






Fluorescence parameters of chlorophyll *a* halophytes as a response to salinity of post mining subsidence reservoirs

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RECEIVED 31.05.2022

ACCEPTED 28.11.2022

AVAILABLE ONLINE 31.12.2022

Abstract: The increasing salinity of water in reservoirs is caused by climate change. On the other hand, an increase in salinity promotes the group species, halophytes that tolerate or need NaCl for growth. The aim of this study was to identify the response of facultative halophytes' photosynthetic apparatus efficiency (*PE*) to water salinity. The study covered the spiny water nymph (*Najas marina* L.) population in four mining subsidence reservoirs. *Najas marina* is a facultative halophyte which means that it can occur in both fresh and salt water. This plant has the characteristics of the species invasive, such as rapid biomass growth, and wide ecological tolerance. Water salinity, described by conductivity, in the reservoirs ranged from 646 to 3061 $\mu\text{S}\cdot\text{cm}^{-1}$. *PE* was expressed in terms of chlorophyll *a* fluorescence parameters, which were collected *in situ* using a Pocket PEA device. Water parameters using a YSI ProDSS probe were identified. Data analysis was performed using OJIP test and *s* the non-parametric Spearman's rank test ($p \leq 0.05$). The relationship between chlorophyll *a* fluorescence parameters and water parameters showed that conductivity, salinity, water clarity, and nitrate content statistically significantly affected *PE* ($p < 0.05$). Generally, the higher salinity e.g. more than 3000 $\mu\text{S}\cdot\text{m}^{-1}$, supports *PE* of facultative halophyte at the stage of optimum development in the vegetation season.

Keywords: chlorophyll *a* fluorescence, climate change, halophyte, *Najas marina*, salinity

INTRODUCTION

Progressing global climate change, less rainfall [JEPPSEN *et al.* 2014], and higher air temperatures [SIERKA, PIERZCHAŁA 2022] will lead to increased salinity of soils [MISHRA, TANNA 2017] and water reservoirs [ABD ELLAH 2020]. It is predicted that by the end of the century, evaporation from water reservoirs will increase by as much as 16% [WANG *et al.* 2018] towards to years 2006–2015 [WOOLWAY *et al.* 2020]. Change in the physical properties of surface waters and an increase in the content of chemical compounds, including NaCl [JEPPSEN *et al.* 2015; VINEIS *et al.* 2011]. These phenomena will cause changes, among others, in the nature of lakes from fresh water to salt water [CHEN *et al.* 2018] and probably will launch the reactions of aquatic organisms [BRUCET *et al.* 2009; HALABOWSKI, LEWIN 2020; KAŠOVSKÁ *et al.* 2014; LAM-GORDILLO *et al.* 2022] to this

environmental factor. Groups of organisms that tolerate increased salinity of water, e.g. algae. The response to salinisation was to replace dominant diatoms in 1988 with dominant cyanobacteria and green algae after 20 years [CHEN *et al.* 2018]. The increase in salinity water significantly decreased species diversity, e.g. of zooplankton, destabilising ecosystems' entire food web [GUTIERREZ *et al.* 2018; JEPPSEN *et al.* 2015].

However, different organism's inherently possessed various measures and other capacities to cope with exposure to high salinity, and salt stress responses and tolerance vary between species [JAMPEETONG, BRIX 2009].

In nature, there are species – halophytes – that have adapted to the salinity of the habitat even salinity above 200 nM NaCl [FLOWERS, COLMER 2008], which supports their growth [KHAN *et al.* 2015]. Halophytes include *Najas marina* L., species of invasive traits [GHAZANFAR *et al.* 2014].

However, different species of plants inherently possess various ways and other capacities to cope with exposure to high salinity, and salt stress responses and tolerance vary between species [JAMPEETONG, BRIX 2009]. Understanding physiological solutions used by the halophytes (facultative too) can contribute to will allow for the studies of traits development resistant species to abiotic stress such as salinity e.g. in the group of crops [UDAWAT *et al.* 2016].

The phenomena of surface water salinization are often associated with industrial human activities. This occurs in regions where underground mining of hard coal accompanies pumped salinity waters to the surface [LI *et al.* 2014; PIERZCHAŁA, SIERKA 2020; TIMPANO *et al.* 2015]. These waters are deposited in land deformations [WOJCIECHOWSKI 2007] and the resulting reservoirs are shallow lakes not more than a few meters deep [SCHEFFER 1998].

