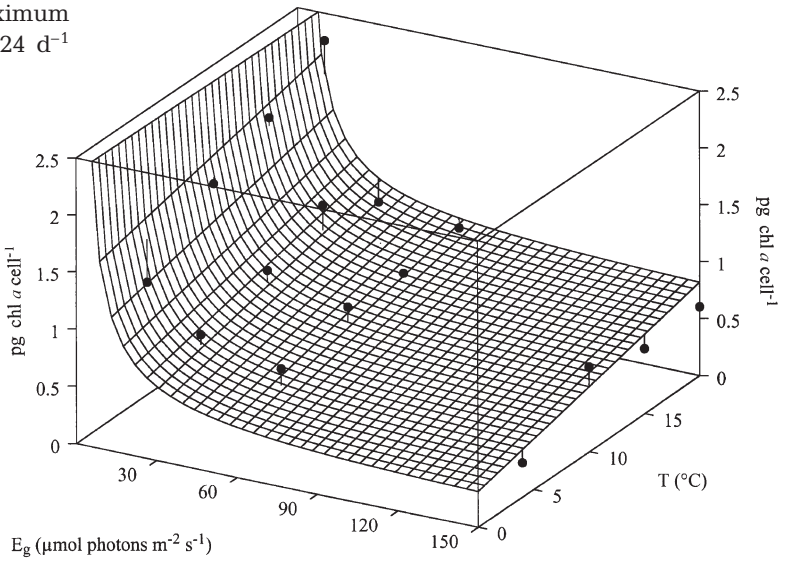

Fig. 3. *Rhodomonas salina*. Chl *a* per cell versus growth irradiance (E_g) and temperature (T) relationship (mean value, n = 3). Fitted by the equation $z = 0.23 + 12.35/x + 0.03y$, $r^2 = 0.88$, SE = 0.20, $F = 47.13$. Error bars = ±SD

The PE/chl *a* ratio (Fig. 4) showed only small irradiance-dependent changes (21% of variances explained, $p < 0.01$), reflecting that for a given temperature both cellular chl *a* and PE (mean of 3.87 pg cell^{-1}) content depend on irradiance in the same way. The negative effect of temperature on PE is more pronounced than on chl *a*, resulting in a sharp decline in PE/chl *a* with decreasing temperature (73% of variances explained, $r^2 = 0.71$, $p < 0.001$).

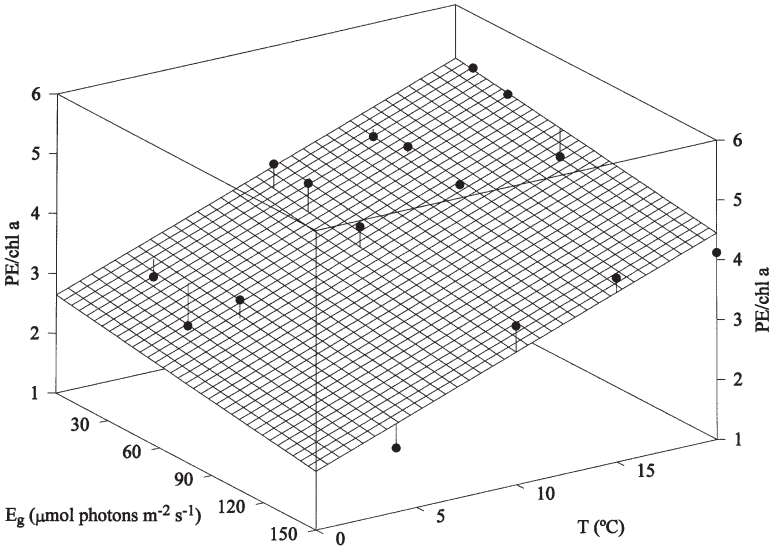

Fig. 4. *Rhodomonas salina.* Phycoerythrin (PE)/chl *a* per cell versus growth irradiance (E_g) and temperature (T) relationship. Fitted by the equation $z = 2.64 - 0.004x + 0.12y$, $r^2 = 0.79$, SE = 0.04, $F = 24.70$. Error bars = ±SD

