Photosynthetic activity of Arctic *Vaucheria* (Xanthophyceae) measured in microcosmos

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Abstract

The xanthophycean alga *Vaucheria* inhabits very variable, and hence extreme, ecosystem of polar river estuaries. Therefore, it represents a unique system for the study of adaptation/acclimatization mechanisms. Since the ecophysiological measurements cannot be performed in the field directly, a piece of *Vaucheria* community collected in the Adventelva estuary, Longyearbyen, Svalbard, was taken to a microcosmos in order to study it. The set of instrumentation for measurement of ecophysiological parameters was constructed for such a field study. The community structure and photosynthetic activity expressed as the dissolved oxygen concentration and effective quantum yield (and hence relative electron transfer rate) were measured. The study defined relations between encountered environmental conditions and *Vaucheria* photosynthetic activity in late Arctic summer. The community consisted almost entirely of *Vaucheria* thalli; small marine pennate diatoms were rare. The microcosmos was proved functional since the structure of the community remained unaffected. Both methods of photosynthetic activity measurement were able to record its diel changes during 10 days lasting incubation. Although only weak correlation was found between the oxygen concentration and variable chlorophyll, probably due to the time necessary for oxygen diffusion, data from fluorometers were highly correlated. The *Vaucheria* expressed diel cycles in the photosynthetic activity, and the photosynthetically active radiation was the main driving factor. The incubation in microcosmos proved to be suitable for ecophysiological studies which will include the addition of tidal cycles and more sophisticated protocols for photosynthetic activity measurement.

Key words: *Vaucheria,* photosynthesis, temperature, PAR, instrumentation, RDA analysis

DOI: 10.5817/CPR2017-1-6

Received December 21, 2016, *accepted* June 23, 2017.
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Acknowledgements: The research was supported by project LM2015078 - CzechPolar2 - Czech Polar Research Infrastructure (MŠMT), and a long-term research development project no. RVO 67985939 (IB). We would also like to thank to the Czech Arctic Research Infrastructure "Josef Svoboda Station" (as a part of the Czech Polar Research Infrastructure, CzechPolar2) for support and to Dr. Linda Nedbalová for providing the Minikin QT datalogger.

Introduction

The Svalbard river estuaries with a dynamic system of lotic and lacustrine environments (flow channels and lagoons) represent very variable and hence extreme, environment *sensu* marginal unstable environment (Elster 1999), or poikilo-environment (Gorbushina et Krumbein 1999). The microenvironment of the flow channels and lagoons may differ in the thermal regime, salinity range, water and nutrients availability, turbidity, light penetration or sediment stability, providing thus various microenvironmental niches. The resulting microenvironmental parameters are driven by the interplay of actual weather conditions (wind, cloudiness), diel (temperature, light) and tidal (salinity, water availability) cycles.

In the estuary of the Adventelva, Longyearbyen, Svalbard, the xanthophycean alga *Vaucheria* sp. forms large amounts of biomass, representing thus the main primary producer in this ecosystem. Since it forms thick mats, it contributes to sediment stabilization. The occurrence of this alga seems to be restricted to the intertidal zone, where it forms a green belt along the coastline and flow channels (Fig. 1). Therefore, it must be adapted to rapid changes in temperature, irradiance (photosynthetically active radiation, PAR, and ultra-violet radiation, UVR), water availability and salinity, especially with respect of tidal rhythms. Therefore, this alga represents suitable model organism for the study of effects of different stress factors and their combinations in the laboratory as well in the field.

However, the positioning of the proposed instrumentation for ecophysiological measurements on *Vaucheria* (dataloggers, oxygen probes, and fluorometers) was found difficult due to technical limitations of the instruments, especially with respect to expected long-term exposition to seawater, bottom sediment stability and effects of tides and high waves on the stable position of the instruments. For this reason, use of isolated microcosmos containing *Vaucheria* community was proposed. The aim of this study was (a) to characterize the *Vaucheria* community, (b) to test the instrumentation for measurement of its photosynthetic activity in *ex situ* conditions, (c) to measure *Vaucheria* photosynthetic activity and (d) to define relations between encountered environmental conditions and *Vaucheria* photosynthetic activity in late Arctic summer.

