Polar Biology (2020) 43:2003–2010
https://doi.org/10.1007/s00300-020-02758-4

ORIGINAL PAPER

The effect of temperature on Antarctic lichen cytochrome and alternative respiratory pathway rates

Mikhail Shelyakin[1] · **Ilya Zakhozhiy**[1] · **Tamara Golovko**[1]

Received: 24 July 2019 / Revised: 25 September 2020 / Accepted: 30 September 2020 / Published online: 9 October 2020

Abstract

Respiration is a crucial process that provides all living organisms with energy and metabolites for growth and cellular maintenance. The processes that control respiration in lichens remain poorly understood. We investigated the effects of short-term temperature changes on the respiration rate, as well as the relative contributions of the cytochrome and alternative pathways of thalli from four green-algal lichen species collected from their natural habitats in Antarctica. Lichen respiration was sensitive to short-term temperature increases over a range of 5–35 °C. The total O_2 uptake rate was increased by fourfold, and the mean respiratory coefficient (Q_{10}) decreased from 2.5 to 1.3 as the temperature increased. An increase in temperature from 5 to 15 °C had a positive effect on cytochrome respiration coupled with energy production. Temperatures above 15 °C stimulated the activation of the alternative (energy-dissipating) respiratory pathway. Hyperthermia led to increased O_2 consumption that was not associated with mitochondrial oxidases. The effects of increased temperature on the respiration rates were more pronounced in the bipolar lichens *Umbilicaria decussata* and *Usnea sphacelata* than in the *Usnea aurantiaco-atra* species with a narrower geographical distribution.

Keywords Lichens · Antarctica · Temperature · Respiration · Cytochrome and alternative respiratory pathways · Residual respiration

Introduction

Lichens are ancient stable entities formed from the symbiotic association between a fungus and a photosynthesizing partner (photobiont) that is a species of green algae and/or cyanobacteria. Green algae supply the heterotrophic mycobiont with reduced carbon in the form of sugar alcohols (ribitol, sorbitol, erythritol), whereas cyanobacteria produce glucose (Elix and Stocker-Wörgötter 2008). The heterotrophic mycobiont accounts for over 90% of the thallus biomass and most of the respiration (Palmqvist et al. 2008).

Respiration of the photoautotrophs breaks down photosynthetic products to release energy in the form of ATP, reducing equivalents, metabolizing components, and intermediates necessary for growth and cellular maintenance. In addition to the cytochrome pathway (CP), which is coupled with ATP synthesis, an alternative (energy-dissipating) pathway (AP) of respiration exists in the mitochondrial electron transport chain (ETC) of plants, algae, and saprotrophic and parasitic fungi (Weger et al. 1990; Umbach and Siedow 2000; Joseph-Horne et al. 2001; Lambers and Ribas-Carbo 2005; van Dongen et al. 2011; Neimanis et al. 2013). In plants, the respiratory rate and distribution of electrons between the two respiratory pathways depend on temperature (Gonzàlez-Meler et al. 1999; Atkin et al. 2002; Campbell et al. 2007). To our knowledge, no studies have yet been performed regarding the role of AP in lichen respiration or regarding the effects of temperature on the activation of different respiratory pathways.

Antarctica has a harsh climate, with a cold summer and frequent precipitation in the form of snow. At night, lichens are often exposed to low negative temperatures. In the day, the average air temperature is slightly higher than 0 °C. A

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00300-020-02758-4) contains supplementary material, which is available to authorized users.

✉ Mikhail Shelyakin
shelyakin@ib.komisc.ru

[1] Institute of Biology of Komi Scientific Centre, Ural Branch of the Russian Academy of Sciences, 28 Kommunisticheskaya Street, Syktyvkar, Komi Republic, Russia 167982

short-term increase in the air temperature of up to 5–10 °C can warm lichen thalli to temperatures of up to 20–35 °C (Øvstedal and Smith 2001).

In the current study, we investigated the effect of short-term changes in temperature on the respiration rate, capacity (maximal activity), and relative contribution of the cytochrome and alternative respiratory pathways in the thalli of four lichen species typical to the Antarctic region.

Materials and methods

The thalli of four chlorolichens (*Ramalina terebrata*, *Umbilicaria decussata*, *Usnea aurantiaco-atra*, and *Usnea sphacelata*) were collected during the summer of 2015, 2016, and 2018 (January–April) in their natural habitats (Table 1). Some lichens were collected on the coast in the eastern part of continental Antarctica, and others were collected from islands near the Antarctic Peninsula.

The lichen samples were delivered to the laboratory and stored in an air-dry state in the dark at 4 °C. Before the start of the experiment, hydrated thalli were acclimated for one week in a Binder KWVF-720 climatic chamber (Binder GmbH, Tuttlingen, Germany) at 15 °C and 60% relative humidity. Photosynthetically active radiation (PAR) intensity was approximately 80 µmol m^{-2} s^{-1}, and lichens were incubated with a photoperiod of 10/14 h (day/night).

