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ECOPHYSIOLOGICAL FEATURES OF POLAR SOIL UNICELLULAR MICROALGAE[1]

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Due to their ecological, physiological, and molecular adaptations to low and varying temperatures, as well as varying seasonal irradiances, polar non-marine eukaryotic microalgae could be suitable for low-temperature biotechnology. Adaptations include the synthesis of compounds from different metabolic pathways that protect them against stress. Production of biological compounds and various biotechnological applications, for instance, water treatment technology, are of interest to humans. To select prospective strains for future low-temperature biotechnology in polar regions, temperature and irradiance of growth requirements (Q_{10} and Ea of 10 polar soil unicellular strains) were evaluated. In terms of temperature, three groups of strains were recognized: (i) cold-preferring where temperature optima ranged between 10.1 and 18.4°C, growth rate 0.252 and 0.344 · d^{-1}, (ii) cold- and warm-tolerating with optima above 10°C and growth rate 0.162–0.341 · d^{-1}, and (iii) warm-preferring temperatures above 20°C and growth rate 0.249–0.357 · d^{-1}. Their light requirements were low. Mean values Q_{10} for specific growth rate ranged from 0.7 to 3.1. The lowest Ea values were observed on cold-preferring and the highest in the warm-preferring strains. One strain from each temperature group was selected for P_N and R_D measurements. The P_N:R_D ratio of the warm-preferring strains was less affected by temperature similarly as Q_{10} and Ea. For future biotechnological applications, the strains with broad temperature tolerance (i.e., the group of cold- and warm-tolerating and warm-preferring strains) will be most useful.

Key index words: **activation energy; dark respiration; growth; net photosynthesis; net photosynthesis:dark respiration ratio; polar soil unicellular microalgae; temperature and irradiance requirements; temperature quotient**

***Abbreviations*: μ, relative growth rate; CCA, canonical correspondence analysis; Ea, activation energy; I, irradiance; P_N, net photosynthesis; Q_{10}, temperature quotient; R_D, dark respiration; T, temperature**

Eukaryotic unicellular microalgae (Trebouxiophyceae, Chlorophyceae) play a key role in Arctic and Antarctic ecology as primary producers (Priscu 1998, Elster 2002, Elster and Benson 2004). They inhabit all aquatic and terrestrial habitats, including biofilms on surface of soil (e.g., Rindi et al. 2009). They were reported from nearly all soil types, including polar desert soils (Elster 1999, Kaštovská et al. 2005, 2007, Fermani et al. 2007). Langhans et al. (2009) recognized unicellular microalgae species as key players for monitoring the succession of temperate biological soil crust formation.

Unicellular microalgae originating from polar terrestrial habitats cope with extreme temperatures, varying irradiances and daylengths, as well as low availability of essential macronutrients and micronutrients and other resources. A very broad spectrum of nutritional requirements of unicellular microalgae was demonstrated (Iwamoto 2004, Shukla

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et al. 2011). Unicellular microalgae have a simple life cycle with asexual reproduction where differences between mother and daughter cells are negligible. Presumably, there was a twofold difference in cell size for binary fission of the phase of cell separation in budding. To survive and grow successfully in cold environments, they have evolved a complex range of biochemical reactions of their cellular constituents, which enable to compensate for the negative effect of low temperatures. Consequently, a wide range of metabolic activities were detected in cold ecosystems (Shukla et al. 1997a,b, Shukla and Kashyap 1999, Rai and Gaur 2001, Pandey et al. 2004, Vonshak and Torzillo 2004, Elster et al. 2008). In this respect, particular attention has been paid to the metabolic facilities of psychrotolerant terrestrial strains of Trebouxiophyceae and Chlorophyceae microalgae (Kvíderová and Lukavský 2005, Shukla et al. 2011, Wong et al. 2015). Their cellular constituents or products could provide a large biotechnological potential (Lang et al. 2011, Olivieri et al. 2011, 2013, Cadoret et al. 2012, Barreiro et al. 2013, Slocombe et al. 2015). Kvíderová et al. (2017) summarized biotechnological uses of polar microalgae, which could be economically profitable. Polar unicellular microalgae developed wide spectrum of ecological, physiological, and molecular defensive and adaptive strategies, which include the synthesis of a tremendous diversity of compounds (e.g., PUFA, asthaxanthin) originating from different metabolic pathways which protect them against the stresses of the harsh polar environment. Production of different biological compounds and various biotechnological applications, for instance, water treatment technology in low-temperature environments and many others, are the perspectives for humans exploring the polar regions.

Possible constructions of photobioreactors for mass cultivation of microalgae are proposed for operations in polar regions. To our knowledge, the economy of the annual mass cultivation of unicellular microalgae in the Arctic has not been evaluated yet. Climate change in the Arctic brings broad opportunities for development of economic activity (urbanization of the Arctic) including establishing of novel industries (mining, fishery, ocean transport, etc.). Today, development of bioprospection and biotechnologies at low temperatures is one of the most urgent global tasks. At present, microalgal research in the Arctic aims to explore the biotechnological potential of polar cyanobacteria and micro-algae adapted to low temperatures that may produce high-value compounds are on the way in cold period of the year in Central Europe (e.g., Shukla et al. 2013). Developed and verified cultivation technology will be modified for Arctic conditions where it will contribute to protection of the Arctic ecosystem and to sustainable urbanization of this region (Callaghan et al. 2004).

Unicellular microalgae of simple morphology are ubiquitous in terrestrial ecosystems (Hodač et al. 2016). Genetic population analyses (molecular techniques of DNA sequencing and strict molecular clock) revealed biogeographical and environmental history of particular species, including existence of cryptic species (Boenigk et al. 2005, Rindi et al. 2008, Dal Grande et al. 2014, Řídká et al. 2014, Ryšánek et al. 2014, Škaloud et al. 2014, 2015). However, up to now, a little is known about phytogeographical distribution of unicellular microalgae in polar (Arctic and Antarctic) in comparison with non-polar regions (Rybalka et al. 2009, Vyverman et al. 2010, Hodač et al. 2016). Despite the evident importance of unicellular microalgae as primary producers in polar environment (Hodač et al. 2016 and references there), surprisingly, there are yet only a few molecular-phylogenetic studies focusing on ubiquity and/or regional endemism of Arctic, Antarctic, and other strains (Huss et al. 1999, Finlay 2002, Finlay and Fenchel 2004, De Wever et al. 2009, Rybalka et al. 2009, Vyverman et al. 2010, Hodač et al. 2016). Within 10 strains of unicellular microalgal species in this ecophysiological research, seven of them were described in respect of their molecular-phylogenetic properties (Hodač et al. 2016).

Unicellular microalgae have an important advantage over many other organisms as they can be extensively cultured for the production and processing of desirable compounds (Watson 2003, Iwamoto 2004). However, one out of the major limitations in biotechnological applications of polar unicellular microalgal strains is their remarkably slow growth (Cao et al. 2016). The growth rate of the most rapidly growing a cold-adapted microalga at the temperature yielding the maximum growth rate is less than that of a micro-alga adapted to "temperate" temperatures (Eppley 1972). The life cycle speed is a critical factor affecting commercial production of long-term solar-powered cultivation of natural microalgal strains (Kenny and Flynn 2017). In view of that above, there is an emphasis to optimize growth conditions for improvement of the growth and biomass yield (quantity of product per culture medium). A higher yield of biomass facilitates a detailed characterization of polar strains (morphological, physiological, biochemical, and molecular-genetic) which could help to develop the further biotechnological applications. In addition, such information could help to develop a successful cultivation set-up and practical biomass production of unicellular microalgae in non-summer conditions in temperate and/or polar regions.

Culturing of unicellular microalgae and their maintenance in a culture collection as a stable renewable resource are a great advantage for biotechnology. One out of promising applications, with a possible impact on both the public health and the safeguard of the environment, is definitely the utilization of unicellular microalgae as a source of bioactive substances. Recently, much effort has

been expended on the search for new therapeutic compounds, demonstrating microalgal antibacterial, antifungal, and anticancer activities (Arun et al. 2012, Dewi et al. 2018). Wells et al. (2017) reviewed health benefits of algae-derived food products. Some prospects for new chemicals have been reported in recent years, the most prominent of which of high nutritional and medical values are carotenoids (Andrade et al. 2018), polyunsaturated fatty acids (PUFAs; Wan et al. 2019), polysaccharides (Barboríková et al. 2019), peptides (Ejike et al. 2017), and radical scavengers (Chen et al. 2016).