Material and Methods

Material collection: The *Vaucheria* community was collected in the estuary of the Adventelva, Longyearbyen, Svalbard (78° 13' 22.51'' N, 15° 40' 7.39'' E) during low tide (Fig. 1).

Community structure: The structure of the community was evaluated using Olympus BX-53 light microscope (Olympus, Japan) and the microphotographs were taken by Olympus DP-72 digital camera (Olympus, Japan). The images were processed using QuickPhoto 2.3 software (ProMicra, Czech Republic).

Ecophysiological measurements: The ecophysiological measurements were performed *ex situ* at the Payer's house, Czech Arctic Research Infrastructure "Josef Svoboda Station from August 12 to August 22, 2016. A piece of the *Vaucheria* community was placed into a plastic dish of 25 cm in diameter and was submerged in ca 5 cm of seawater ("The Yard"). The dish was positioned in front of the house (Fig. 2).

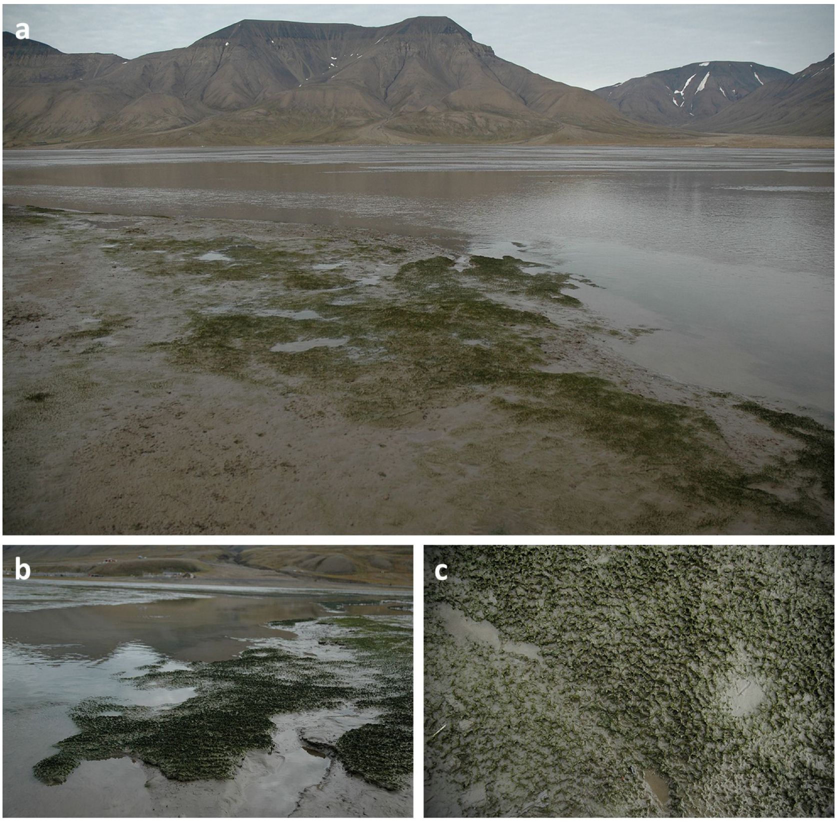

Fig. 1. *Vaucheria* locality in Adventelva estuary, Longyearbyen, Svalbard, in August 2016.