The functional state of the photobiont was estimated by measuring the maximum quantum yield (F_v/F_m) of photosystem II (PSII) (Genty et al. 1989). F_v/F_m was used as a vitality indicator in the transported lichens. Chlorophyll *a* (Chl *a*) fluorescence parameters were determined using a PAM-2100 portable fluorimeter (Walz, Effeltrich, Germany). Hydrated thalli were pre-adapted to the given temperature (− 1, + 5, or + 15 °C) for 30 min. Then, the Chl *a* fluorescence parameters were measured using a specialized cooling unit based on Peltier modules. The maximum quantum yield value was calculated using the following equation:

$$F_v/F_m = \left(F_m - F_o\right)/F_m \quad (1)$$

where F_o and F_m are the background and maximum levels of Chl *a* fluorescence, respectively. These parameters were measured after pre-adapting thalli in the dark for 30 min before analysis.

The respiration rate was determined by measuring O_2 uptake by polarography using an Oxytherm System Clark electrode (Hansatech Instruments, Pentney, UK). Data were expressed as nmol O_2 (g dry weight min)$^{-1}$. The upper parts of the fruticose lichen thalli or the marginal regions of the foliose lichen thalli were cut into 2–3-mm^2 strips. Samples weighing 15–20 mg were placed in a 4-ml reaction vessel containing 1.5 ml 50-mM Hepes buffer (pH 7.2; Helicon, Moscow, Russia) and were constantly agitated during measurements. The O_2 uptake rate was measured at temperatures of 5, 15, 25, and 35 °C. A fresh sample was used at each temperature. Each measurement included 4–7 biological replicates.

The CP and AP respiration rates were estimated using specific inhibitors (Bahr and Bonner 1973; Møller et al. 1988). To facilitate the penetration of solutions into cells and avoid the use of the high concentrations of inhibitors, the lichen thalli were cut. The high concentrations of inhibitors can have a damaging effect and disrupt cellular functions (van der Plas and Wagner 1980). The optimal concentrations of inhibitors were selected in the preliminary experiments, using the method suggested by Møller et al. (1988), and they did not differ from those recommended for plant tissues. A solution of 6 mM benzhydroxamic acid (BHAM) (Lancaster, UK) was used as an alternative oxidase (AOX) inhibitor. The activity of cytochrome oxidase (COX) was suppressed with 2 mM KCN (Sigma Aldrich, St. Louis, MO, USA). The rates of each respiratory pathway were calculated in absolute (nmol O_2) and relative (% of total respiration) units.

Inhibitors were added successively after measuring the total O_2 uptake rate. For each sample, the total respiration rate was measured for 10 min at each temperature. The total O_2 consumption rate (without inhibitors addition) plus CP, AP, and residual respiration capacities (after addition of the respiratory pathways inhibitors) were measured over 30 min for each sample at each temperature. In preliminary experiments, it was found that the total O_2 uptake rate by thallus samples remained stable within this period.

The rate of oxygen consumption was calculated as follows:

Table 1 Sample collection sites and characteristics of the Antarctic lichens

Species	Coordinates of sampling locations	Life-form	Ecological group
Ramalina terebrata Hook f. & Taylor	62° 13.203′ S 59° 01.661′ W 62° 13.924′ S 59° 00.617′ W	Fruticose	Epilithic
Umbilicariadecussata (Vill.) Zahlbr	69° 44.533′ S 73° 42.600′ E	Foliose	Epilithic
Usnea aurantiaco-atra (Jacq.) Bory	62° 11.416′ S 58° 55.604′ W 61° 13.139′ S 55° 21.224′ W 62° 12.319′ S 58° 57.277′ W	Fruticose	Epilithic
Usnea sphacelata R. Br	69° 44.533′ S 73° 42.600′ E 67° 40.052′ S 45° 50.122′ E	Fruticose	Epilithic

$$V_t = V_{alt} + V_{cyt} + V_{res} \quad (2)$$

where V_t is total respiration, V_{alt} is alternative respiration suppressed by AOX inhibitor, V_{cyt} is cyanide-sensitive (cytochrome) respiration, and V_{res} is residual respiration recorded in the presence of the inhibitors of CP and AP.

V_{alt} was calculated based on the rate of oxygen consumption in the presence of the CP inhibitor KCN. V_{cyt} was calculated based on the rate of oxygen consumption in the presence of the AOX pathway inhibitor benzhydroxamic acid. V_{res} was calculated as the rate of oxygen consumption after adding both the AOX and COX inhibitors.

Statistical analysis of the obtained data was performed using Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA). Data are shown as the mean ± standard error (SE). Normal distribution was confirmed using the Shapiro–Wilk test. Means were compared using analysis of variance (one-way ANOVA) and Duncan's test. The significance of the influence of different factors on the total respiration and the capacity and ratio of different respiratory pathways was shown using F- and Wilks's test values (two-way ANOVA). A value of $p \leq 0.05$ was considered statistically significant.

Results

Photochemical activity in green-algal lichens

All species investigated in this study were green-algal lichens. To assess the state of the thalli collected in Antarctica, we investigated the photochemical activity of the photobionts, which play a central role in providing nutrition for the heterotrophic mycobiont. Temperatures of −1 to +15 °C did not affect the maximum photochemical efficiency index, showing an F_v/F_m value of 0.68–0.71 (Table 2).

Impact of short-term changes in temperature on respiratory O_2 uptake in lichen thalli

Figure 1 shows the respiratory activity of lichen thallus cuts exposed to temperatures of 5–35 °C. *U. decussata* and *U. sphacelata* thalli had higher O_2 uptake than did thalli from *U. aurantiaco-atra* or *R. terebrata*. The average change in sensitivity over a range of 10 °C (Q_{10}) for 5–35 °C was 1.8 (Table 3). Detailed analysis showed that the $Q_{5\text{-}15\ °C}$ was 2.2–3.2, while the $Q_{25\text{-}35°C}$ was only 1.2–1.6. This decrease in the Q_{10} values indicates a decreased temperature sensitivity for lichen respiration at higher temperatures. It should be noted that, compared to those for *U. sphacelata* respiration, the Q_{10} was higher for *U. aurantiaca-atra* respiration at 5–15 °C, and lower at 15–35 °C.