This study follows previous research of polar soil unicellular microalgae, where the selected strains from a polar collection were used. These studies were focused on their mineral nutrient requirements (Shukla et al. 2011), biogeographical origin (Hodač et al. 2016), and finally, on *Chlorella mirabilis* (at present *Edaphochlorella mirabilis*; Darienko et al. 2016) potential biomass and biotechnologically important compound production in low-temperature environment (Shukla et al. 2013). The aim of this study was to find out comprehensive pieces of ecophysiological information on unicellular strains, which were collected and isolated from various soil habitats in polar regions. The second aim was to consider a hypothetical biotechnological outcome of studied polar soil unicellular microalgae. Ecophysiological experiments, such as (i) temperature-light cross gradient for algal growth, (ii) temperature coefficient (Q_{10}) and activation energy (Ea), and (iii) photosynthesis-respiration temperature dependence, were performed for strains of unicellular microalgae from polar regions in this study. For future cultivation experiments, the following strain's selection criteria were tested: (a) high growth rate at low temperatures at the range between 0 and 10°C); (b) tolerance of high temperatures above 20°C; and (c) high photosynthetic rates across the temperature gradient from 0 to 25°C.

MATERIALS AND METHODS

Unialgal strain isolation and cultivation. In all, 10 unicellular strains originating from various polar regions and habitats (Table 1) were isolated in the Centre for Phycology of the Institute of Botany CAS in Třeboň and the Institute of Soil Biology CAS in České Budějovice, Czech Republic. Micro-algal samples collected in the field were transported to the laboratory in a frozen state. Natural samples of the soil collected and/or micro-algal biomass were spread on agar plates on Petri dishes (solid media in 1.5% agar contained the Z mineral nutrient medium; Staub 1961). The dilution plate method was used for isolation of strains. All examined strains are kept in the Culture Collection of Algae at the Laboratory of Algology (http://www.butbn.cas.cz/CCALA/) in Třeboň at a temperature of 8°C and an irradiance of 80 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} of PAR. Several strains (strains marked as [e] in Table 1) were analyzed for their phylogenetic characterization (Hodač et al. 2016).

Temperature and irradiance requirements. To find the temperature and light demands of 10 unicellular microalgae (see Table 1, strains marked as [a]), a method of cross gradients of temperature and light was used as described by Kvíderová and Lukavský (2001). The temperature ranged from −4 to 24°C and the irradiance from 5 to 65 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} of PAR to cover the expected range of optimum irradiances for soil algae (Karsten and Holzinger 2012, Karsten et al. 2013, 2016). The lower level of irradiance was chosen because the strains originated from soil. In soil, algae did not grow on its surface. They always grew in soil upper layer, up to 2 cm depth. There is lower irradiance, but more stable environmental conditions in respect to water content, temperature, and even irradiance (Elster et al. 1999). The irradiance and temperature gradients were chosen according to mean microclimatic conditions that occurred on the top soil or slightly below during the sampling seasons (Elster et al. 1999, Tscherko et al. 2003, Kaštovská et al. 2007). The irradiance was measured by a digital luxmeter PU-550 (Metra Blansko, Czech Republic) equipped with a modified quantometric PAR sensor and the temperature was measured by a digital Omega thermometer (USA) at each pre-calibrated marked area selected for a microplate position.

The homogenous microalgal suspension in the Z mineral nutrient medium (Staub 1961) of the volume of 0.2 mL and the initial cell density of 10^5 cells $\cdot$ mL^{-1} was inoculated in wells of 36 serological plates used for 36 different combinations of temperature and irradiance. There were six replicates (in six wells of one column) for each strain on every plate. Following the inoculation, the plates were covered with a thin translucent polyethylene foil to prevent the water evaporation from the algal suspension. The plates were transferred to a metallic platform of the unit for crossed gradients of temperature and irradiance (Labio, Prague, Czech Republic; Kvíderová and Lukavský 2001) with the pre-calibrated marked areas for particular temperature-irradiance combinations. They were covered with a Perspex cover with strips of translucent paper to create different irradiances of white fluorescent light provided by fluorescent tubes fixed above the platform. During the cultivation, the air mixed with CO_2 was permanently pumped under the Perspex cover to prevent from inorganic carbon limitation (as known from previous studies the final CO_2 concentration was 2% v/v; Kvíderová and Lukavský 2001). A thin layer of water was applied on the metallic platform to increase the heat transfer between the plates and the platform. Moreover, it kept high the humidity level under the cover and, together with the polyethylene foil, prevented rapid loss of water from algal suspensions. To synchronize the algal population, the plates were incubated in darkness for 1 day. The absorbance (light scattering) at 750 nm (A_{750}) was measured instantly on every other day by the iEMS Plate Reader (LabSystems, Ltd., Finland). The growth experiments lasted 14 d. The measured values were converted to the number of cells (N; cells $\cdot$ mL^{-1}) and dry weight per unit volume (DW; mg $\cdot$ mL^{-1}) according to the individual conversion curves and equations. The growth rate (d^{-1}) was calculated as the slope of linear regression of dependence of N on time during exponential growth phase (Kvíderová and Henley 2005).

To get the conversion equation, a sample of dense culture of individual strains was diluted creating 0.5, 0.3, 0.1, 0.05, 0.03, 0.01, 0.005, 0.003, 0.001, 0.0005, 0.0003, and 0.0001strength solutions, each of volume of 5 mL. The A_{750} was measured in six wells for every solution in immunological plates of the suspension volume of 0.2 mL by the plate reader. The number of cells in the undiluted cultures was counted in the Bürker's counting chamber and the number of cells in the diluted ones was calculated by multiplying by the dilution factor. The conversion equation parameters of individual strains were calculated using SigmaPlot 10.0. (Systat Software, USA) from the relationship between the A_{750} and the number of cells.

TABLE 1. List of experimental polar unicellular microalgal strains, their original localities, and habitats: [a]temperature and irradiance requirements, [b]temperature quotient (Q_{10}), [c]activation energy (Ea), [d]temperature dependence of photosynthesis and respiration, [e]strains phylogenetically analyzed (Hodač et al. 2016).

Species determination	Strain numbe r Alternative	Polar region GPS coordinates	Location	Collection Year	Habitat	Isolator Reference
Bracteacoccus sp.	N2[a,b,c,d]	Arctic	SvalbardNy-Ålesund	1999	Deglaciated soil	Řeháková, Kaštovská et al. (2005), Lepka (2007)
	1999/2	79°580 N, 11°210 E				
Muriella terrestris	N5[a,b,c,e]	Antarctic	Adelaide Island	2003	Rookeries	Elster, Lepka (2007)
	2003/1	67°34′ S. 68°08′ W				
Chlorella sp.	N6[a,b,d]	Antarctic	Adelaide Island	2003	Rookeries	Elster, Lepka (2007)
	2003/2	67°34′ S. 68°08′ W				
Pseudomuriella sp.	N7[a,b,d]1998/7	Arctic	SvalbardNy-Ålesund	1998	Deglaciated soil	Elster, Kaštovská et al. (2005), Lepka (2007)
		79°58′ N. 11°21′ E				
Chlorella sp.	L3[a,b,e]	Antarctic	King George Island	1996	Deglaciated soil	Lukešová, Lepka (2007)
	1996/15, SPH4	62°10′ S. 58°30′ W				
Chlorella vulgaris	L5[a,b,c,e]	Arctic	Ellesmere Island	1994	Soil close to river	Lukešová, Elster et al. (1999), Lepka (2007)
	1994/5	79°08′ N. 80°30′ W				
Edaphochlorella mirabilis	L10[a,b,e]	Antarctic	King George Island	1997	Deglaciated soil	Lukešová, Lepka 2007
	1997/10, EG/B-11	62°10′ S. 58°30′ W				
Marvania sp.	L15[a,b,e]	Antarctic	Anchorage Island	1999	Bare ground	Lukešová, Lepka (2007)
	1999/15	67°36′ S. 68°15′ W				
Chlorella sp.	L24[a,b,e]	Antarctic	King George Island	1997	Deglaciated soil	Lukešová, Lepka (2007)
	1997/24	62°10′ S. 58°30′ W				
Marvania sp.	L32[a,b,e]	Antarctic	King George Island	1996	Deglaciated soil	Lukešová, Lepka (2007)
	1996/109, EG-1	62°10′ S. 58°30′ W				

Determination of temperature coefficient for growth (Q_{10}). Temperature coefficient (Q_{10}) was calculated for all strains evaluated in temperature-irradiance cross gradient experiments (strains marked as [b] in Table 1). The temperature coefficient (Q_{10}) was calculated using the formula of van't Hoff (1884) with assumptions according to Eppley (1972),

$$Q_{10} = \left(\frac{\mu_2}{\mu_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad (1)$$

where μ_1 is growth rate at temperature T_1 and μ_2 is growth rate at temperature T_2, and $T_2 > T_1$. The Q_{10} values for specific growth rate were calculated in the temperature range of 8–20°C. At lower temperatures, the calculations were not possible for several strains due to negative growth rates.