The result of years of searching for tools to identify plant responses to environmental factors is chlorophyll fluorescence parameters. Because photosynthesis is one of the fundamental processes in plants and is extremely sensitive to environmental changes so its course is used to quickly diagnose *in situ* the state e. g. of aquatic macrophytes cells under the influence of factors directly in their environment, non-destructive, and in real-time [DĄBROWSKI *et al.* 2021; KALAJI *et al.* 2017]. The increase in fluorescence during the first second of exposure for light from initial values (F_0) to maximum values (F_m) is represented as a curve with more than a few points (denoted as O, K, J, I, P) [STRASSER, STRASSER 1995].

So far, chlorophyll *a* fluorescence has been used to identify the condition of plants under conditions of artificial [DĄBROWSKI *et al.* 2021] or natural salinity [XIA *et al.* 2004]. *In situ* studies on water halophyte species in areas created as a result of human industrial activity have not been conducted. Scientists have concluded that salinity is not compulsorily required for the growth of halophytic species [GRIGORE *et al.* 2012].

On the other hand, the study of the efficiency of the photosynthetic apparatus of halophytes [GHAZANFAR *et al.* 2014; KRAWCZYK *et al.* 2016] in reservoirs with different salinity will answer the following questions: 1) does different salinity of the habitat affect the changes in photosynthetic activity of facultative halophytes?; 2) what salinity of water reservoirs will be conducive to the spread of halophytes in the era of climate change?

MATERIALS AND METHODS

THE STUDY SPECIES

Spiny water nymph (*Najas marina* L.) is an annual facultative halophytic plant [AGAMI *et al.* 2006] found in ponds, lakes, and marine bays. The plant has characteristics of an invasive species such as rapid biomass growth, wide ecological tolerance, and high seed production. Despite being a submerged plant, it grows very shallowly, in water depths of 1–3 m, and by means of simple, unbranched roots of varying lengths (from a few centimetres to half a meter) it attaches itself to the bottom of the body of water in which it is found [RÜEGG *et al.* 2017].

THE STUDY AREA

Study objects (Photo 1) were located in the southern part of Poland, in the area of the Silesian Upland [KONDRACKI 2002]. Underground hard coal mining led to the lowering of the ground surface by as much as 18 m in relation to its original position [WOJCIECHOWSKI 2007]. In the resulting depressions – subsidence basins – water accumulation precipitation and groundwater [CHYLAT *et al.* 2003]. Those waters are generally saline because are pumped out while already salinised in the dewatering of hard coal deposits and due to reservoir edge stabilisation by waste materials that follow hard-coal mining [SIERKA *et al.* 2009]. Reservoirs were selected for the study that: 1) were formed as a result of land subsidence and surface water accumulation, 2) with presence of *Najas marina*, 3) had water salinity (Tab. 1). The reservoir with the lowest salinity was treated as the reference one.

SAMPLING AND ANALYSIS TECHNIQUES

The research in the terrain was carried out in transects at right angles to the shore of the reservoir. Field studies were conducted in transects perpendicular to the shore of the reservoir, located at a distance of at least 10 m from each other. The functioning of the photosynthetic apparatus was assessed on selected chlorophyll *a* fluorescence parameters. Data were collected two times in 2019: I – the 2nd week of July (optimal development of the study species) and II – the last week of August (seed sowing stage time) [RÜEGG *et al.* 2017]. In I and II collection terms, 640 measurements of chlorophyll fluorescence parameters in study



Photo 1. Object of the study – mining water subsidence reservoirs; N = Nieborowice, K = Książenice, S = Świętochłowice, L = Lignozy (phot. M. Bujok)

Table 1. Characteristics of the study reservoirs

Parameter	Reservoir			
	Nieborowice (N)	Książenice (K)	Martyn II (M)	Ligęzy (L)
Coordinates of measuring points	N 50°19'1" E 18°62'07"	N 50°18'34" E 18°62'60"	N 50°29'72" E 18°89'24"	N 50°20'07" E 18°63'68"
Conductivity of water ($\mu\text{S}\cdot\text{cm}^{-1}$)	646.2	743.6	2005.4	3061.1
Area (ha)	20	8.45	2.74	1.22
Cover of <i>Najas marina</i> (%)	40	40	40	40
Shores of the reservoir fortified with waste rock (%)	27	4,5	28	46

Source: own study.

species were made *in situ*. The plant was taken out of the water, without losing contact with water through the roots, and then clips were applied for 20 min to quench the photosynthetic process and then measurements were made using Hansatech's Pocket PEA.