Table 2 Values of the maximum potential quantum yield (F_v/F_m) of Antarctic lichens at different temperatures

Species	F_v/F_m (relative units, mean ± SE)		
	−1 °C	+5 °C	+15 °C
Ramalina terebrata	0.68 ± 0.01[a]	0.69 ± 0.01[a]	0.71 ± 0.01[a]
Umbilicaria decussata	0.71 ± 0.01[a]	0.73 ± 0.01[a]	0.68 ± 0.01[b]
Usnea aurantiaco-atra	0.68 ± 0.02[a]	0.71 ± 0.03[a]	0.71 ± 0.02[a]
Usnea sphacelata	0.71 ± 0.02[a]	0.69 ± 0.01[a]	0.67 ± 0.02[a]

F_v/F_m values were calculated for photosystem II in the thalli of each lichen species (n = 5–11 per species). Different superscript letters denote statistically significant changes owing to temperature (ANOVA, $F_2 = 1.41$ $p = 0.278$ for *R. terebrata*; $F_2 = 13.74$ $p = 0.0003$ for *U. decussata;* $F_2 = 0.32$ $p = 0.734$ for *U. aurantiaco-atra;* and $F_2 = 0.76$ $p = 0.484$ for *U. sphacelata*)

Impact of short-term temperature changes on respiratory pathways activity

Next, we measured the temperature-dependent activity and contribution of each respiratory pathway to O_2 uptake (Fig. 1). At 5 °C the respiratory pathways ratio did not differ significantly between the lichen species (*Wilks* test = 0.58, ANOVA $F_6 = 1.36$, $p = 0.27$). CP was dominant for the respiration of all investigated lichen species. The proportion of CP respiration was 50–53% of the total rate of O_2 consumption, while that of AP was 29–34%. The proportion of residual (non-mitochondrial) respiration did not exceed 15% of the total O_2 uptake rate at this temperature.

An increase in the temperature from 5 to 15 °C led to enhanced respiration in lichens, mainly due to CP activation. At this temperature, CP capacity (V_{cyt}) increased by 2.5– to threefold. However, changes in the CP contribution to total O_2 uptake were less pronounced. At 15 °C, the CP contribution in *R. terebrata*, *U. decussata*, and *U. sphacelata* respiration was 60–67%. In *U. aurantiaco-atra*, this parameter remained unchanged compared to that at 5 °C.

A statistically significant increase in AP capacity (V_{alt}) in *U.aurantiaco-atra* did not lead to an increase in the contribution of AP to total respiration. In the other studied species, V_{alt} remained the same, but its contribution to total respiration decreased significantly. Non-mitochondrial respiration accounted for less than 20% of the total O_2 uptake.

When the temperature was increased from 15 to 25 °C, V_{cyt} increased by an average of 1.5- to twofold in all lichens except *U. aurantiaco-atra*. A significant increase in V_{alt} (2.6-fold) was noted only in *U. sphacelata*. Non-mitochondrial respiration was notably activated in the *U. decussata* thalli, whereas V_{res} in *U. sphacelata* showed a decrease.

The increase in temperature led to species-specific changes in the ratio of the respiratory pathways. In *R. terebrata*, the contribution of CP to total respiration increased along with decreased AP and residual respiration