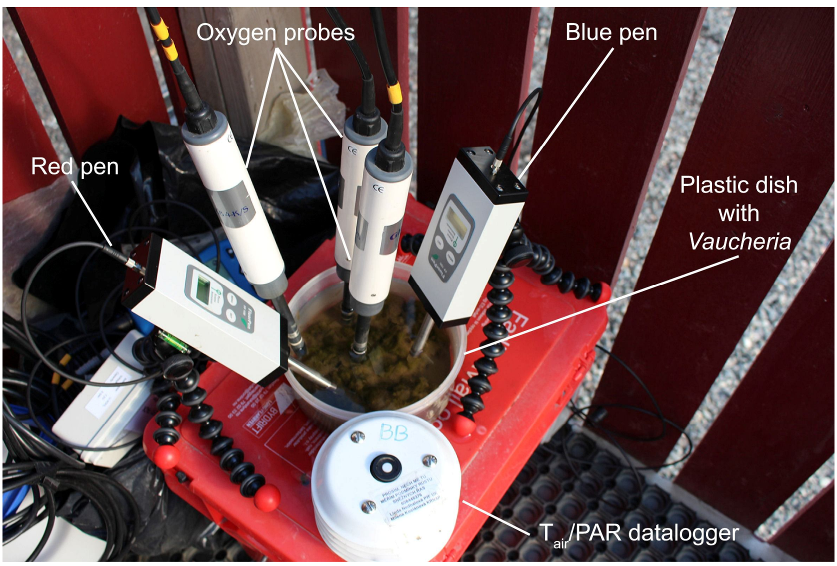

Fig. 2. „The Yard" experimental set-up.

The air temperature (T_{air}) and PAR were measured using Minikin QT datalogger (EMS Brno, Czech Republic). The photosynthetic activity was measured as the actual quantum yield using Monitoring Pens handheld fluorometers (blue and red versions abbreviated as “blue pen” and “red pen” in this study, Photon Systems Instruments, Czech Republic), and as dissolved oxygen concentration (DOC) using three Clark electrodes with thermometers positioned in the sediment, near the alga at depth of ca 3 cm and just under the water surface. The electrodes were connected to a datalogger (Gryf, Czech Republic). The environmental and physiological data were collected in 15 min. intervals, and the UTC was used as the reference time.

The effective quantum yield (Φ_{II}) was calculated according to equation (Roháček et al. 2008):

$$\Phi_{II} = (F_M'-F_S)/F_M' \qquad \textit{Eqn. } 1$$

where F_M' is the maximum fluorescence in light and F_S is the steady-state fluorescence in light.

The relative electron transfer rate (rETR) was calculated according to equation (Maxwell et Johnson 2000):

$$rETR = \Phi_{II} \times PAR \times 0.5 \qquad \textit{Eqn. } 2$$

where Φ_{II} is the effective quantum yield, PAR is the value of incoming PAR and 0.5 is a factor reflecting the partitioning the energy between photosystems.

Statistics: The statistical analyses were performed using Statistica 13 ([1] - Dell 2015) and CANOCO 5 (Ter Braak et Šmilauer 2012) software. Descriptive statistics was used to characterize the environmental conditions. The paired t-test was applied to evaluate the differences between “blue” and “red” pens. The RDA (redundancy analysis) was used to evaluate the effects of environmental variables on photosynthetic activity, and the statistical significance was tested using Monte Carlo permutation test. The results were considered statistically significant at $P < 0.05$.

Results

Community structure: The *Vaucheria* sp. was dominant in the community. The green, photosynthetically active, parts formed shrub-like structures, while the colorless rhizoids formed dense mat stabilizing the photosynthetically active parts. Small numbers of marine pennate diatoms were also observed among *Vaucheria* thalli (Fig. 3).

Environmental conditions: During the ecophysiological measurements, the air temperature ranged from 3.8°C (recorded in the morning on August 21) to 13.2°C (recorded in the afternoon on August 18); the temperature mean value was 7.8°C. The PAR dropped to 0.7 µmol m^{-2} s^{-1} at local midnight on August 18 and reached up to 817 µmol m^{-2} s^{-1} at local mid-day on August 1; the PAR mean of 67 µmol m^{-2} s^{-1} indicated prevailing low-light conditions. In “The Yard”, the temperature in bottom sediment (mean 8.0°C, minimum 3.5°C, maximum 13.4°C) was comparable to the air temperature. The temperature near the *Vaucheria* thali was slightly higher (mean 8.7°C, minimum 4.3°C, maximum 17.0°C) and temperature at the water surface was even higher (mean 11.0°C, minimum 6.5°C, maximum 22.2°C) (Fig. 4).

Instrumentation tests and comparisons: The positioning of *Vaucheria* mat into “The Yard” did not affect the mat structure significantly.

