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Chlorophyll fluorescence changes, as plant early state indicator under different water salinity regimes' on invasive macrophyte *Elodea canadensis* (Michx., 1803).

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Abstract

Photosynthetic apparatus analysis provides information about the physiological state of plants. Changes analyzed by the pulsed chlorophyll-fluorometer allow determining plant cells' metabolites' modifications even at inconsequential cellular damage. It was assessed the possible impact of various salinity levels: 0.584, 1.461, 2.922, and 5.844 PSU (Practical Salinity Unit) on the fluorescent characteristics of the pigment complexes of photosynthetic apparatus on the alien invasive waterweed species (*Elodea canadensis* (Michx., 1803)) of the most serious concern in Europe. The information about aquatic macrophyte photosynthetic systems: PSI and PSII was obtained. The results indicate that a high salinity level: 2.922 and 5.844 PSU, after a prolonged time of impact, seriously affect photosynthetic apparatus inhibition. The decrease in $\Delta F_v/F_m´$ values at 2.922 and 5.844 PSU indicates deterioration in macrophytes' physiological state in general. In the post-stress period, photosynthesis intensified. The interesting feature was noted, that a small water salinity level (0.584) stimulates the chlorophyll formation and increases the F_vF_m index.The research revealed the dependence of photosynthesis processes in plants on salinity levels. A rapid increase in the sensitivity of the PS II system of submerged macrophytes to high salinity was detected, which probably is related to the inhibition of protein synthesis. This data provides information for the further bio-diagnostics of the plant's overall condition and prediction of the exposure degree and the possibility of developing forecasts of growth and invasive plant distribution.

Keywords

invasive species, Canadian waterweed, salinity, freshwater macrophyte, photosynthesis, bio-testing.

Introduction

After analyzing the latest data from the various international studies, it can be concluded that *E. canadensis* (Michx., 1803) has been established in more than 26 European countries, while its congeners the non-native *E. nuttallii* (Planch.) H. St. John and *E. callitrichoides* (Rich.) Casp. are very rare or absent in most of Europe. Due to their morphological similarities, similar habitat preferences, and weedy growth, *Elodea* sp. are often misidentified, particularly in the early invasion phases (Lambdon et al. 2008 Nichols and Shaw 1986).

The effects of dissolved sodium chloride stress on freshwater plants have been little studied up to now, although it is expected to present different levels of salt sensitivity or salt resistance betting on the plant species. It is noted that one of the generally accepted indicators for the practical study of the overall condition of terrestrial and aquatic plants is the assessment of changes in the primary processes of photosynthesis and the formation of photosynthetic pigments in response to environmental factors (Grinberga and Priede 2010). This indicator's general value for the plant's overall state analysis is the high sensitivity to processes in the photosynthetic apparatus under the earliest stages' adverse factors .

Salt-sensitive plants have reduced survival, growth, and development when exposed to even low to moderate salinities. In contrast, salt-tolerant species can grow and reproduce even at oceanic salinities. High concentrations of salt impose both osmotic and ionic stresses on the plants, leading to several morphological and physiological changes, such as interruption of pigment synthesis and overall decreasing of photosynthetic activities. However, different species of plants inherently possess various measures and other capacities to cope with exposure to high salinity, and salt stress responses and tolerance vary between species (Jampeetong and Brix 2009, Misra et al. 1998).

The F_v/F_m parameter that indicates Maximum Quantum Efficiency (MQE) is the most used chlorophyll fluorescence measuring parameter. F_v/F_0 - while it does not directly correlate with carbon assimilation, it is a very sensitive stress detector, and it allows compared samples in the dark-adapted state. This protocol for measuring the MQE of PSII in plants has withstood time and was developed by (Butler and Kitajima 1975). Disturbances in photosynthesis's primary functions are directly reflected in chlorophyll fluorescence changes and appear long before the plant's physiological state's visible deterioration. It was also noticed that the measurement of chlorophyll fluorescence is the fastest, informative, and convenient in comparison with other experimental methods that also apply for the ecological monitoring of plants (Jiang et al. 2018, Murata et al. 1966). The photosynthesis process is susceptible to high salinity levels, which affects many aspects of this process. The electron transfer along the photosynthetic electron transport chain (ETC) suggests the sequential participation of two photosystems: PSII and PSI, but the carriers reduced in PSII serve as electron donors for PSI. The activity of photosynthetic systems contemporaneously affects the redox state of plants another photosystem. This

relationship between PSII and PSI manifested in the fluorescence of chlorophyll, the level of which depends on the redox state of the quinone acceptor (QA). The photoreaction of PSII restores QA, increasing the level of fluorescence, and the activity of PSI leads to the oxidation of QA and a decrease in fluorescence (Herlory et al. 2007, Falkowski et al. 1986, Trissl et al. 1993).