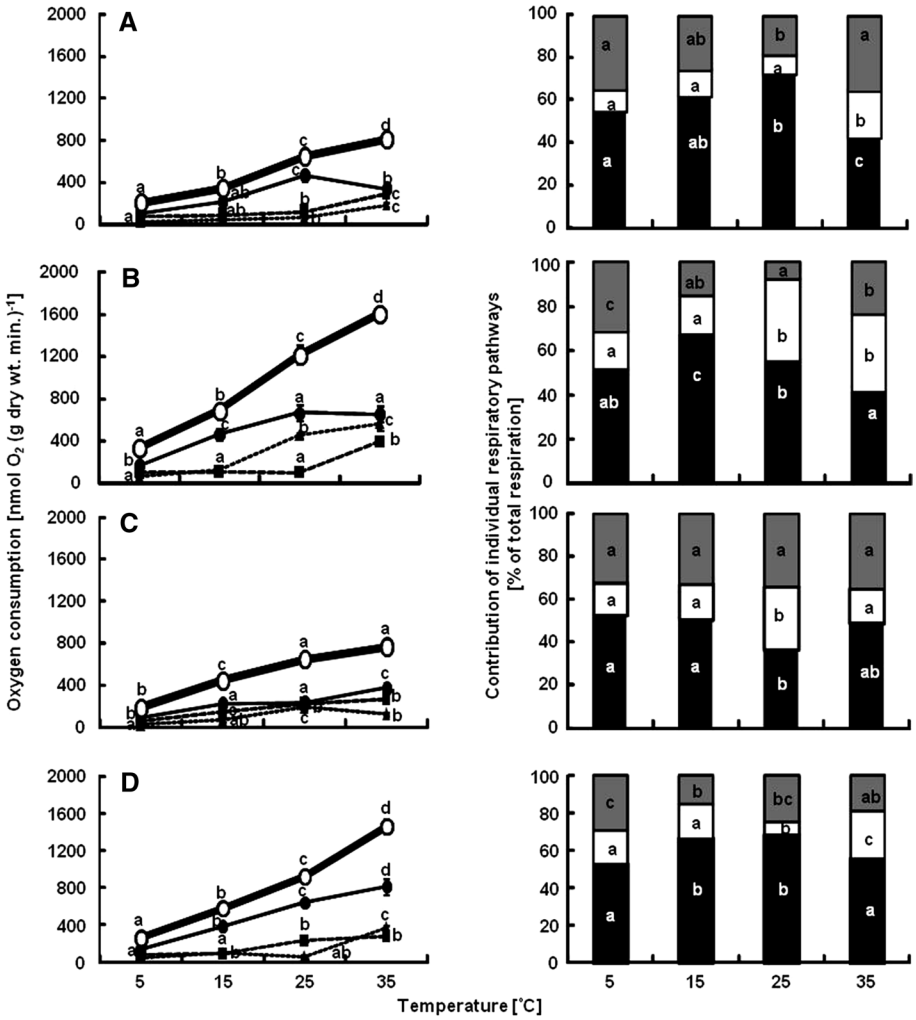

Fig. 1 Effect of temperature on total respiration and respiratory pathways rates and the relative contribution of each respiratory pathway to total respiration in the Antarctic lichens. **a** *Ramalina terebrata*; **b** *Umbilicaria decussata*; **c** *Usnea aurantiaco-atra*; and **d** *Usnea sphacelata*. Data are shown as the mean ± SE. The total respiration rate (open circle) and each respiratory pathway: cytochrome respiration (filled circle), alternative respiration (filled square), and residual respiration (filled triangle) are shown on the left. The contributions of the cytochrome (black) and alternative pathways (grey), and residual respiration (white), to total respiration are shown on the right. Different letters at each data point and column indicate significant differences between temperatures. Changes in the activity and contribution of individual respiratory pathways in lichen thalli with increasing temperature were statistically significant (*Wilks* test = 0.05, ANOVA $F_9 = 43.12$, $p < 0.001$ and *Wilks* test = 0.46, ANOVA $F_6 = 9.55$, $p < 0.001$, respectively). Respiratory pathways capacity (*Wilks* test = 0.12, ANOVA $F_9 = 24.74$, $p < 0.001$) and ratio (*Wilks* test = 0.28, ANOVA $F_6 = 17.74$, $p < 0.001$) also depended on lichen species

contribution. In *U. sphacelata* thalli, CP contribution was unchanged, while AP contribution increased and residual respiration was decreased. In *U. decussata*, a significant increase in the residual respiration contribution was noted due to the decline of CP and AP. *U. aurantiaco-atra* showed a slight decrease in the CP contribution and an increase in the residual respiration contribution.

A temperature of 35 °C strongly affected the AP and residual respiration capacity. V_{alt} was increased in *R. terebrata* and *U. decussata*, while V_{res} increased in all species except *U. aurantiaco-atra*. The contribution of residual respiration increased by 1.5- to twofold and reached 37% of the total respiratory rate. The increased role of AP and the activation of non-mitochondrial respiration led to a decrease in the CP contribution to total O_2 uptake.

Discussion

As expected, the transportation and storage of dry thalli in a cool dark chamber had no negative effect on their viability. The F_v/F_m value averaged 0.7, which indicates a high

Table 3 The temperature coefficient Q_{10} for the total respiration rate (V_t) and the capacities of the cytochrome (V_{cyt}), alternative (V_{alt}), and residual (V_{res}) respiratory pathways in Antarctic lichens at different temperature ranges

Species	V_t			V_{cyt}			V_{alt}			V_{res}		
	$Q_{(5-15\,°C)}$	$Q_{(15-25\,°C)}$	$Q_{(25-35\,°C)}$	$Q_{(5-15\,°C)}$	$Q_{(15-25\,°C)}$	$Q_{(25-35\,°C)}$	$Q_{(5-15\,°C)}$	$Q_{(15-25\,°C)}$	$Q_{(25-35\,°C)}$	$Q_{(5-15\,°C)}$	$Q_{(15-25\,°C)}$	$Q_{(25-35\,°C)}$
Ramalina terebrata	2.6 ± 0.4^{b}	1.6 ± 0.2^{a}	1.3 ± 0.03^{a}	2.6 ± 0.7^{b}	1.9 ± 0.2^{b}	0.8 ± 0.1^{a}	1.3 ± 0.2^{a}	1.4 ± 0.3^{a}	2.7 ± 0.6^{b}	1.0 ± 0.3^{a}	1.7 ± 0.4^{a}	2.9 ± 0.4^{b}
Umbilicaria decussata	2.4 ± 0.3^{b}	1.5 ± 0.2^{a}	1.3 ± 0.1^{a}	3.0 ± 0.4^{b}	1.3 ± 0.2^{a}	1.0 ± 0.1^{a}	1.0 ± 0.2^{a}	1.1 ± 0.2^{a}	3.9 ± 0.1^{b}	2.2 ± 0.3^{b}	3.0 ± 0.2^{c}	1.2 ± 0.1^{a}
Usnea aurantiaco-atra	3.2 ± 0.5^{b}	1.0 ± 0.1^{a}	1.2 ± 0.1^{a}	3.0 ± 0.5^{b}	1.6 ± 0.3^{a}	1.1 ± 0.1^{a}	3.0 ± 0.3^{b}	1.6 ± 0.2^{a}	1.4 ± 0.1^{a}	3.9 ± 0.9^{b}	1.5 ± 0.3^{a}	0.7 ± 0.02^{a}
Usnea sphacelata	2.2 ± 0.2^{b}	1.6 ± 0.1^{a}	1.6 ± 0.1^{a}	$2.9 \pm .02^{b}$	1.6 ± 0.1^{a}	1.3 ± 0.1^{a}	1.0 ± 0.2^{a}	3.2 ± 0.5^{b}	1.2 ± 0.1^{a}	2.5 ± 0.2^{b}	0.5 ± 0.1^{a}	4.4 ± 0.4^{c}