Photosynthetic parameters

In all *P* versus *E* curves, the maximum photosynthetic rate (P_{max}) was reached at ≥60 µmol photons m^{-2} s^{-1}, followed by a plateau without any signs of photoinhibition (single *P-E* curves not shown). Maximum photosynthetic rates varied for the entire data set between 78 and 146 mmol O_2 g chl *a*$^{-1}$ h^{-1} (mean of 96 mmol O_2 g chl *a*$^{-1}$ h^{-1}; Table 1). However, no significant variability with irradiance could be found within each temperature regime ($p > 0.05$). P_{max} increased significantly ($p < 0.01$, Tukey's test) from 15 to 20°C. The photosynthetic energy conversion efficiency, α, did not vary significantly with irradiance ($p > 0.05$), whereas a variation in temperature could be observed (70% of variances explained, $p < 0.01$) with values at 15°C significantly higher than at the other temperatures ($p < 0.01$,

Table 1. Photosynthetic characteristics of *Rhodomonas salina* cultured in different growth irradiance (E_g); temperature (T) regimes: maximum gross photosynthesis per chl *a* ($P_{max\cdot chl\ a}$), initial slope per chl *a* ($\alpha_{chl\ a}$) of the *P-E* curve, light saturation constant (E_k), gross photosynthetic performance (P_i) and dark respiration (R) per chl *a* and per cell, and compensation irradiance of photosynthesis (E_c). Means of P_{max}, P_i and R from exponentially growing cultures were calculated from triplicate cultures. Coefficient of variation ranged from 5 to 10%. Chl *a* contents were insufficient to allow oxygen electrode measurements of the irradiance-temperature regimes 60/150 µmol photons m^{-2} s^{-1} and 5°C

E_g	T (°C)	$P_{max\cdot chl\ a}$	$\alpha_{chl\ a}$	E_k	Gross $P_{i\cdot chl\ a}$	Gross $P_{i\cdot cell}$	$R_{chl\ a}$	R_{cell}	R/P_i	E_c
10	5	87.9	1.3	65	12.6	15.0	26.05	31.0	2.1	19
	10	87.2	1.9	45	18.2	30.3	32.90	54.7	1.8	18
	15	77.9	3.6	22	29.1	55.6	44.18	84.5	1.5	25
	20	80.0	1.1	71	9.3	21.1	30.28	68.4	3.2	14
30	5	115.6	2.5	46	51.7	42.9	33.33	27.7	0.6	22
	10	94.0	2.1	45	50.7	50.5	41.48	41.3	0.8	28
	15	87.1	3.8	23	64.8	80.3	50.44	62.5	0.8	19
	20	125.4	1.5	84	30.6	28.6	37.97	35.5	1.2	39
60	5	–	–	–	–	–	–	–	–	
	10	89.5	2.0	44	63.4	52.0	45.25	37.1	0.7	39
	15	82.4	4.1	20	81.6	64.0	47.92	37.6	0.6	17
	20	102.3	2.8	37	79.8	68.0	51.04	43.5	0.6	25
150	5	–	–	–	–	–	–	–	–	–
	10	83.1	1.8	47	73.9	54.4	45.41	33.4	0.6	27
	15	91.2	2.6	35	92.5	52.3	49.41	28.0	0.5	27
	20	146.7	1.9	77	100.8	60.5	64.12	38.5	0.6	55

E_g, E_k, E_c = µmol photons m^{-2} s^{-1}
$P_{max\cdot chl\ a}$, $P_{i\cdot chl\ a}$, $R_{chl\ a}$ = mmol O_2 g chl *a*$^{-1}$ h^{-1}
$\alpha_{chl\ a}$ = mmol O_2 g chl *a*$^{-1}$ h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$
$P_{i\cdot cell}$, R_{cell} = mmol O_2 (10^{12} cell)$^{-1}$ h^{-1}