The Hansatech Pocket PEA fluorimeter uses a LED diode with an intensity of $3500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a wavelength of 627 nm to excite the fluorescence. It collects data at the rate of 1 measurement every 10 μs for the first 300 μs and then performs one measurement every 100 μs which gives a total of 118 measurements. For the measurement of chlorophyll *a* fluorescence, non-overlapping leaves were selected, without signs of disease, while trying to be as random as possible in the selection. Physicochemical parameters of water were carried out by YSI ProDSS probe in the places where *Najas marina* occurs. A total of 160 measurements were taken on 6 parameters.

CHLOROPHYLL FLUORESCENCE PARAMETERS

Fluorescence parameters (after STRASSER *et al.* [2004; 2010]) showed in Table 2 were measured *in situ*. Parameters $t(F_m)$, PI_{total} , F_v/F_0 , Area, F_0 , F_v/F_m , F_m were used for detailed analyses.

The $t(F_m)$ parameter determines the time to reach maximum F_m fluorescence. It is usually in the range of 500–800 ms. The PI is an indicator of photosystem II (PSII) functioning and relates to overall PSII performance. The lower the PI value the worse the PSII functioning [KALAJI 2011]. The is maximum fluorescence variation, $F_v = F_m - F_0$. The F_v/F_0 ratio indicates the maximum efficiency of oxygen release (due to water splitting) on the donor site of the PSII. The Area parameter is defined as the product of the fluorescence signal (measured in bits) by the transition time from minimum (F_0) to maximum (F_m) fluorescence. The F_0 parameter represents the minimum fluorescence, measured before photosynthesis starts, under weak red light and open PSII reaction centres (darkened leaf). The parameter F_v/F_m is the parameter of the maximum photochemical efficiency of PSII. It is the ratio of the difference between the maximum and minimum fluorescence to maximum fluorescence $-(F_m - F_0)/F_m$. The F_m parameter defines the maximum fluorescence when exposed to high-intensity light and all PSII reaction centres are closed.

PHYSICO-CHEMICAL PROPERTIES OF WATER

The YSI ProDSS probe was used to measure selected physico-chemical parameters such as electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$), SALT (ppt), TDS ($\text{mg}\cdot\text{dm}^{-3}$), pH, and ammonium content (NO_3^+N), and the total amount of dissolved substances ($\text{mg}\cdot\text{dm}^{-3}$).

The varying salinity of the water in the studied reservoirs allowed the arrangement of an experimental system *in situ* with a salinity gradient. Reservoir N, with the lowest salinity, was taken as the reference object. In reservoirs formed in mining subsidence basins, fortify water with soluble chemical compounds by hard coal mining waste material, including NaCl. The waste material is used to firm up the edges of the reservoir [SIERKA *et al.* 2012] from which chemicals flow into the water.

STATISTICAL ANALYSIS

The analysis of the physicochemical parameters of the water was performed using the Statistica 13 package, by means of which the significance of the differences between the water parameters of the reservoirs was determined by ANOVA (Welch's F test) and the post hoc test of the reasonable significant difference (RIR) by Tukey. For the results assumed that $p \leq 0.05$. Correlations between water parameters and the efficiency of the photosynthetic apparatus was verified with the non-parametric Spearman's rank test $p \leq 0.05$.

The program Microsoft Excel 2016 (Microsoft, Inc., Redmond, WA, USA) was used to present the main parameters of chlorophyll *a* fluorescence. Data is presented using radar, tables, and histograms.