Data are shown as the mean ± SE ($n = 4$–6 per species). Q_{10} is the proportional change in respiration rate per 10 °C temperature increase. Different superscript letters denote statistically significant changes owing to temperature (ANOVA, $F_8 = 11.9$ $p = 0.015$ for *R. terebrata*; $F_8 = 27.1$ $p < 0.001$ for *U. decussata;* $F_8 = 5.2$ $p = 0.03$ for *U. aurantiaco-atra;* and $F_8 = 16.3$ $p < 0.001$ for *U. sphacelata*)

potential photochemical activity of the photosynthetic partner in the studied lichen species.

In hydrated thalli preliminarily kept in a climatic chamber to fully restore their functional activity, the respiration rate at a temperature of 15 °C varied from 380 (*R. terebrata*) to 690 nmol O_2 (g dried weight min)$^{-1}$ (*U. decussata*). We don't found significant differences in the O_2 consumption rate between *R. terebrata* and *U. aurantiaco-atra* (Fig. 1 A and C). It is in contradiction with data reported earlier (Kappen and Redon 1987). The authors of this study showed that *R. terebrata* thalli had more active respiration rate (measured by the release of CO_2) than *U. fasciata* (synonym of *U. aurantiaco-atra)* in the temperature range from -2 to 30 °C. They noted that the samples of *R. terebrata* thalli were selected in the habitats with large amount of organic nitrogen. Previously, we founded a strong statistically significant positive correlation between the respiration activity and nitrogen content in the 9 species of Antarctic lichens (Shelyakin et al. 2019). The samples of *R. terebrata* and *U. aurantiaco-atra* used in our study were both from bird sites, which are rich in organic nitrogen.

Previous studies showed that Antarctic lichen respiration is sensitive to temperature changes (Kappen 1983, 1985; Ino 1985; Laguna-Defior et al. 2016) and that Antarctic lichens cannot acclimate their respiration rates in response to temperature increases (Colesie et al. 2018). Our findings showed that the total O_2 uptake rate in thalli increased fourfold and that the mean respiratory coefficient (Q_{10}) decreased from 2.5 to 1.3 with a rise in temperature from 5 to 15 °C. The changes in the Q_{10} respiration coefficient for Antarctic lichens are consistent with the existing models of temperature-dependent plant respiration (Atkin and Tjoelker 2003).

To the best of our knowledge, this is the first study to investigate the temperature-dependent changes in the respiratory pathways activities and the CP and AP ratio in Antarctic lichens. We found that the lichen species did not differ significantly with regard to the pathways ratio at 5 °C. At this temperature, the AP contribution to the total O_2 uptake rate was approximately 30% (Fig. 1). For Antarctic lichens, the temperature of 5 °C is optimal (Schroeter et al. 2017). The involvement of AP in respiration at a low positive temperature may be a constitutive feature of Antarctic lichens. For most plants, the proportion of AP in respiration is 15–20% in optimal growth conditions and increases 1.5–2 times during cold hardening (Purvis and Shewfelt 1993; Atkin et al. 2002; Fiorani et al. 2005). The high AP contribution to total respiration has been found to play a role in the formation of stress tolerance in various plant species living under extreme environmental conditions (Umbach et al. 2009; Kornfeld et al. 2013; Del-Saz et al. 2018).

The increase in temperature caused the species-specific changes in the respiratory pathways' activity. We found that in all the studied lichens, the increase in temperature from