Determination of activation energy for growth (Ea). Similarly, the activation energy for growth (Ea) was calculated for strains (strains marked as [c] in Table 1) from the slope of linear regression of the dependence of growth rate on temperature.

$$\ln \mu = a\frac{1}{T} + c \quad (2)$$

where

$$a = -\frac{\mathrm{Ea}}{R} \quad (3)$$

and therefore

$$\mathrm{Ea} = -\mathrm{Ra} \quad (4)$$

where μ is the growth rate, a is the slope of the linear regression, T is the temperature in K, R is the gas constant of 8314 J · mol^{-1} · K^{-1}, and c is a constant corresponding to the intercept with the y-axis. The Ea values for specific growth rate were calculated in the temperature range of 8–20°C. As in the case of Q_{10}, the calculations were not possible for several strains due to negative growth rates at lower temperatures.

Temperature dependence of photosynthesis and respiration. The rate of net photosynthesis (P_N) and dark respiration (R_D) as criteria of the metabolism and growth rates in three unicellular strains (see Table 1, strains marked as [d]) were measured as an oxygen production or consumption rate. These strains represented each group distinguished by the CCA. Algal suspension was placed into a magnetically stirred, thermostatically controlled (± 0.1°C) closed chamber (8.2 mL); and a Clarke-type oxygen sensor (Labio, Czech Republic) was used. The rate of oxygen production and/or consumption was recorded using a TZ 4200 linear chart recorder (Laboratory instruments, Prague, Czech Republic – for details see Adamec 1997 and Machová et al. 2008). Before measurements, the suspension of unicellular microalgae was centrifuged (3,000 rpm, 4°C, 15 min) and then transferred to a working solution (0.88 mM $NaHCO_3$ + 0.05 mM KCl; Allen and Spence 1981, Adamec and Ondok 1992). Initial pH of the working solution was set to 6.92, and corresponded to 0.25 mM free CO_2 (Helder 1988). The concentration of CO_2 in the solution was high enough to prevent a CO_2 limitation during the P_N measurements. Moreover, this corresponded roughly to the CO_2 concentrations at which the algae were grown in the field (Sand-Jensen 1989). A 55-W halogen lamp provided a constant irradiance of 300 μmol photons · m^{-2} · s^{-1} in the experimental chamber (the irradiance reaching the top soil during the polar summer ranged between 40 and 700 μmol photons · m^{-2} · s^{-1} (Elster et al. 1995). The light was homogenized by a neutral dispersion filter. First, the dark respiration rate was measured for about 20 min and the photosynthetic rate in the same algal sample was measured afterwards, within about the next 20 min. After each individual

measurement, the concentration of chlorophyll *a* was estimated spectrophotometrically by methanol–acetone extraction method (Pechar 1987). The P_N and R_D were measured at five different temperatures (3, 8, 14, 20, and 26°C) to find the temperature curve for photosynthesis. Four independent measurements were performed for each temperature in each strain.

Statistical analyses. In the crossed-gradient growth experiment, the raw data were subjected to Grubbs test (Grubbs 1969) for elimination of outlying values for six samples at the P-level of 0.05 and the outlying values were excluded from the next calculations. The optimum and growth limits were estimated from contour graphs where the data were smoothed by the least-square method. The statistical significance was evaluated by two-way ANOVA with temperature and irradiance as factors using Statistica 13.0 software (Dell 2015). The canonical correspondence analysis (CCA) was performed using Canoco software (Ter Braak and Šmilauer 2012). The effect of temperature on P_N and R_D was evaluated by one-way ANOVA (Tukey HSD test for multiple comparisons) using Statistica 13.0 software (Dell 2015). The homologous groups were determined for P = 0.05. The differences Q_{10} and Ea for growth, net photosynthesis, and dark respiration were evaluated by Kruskal–Wallis test using Statistica 13.0 software (Dell 2015). The effects were considered statistically significant for $P < 0.05$.

RESULTS

Temperature and irradiance requirements. The cultivation in the crossed gradients of temperature and irradiance revealed specific growth requirements for each strain. The combined effects of temperature and irradiance significantly affected the growth rate (two-way ANOVA, n = 216 in each strain, Table S1 in the Supporting Information). All experimental strains were able to grow at low temperatures (<10°C). The CCA analyses showed, the first and second ordination axis explained 17.81% and 0.92% of variability, respectively (Fig. 1).

According to the temperature requirements, three groups of strains were recognized (Table 2, Fig. 1). Three strains, *Pseudomuriella* sp. N7, *Chlorella* sp. L3, and *Chlorella vulgaris* L5, were considered cold-preferring strains (Fig. 1), since the temperature optimum ranged between 10.1 and 14.3°C in *Pseudomuriella* sp. N7 and *Chlorella* sp. L3, and between 10.1 and 18.4°C in *Chlorella vulgaris* L5, respectively. The cold-preferring strains *Pseudomuriella* sp. N7 and *Chlorella* sp. L3 were isolated from freshly deglaciated soils in the Arctic and the Antarctic, respectively, while the strain *Chlorella vulgaris* L5 originated from a soil near a river in the Arctic. Five of strains were cold- and warm-tolerating (Fig. 1) with growth temperature optimum above 10°C and/or tolerating temperature above 20°C. All cold- and warm-tolerating strains originated from the Antarctic. Finally, two warm-preferring strains, *Muriella terrestris* N5 and *Bracteacoccus* sp. N2, originated from the Arctic and Antarctic, respectively. No correlation between the polar region (Arctic or Antarctic) or habitat type and temperature requirement was detected (Fig. 1).

In upper part of Figure 2, a–c, there are figures of strains' growth rates in measured range of temperatures. Warm-preferring strains *Bracteacoccus* sp. N2 and *Muriella terrestris* N5 (Fig. 2a) continuously increased their growth rate at temperatures higher than 15°C. Their growth rate in optimal temperatures ranged from 0.249 to 0.357 $\cdot$ d^{-1} (Table S2 in the Supporting Information) The cold- and warm-tolerating strains *Edaphochlorella mirabilis* L10, *Marvania* sp. L15, *Chlorella* sp. N6, *Marvania* sp. L32, *Chlorella* sp. L24 (Fig. 2b) increased or slightly decreased their growth at temperatures higher than 15°C. Their growth rate in optimal conditions ranged from 0.162 to 0.341 $\cdot$ d^{-1}; Table S2). The temperature optima of the cold-preferring strains (N7, L3, L5) ranged between 10.1 and 14.3°C and 10.1 to 18.4°C, respectively (Fig. 2c). The growth rate at optimal temperature ranged from 0.252 to 0.344 $\cdot$ d^{-1} (Table S2) and these temperature optima lay within a narrow range of 10–15°C and 10–18°C, respectively.

Since all strains originated from the upper layer of soil (in depth ~0.5 cm to 2 cm), their light requirements were similar. The strains were able to grow at very low irradiances within the range from 10 to 25 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} (Fig. 2, d–f). Approximately half of them preferred low-light conditions (Tables 2, S3 in the Supporting Information), thus indicating low-light conditions in the soil. The cold- and warm-tolerating and warm-preferring strains (*Edaphochlorella mirabilis* L10, *Marvania* sp. L15, *Chlorella* sp. N6, *Marvania* sp. L32, *Chlorella* sp. L24, *Bracteacoccus* sp. N2, and *Muriella terrestris* N5) did not increase their growth rate in response to increasing irradiance (Fig. 2, d and e). Positive effect of increasing irradiance was recorded only for cold-preferring strains *Pseudomuriella* sp. N7, *Chlorella* sp. L3, and *Chlorella vulgaris* L5 (Fig. 2f).