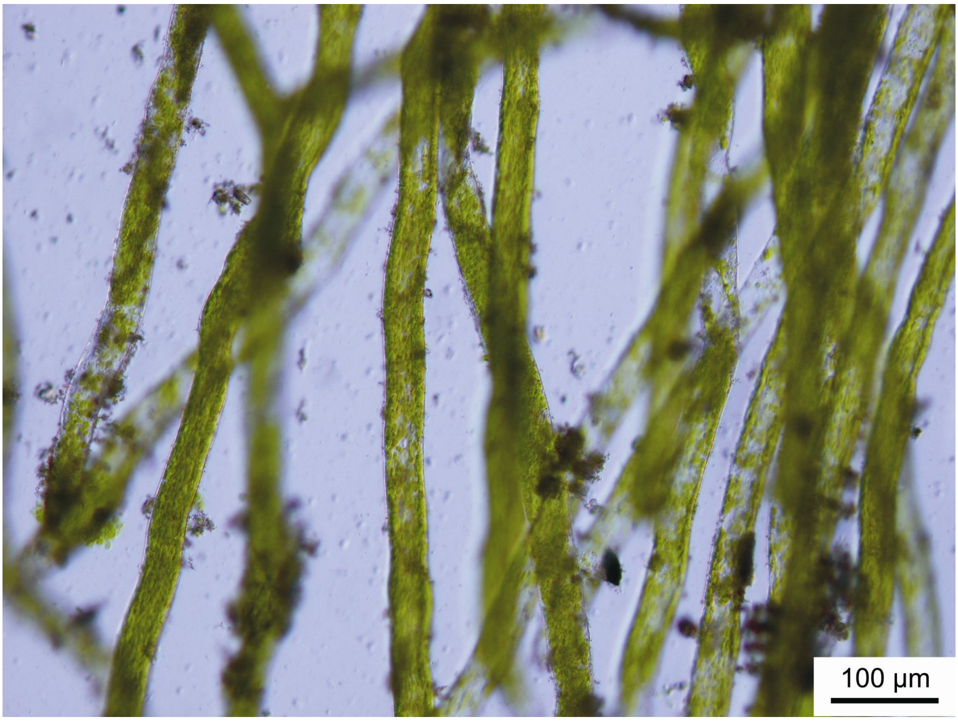

Fig. 3. The microphotograph of *Vaucheria* thallus.

The DOC measurement revealed anoxic conditions in the sediment, reflecting thus real conditions in the field and integrity of the microbial mat. The increased DOCs at the surface could be caused by emerging of the probe from water (Fig. 4).

Both proposed methods, variable chlorophyll fluorescence and DOC measurements, provided data on photosynthetic activity in *Vaucheria*. The correlation of the rETR and oxygen concentrations were relatively weak (Table 1). The data from red and blue Monitoring Pens were correlated tightly, however, the quantum yield measured by the blue version of the instrument were slightly, but significantly, lower (paired t-test, n=963, t=-9.776, $P < 0.001$).

Photosynthetic measurements: The DOC in *Vaucheria* community ranged -0.2 to 23.7 mg l^{-1}, corresponding to -1.8 and 233% of saturation. The lowest DOC values were observed during “night”. The Φ_{II} measured by both pens varied from 0 to 0.7, and the maximum values were observed during nighttime. The rETR ranged from 0 to 124.4 and 110 when measured by blue and red pens, respectively with maximum values occurring around mid-day (Fig. 5a).

The diel changes in the rETR and DOC in *Vaucheria* thali followed the temperature and PAR course (Fig. 5b). The values of the effective quantum yields were negatively correlated to PAR and water temperature, while the changes in oxygen concentration were not dependent significantly on any of studied environmental variables.

		Oxygen		Φ_{II}		rETR	
		DO	Saturation	Blue	Red Pen	Blue Pen	Red Pen
Oxygen	DO	---	0.9875	-0.2227	-0.1073	0.1519	0.1400
			P<0.001	*P<0.001*	*P=0.001*	*P<0.001*	*P<0.001*
	Saturation		---	-0.2988	-0.1614	0.2360	0.2155
				P<0.001	*P<0.001*	*P<0.001*	*P<0.001*
Φ_{II}	Blue Pen			---	0.8365	-0.7371	-0.6800
					P<0.001	*P<0.001*	*P<0.001*
	Red Pen				---	-0.4896	-0.6145
						P<0.001	*P<0.001*
rETR	Blue Pen					---	0.8438
							P<0.001
	Red Pen						---

Table 1. The correlations expressed as correlation coefficient (r) and statistical significance (P – value) among different methods of photosynthetic activity measurement. The statistically significant correlations are marked bold. *Abbreviation*: DO – dissolved oxygen.