5 to 15 °C led to the activation of the cytochrome respiration capacity. At the same temperature range, the significant increase in alternative respiration was observed in *U. aurantiaco-atra*. The increase in AP and / or residual respiration activities was observed in the 15–35 °C range in *R. terebrata, U. decussata,* and *U. sphacelata* thalli. The cytochrome respiratory pathway is coupled with ATP synthesis; therefore, an increase in the V_{cyt}/V_{alt} ratio is favorable for lichen metabolism. The alternative respiratory pathway, which gives less ATP synthesis, separates from the main respiratory pathway at ubiquinone. The reduction level of the ubiquinone pool is one of the factors determining AP activity in plant mitochondria (Vanlerberghe and McIntosh 1997). The high temperature stimulates mitochondrial respiration and increases the reduction level of the ubiquinone pool. The over-reduction of ubiquinone is a major source of mitochondrial reactive oxygen species (ROS), which are harmful to living cells. The activation of the alternative respiratory pathway may prevent an excessive reduction of the ubiquinone pool and the development of oxidative stress in the cells of these Antarctic lichens under short-term high temperature exposure. The AP is an important component of the antioxidative system in plants (Maxwell et al. 1999) and fungi (Joseph-Horne et al. 2001). According to several studies, during the day and under direct sunlight, the air in Antarctica can warm up to 16–18 °C and the temperature of thalli can reach 20 °C or higher (Pannewitz et al. 2003; Cao et al. 2015; Sadowsky and Ott 2016). The effect of these relatively high temperatures on lichens depends on their state. The dry lichens are inactive and resistant to extreme conditions. Although the rate of desiccation of lichens depends on temperature, complete inactivation of various processes in lichen thalli occurs within a few hours after desiccation begins (Kranner et al. 2008; Cho et al. 2020). In the literature, there are direct data on maintenance of the functional activity of lichens habited in the natural conditions of Antarctica when the temperature rises above 20 °C (Schroeter et al. 2017). It is known that the respiration is one of the most resistant functions. We found that the respiration of hydrated thalli, pre-acclimated in favorable for life conditions, is sensitive to short-term (about 30 min) action of high temperature. Thus, the changes in the ratio of respiratory pathways can play an important role in the functional adaptation and optimization of energy exchange processes of Antarctic lichens in the first minutes or hours of exposure to high temperatures before the onset of cryptobiosis.

The O_2 uptake, which was not suppressed by inhibitors of mitochondrial oxidases, was markedly activated at high temperatures. The increased contribution of residual respiration to total O_2 uptake may have been caused by the activation of cytosolic and apoplastic oxidases due to increased oxidative stress and impaired mitochondrial function. The increase in O_2 uptake under high temperatures, which is not associated with respiratory activity, has been observed in several different plants (Ivanova et al. 1989). This was thought to be due to cellular and mitochondrial damage. Our study demonstrates the species specificity in terms of the reaction of each lichen's mitochondrial respiration to temperature increases.

Compared to the ratios of *U. sphacelata*, *U. decussata*, and *R. terebrata,* the V_{cyt}/V_{alt} ratio of *U. aurantiaco-atra* was lower and varied from 1.1 to 1.7 across a wide range of temperatures. The respiratory pathways ratio was also more constitutive. The results of the two-way ANOVA analysis confirmed the species-specific temperature dependence of Antarctic lichen respiration (Fig. 1). Both factors, temperature and species, had significant effects on the respiratory metabolism of the lichens. The ANOVA *F* and *Wilks's* test values indicated that the changes in the total O_2 uptake and individual respiratory pathways rates depended more on the temperature than on the lichen species. At the same time, changes in the ratio of the respiratory pathways and their relative contribution to total respiration were more dependent on the species than on the temperature.

We tested the interaction effect of temperature and species on the respiratory pathways ratio in lichens thalli. As presented in the tables (Online Resource 1), the number of significant differences (p-level value ≤ 0.05) in the total respiration rate and respiratory pathways capacity and ratio is greater between species at the given temperature than that in the same species at different temperatures. In other words, the combined effect of temperature and species was statistically significant, which was expressed in the fact that with the increasing temperature, the interspecific differences in the studied respiratory characteristics increased. This pattern is especially pronounced between *U. aurantiaco-atra* and other three investigated lichens. In this regard, it is relevant to note that according to the Consortium of North American Lichen Herbaria (CNALH, https://lichenportal.org/portal/) and Global Biodiversity Information Facility (GBIF, https://www.gbif.org/) data, *U. aurantiaco-atra* is mostly distributed along the Northern border of the Antarctic circle and only partially covers the southern part of the temperate zone of South America (the Eastern slopes of the Southern Andes and Tierra del Fuego), where macroclimatic conditions are close to those in Marine Antarctica. *U. decussata* and *U. sphacelata* can be found in various climatic zones within both the northern and southern hemispheres. In the south, the area of *R. terebrata* distribution often overlaps with that of *U. aurantiaco-atra*. The northern border of the distribution of *R. terebrata* is located on the border of the subtropical and tropical zones of South America, where the lichen is found in the cloudy forests and on ocean coasts. *R. terebrata* has also been found in Hawaii (Lamb 1964). It is likely that the high and stable contribution of the alternative respiratory pathway to the total respiration at the range of temperatures is one of the constitutive physiological mechanisms of *U.*

aurantiaco-atra tolerance to the short-term temperature changes in its habitats. The information on the alternative respiratory pathway in lichens is very scanty. Therefore, even indirect data are of interest. Beckett et al. (2011) showed that lichens in the desert are more resistant to desiccation and are characterized by a higher rate of metabolic heat dissipation as opposed to lichens in wet habitats. Moreover, the activation of heat release after rehydration in desert lichens was less pronounced than in lichens from wet habitats. It is well known, that alternative respiration is an energy-dissipating pathway, and the metabolic heat release rate correlates with the AP capacity (Garmash et al. 2013). We have to assume the lichens with increased AP involvement are more successful in extreme habitats, whereas lichens with a plastic respiratory pathways ratio adapt better to a wide range of conditions.