RESULTS

CHLOROPHYLL FLUORESCENCE PARAMETERS ANALYSIS

Najas marina photosynthesis parameters are statistically significantly different between plant populations performed at I term optimum growth phase and II term seed release phase in terms of $t(F_m)$ ($F = 5.2327$, $p < 0.0275$), PI_{total} ($F = 26.1147$, $p < 0.0000$), F_v/F_0 ($F = 9.9664$, $p < 0.0016$) (Fig. 1). The trend is the same in other parameters, such as Area. The response of plants varies according

Table 2. Measurable fluorescence parameters¹⁾

Parameter's symbol and formula	Parameter's explanation
$V_J = (F_J - F_0)/(F_m - F_0)$	relative variable fluorescence at the <i>J</i> -step
$V_I = (F_I - F_0)/(F_m - F_0)$	relative variable fluorescence at the <i>I</i> -step
$\varphi_{Po} = 1 - F_0/F_m$	maximum quantum yield of primary photochemistry (at <i>t</i> = 0)
$\varphi_{Eo} = (1 - F_0/F_m)(1 - V_J)$	quantum yield of electron transport (at <i>t</i> = 0)
$\varphi_{Ro} = (1 - F_0/F_m)(1 - V_I)$	quantum yield of reduction of end electron acceptors at the photosystem I (PSI) acceptor side (RE)
$\varphi_{Do} = F_0/F_m$	quantum yield (at <i>t</i> = 0) of energy dissipation
$\Psi_{Eo} = 1 - V_J$	probability (at <i>I</i> = 0) that a trapped exciton moves an electron into the electron transport chain beyond <i>Q_A</i>
$\delta_{Ro} = (1 - V_I)/(1 - V_J)$	efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE)
<i>t</i> (<i>F_m</i>)	time to reach the maximal fluorescence intensity <i>F_m</i>
$PI_{abs} = \gamma_{RC}/(1 - \gamma_{RC}) \cdot \varphi_{Po}/(1 - \varphi_{Po}) \cdot \Psi_{Eo}/(1 - \Psi_{Eo})$	performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
$PI_{total} = PI_{ABS} \cdot \delta_{Ro}/(1 - \delta_{Ro})$	performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors
$ABS/RC = (1 - \gamma_{RC})/\gamma_{RC}$	absorption flux (of antenna Chls) per reaction centre (RC)
$TR_o/RC = M_o(1/V_J)$	trapping flux (leading to <i>Q_A</i> reduction) per RC
$ET_o/RC = M_o[1/V_J]\Psi_o$	electron transport flux (further than <i>Q_A</i> ⁻) per RC
$RE_o/RC = M_o(1/V_J)(1 - V_I)$	electron flux reducing end electron acceptors at the PSI acceptor side per RC
$DI_o/RC = (ABS/RC - TR_o/RC)$	dissipated energy flux per RC (at <i>t</i> = 0)
$\varphi_{Po} = 1 - F_0/F_m$	maximum quantum yield of primary photochemistry (at <i>t</i> = 0)
$\varphi_{Eo} = (1 - F_0/F_m)(1 - V_J)$	quantum yield of electron transport (at <i>t</i> = 0)
$\varphi_{Ro} = (1 - F_0/F_m)(1 - V_I)$	quantum yield of reduction of end electron acceptors at the PSI acceptor side (RE)
$\varphi_{Do} = F_0/F_m$	quantum yield (at <i>t</i> = 0) of energy dissipation
$\Psi_{Eo} = 1 - V_J$	probability (at <i>t</i> = 0) that a trapped exciton moves an electron into the electron transport chain beyond <i>Q_A</i> ⁻
$\delta_{Ro} = (1 - V_I)/(1 - V_J)$	efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE)
Area	area above the induction curve

¹⁾ All parameters are expressed in relative units.
Source: own elaboration based on STRASSER *et al.* [2004; 2010].