Q_{10} and activation energy. The mean values Q_{10} for specific growth rate ranged from 0.7 (L3) to 3.1 (N5), (Table 3, Fig. 3). The mean Q_{10} values differed significantly among the strains, as well as at individual irradiances (Figs. 3, S1 in the Supporting Information). The effect of irradiance on the Q_{10} values, evaluated by one-way ANOVA, was observed in strains of *Chlorella* sp. N6 (one-way ANOVA, $F_{5,35} = 25.80$, $P < 0.001$), *Chlorella vulgaris* sp. L5 (one-way ANOVA, $F_{5,35} = 6.098$, $P < 0.001$), and *Marvania* sp. L32 (one-way ANOVA, $F_{5,35} = 5.069$, $P < 0.002$; Fig. S1).

The highest Q_{10} values (around 3) were found in warm-preferring strains *Bracteacoccus* sp. N2 and *Muriella terrestris* N5. In other strains, the Q_{10} ranged around 1.0, and these differences were statistically significant. The observation indicates that the key metabolic processes for triggering the growth may be common in all strains and may remain arrested in a 10–20°C range (Figs. 3, S1).

The mean Ea values for specific growth rate ranged between −52.89 (L3) and 101.30 (N5; Table 3,

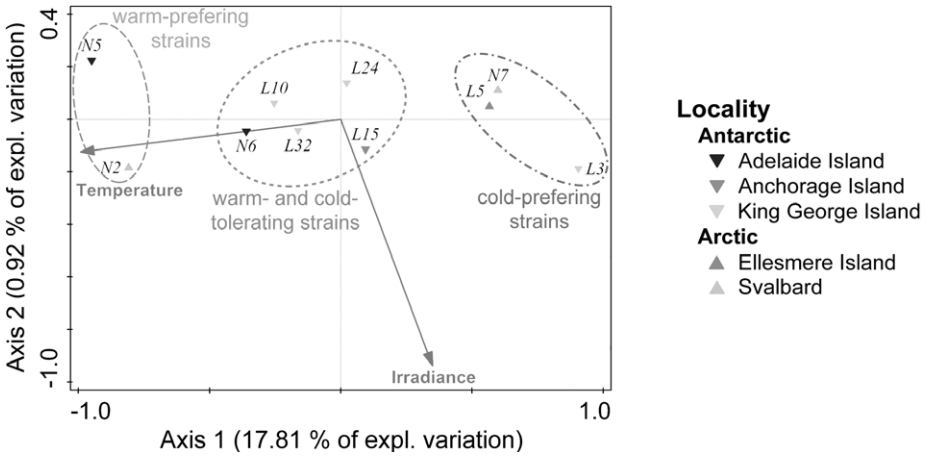

FIG. 1. The CCA results (pseudo-$F = 6.7$, $P < 0.002$ for the first canonical axis; pseudo-$F = 3.5$, $P < 0.002$ for all canonical axes) indicating different temperature requirements, but similar irradiance requirements. The dotted lines determine three groups of strains: warm-preferring strains, cold- and warm-tolerating strains, and cold-preferring strains. The number corresponds to the strain number mentioned in Table 1. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2. The ecological requirements of selected polar unicellular microalgal strains. More detail information on growth rates corresponding to limits and optima are introduced in Tables S2 and S3.

	Temperature [°C]			Irradiance [μmol photons · m^{-2} · s^{-1}]		
	Lower limit	Optimum	Upper limit	Lower limit	Optimum	Upper limit
Bracteacoccus sp. N2	4.5–10.1	14.3–20.5	>20.5	<12.3	<12.3 to >50.5	>50.5
Muriella terrestris N5	4.5	>20.5	>20.5	<12.3	12.3–15.9	>50.5
Chlorella sp. N6	1–4.5	14.3–18.4	>20.5	<12.3	<12.3 to >50.5	>50.5
Pseudomuriella sp. N7	1–4.5	10.1–14.3	>20.5	<12.3	15.9–21.5	>50.5
Chlorella sp. L3	1–4.5	10.1–14.3	20.5	15.9–21.5	21.5 to >50.5	>50.5
Chlorella vulgaris L5	1–4.5	10.1–18.4	>20.5	<12.3	<12.3 to >50.5	>50.5
Edaphochlorella mirabilis L10	4.5–10.1	10.1–20.5	>20.5	12.3–15.9	15.9–21.5	27.8
Marvania sp. L15	1–10.1	10.1–18.4	>20.5	<12.3	<12.3 to >50.5	>50.5
Chlorella sp. L24	1–4.5	10.1 to >20.5	>20.5	<12.3	<12.3 to >21.5	>50.5
Marvania sp. L32	4.5–10.1	10.1–18.4	>20.5	<12.3	15.9	>50.5

Fig. 4). The significant effects of irradiance on Ea, revealed by one-way ANOVA, were observed in strains *Chlorella* sp. L3 (one-way ANOVA, $F_{5,35} = 4.727$, $P = 0.003$), *Chlorella vulgaris* L5 (one-way ANOVA, $F_{5,35} = 2.704$, $P = 0.039$), *Marvania* sp. L15 (one-way ANOVA, $F_{5,35} = 2.690$, $P = 0.043$), and *Chlorella* sp. L24 (one-way ANOVA, $F_{5,35} = 3.035$, $P = 0.025$; Fig. S2 in the Supporting Information).

The lowest Ea values were observed on cold-preferring strains, and the highest Ea in the warm-preferring ones (Figs. 4, S2). Contrary to Q_{10}, a continuous gradient from warm-preferring to cold-preferring strains was observed, probably due to large internal variability of Ea in individual strains.

The analyses of Arrhenius plots of dependence of growth rates (μ) on temperature were performed for strains used in the photosynthesis-respiration measurements (i.e., *Bracteacoccus* sp. N2, *Chlorella* sp. N6, and *Pseudomuriella* sp. N7; Fig. 5). While the regression courses had similar patterns in strains *Bracteacoccus* sp. N2 and *Pseudomuriella* sp. N7, the regression slope, and hence Ea, may change at different irradiances, as was observed in *Chlorella* sp. N6 (t test for irradiances of ~25 and ~50 μmol photons · m^{-2} · s^{-1}, $t_{10} = 4.036$; $P = 0.002$; Fig. 5). Since the irradiance effect was not significant (one-way ANOVA, $F_{5,35} = 2.144$, $P = 0.087$) in *Chlorella* sp. N6, detailed investigation of combined effects of cultivation temperature and irradiance in larger scales of irradiances is needed.

Temperature dependence of photosynthesis and respiration. For measurements of the rate of net photosynthesis (P_N) and dark respiration (R_D), three strains (*Bracteacoccus* sp. N2, *Chlorella* sp. N6, and *Pseudomuriella* sp. N7; Fig. 6) fitted into three different temperature ecological groups introduced above (Fig. 1). The measurements proved a P_N slightly increase at higher temperatures in all tested strains (Fig. 6, Table S4 in the Supporting Information). R_D remained stable in the warm-preferring strain *Bracteacoccus* sp. N2, but slightly increased at higher temperatures in cold- and warm-tolerating or cold-preferring strains *Chlorella* sp. N6 and *Pseudomuriella* sp. N7, respectively (Fig. 6, Table S4). However, the character of the P_N:R_D ratio to the temperature gradient was specific for each strain. The P_N:R_D ratio of the warm-preferring *Bracteacoccus* sp. N2 was less affected by temperature than in other experimental strains. In the cold- and warm-tolerating *Chlorella* sp. N6, the P_N:R_D ratio decreased continuously with rising temperature. The high P_N:R_D ratio at low temperature could suggest high primary production at