Induction changes include primary nitrogen reduction and subsequent reduction due to electrons' appearance in PSII and resulting in changes of the acceptor part of PSI during the photo-activation of ferredoxin-NADP reductase (PNR) (Chekalyuk and Hafez 2008, Brack and Frank 1998, Heidbuchel et al. 2019, Jassby and Platt 1976). An essential advantage of fluorescence methods is their speed and high sensitivity, making it possible to quickly diagnose *in situ* the state of aquatic macrophytes cells under the influence of different adverse factors directly in their environment, non-destructive, and in real-time.

Thus, our study aimed to investigate the effect of different salt concentrations (close to sea ones) on the invasive aquatic plant. This paper presents data about the salinity impact on *E. canadensis* leaves and obtains insights about photosynthetic activity changes for understanding the possible effect on the invasive freshwater plant after salinity level increases and subsequent saline water intrusion into the freshwaters.

Material and methods

Study site and Sampling

The experiment's plant: Canadian waterweed or pondweed *Elodea canadensis* (Michx., 1803), has transferred from the natural environment (Lielais Stropu Lake: Daugavpils, Latvia) to a laboratory aquarium for further experiment.

Cultivation of plant material

The plant for the experimental part propagated in the laboratory aquarium tank. For the analysis, we used a bi- distilled water solution mixed with 1.6 g/L (Merck, Hoagland's No. 2 Basal Salt Mixture) as a nutrient and various salt concentrations: 0.584, 1.461 , 2.922, 5.844 PSU, and control. Changes in pigment content and photosynthetic activity were carried out every week for three weeks. The plants were grown under the optimal conditions in a Versatile Environmental climate test chamber, with photoperiodicity: 16 hours a day and 8 hours a night, relative illumination 30 $\mu E/m^2$, temperature 18+/- 1 °C, and ambient relative humidity (RH) in climate chamber ≈80% .

Measurement of Chl fluorescence

All measurements were performed at room temperature (20°C) and carried out on a hand-held pulse modulated chlorophyll fluorometer OS–30p (Opti-Sciences, US) with a leaf-clip holder for plant leaves. It allowed simultaneously record fluorescence and measures the pigment's redox state of the PSI reaction-centre, monitoring the reactions of PSII and PSI simultaneously and recording the induction of changes in delayed fluorescence.

Measurements of fluorescence and determination of the redox state by the change in absorbance were accomplished at 700–750 nm wavelength, beam saturation intensity was exposed at 6000 µM, as a saturation light source: an array of red LEDs 660 nm. Detection method: pulse-modulated, test duration: 0.1–1.5 seconds, and automatic light calibration set to 3500 µM. The default saturation pulse duration was set at 2.0 seconds; however, the software takes a rolling eight-point 25 ms average to determine F_0 and F_m. Before dark adaptation measuring the fluorescence indices, the samples were kept in the dark until 150 minutes as the plant dark adaptation in the wet state. The photochemical efficiency (F_v/F_m) of dark-adapted plants calculated according to the formula: $F_v/F_m = (F_m–F_0)/F_m$ (Murchie and Lawson 2013, Hader et al. 1997, Antal et al. 2011, Franklin and Badger 2001).

Statistical analysis

All measurements were analyzed statistically and presented as means and standard deviations (SD). Statistical variance analysis of the independent data with three replicates (n = 3) for *Chl* fluorescence parameters was analyzed using *Statistica* ver.13.3 (StatSoft, Palo Alto, California, USA) and compared with the minor significant differences at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Results and Discussion

Measurements are done employing a weak modulated light, which is just too low to drive photosynthesis but enough to excite pre-photosynthetic antenna fluorescence. After dark adaptation, the fluorometer allows the accurate measurement of minimum fluorescence (F_0). In this state, photosystem II maximally oxidized. The xanthophyll cycle, ΔpH of the thylakoid lumen, state transitions, chloroplast migration have relaxed in their inactive forms. The modulated fluorometer then irradiates the plant leave-plates with an intense saturation light that is high and long enough to reduce or close all available PSII reaction centres entirely (Misra et al. 1998, Van Kooten and Snel 1990). Our studies have shown that high salinity levels: 2.922 and 5.844 PSU after a prolonged time at the third week of influence substantially reduce the growth of *E. canadensis* (data not shown). The F_v/F_m ratio reflects the maximum quantum yield of charge separation in PSII. Toward the donor side, partially inhibit the oxygen-releasing complex, while on the acceptor side, it inhibits electron transport. This process also manifests itself in a negative effect on the efficiency of non-cyclic electron transport. The combined parameters serve as indicators of the functional activity of PSII, referred to as the absorbed energy (ABS). These parameters showed the high sensitivity of plants to increasing salinity level (Ilik et al. 2003, Kolber et al. 1998, Krause and Weis 1991).