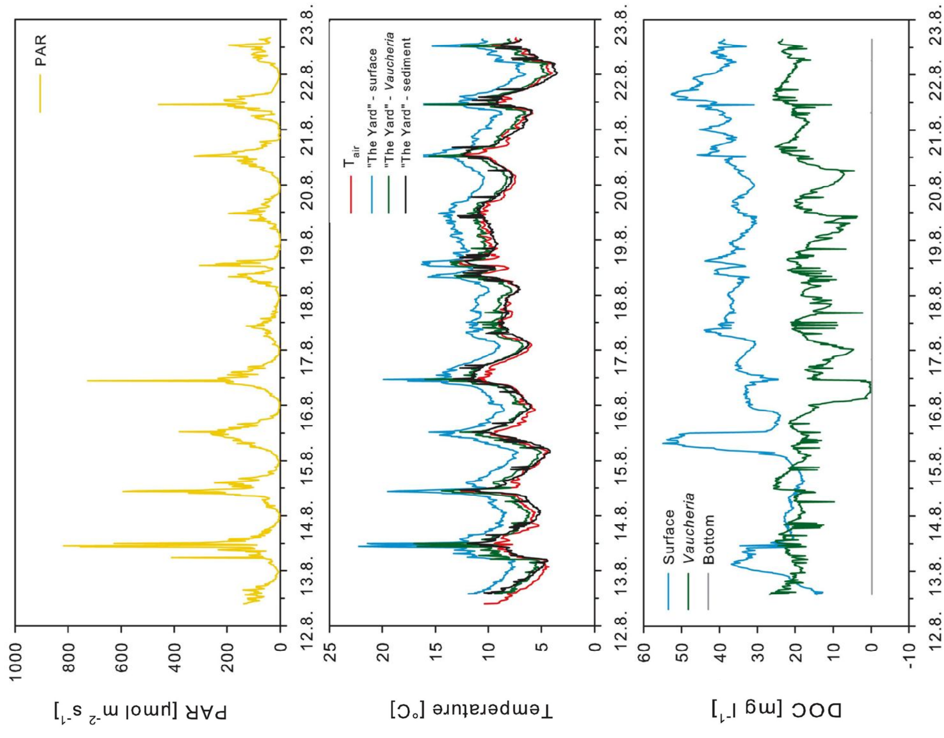

Fig. 4. The courses of PAR, temperatures, and dissolved oxygen concentration (DOC) at different depths in "The Yard" during the experiment.