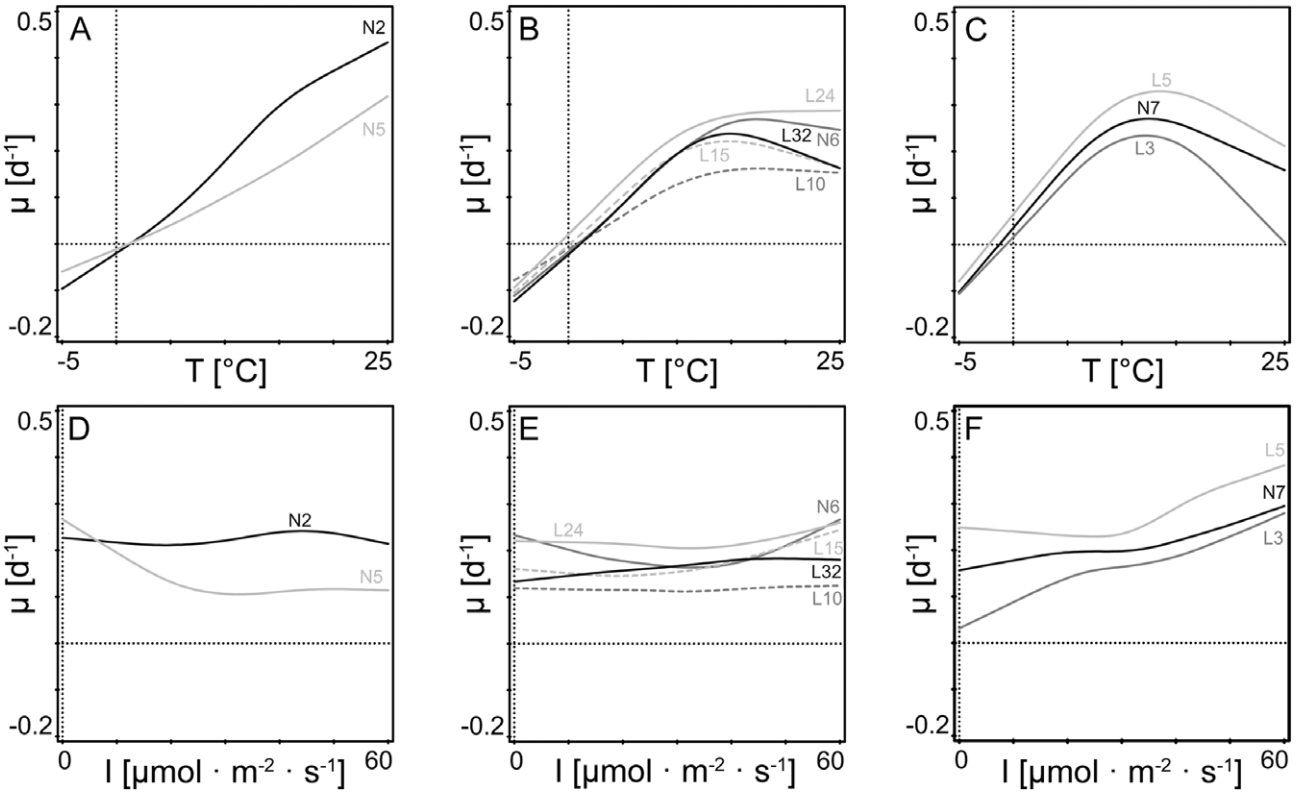

FIG. 2. The fitted generalized additive models of response of growth rate of individual strains to temperature (T) and irradiance (I). (a) response of warm-preferring strains to temperature, (b) response of cold- and warm-tolerating strains to temperature, (c) response of cold-preferring strains to temperature, (d) response of warm-preferring strains to irradiance, (e) response of cold- and warm-tolerating strains to irradiance, and (f) response of cold-preferring strains to irradiance. The number corresponds to the strain number mentioned in Table 1. [Color figure can be viewed at wileyonlinelibrary.com]

low temperatures. In the cold-preferring *Pseudomuriella* sp. N7, the P_N:R_D ratio was significantly higher at temperatures between 14 and 20°C, thus indicating a primary production optimum in this range (Fig. 6, Table S4).

The comparison of Q_{10} and Ea for growth, net photosynthesis, and dark respiration was performed for three strains *Bracteacoccus* sp. N2, *Chlorella* sp. N6, and *Pseudomuriella* sp. N7 (Table 3). In *Bracteacoccus* sp. N2, the values of Q_{10} and Ea for growth were significantly higher than those for photosynthesis and respiration. Contrary, no significant differences were observed for the *Chlorella* sp. N6. Finally, the Q_{10} values were similar, but the Ea for growth was significantly lower than that for photosynthesis and respiration, indicating thus strain-specific response or influence of cultivation and measurement conditions (Table S5 in the Supporting Information).

DISCUSSION

We measured ecophysiological features (temperature and irradiance requirements, Q_{10} and Ea for growth and temperature dependence of photosynthesis and respiration) of unicellular microalgal strains originated from both Arctic and Antarctic soils with the aim to evaluate the level of their acclimation/adaptation to polar environment. We accept the term "adaptation" as a genetically fixed response to outer environmental conditions and "acclimation" as a response to sporadic extremes (fluctuations) of the environment that is not genetically fixed, but comprises biochemical (e.g., synthesis of screening pigments), morphological (e.g., cell wall modification), or physiological (e.g., state transitions) changes (Elster 1999). Unicellular green microalgae of simple morphology are considered airborne microalgae, which are supposedly easily dispersed around the globe via air (Herbold et al. 2014) and ocean (Hellweger et al. 2014) transport. However, it does not impede the genetic structuring of their global population (Bottos et al. 2014, Hellweger et al. 2014, Hodač et al. 2016). On the basis of previous phylogenetic analyses (De Wever et al. 2009, Vyverman et al. 2010, Kochkina et al. 2014, Hodač et al. 2016) of SSU and ITS2 rDNA sequence analyses, the studied polar soil unicellular microalgae strains consisted of different species of Antarctic Chlorellaceans: *Chlorella* sp. L3 (King George Island), *Chlorella* sp. L24 (King George Island), *Marvania* sp. L15 (Anchorage Island), *Marvania* sp. L32

TABLE 3. Values of Q_{10} and Ea reported for various metabolic processes and growth in microalgae (mean ± SD, n = 6 for Growth rate and n = 4 for Net photosynthesis and Respiration). In this study, the mean growth irradiance was 27.3 μmol photons · m^{-2} · s^{-1}, while the photosynthesis and respiration measurements were performed at 300 μmol photons · m^{-2} · s^{-1}.

Organism (Temperature range)	Process	Q_{10}	Ea	Reference
Anabaena sp. (5–40°C)	Nitrogen fixation	5.3 (10–20°C)	54.42	Shukla et al. 1997b,
	Ammonium uptake	4.8 (10–20°C)	41.80	Shukla et al. 1997b,
	Glutamine synthetase	2.2 (10–20°C)	56.30	Shukla et al. 1997b,
Thalassionema pseudonana	Specific growth rate	3.1 (8–17°C)	76.3	Berges et al. 2002
	^{15}N – uptake	2.9 (8–17°C)	71.6	Berges et al. 2002
	Nitrate reductase	2.7(8–17°C)	67.30	Berges et al. 2002
Chlorella sacharophila (5–15°C)	Gross photosynthesis	2.3	55.7	Vona et al. 2004
Chlorella sorokiniana (10–20°C)	Gross photosynthesis	3.0	77.8	Vona et al. 2004
Koliella antarctica (−10 to 15°C)	Methyl Viologen: NR	1.9	43.35	Di Martino Rigano et al. 2006
Bracteococcus sp. N2 (8–20°C)	Growth rate (μ)	3.0 ± 1.4	78.27 ± 41.66	This study
	Net photosynthesis	1.1 ± 0.8	8.43 ± 10.82	This study
	Respiration	1.1 ± 0.4	4.87 ± 21.98	This study
Muriella terrestris N5 (8–20°C)	Growth rate (μ)	3.1 ± 2.5	101.30 ± 77.8	This study
Chlorella sp. N6 (8–20°C)	Growth rate (μ)	1.4 ± 1.8	3.87 ± 78.73	This study
	Net photosynthesis	1.9 ± 0.1	39.82 ± 23.51	This study
	Respiration	0.9 ± 0.1	−6.63 ± 5.02	This study
Pseudomuriella sp. N7 (8–20°C)	Growth rate (μ)	1.2 ± 1.9	−38.24 ± 57.37	This study
	Net photosynthesis	1.5 ± 0.5	23.23 ± 34.67	This study
	Respiration	1.0 ± 1.1	−12.07 ± 41.92	This study
Chlorella sp. L3 (8–20°C)	Growth rate (μ)	0.7 ± 0.8	−52.89 ± 86.96	This study
Chlorella vulgaris L5 (10–20°C)	Growth rate (μ)	0.9 ± 0.8	−15.51 ± 62.28	This study
Edaphochlorella mirabilis L10 (8–20°C)	Growth rate (μ)	1.1 ± 0.8	−8.77 ± 45.36	This study
Marvania sp. L15 (8–20°C)	Growth rate (μ)	1.1 ± 1.0	−11.95 ± 59.41	This study
Chlorella sp. L24 (8–20°C)	Growth rate (μ)	1.3 ± 0.6	−3.92 ± 28.24	This study
Marvania sp. L32 (8–20°C)	Growth rate (μ)	1.1 ± 0.9	−10.20 ± 32.23	This study

(King George Island), *Edaphochlorella mirabilis* L10 (King George Island), and *Muriella terrestris* N5 (Adelaide Island). Hodač et al. (2016) also confirmed relatives of Arctic and Antarctic *Chlorella vulgaris* L5 (Ellesmere Island) mainly based on that the most of the studied unicellular microalgal strains (7 out of 10) exhibit ≥ 99.5% similarity to strains from the temperate zone and are widespread, on the one hand, but, on the other hand, differ in their temperate-polar distribution.