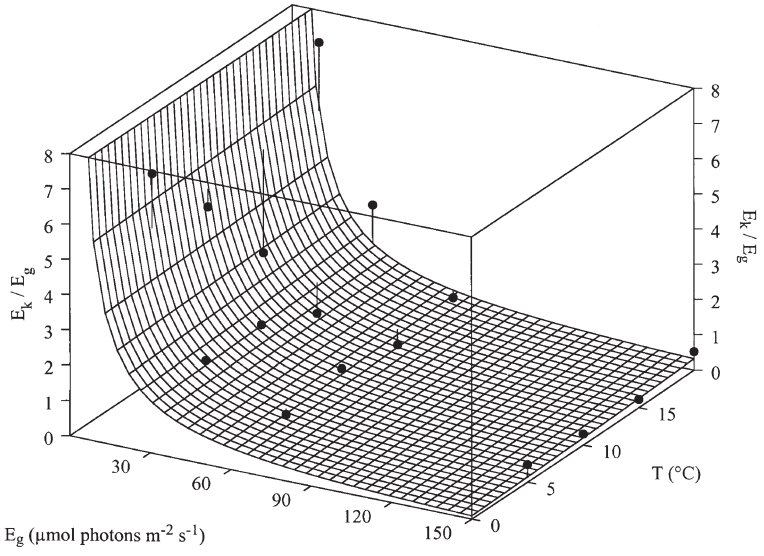

Fig. 5. *Rhodomonas salina.* E_k/E_g versus growth irradiance (E_g) and temperature (T) relationship. Fitted by the equation $z = -0.20 + 51.85/x + 0.01y$, r^2 = 0.77, SE = 1.16, F = 21.24. Error bars = ±SD. A value of $E_k/E_g < 1$ indicates light limitation

Tukey's test; Table 1). Consequently, the calculated light saturation constant, E_k (P_{max}/α), was relatively constant over the range of growth irradiances (mean of 47 µmol photons m^{-2} s^{-1}) for each temperature regime and was in all cases above the lowest examined growth irradiance (10 µmol photons m^{-2} s^{-1}; Table 1). This is depicted in Fig. 5, where a value of $E_k/E_g \leq 1$ occurred only in cultures growing at $E_g \geq 60$ µmol photons m^{-2} s^{-1}, thus indicating light limitation below this level of irradiance. Regardless of E_g, E_k was always lowest at 15°C (mean 25 µmol m^{-2} s^{-1}) (Table 1), which reflects the highest α at this temperature (Table 1).

Respiration

An approximately 2-fold increase in respiration rates normalised to chl *a* ($p < 0.01$) was observed over the examined range of growth irradiances/temperatures (Table 1). Respiration per cell was inversely related to irradiance because of decreases in cell chl *a* with growth irradiance (Fig. 3). The ratio of respiration to gross photosynthesis at the growth irradiance E_g (R/P_i) decreased with increasing irradiance, and P_i exceeded R only for levels above approximately 30 µmol photons m^{-2} s^{-1} (Table 1). The compensation irradiance (E_c; Table 1) was not significantly affected by light or by temperature changes ($p > 0.05$), reaching a mean of 26 (±5.5 SD) µmol photons m^{-2} s^{-1}. This indicated that in low-light cultures (10 µmol photons m^{-2} s^{-1}), no positive net photosynthetic growth could occur. This is confirmed by calculating the net photosynthesis at E_g (net P_i; Fig. 6) with positive values above approximately 26 µmol photons m^{-2} s^{-1}.