Conclusion

Our study shows that the respiration rate of Antarctic lichens is sensitive to short-term temperature increases in the 5–35 °C range. The total O_2 uptake rate in thalli increased by fourfold, and the mean respiratory coefficient Q_{10} decreased from 2.5 to 1.3 as temperature increased. In the thalli of *R. terebrata*, *U. decussata*, and *U. sphacelata* with wider distribution areas, the increase in temperature from 5 to 15 °C had a positive effect on the cytochrome respiratory pathway capacity, which is associated with energy synthesis. Furthermore, temperatures higher than 15 °C stimulated the energy-dissipating alternative respiratory pathway. Hyperthermia enhanced the residual respiration independent of mitochondrial oxidases. In *U. aurantiaco-atra* species with narrower geographical distribution, the respiratory pathway ratio was more stable at temperatures ranging from 5 to 35 °C. At the same time, the relative contribution of the energetically ineffective alternative respiratory pathway to the total O_2 uptake rate was higher in *U. aurantiaco-atra*. Based on the experimental data, we can conclude that the observed short-term temperature increases in the thalli of Antarctic lichens affect the contribution rate of respiratory pathways, resulting in an energy imbalance.

Acknowledgements We thank Mikhail Andreev (Komarov Botanical Institute, Russian Academy of Sciences) for the opportunity to collect the lichen samples used in this study. Furthermore, we would like to thank Editage (www.editage.com) for providing professional English-language editing.

Funding This work was carried out as part of the "Physiology and Stress-Resistance of Photosynthesis of Plants and Poikilohydric Photoautotrophs in Conditions of the North" project (No. AAAA-A17-117033010038-7) and was partly funded by RFBR (research project No. 18-34-00346 mol_a).

Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest to declare.

Ethical Approval To ensure objectivity and transparency in research, we have ensured that the accepted principles of ethical and professional conduct have been followed.

References

Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. Trends Plant Sci 8:343–351. https://doi.org/10.1016/S1360-1385(03)00136-5

Atkin OK, Zhang Q, Wiskich JT (2002) Effect of temperature on rates of alternative and cytochrome pathway respiration and their relationship with the redox poise of the quinone pool. Plant Physiol 128:212–222. https://doi.org/10.1104/pp.010326

Bahr JT, Bonner WD (1973) Cyanide-insensitive respiration. I. The steady states of skunk cabbage spadix and bean hypocotyl mitochondria. J Biol Chem 248:3441–3445

Beckett RP, Alyabyev AJ, Minibayeva FV (2011) Patterns of heat production during desiccation and rehydration in lichens differing in desiccation tolerance. Lichenologist 43:178–183. https://doi.org/10.1017/S0024282910000769

Campbell C, Atkinson L, Zaragoza-Castells J et al (2007) Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. New Phytol 176:375–389. https://doi.org/10.1111/j.1469-8137.2007.02183.x

Cao S, Zhang J, Zheng H et al (2015) Photosynthetic performance in Antarctic lichens with different growth forms reflect the diversity of lichenized algal adaptation to microhabitats. Pol Polar Res 36:175–188. https://doi.org/10.1515/popore-2015-0012

Cho SM, Lee H, Hong SG, Lee J (2020) Study of ecophysiological responses of the Antarctic fruticose lichen *Cladonia borealis* using the PAM fluorescence system under natural and laboratory conditions. Plants 9:85. https://doi.org/10.3390/plants9010085

Colesie C, Büdel B, Hurry V, Green TGA (2018) Can Antarctic lichens acclimatize to changes in temperature? Global Change Biol 24:1123–1135. https://doi.org/10.1111/gcb.13984

Del-Saz NF, Ribas-Carbo M, McDonald AE et al (2018) An in vivo perspective of the role(s) of the alternative oxidase pathway. Trends Plant Sci 23:206–219. https://doi.org/10.1016/j.tplants.2017.11.006

Elix JA, Stocker-Wörgötter E (2008) Biochemistry and secondary metabolites. In: Nash TH (ed) Lichen Biology, 2nd edn. Cambridge University Press, Cambridge, pp 104–133

Fiorani F, Umbach AL, Siedow JN (2005) The alternative oxidase of plant mitochondria is involved in the acclimation of shoot growth at low temperature. A study of *Arabidopsis* AOX1a transgenic plants. Plant Physiol 139:1795–1805. https://doi.org/10.1104/pp.105.070789

Garmash EV, Dymova OV, Malyshev RV et al (2013) Developmental changes in energy dissipation in etiolated wheat seedlings during the greening process. Photosynthetica 51:497–508. https://doi.org/10.1007/s11099-013-0044-z

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta Gen Subj 990:87–92. https://doi.org/10.1016/S0304-4165(89)80016-9

Gonzàlez-Meler MA, Ribas-Carbo M, Giles L, Siedow JN (1999) The effect of growth and measurement temperature on the activity of

the alternative respiratory pathway. Plant Physiol 120:765–772. https://doi.org/10.1104/pp.120.3.765

Ino Y (1985) Comparative study of the effects of temperature on net photosynthesis and respiration in lichens from the Antarctic and subalpine zones in Japan. Bot Mag Tokyo 98:41–53. https://doi.org/10.1007/BF02488905

Ivanova TI, Semikhatova OA, Yudina OS, Leina GD (1989) The effect of temperature on plants respiration in natural ecosystems of different botanical and geographical zones. In: Semikhatova OA (ed) Ekologo fiziologicheskiye issledovaniya fotosinteza I dykhaniya rasteniy (Ecological physiological studies of plant photosynthesis and respiration). Nauka, Leningrad, pp 140–167 **(in Russian)**