Temperature and irradiance requirements. The growth of micro-algae is limited by many environmental factors but temperature and light belong to the most important (Rai and Gaur 2001). Nutrients including inorganic carbon may contribute significantly to growth rate; however, in our experiments, the nutrient concentrations in the culture medium reached saturation levels. Every strain has adapted to the microenvironment of the original location. Temperature variation in habitats where unicellular microalgae occurs is usually large (euthermal; e.g., all types of polar freshwater and soil environments; Elster et al. 1999, Teoh et al. 2004, Hu et al. 2008, Chong et al. 2011). The response and adaptation of microalgae to low-temperature stress and frost involve changes in physiological processes and biochemical composition which is connected with the production of bioactive substances (Arun et al. 2012, Dewi et al. 2018). For instance, selected micro-algae adapt to low-temperature conditions by producing PUFA and cryoprotectants, and by having high light-harvesting capacity (Morgan-Kiss et al. 2006). According to the temperature requirements, the studied cold-preferring Antarctic strain *Chlorella* sp. L3 is rather psychrophilic, with temperature optimum between 10.1 and 14.3°C and upper growth limit of 20.5°C. Ecologically similar psychrophilic *Chlorella* BI sp. was also isolated from mats of freshwater ponds located within an ablation zone on the McMurdo Ice Shelf on the Ross Sea, south of Bratina Island, Antarctica (Morgan-Kiss et al. 2008). An optimal growth temperature was approximately 10°C and cultures were unable to grow at temperatures > 20°C. The cold-preferring strains *Pseudomuriella* sp. N7 and *Chlorella vulgaris* L5, both from the Arctic, are also considered rather psychrophilic and their temperature optimum is between 10.1 and 14.3°C and 10.1 and 18.4°C, respectively. Both of them sharply decrease their growth rate at temperatures > 20.5°C. Besides cold-preferring strains, also cold- and warm-tolerating rather psychrotolerant strains (*Chlorella* sp. N6, *Edaphochlorella mirabilis* L10, *Marvania* sp. L15, and *Marvania* sp. L32) were recognized. Their growth rate mildly decreased at temperatures > 20.5°C. Warm-preferring but still cold-tolerant strains (*Bracteacoccus* sp. N2 and *Muriella terrestris* N5) were considered as mesophilic. Their thermal properties again are related to environmental parameters of habitats from where they were isolated (Kvíderová and Lukavský 2005). Similar results of temperature preferences of Antarctic isolates were documented by Teoh et al. (2004).

Teoh et al. (2004) also analyzed the growth rates of six Antarctic strains, including unicellular microalgae *Chlorella* and *Stichococcus*. They both

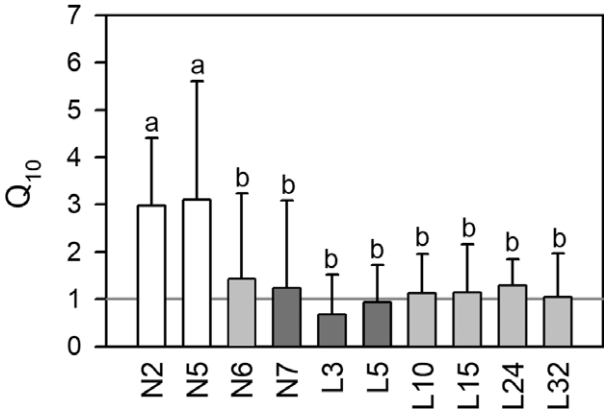

FIG. 3. The Q_{10} values for growth rates (mean ± SD, $n = 6$) for polar algal strains in the temperature range 10–20°C summarized for all irradiances. The F and P values corresponds to results of one-way ANOVA (n = 36 for each strain). The letters indicate homologous groups recognized by the Tukey's HSD test at $P = 0.05$. The number corresponds to the strain number mentioned in Table 1. [Color figure can be viewed at wileyonlinelibrary.com]

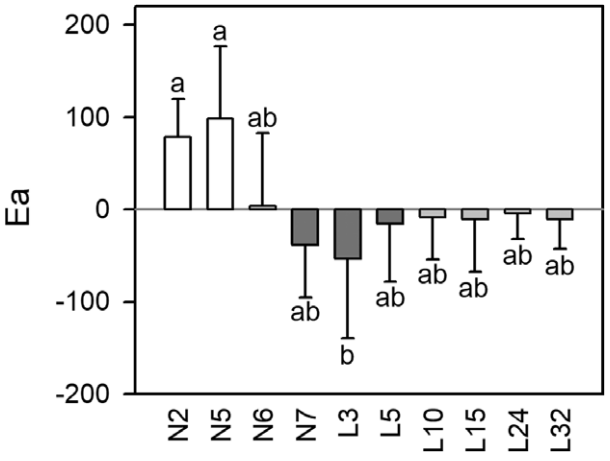

FIG. 4. The Ea values for growth rates (mean ± SD, $n = 6$) for polar algal strains in the temperature range 10–20°C summarized for all irradiances. The letters indicate homologous groups recognized by the Tukey's HSD test at $P = 0.05$. The number corresponds to the strain number mentioned in Table 1. [Color figure can be viewed at wileyonlinelibrary.com]

showed broad optimum temperature for growth, ranging from 6 to 20 and 4 to 14°C, respectively. Their growth rates at the temperature of 6, 9, and 14°C were 0.19, 0.23, 0.24 · d^{-1}, and 0.20, 0.19, and 0.20 · d^{-1}, respectively. Our results in a similar range of temperatures oscillated from 0.252 to 0.344 · d^{-1}. In contrary to our results, Cao et al. (2016) observed higher growth rates of 0.85 · d^{-1} at 15°C. However, it was also shown (Shukla et al. 2013) that *Edaphochlorella mirabilis* L10 increases the growth rate under nitrogen and carbon source manipulation at low temperatures. Its growth rate in nutrient manipulation conditions and at temperature of 15°C and irradiance 75 μmol photons · m^{-2} · s^{-1} fluctuated from 0.13 to 0.23 in indoor and outdoor 0.28 to 0.44 · d^{-1} conditions, respectively. However, only the effect of 5% glycerol addition was statistically significant. There are convincing literature data to support the fact that biomass yield of strains of microalgae can be significantly increased by subjecting the culture to mixotrophic growth conditions (Samejima and Myers 1958). However, information is still scarce on polar strains of microalgae with respect to culture conditions to enhance growth rate and production of useful compounds.

Special ecophysiological features strains studied here also confirm data of photosynthetic characteristics and the effect of UV radiation and elevated temperature on *Chlorella* sp. isolated from snow at King George Island, Maritime Antarctica (Rivas et al. 2016). The irradiance 10 cm below the snow surface did not exceed 350 μmol photons · m^{-2} · s^{-1}, which matches well the average irradiance required for saturation of photosynthesis determined for *Chlorella* sp. (276 μmol photons · m^{-2} · s^{-1}). In alpine soil algae, the saturation irradiances of photosynthesis varied around 20–40 μmol photons · m^{-2} · s^{-1} (Karsten and Holzinger 2012, Karsten et al. 2013). The studied strains were able to grow at very low irradiances indeed, with optimum irradiance between ~15 to 20 μmol photons · m^{-2} · s^{-1}, thus indicating an adaptation to low-light conditions in the soil. The low explained variation on the second ordination axis (Fig. 2) confirmed the similarity of light requirements of tested strains. The long-term tolerance of high irradiance (above 300 μmol photons · m^{-2} · s^{-1}) remains to be determined; however, short-term (20 min) tolerance of irradiance of 300 μmol photons · m^{-2} · s^{-1} was proven during the measurement of temperature dependence of photosynthesis and respiration in the strains *Bracteacoccus* sp. N2, *Chlorella* sp. N6, and *Pseudomuriella* sp. N7. These light requirements are in the lower range described for *Chlamydomonas* sp. from the Giant Mountains, Czech Republic (Kvíderová 2010), and clearly lower than light requirements of 523–826 μmol photons · m^{-2} · s^{-1} measured in Arctic population of *Chlamydomonas nivalis* (Stibal et al. 2007).