DISCUSSION

It has often been shown that in sea ice communities where irradiance is low, microalgal inhabitants are remarkably shade adapted (e.g. Cota 1985, Palmisano et al. 1985, Robinson et al. 1995). The energy limitation in such environments may be partially offset by an increase in the amounts of photosynthetic pigments, and thus, in an increase in light-capturing ability per cell, as is commonly observed in ice algae (Antia et al. 1969, Reynolds 1984). In fact, the chl *a* content of *Rhodomonas salina* cells growing at 10 µmol photons m^{-2} s^{-1} was 2 to 3 times as high as that of cells growing at 150 µmol photons m^{-2} s^{-1}. However, this difference was reduced for low-temperature cultures (Fig. 3) and was even smaller if chl *a* was expressed on a cell volume basis because of the decrease in biovolume per cell with increasing irradiance and temperature (Fig. 2).

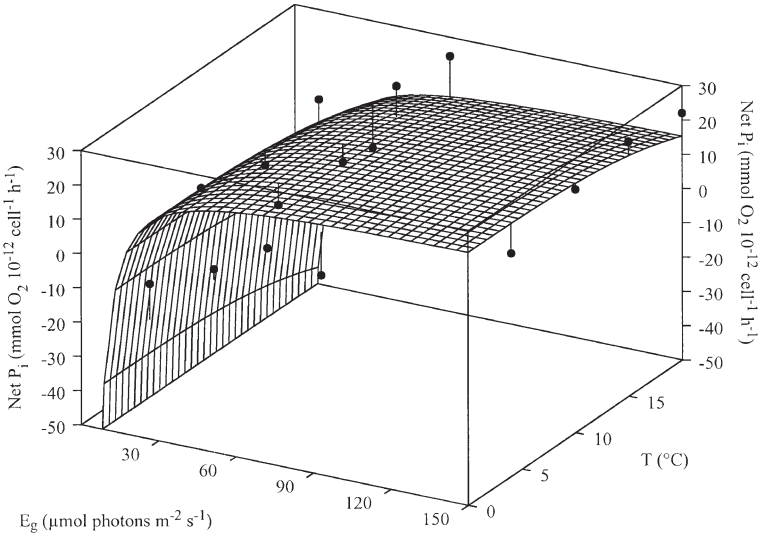

Fig. 6. *Rhodomonas salina.* Net photosynthesis (P_i) per cell versus growth irradiance (E_g) and temperature (T) relationship. Fitted by the equation $z = 30.04 + 0.12x - 491.92/y$, $z = 30.35 - 548.36/x - 0.34y$, r^2 = 0.86, SE = 8.94, F = 40.78. Error bars = ±SD

Compared to other cryptophytes, which acclimate by changing their relative chl *a* content by a factor of 5 or more (Morgan & Kalff 1979, Ojala 1993b), this was a rather limited increase.

Besides chl *a*, the photosystem II of cryptophytes receives excitation energy through the phycobiliprotein light-harvesting complex (LHC; MacColl et al. 1976, reviewed in Larkum & Barrett 1983). By increasing the amount of LHC pigments by more than the cellular chl *a* content, this light-harvesting system allows photosynthesis to function with high efficiency under limited irradiance. This acclimation, i.e. the increase of the phycobiliprotein/chl *a* ratio at low irradiance, as shown for Cyanophyceae (Halldal 1958) or Cryptophyceae (Faust & Gantt 1973), did not occur in our experiments. The PE/chl *a* ratio of *Rhodomonas salina* was constant for all light intensities within temperature regimes (Fig. 4), indicating a reduction in the number of all photosynthetic units per cell with increasing irradiances (Bernard et al. 1996). An inhibitory effect of nitrogen deficiency on PE production and phycobiliprotein degradation (Lichtlé 1979, Rhiel et al. 1985) can be excluded because we used a nitrogen-enriched medium and inocula from exponentially growing (i.e. nitrogen-sufficient) cultures. Furthermore, constant C:N ratios of 4.1 (±0.52, not shown) were measured for the entire experimental set.