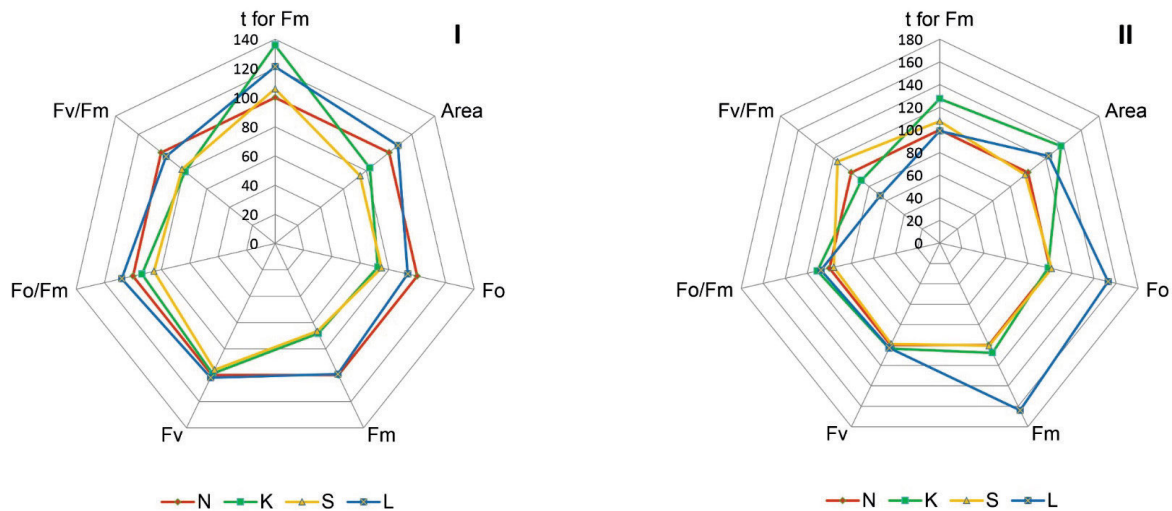


Fig. 1. Radar charts showing changes in the values of seven selected parameters of chlorophyll fluorescence in July (I) and in August (II); N, K, S, L as in Photo 1; source: own study

to the number of mineral salts in the water. The F_v/F_m parameter considered a reliable measure of photosynthetic apparatus photochemical activity [CETNER *et al.* 2016] indicates that photosynthetic activity was the best in I term in the most saline reservoir. On the other hand, in II term photosynthetic activity was the best in more reservoirs, with lower salinity.

The OJIP test values indicate that the efficiency of photosynthetic apparatus (EPA) decreases with plant development in vegetation season I – K reservoir vs II, L reservoir (Fig. 2). Lower salinity sustains EPA in II term, K reservoir.

WATER PARAMETERS ANALYSIS

The statistical analysis results showed that the differences between the water parameters of the studied reservoirs are statistically significant on the level $p \leq 0.0000$. They differ in electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$), SALT (ppt), TDS ($\text{mg}\cdot\text{dm}^{-3}$), pH, ammonium content (NO_3^+N), and the total amount of dissolved substances ($\text{mg}\cdot\text{dm}^{-3}$) both in I and II terms.

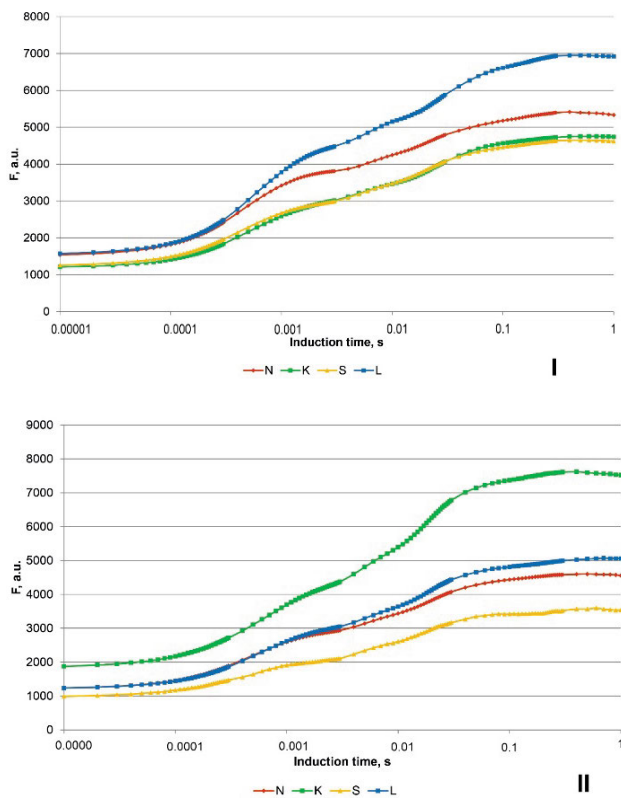


Fig. 2. Statistically significant differences ($p < 0.05$) between the salinity of water (expressed by conductivity in $\mu\text{S}\cdot\text{cm}^{-1}$) in reservoirs, both in I and II terms; N, K, S, L as in Photo 1; source: own study

DISCUSSION

Halophytes have developed many ways to cope with excess dissolved ions in water, including their removal by roots, accumulation of ions in shoots and vacuoles, and shedding and dropping of specialised leaves [VOLKOV 2015]. Another mechanism for coping with high concentrations of Na^+ and Cl^- ions are in the osmoprotectant or SOS (salt overly sensitive) signalling

pathway and antioxidant regulation [GUPTA, HUANG 2014]. In the case of *Najas marina*, as a facultative halophyte, it is not fully understood [RÜEGG *et al.* 2017]. This is also because aquatic plant research, e.g. on chlorophyll fluorescence, has been gaining momentum in recent years [PETJUKEVIC, SKUTE 2022; ROSZKOWSKA 2019]. Assuming that the salinity of water in reservoirs formed in subsidence basins is a stress factor for organisms [SILVA *et al.* 2010] the efficiency of the photosynthetic apparatus of *Najas marina* was studied.