Temperature coefficient and activation energy. Q_{10} and Ea are important parameters for describing the effect of temperature on individual metabolic processes and overall growth (Raven and Geider 1988). The Q_{10} and Ea values of the growth provide an indirect evidence for the threshold of temperature and energy required for the activation of key metabolic activities (Shukla et al. 1997a,b). The values recorded for the selected polar strains except for *Bracteacoccus* sp. N2 and *Muriella terrestris* N5 of Arctic and Antarctic origin, respectively, show about half the value (Table 3) reported for a marine diatom *Thalassiosira weissflogii* ($Q_{10} = 2.46$) in a similar temperature range 10–20°C (Lomas and Glibert 1999). However, the Antarctic strain of *Bracteacoccus* sp. N2 exhibited 40.6% higher value of Q_{10} as compared to *T. weissflogii* (Table 3).

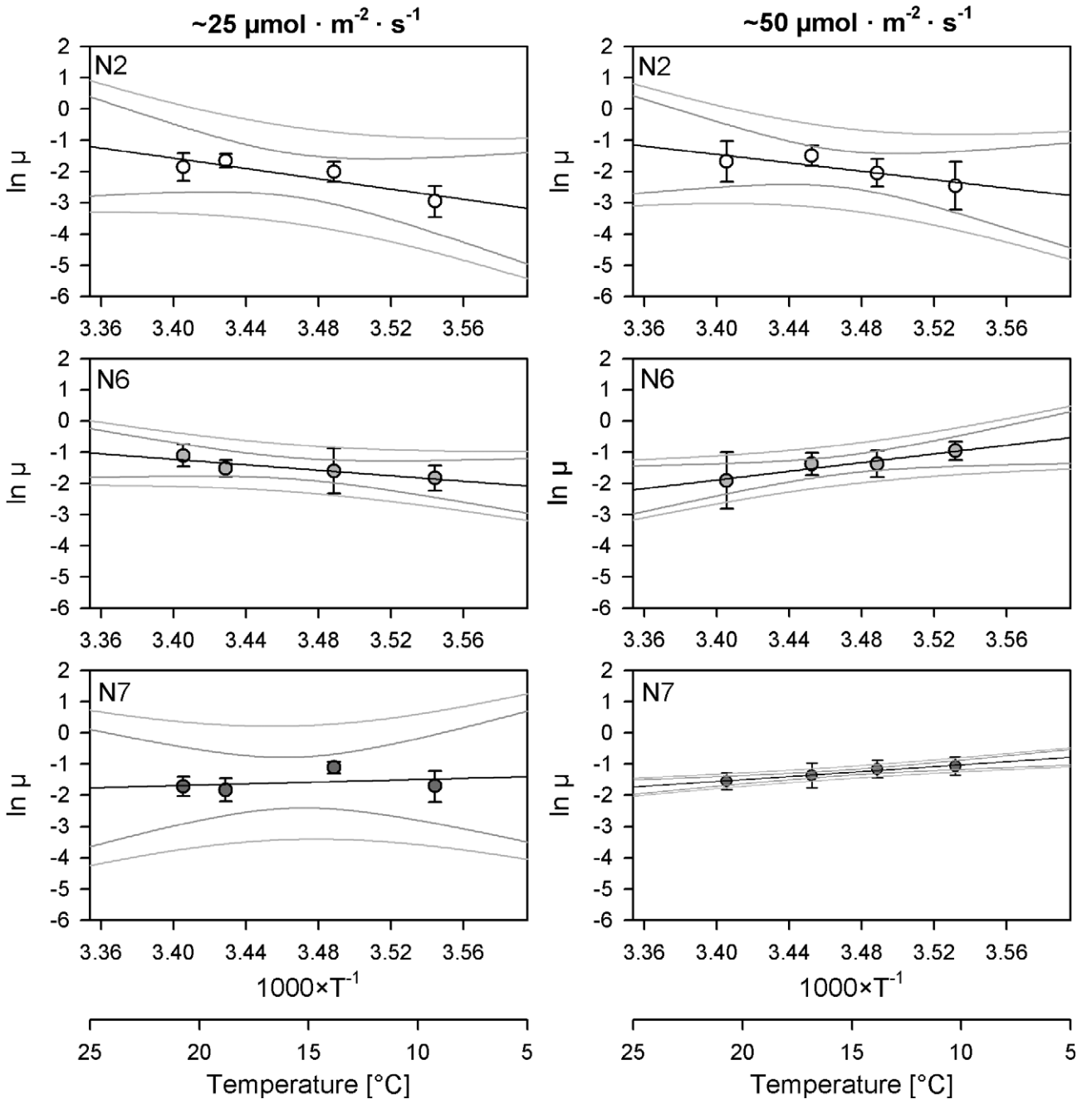

FIG. 5. Arrhenius plot of dependence of specific growth rate µ ($n = 6$, mean ± SD) on temperature in range 8–20°C for three strains used in photosynthesis/respiration measurements at two irradiances. The regression line is black. The dark grey lines indicate 95% confidence band, and the grey ones 95% prediction band. The number corresponds to the strain number mentioned in Table 1. [Color figure can be viewed at wileyonlinelibrary.com]

Descolas-Gros and de Billy (1987) in an earlier investigation on a temperate diatom *Phaeodactylum tricornutum* and Antarctic *Nitzschia kerguelensis* recorded a value of 72 kJ · mol^{-1} for the activation energy of carboxylase activity of RuBisCO (in temperature range of 0–40°C). Li et al. (1984) reported Ea value of 70.6 kJ · mol^{-1} for light-saturated photosynthesis in *Phaeodactylum tricornutum*. Energy of activation required for activating respiratory electron transport was 51.0, 68.6, and 51.0 kJ · mol^{-1} for *Chaetoceros debile*, *Cyclotella* sp. and *Dunaliella tertiolecta*, respectively (Ahmed and Kenner 1977). The values obtained in the present study ranged from 8.43 to 39.82 kJ · mol^{-1} for the net photosynthesis of selected polar strains N2, N6, and N7. This indicates that single vital metabolic activities such as nutrient transport, photosystem II activity, and carbon fixation are not triggered until the above