Joseph-Horne T, Hollomon DW, Wood PM (2001) Fungal respiration: a fusion of standard and alternative components. Biochim Biophys Acta Bioenergy 1504:179–195. https://doi.org/10.1016/S0005-2728(00)00251-6

Kappen L (1983) Ecology and physiology of the Antarctic fruticose lichen *Usnea sulphurea* (Koenig) Th. Fries. Polar Biol 1:249–255. https://doi.org/10.1007/BF00443196

Kappen L (1985) Lichen-habitats as micro-oases in the Antarctic: the role of temperature. Polarforschung 55:49–54

Kappen L, Redon J (1987) Photosynthesis and water relations of three Maritime Antarctic lichen species. Flora 179:215–229. https://doi.org/10.1016/S0367-2530(17)30240-2

Kornfeld A, Heskel M, Atkin OK et al (2013) Respiratory flexibility and efficiency are affected by simulated global change in Arctic plants. New Phytol 197:1161–1172. https://doi.org/10.1111/nph.12083

Kranner I, Beckett R, Hochman A, Nash TH (2008) Desiccation-tolerance in lichens: a review. Bryologist 111:576–593. https://doi.org/10.1639/0007-2745-111.4.576

Laguna-Defior C, Pintado A, Green TGA et al (2016) Distributional and ecophysiological study on the Antarctic lichens species pair *Usnea antarctica*/*Usnea aurantiaco-atra*. Polar Biol 39:1183–1195. https://doi.org/10.1007/s00300-015-1832-7

Lamb IM (1964) Antarctic lichens: I. The genera *Usnea*, *Ramalina*, *Himantormia*, *Alectoria*, *Cornicularia*. British Antarctic Survey, London

Lambers H, Ribas-Carbo M (eds) (2005) Plant respiration: from cell to ecosystem. Springer, Dordrecht

Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci USA 96:8271–8276

Møller IM, Bérczi A, van der Plas LHW, Lambers H (1988) Measurement of the activity and capacity of the alternative pathway in intact plant tissues: identification of problems and possible solutions. Physiol Plant 72:642–649. https://doi.org/10.1111/j.1399-3054.1988.tb09176.x

Neimanis K, Staples JF, Hüner NPA, McDonald AE (2013) Identification, expression, and taxonomic distribution of alternative oxidases in non-angiosperm plants. Gene 526:275–286. https://doi.org/10.1016/j.gene.2013.04.072

Øvstedal DO, Smith RIL (2001) Lichens of Antarctica and South Georgia: a guide to their identification and ecology. Cambridge University Press, Cambridge

Palmqvist K, Danlman L, Jonsson A, Nash THI (2008) The carbon economy of lichens. Lichen Biology, 2nd edn. Cambridge University Press, Cambridge, pp 183–215

Pannewitz S, Schlensog M, Green TGA et al (2003) Are lichens active under snow in continental Antarctica? Oecologia 135:30–38. https://doi.org/10.1007/s00442-002-1162-7

Plas LHWVD, Wagner MJ (1980) Influence of ethanol on alternative oxidase in mitochondria from callus-forming potato tuber discs. Physiol Plant 49:121–126. https://doi.org/10.1111/j.1399-3054.1980.tb02639.x

Purvis AC, Shewfelt RL (1993) Does the alternative pathway ameliorate chilling injury in sensitive plant tissues? Physiol Plant 88:712–718. https://doi.org/10.1111/j.1399-3054.1993.tb01393.x

Sadowsky A, Ott S (2016) Symbiosis as a successful strategy in continental Antarctica: performance and protection of *Trebouxia* photosystem II in relation to lichen pigmentation. Polar Biol 39:139–151. https://doi.org/10.1007/s00300-015-1677-0

Schroeter B, Green TGA, Pintado A et al (2017) Summer activity patterns for mosses and lichens in Maritime Antarctica. Antarct Sci 29:517–530. https://doi.org/10.1017/S095410201700027X

Shelyakin MA, Andreev MP, Tabalenkova GN, Golovko TK (2019) Respiratory activity of some lichen species—representatives of Antarctic flora. Contemp Probl Ecol 12:332–338. https://doi.org/10.1134/S1995425519040115

Umbach AL, Lacey EP, Richter SJ (2009) Temperature-sensitive alternative oxidase protein content and its relationship to floral reflectance in natural *Plantago lanceolata* populations. New Phytol 181:662–671. https://doi.org/10.1111/j.1469-8137.2008.02683.x

Umbach AL, Siedow JN (2000) The cyanide-resistant alternative oxidases from the fungi *Pichiastipitis* and *Neurospora crassa* are monomeric and lack regulatory features of the plant enzyme. Arch Biochem Biophys 378:234–245. https://doi.org/10.1006/abbi.2000.1834

van Dongen JT, Gupta KJ, Ramírez-Aguilar SJ et al (2011) Regulation of respiration in plants: a role for alternative metabolic pathways. J Plant Physiol 168:1434–1443. https://doi.org/10.1016/j.jplph.2010.11.004

Vanlerberghe GC, McIntosh L (1997) Alternative oxidase: from gene to function. Annu Rev Plant Physiol Plant Mol Biol 48:703–734. https://doi.org/10.1146/annurev.arplant.48.1.703

Weger HG, Guy RD, Turpin DH (1990) Cytochrome and alternative pathway respiration in green algae: measurements using inhibitors and $^{18}O_2$ discrimination. Plant Physiol 93:356–360. https://doi.org/10.1104/pp.93.1.356