The examined chlorophyll fluorescence parameters and in the case of reservoir S – higher salinity, such as F_m , Area or F_v/F_m had low values. Thus, this indicates a weakened physiological condition of the tested plant, which persisted during the two months of the study. In the other reservoirs, the condition was much better assessed. The visualisation of the OJIP test results showed, as indicated in the results, that salinity contributes to better performance in the optimum development of *Najas marina* [RÜEGG *et al.* 2017] and lower salinity in the final phase. According to studies, it develops best in waters with salinity between 2 and 20‰ [SILVA, WIJAYARATNE 2017].

From the F_m values, it can be seen that in I term the level of maximum fluorescence under high-intensity light irradiation and with all PSII reaction centres closed was the same for all populations studied regardless of salinity. Stress was recorded in the optimal phase of plant growth in reservoirs with lower salinity. Perhaps the high salinity of the water resulted in its higher transparency [PIERZCHAŁA, SIERKA 2020] and maintenance of better light conditions in the reservoir.

In the case of photosynthesis parameters, F_0 , the values indicate an increase in the efficiency of excitation energy transfer between individual dye molecules at the PSII antenna. Thus, high water salinity (reservoir L) contributes the least to thylakoid damage and inactivation of PSII, whose value increases with seasonal plant growth. The parameter $t(F_m)$ determines the time to reach maximum F_m fluorescence. It is usually in the range of 500–800 ms under laboratory conditions [KALAJI, LOBODA 2007]. In our study, it did not exceed 140 ms. It may have been the action of stress factors on the studied object, which may have translated into slowing down the transport of electrons from the reaction centres towards the plastoquinones. The value of the Area parameter fluctuated between the most saline reservoirs at the studied times. Thus, the transport of electrons to the plastoquinone may have been blocked as a result of the salinity factor [BIBER 2009].

The reason for the differences between the OJIP test values in I and II terms for *Najas marina* fluorescence may be due to the fact that at the end of II term the water temperature decreases (shorter insolation time during the day) and the physiological state of *Najas marina* is temperature dependent. The higher the temperature, the better the plant development [HOFFMANN *et al.* 2014]. The statistically significant correlations found between the studied fluorescence parameters of photosynthesis and water (salinity and transparency) indicate that facultative halophytes respond to the presence of salts in the living environment. HAO *et al.* [2021] have studied these responses' spectrum and mechanisms. Studies *in situ* allow small reference to the results obtained. Results from the fact that the individuals for the measurements had to be taken out of the water each time, which could cause stress resulting in the disturbance of the efficiency of the photosynthetic apparatus. Nevertheless, the results obtained

do not provide a basis for generalising the response of plants, which are facultative halophytes, to a factor such as salinity. Salinity increase it contributes to further deprivation of mechanisms of occurrence in environments with significant salt content, which will become more common due to climate change [WOOLWAY *et al.* 2020].

Statistical analyses showed that the amount of nitrate dissolved in the water can affect plant performance according to HOFFMANN *et al.* [2014] the more nitrate, the better the plant grows. However, this does not provide a basis for generalisation to all species of aquatic environments, as the study was conducted within a narrow range of salinity, within a specific range of water conductivity, and on a single taxon.

CONCLUSIONS

1. Photosynthesis efficiency of facultative halophytes increases with increasing water salinity.
2. Optimum efficiency of the photosynthetic apparatus is the highest in waters with salinity above 3000 $\mu\text{S}\cdot\text{cm}^{-1}$ in plants in the full phase of vegetative development.
3. Water salinity below 1000 $\mu\text{S}\cdot\text{cm}^{-1}$ supports the photosynthesis of facultative halophytes during the sowing period.
4. With progressing climate changes and the increasing salinity of water reservoirs, the spread of facultative halophytes will increase is not clear.

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