The influence of salinity level (5.844 PSU) on *E. canadensis* was manifested in a decrease of chlorophyll F_v/F_m's fluorescence by more than 42 % and, after dark adaptation phase, on 86%, but in the substrate with a 2.922 PSU concentration, observed a decrease of 21% and after dark adaptation phase, on 78% compared to control samples on the third week (Fig. 1). The observed reduction in the F_v/F_m value was mainly associated with a decrease in the maximum fluorescence ($F_{m)}$ value, which is characteristic of the process of photo-

inhibition of PSII. A typical fluorescence trace made on a dark-adapted leaf showing how minimum fluorescence intensity (F_0) and maximum fluorescence intensity (Fm) are formed. The measuring beam excites chlorophyll but is not of sufficient intensity to induce electron transport through PSII. This reaction gives (F_0) the minimal level of fluorescence, and reaction centres are said to be open. A brief saturating pulse of light results in the maximum possible yield of fluorescence (F_m). During this, pulse reaction centres are effectively closed. The data shows how the values of the indicators: F_0, F_m, and F_v have changed over three weeks at different salt concentrations presented in the graphs (Fig. 2). A short-lag phase probably preceded the exponential reduction of F_v/F_m to the initiation level due to the accumulation of the active concentration of salt inside the cells. Increased salt levels also affect the donor/ acceptor part of PSII (Murchie and Lawson 2013, Butler and Kitajima 1975, Genty et al. 1989, Kuster et al. 2007). The F_m output during this saturating light radiation represents a sufficiently reduced PSII. It has been found that healthy aquatic macrophytes have an F_v/F_m value in the range of 0.7 to 0.75; lower and higher values possibly indicate plant stress, and these indicators differ significantly from plants that grow in soil on the land surface (Maxwell and Johnson 2000).

The presence of photosynthetically active cells in the leaf may indicate their resistance to the effect of small salt concentrations (1.461 PSU) and give the possibility of participation in further plant development (data not shown) . Indeed, the F_v/F_m ratio initially slowly decreased from 0.72 to 0.68, after which it returned to its initial state or slightly exceeded the initial value. The result obtained due to microfluorimetry is consistent with the assumption that aquatic plant can adapt to the minor and short-lived negative effect of salt by preserving individuals existing resistance and eliminating the unstable part of the plant population. Photo-inhibition of photosynthesis associated with the development of photo-oxidative stress in cells subjected to salinity level may be enhanced by combining other adverse factors of different natures. The profound inhibition of photosynthesis is mainly associated with the photooxidation of the D_1 proteins in the PSII reaction centres (Kuster et al. 2007, Batjuka et al. 2016). The restoration of the activity of PSII is accompanied by resynthesizes of this protein in the chloroplast. The concentration of active centres of PSII in plant cells mainly depends on the ratio of its photo-oxidative destruction and repair level. It can be determined by reducing the F_v/F_m value in the light conditions and subsequent relaxation in the dark phase, respectively (Baker 2008, Aro et al. 1993).

The low values of the F_v/F_m parameter for samples grown with 2.922 and 5.844 PSU salt concentration indicate a decrease in the functional activity of PSII, mainly due to a reduction in the proportion of active reaction centres and an increase in the quenching of excited states in the antenna due to heat generation. A reduction in the efficiency of transfer of excitation energy from the light-harvesting complex to the reaction centre is accompanied by an increase in unused light energy dissipation. It also noted that the quantum efficiency of energy dissipation in cells exposed to high salt concentrations is high. Changes in the redox state of the reaction centre of PSI were less sensitive to elevated concentrations of salt. However, a decrease in the recovery rate of PSII was observed. The appearance of delayed fluorescence is due to the accumulation of certain emitted redox states responsible for the reverse recombination of charges and quanta's

emission (Massachiro and Takuo 1994, Riis and Hawes 2003, Schreiber 2004). Small concentrations of salt increase the photosynthesis rate and stimulate the growth and development of the aquatic plant. However, at the same time, increased concentrations of dissolved sodium chloride could lead to photo-inhibition and cause photo-oxidative destruction of plant photosynthetic pigments and even death of the organism after prolonged exposure (Petjukevičs et al. 2015, Savicka et al. 2018, Sigaud-Kutner et al. 2005).