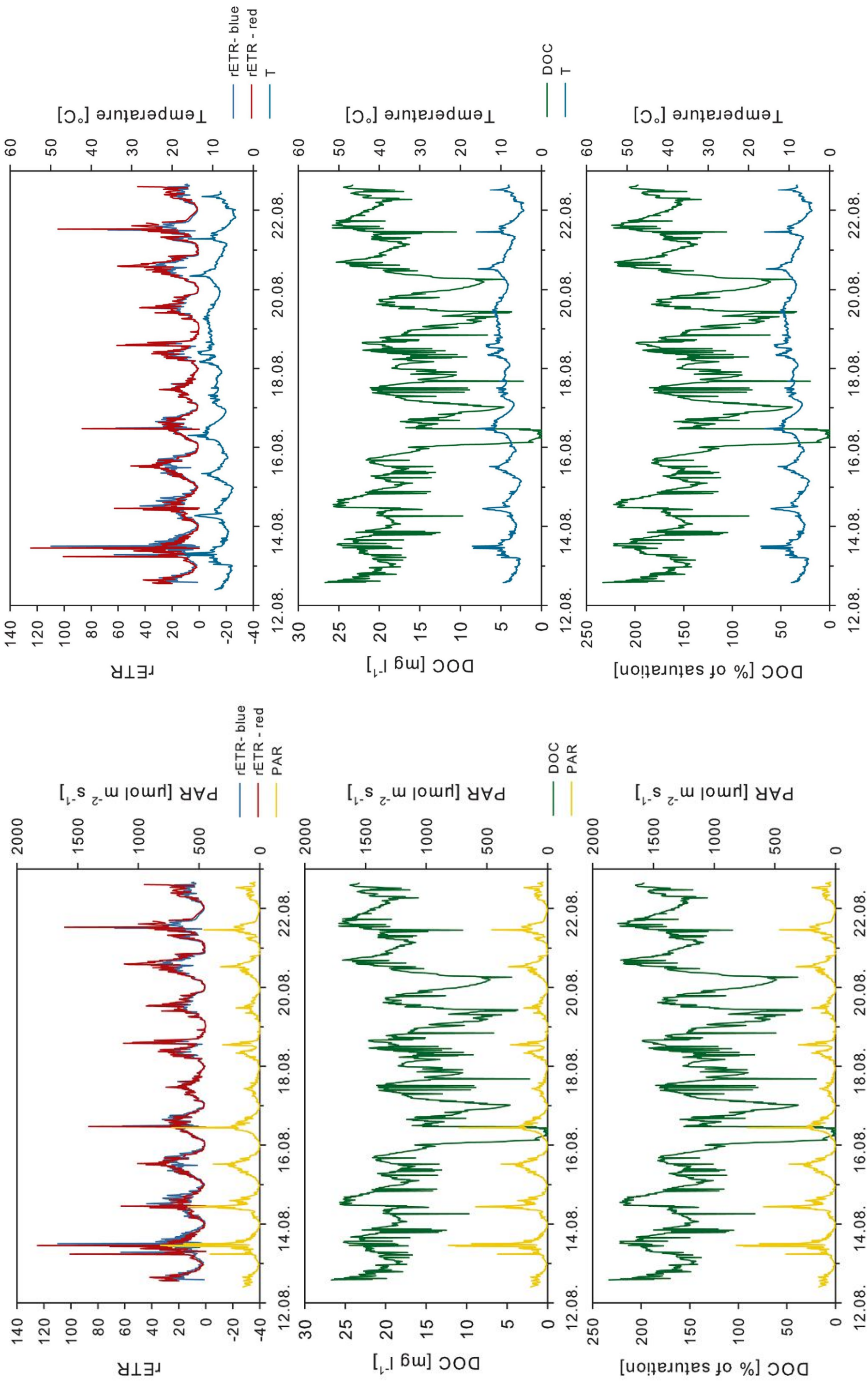

Fig. 5. The record of rETR and DOC in the vicinity of *Vaucheria*, together with (**a**, left) PAR and (**b**, right) temperature.

Nevertheless, all environmental variables explained 37.15% of variation (Monte Carlo permutation test: Axis 1: pseudo-F = 361.0, p = 0.002; All axes: pseudo-F = 113.0, p = 0.002) (Fig. 6), and the PAR was the most important one explaining 25.5% variation (Monte Carlo permutation test, pseudo-F = 329.0, P = 0.002).