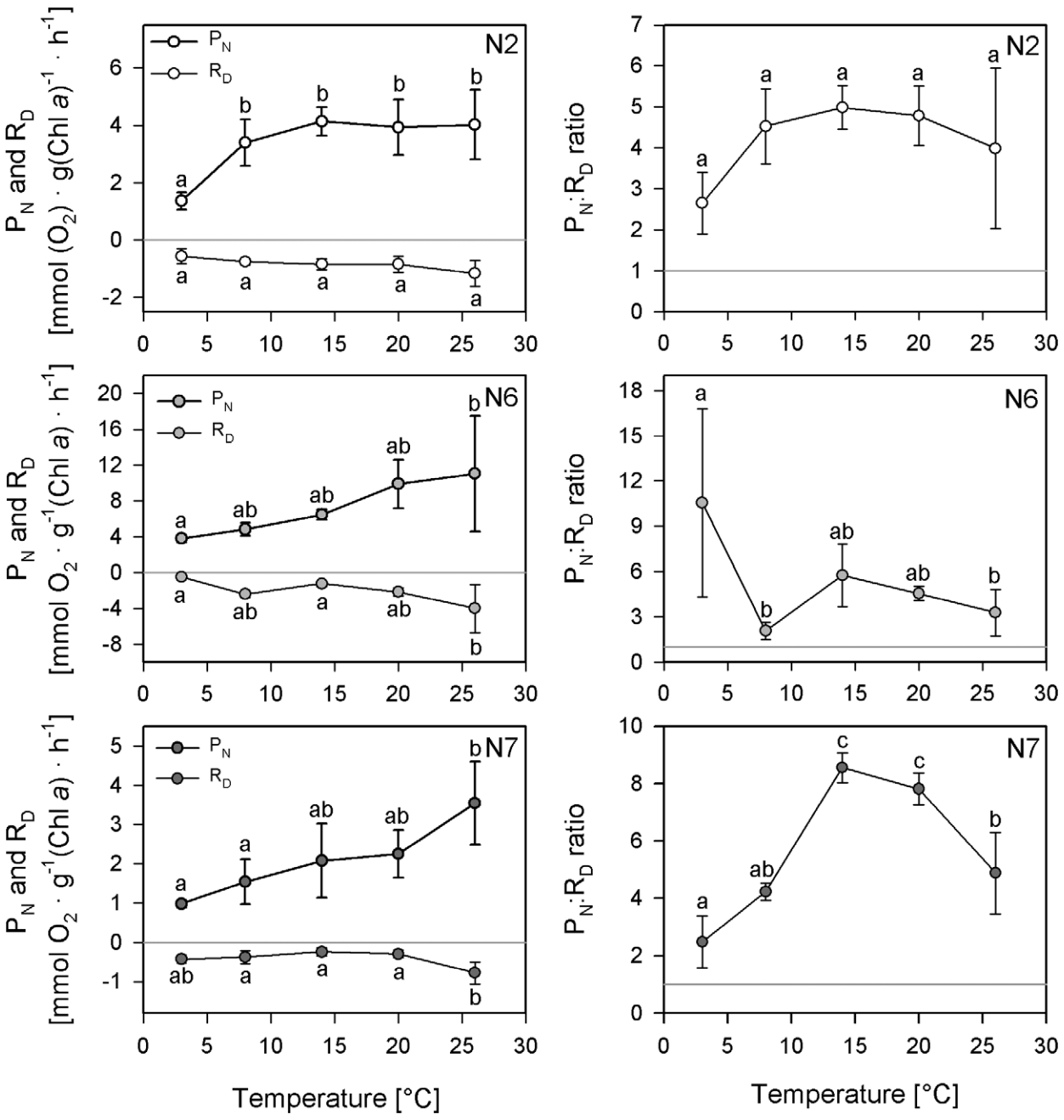

FIG. 6. The effects of temperature on net photosynthesis (PN), dark respiration (R_D), and photosynthesis:respiration (P_N:R_D) ratio in warm-preferring strain *Braceacoccus* sp. N2, warm- and cold-tolerating strain *Chlorella* sp. N6 and cold-preferring strain *Pseudomuriella* sp. N7 (mean ± SD; $n = 4$ in each treatment). The letter indicates homologous groups distinguished by Tukey's HSD test at $P = 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

threshold of energy barrier is crossed. Overall observations reveal an interesting phenomenon of variation in Ea values at low (25 ± 5 μmol photons · m^{-2} · s^{-1}) and higher irradiances (58 ± 5 μmol photons · m^{-2} · s^{-1}) for the polar strains (all data not shown). Therefore, the activation of metabolic processes and the Arrhenius plot of the increase in the rate of the activity depend upon an interplay of the light and temperature and the temperature is not the sole factor for crossing the threshold energy required for the growth.

The findings indicate that activation energy of growth in the microalgal isolates studied depends upon two variables (i.e., irradiance and temperature) contrary to heterotrophic bacteria where activation energy of growth is dependent non-linearly on the temperature, and the temperature is the only growth limiting factor (Ratkowsky et al. 1982). The overall

effects of temperature and irradiance depend on strain history and the thermal and light environment in the habitat where the organism was growing. Therefore, in spite of a favorable temperature, growth may remain arrested due to sub-optimal light conditions. The contribution of mineral nutrients including inorganic carbon is discussed above in case of the growth rate.

Temperature dependence of photosynthesis and respiration. Rates of photosynthesis and respiration in aquatic plants are influenced by many ecological parameters including time of the day, temperature, and irradiance (Azcón-Bieto and Osmond 1983, Davison 1991). The dynamic processes of photosynthesis are associated with three different time scales: rapid photoresponses (min), photoinhibition (h), and photoadaptation (d; Falkowski 1992, Han et al. 2000). Since both aquatic and soil unicellular microalgae grow in liquid water and the ecophysiological studies are performed in liquid media; therefore, we presume that the results should be comparable. In our experiment, R_D and P_N were measured within 20 min. During the rapid photoresponse, photosynthesis usually reaches a steady state with a time lag of several minutes, and is nearly constant afterwards (Han et al. 2000). In our measurements, the P_N and R_D and their ratio followed similar temperature limits of growth measured in the cross-gradient experiments and also fitted into three different temperature ecological groups (Fig. 1). However, the studied strains (warm-preferring *Bracteacoccus* sp. N2, cold- and warm-tolerating *Chlorella* sp. N6, cold-preferring *Pseudomuriella* sp. N7), for which the temperature dependence of photosynthesis and respiration was measured, contained also bacteria. We think that these bacteria were also partly responsible for measured values of R_D. We assume that these bacteria were acclimated or adapted to low temperatures and we were not able to remove them when we isolated and cultivated strains. In comparison with an ecophysiological study of Xanthophyceae (*Tribonema fonticolum* and *T. monochloron*) from the inundation area of the Lužnice River (Třeboňsko Biosphere Reserve, Czech Republic) during winter-spring flood (Machová et al. 2008), our data on R_D were more stable and did not react on temperature increase. In our recent measurements, P_N increased at higher temperatures in all tested strains (Fig. 6) and their photosynthetic temperature optima were higher than those in the environment from which they were collected and isolated. Similar results were reported also by, for example, Tang and Vincent (1999), Kvíderová and Lukavský (2005), Stibal and Elster (2005), Machová et al. (2008).

CONCLUSIONS

This article presents information about the ecophysiological features (temperature/light demand for growth, temperature coefficient-activation energy, and photosynthesis/respiration temperature dependence) of unicellular soil cold-preferring/cold- and warm-tolerating/warm-preferring microalgae isolated in the Arctic and Antarctica. To survive in an extreme climatic environment, microalgae often have to survive wide fluctuations in chemical and physical parameters. They have developed defensive and adaptive strategies, including the synthesis of a tremendous diversity of compounds originating from different metabolic pathways. Polar and low temperature adapted microalgae can be rapidly cultured to explore their biotechnological potential for the production and processing of desirable compounds. However, one of the major limitations in biotechnological applications of polar microalgae is their remarkably slow growth. There is interest in optimizing growth rate and/or physico-chemical conditions during cultivation to improvement the biomass yield and/or activate the production of bioactive molecules. A higher biomass yield facilitates a detailed characterization of polar unicellular microalgal strains (morphological, physiological, biochemical, and molecular-genetic), which could help further develop biotechnological applications. The aim of this study was to obtain knowledge about the ecophysiological features of cold-preferring/cold- and warm-tolerating/warm-preferring unicellular soil microalgae and how to optimize their growth conditions including conditions under which they could produce bioactive molecules. This study provides information related to the biotechnological potential of polar low temperature adapted microalgae to produce valuable metabolites in a polar environment and in Central European non-summer conditions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. The Q_{10} values for growth rates (mean ± SD, $n = 6$) for polar unicellular micro-algal strains in the temperature range 10–20°C at the different growth irradiances.

Figure S2. The Ea values for growth rates (mean ± SD, $n = 6$) for polar unicellular micro-algal strains in the temperature range 10–20°C at the different growth irradiances

Table S1. The statistical significance of effects of temperature (T), irradiance (I) and their combination (T×I) on growth rate of ten polar unicellular micro-algal strains determined by two-way ANOVA ($n = 216$ for each strain). Statistically significant effects are marked bold. d.f. = degrees of freedom.

Table S2. The temperature requirements of selected polar unicellular micro-algal strains and corresponding growth rates (mean ± SD, n = 36). The mean growth rates was calculated from given ANOVA temperature category across all irradiances. The superscript indicates the mean temperature (°C) in the given ANOVA category.

Table S3. The irradiance requirements of selected polar unicellular micro-algal strains and corresponding growth rates (mean ± SD, n = 36). The mean growth rates was calculated from given ANOVA irradiance category across all temperatures. The superscript indicates the mean irradiance [μmol. m^{-2}. s^{-1}] in the given ANOVA category.

Table S4. The statistical significance of effects of temperature net photosynthesis (P_N), dark respiration (R_D) and P_N:R_D ratio of three polar unicellular micro-algal strains determined by one-way ANOVA (n = 20 for each strain). Statistically significant effects are marked bold. d.f. = degrees of freedom

Table S5. Statistical significance of differences among Q_{10} and Ea determined for growth rate (μ), net photosynthesis (P_N) and dark respiration (R_D). (Kruskal–Wallis test, n = 44 for each strain). Statistically significant effects are marked bold. d.f. = degrees of freedom