F_v/F_m analysis could be confirmed as a method with high sensitivities, performance, and accuracy. It allows non-destructive *in situ* measurements to be carried out in real-time, which is important for solving various environmental tasks. The basis of fluorescence methods is that chlorophyll, located in photosynthetic membranes, serves as a natural indicator of plant cells' state when cells are disturbed under adverse conditions. Our data have proven its robust way to measure aquatic plant early-stress that affects photosystem II, and *Chl* fluorescence changes serve as a source of vital information. P robably high salt concentration also leads to the destruction of chlorophyll-*a* and chlorophyll-*b* while increasing the synthesis of carotenoids as plant protection mechanisms occur until the complete destruction of chloroplasts. These processes can be considered responses to ROS generation in the cell under adverse abiotic factors in the first stage in protecting plant chloroplasts (Loeblich 1982, Petjukevičs and Škute 2017, Yang et al. 2008, Skute et al. 2020).

The destructive effect of salt stress on chlorophyll biosynthesis may be due to the formation of proteolytic enzymes, such as chlorophyllase, but chlorophyllase responsible for photosynthetic apparatus damage and chlorophyll degradation (Tanaka et al. 2008, Lichtenthaler 1987). Reducing photosynthesis rate under high salt levels associated with decreased plant stomatal conductance and absorption of carbon dioxide occurs outside the stoma. As a result, carbon dioxide content in the intercellular space is reduced (Hasegawa et al. 2000). The photosynthesis reaction rate in the dark phase is reduced, and absorbed light negatively affects chloroplasts' reaction centres. The impaired growth and development of plants under sodium chloride stress is a consequence of physiological response reactions and involves changes in the cellular ionic balance, mineral nutrition, transfer of water through the plant's stomata conductance, photosynthetic rate, and ultimately in the fixation and utilization of carbon dioxide. The pigment-protein complex's instability, together with disrupted photosynthetic electron transport chain, increased chlorophyllase activity, which is the main reason for chlorophyll destruction under salinity (Brugnoli and Bjorkman 1992).

The effect of various salt levels on the aquatic, invasive plant *E. canadensis* was studied. Results suggest that F_v/F_m distribution pattern in an individual plant may change according to the imposed stress factor. Analyzing F_v/F_m parameter fluctuations may be an effective pattern of identifying stress factors. Our study results suggest that the long-term influence of salt in high concentrations suppressed the *E. canadensis* synthesis of chlorophyll and photosynthetic activity in general, and disturbs physiological processes, apparently directly affecting the activity or metabolic enzyme synthesis of plant pigments.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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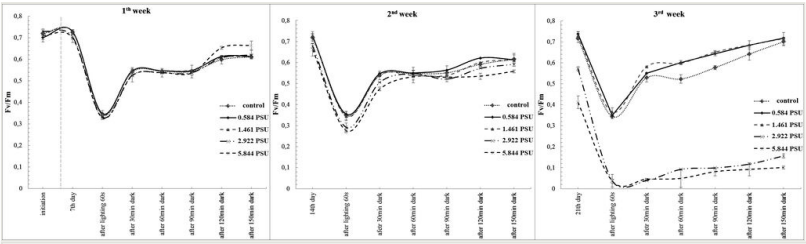

Figure 1.

Measurement of changes in maximum quantum efficiency of PSII photochemistry (F_V/F_m) at different salinities during different exposure days (7, 14 & 21). The error bar represents the standard deviation, n = 3, ($P \leq 0.01$).

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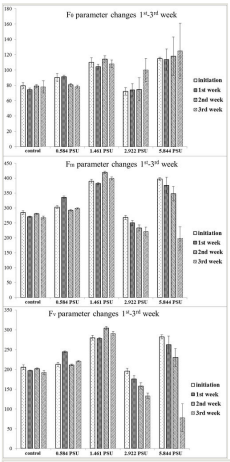

Figure 2.

Changes in maximum fluorescence intensity (F_m), minimum fluorescence intensity (F_0), and variable fluorescence (F_v) at different salinities during different exposure days (7, 14 & 21). The error bar represents the standard deviation, n = 3, ($P \leq 0.05$).