Phycobiliproteins were selectively reduced with decreasing temperature, which resulted in a significantly lower PE/chl *a* ratio ($p < 0.001$; Fig. 4). This reflects a strong decline in antennae size decreasing the amount of light energy absorbed and transferred to the reaction centres (Richardson et al. 1983, Raven & Geider 1988). Low temperatures therefore seem to limit the ability or need to invest biosynthetic energy in light-harvesting pigments, leading to a pigmentation signature similar to that of high-light acclimated cells.

Low-light acclimation typically results in a decrease of the light saturation point E_k, which can be caused either by a decrease in P_{max} (Falkowski 1980, Richardson et al. 1983), by an increase in α or by a combination of both. In our laboratory experiments, the very low light intensities to which *Rhodomonas salina* cultures were exposed did not depress E_k below the level obtained for high-light cultures (Table 1). Values were higher than in comparable field studies and never reached values below 10 µmol photons m^{-2} s^{-1} (Table 1), as frequently recorded for low-light adapted algae and/or sea ice communities (reviewed in Cota & Smith 1991, Thinh 1993, Robinson et al. 1995). The comparison between E_k and growth irradiance leads to the conclusion that available light was limited for cultures growing at irradiances <60 µmol photons m^{-2} s^{-1} and, in all cases, for the 10 µmol photons m^{-2} s^{-1} cultures (Fig. 5). It did not show any significant variation over the entire range of growth irradiances at any temperature regime ($p > 0.05$). Thus, if the change in E_k is the result of photoadaptation of algae to changing growth irradiance (Talling 1957, Henley 1993, Falkowski & Raven 1997), then *R. salina* did not photoadapt to the very low light intensities (maximum 10 µmol m^{-2} s^{-1} at noon, Schubert et al. 2001) observed under snow-covered ice in the DZBC. Nevertheless, *R. salina* is a relatively low-light adapted species with an average E_k value of 49 µmol m^{-2} s^{-1}.

In contrast, E_k varied with temperature. With temperature increasing over the ambient range of 5 to 20°C, P_{max} is enhanced at saturating irradiances, albeit significantly only in the range of 15 to 20°C ($p < 0.05$, Tukey's test; Table 1). Previous studies have also reported an increase of P_{max} with temperature for a wide range of environments and algal species (e.g. Li et al. 1984, Priscu & Goldman 1984, Tilzer et al. 1986). The photosynthetic efficiency, α, appeared to be more temperature-dependent than P_{max}, reaching a clear maximum at moderate temperatures of 15°C (Table 1). This implies minimum E_k values at this temperature and thus, our conjecture of best acclimation at this temperature. The increase of E_k with increasing temperature, which has often been described (Collins & Boylen 1982, Palmisano et al. 1987, Henley 1992), was found in our study only in the range of 15 to 20°C (Table 1). Furthermore, E_k seems to be controlled by variations in α rather than in P_{max} (Beardall & Morris 1976, Henley 1993).

With low-light-acclimation being virtually absent in *Rhodomonas salina*, reduced respiratory losses should be considered as an alternative cause for the existence of under-ice populations (e.g. Morgan & Kalff 1979). For *R. salina*, in fact, respiration per chl *a* was reduced by about ½ with decreasing light and temperature level (Table 1). Nevertheless, this reduction was insufficient to allow positive net photosynthesis P_i at light intensities below 26 µmol photons m^{-2} s^{-1} (±5.5; Fig. 6). It is possible that changes in the photosynthetic/respiration data might be partially attributed to a downward signal drift in the O_2 electrode, which would systematically increase apparent respiration and decrease net photosynthesis. As pointed out by Falkowski et al. (1985), small differences in the experimental light field could lead to large differences in the measurements of gross photosynthesis, particularly at subsaturating levels. However, one advantage of the photosynthetic light dispensation system over the microelectrode method is the precise control of environmental conditions such as irradiance and temperature as well as the supply of reproducible and rapid measurements of *P-E* curves (Wolfstein & Hartig 1998). We therefore believe our results to be accurate, implying that the compensation point for algal photosynthesis (E_c) was at a rela-