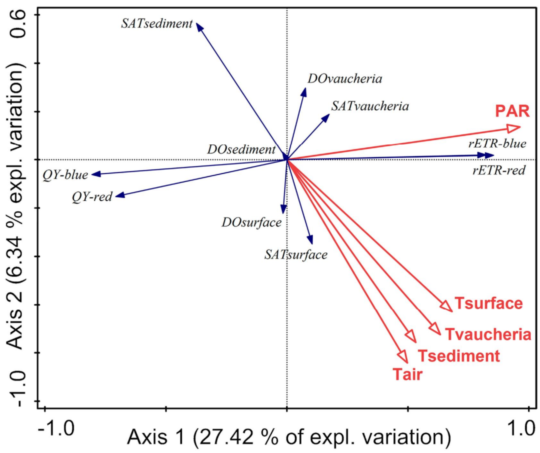

Fig 6. The RDA analysis.
Symbols: PAR – photosynthetically active radiation, Tair – air temperature, Tsurface – temperature at the water surface, Tvaucheria – temperature in the vicinity of *Vaucheria* thalli, Tsediment – temperature in the bottom sediment, DOsurface – DOC at the surface in mg l^{-1}, DOvaucheria – DOC in the vicinity of *Vaucheria* thalli in mg l^{-1}, DOsediment – DOC in the bottom sediment in mg l^{-1}, SATsurface – DOC at the surface in % of saturation, SATvaucheria – DOC in the vicinity of *Vaucheria* thalli in % of saturation, SATsediment – DOC in the bottom sediment in % of saturation, QY-blue – effective quantum yield measured by the blue pen, QY-red – effective quantum yield measured by the red pen, rETR-blue – rTER mesured by the blue pen, rETR-red – rETR measured by the red pen.

Discussion

In the field, the *Vaucheria* sp. formed dense mats. The biofilm or mat formation is considered as a stress avoidance strategy in extreme environments (Kvíderová 2015). So far, only one or two species, *Vaucheria borealis* (Kim et al. 2008, Kim et al. 2011, Skulberg 1996) or *Vaucheria* sp. (Fredriksen et al. 2015), were recorded in Svalbard. However, molecular analyses will be necessary to distinguish if the community consists of one genotype (clone) or species, or if there are different genotypes and/or species adapted to different microclimate conditions (*e.g.* salinity gradient).

The values of PAR and air temperature corresponded to late Arctic summer (Elster et al. 2012, Láska et al. 2012). The air temperatures measured in "The Yard" were comparable to the ground temperatures recorded in Petunia at the same period of the year (Elster et al. 2012, Láska et al. 2012). During the experiments, the *Vaucheria* community was not exposed to freezing temperatures that might be expected in August (Láska et al. 2012). The mean PAR value was lower than August mean values reported by Láska et al. (2012), after recalculation where PAR was considered as 50%

of global solar radiation (Szeicz 1974) and 1 W m^{-2} = 4.57 μmol m^{-2} s^{-1} (Thimijan et Heins 1983), that may be caused different position of the PAR sensor with respect of possible shading. While the sensor was positioned in an exposed site in Petuniabukta (Láska et al. 2012), the sensor in Longyearbyen was positioned in the middle of the settlement, and hence affected by house shades.

"The Yard" proved to be suitable for photosynthetic activity measurements in *Vaucheria*, since the community structure was unaffected by the transport from the original locality. However, the tides that further affect the photosynthetic activity *in situ* due to thali desiccation and interference with the diel courses of temperature and PAR, cannot be simulated in "The Yard" experimental set-up. Since the used instrumentation cannot be applied in the intertidal zone directly, we plan to develop more sophisticated cultivation unit for tide simulations.

The weak correlation between the oxygen concentration and fluorescence parameters (Φ_{II} and rETR) may reflect either the delay caused by oxygen diffusion in water or signal integration and lower sensitivity of used oxygen probes. The slightly higher Φ_{II} measured by the red pen may reflect the difference in physiological status of the examined thali, indicating possible spatial heterogeneity in *Vaucheria* mat.

Despite of continuous light, the diel changes in PAR result in diel changes of the photosynthetic activity in different algal communities in the Arctic (Sehnal et al. 2014, Stibal et al. 2007), and these diel cycles were observed in *Vaucheria* in this study. The sub-saturation DOCs in the "night" indicate that the respiration might prevail in very low irradiances. Since the light saturation of photosynthesis in *Vaucheria* sp. occurs between 20 and 190 μmol m^{-2} s^{-1} depending on light conditions (Leukart et Hanelt 1995) and considering the prevailing light of 67 μmol m^{-2} s^{-1} observed in this study might indicate a low-light adaptation of *Vaucheria* in general. However, a detailed study focused on P-E curves will be necessary to reveal the mechanisms of photoacclimation.

In conclusion, the method of microcosmos incubation and instrumentation for photosynthesis activity measurements seems to be suitable for ecophysiological studies in *Vaucheria*. However, for detailed and complex studies, improved unit for microcosmos should be made to include the tidal cycles, and more sophisticated protocols like for P-E curves should be used.

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