tively low and more or less constant light level (26 µmol photons m^{-2} s^{-1} ± 5.5), but nevertheless above the lowest irradiance examined (10 µmol photons m^{-2} s^{-1}). Thus, a reduced respiration rate might at best allow a long survival of *R. salina* under subcompensatory light intensities and suboptimal temperatures. However, it could not explain the observed significant growth at 10 µmol photons m^{-2} s^{-1} for all temperature regimes (Fig. 1). As opposed to Cloern (1977) and Ojala (1993a), who pointed out that, in general, the growth rate of cryptophytes increases exponentially with temperature and irradiance, we observed a rather flat response to light changes. Growth rate changes in biovolume could be omitted as the cell volume of *R. salina* showed insignificant variations in irradiance (Fig. 2). According to various authors (e.g. Morgan & Kalff 1979, Lewitus & Caron 1990), however, a reduction in irradiance often gives rise to a decrease in carbon quotas and cell volume. Thompson et al. (1991) pointed out that species in which carbon quota and/or cell volumes increase at low irradiance (e.g. Sakshaug & Andresen 1986) respond to a low energy environment in a way which is markedly different from species reducing the cell carbon at low irradiance. Judging from our results, *R. salina* also seems to pursue a different strategy to respond to low-light environments.

Changes with temperature in growth as well as in biovolume were comparable to literature data. For the low temperature culture (5 and 10°C), a significant negative correlation could be found between growth rate and biovolume (Spearman $r = -0.93$, $p < 0.001$). Likewise, Morgan & Kalff (1975) have shown that low temperature adversely affects cell division of cryptophytes more than photosynthesis, resulting in accumulation of carbohydrate and increase in cell volume. Overall growth rates of *Rhodomonas salina* were small similar to those for various cryptophytes (e.g. Cloern 1977). The experimental irradiances for maximum growth of *R. salina* (E_g = 60 µmol photons m^{-2} s^{-1} = 2.59 mol m^{-2} d^{-1} for the experimental 12:12 h cycle) corresponded to the field irradiance for maximum cryptophyte occurrence in the DZBC (2.81 mol m^{-2} d^{-1}, Wasmund 1994). The discrepancy in maximum growth temperature between laboratory (20°C) and field (2.1°C, Wasmund 1994) is easily explained as follows. Cryptophyceae are intensively grazed by zooplankton (review in Stewart & Wetzel 1986) and out-competed by faster growing species. According to Wasmund (1994), during mild winters their biomass in the DZBC remained low due to the competition with Chlorophyceae.

In summary, we observed that *Rhodomonas salina* exhibited an incomplete low-light acclimation in terms of pigmentation and acclimation of E_k and E_c. With respect to temperature acclimation, a distinct optimum at 15°C was observed for α and E_k. However, acclimation of these parameters is insufficient to explain the positive growth rates measured at low light/low temperature conditions. McKnight et al. (2000) have shown that the concentration and the quality of labile DOC can affect the heterotrophic growth of overwintering algae. We therefore hypothesised that DOC assimilation from the seawater based medium must have been the means by which *R. salina* captured energy under low-light conditions. Measurements revealed a significant DOC decline ($p = 0.01$, *t*-test) during the experiment from 13.9 ± 2.9 to 8.9 ± 4.4 mg C l^{-1}, varying with irradiance and temperature conditions (data not shown). Following recent research about osmotrophy (McKnight et al. 2000) and phagotrophy (Roberts & Laybourn-Perry 1999) in overwintering cryptophytes, we will investigate these mechanisms as an explanation for cryptophyte dominance in the DZBC at low light/low temperature conditions (Hammer et al. unpubl.).

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