Arch Biol Sci. 2018;70(3):551-557 https://doi.org/10.2298/ABS180119016S

Impact of moderate heat stress on the biochemical and physiological responses of the invasive waterweed *Elodea canadensis* (Michx. 1803)

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Received: January 19, 2018; **Revised**: April 10, 2018; **Accepted**: April 10, 2018; **Published online**: April 18, 2018

Abstract: Global warming can negatively affect freshwater macrophytes. However, the degree to which freshwater plants can survive after long-term or short-term warming and the underlying molecular mechanisms are not fully understood. The aim of our study was to analyze the responses of an invasive plant to moderate heat stress (HS). Biochemical and physiological stress responses to experimental warming (30±1.0°C/25±1.0°C, day/night) were assessed in the invasive waterweed *Elodea canadensis*. The effect of the moderate HS on the macrophyte was evaluated through changes in the total protein content, catalase activity, lipid peroxidation, cellular membrane permeability by electrolyte leakage and the concentrations of carotenoids and photosynthetic pigments. Catalase activity and carotenoid concentrations increased significantly ($p<0.01$) in comparison to the control. A significant increase ($p<0.05$) in malondialdehyde concentration was observed. However, at the same time there was a persistent low level of electrolyte leakage in heat-treated plants as compared to the control. The results demonstrated that moderate HS improved membrane stability and increased the concentration of photosynthetic pigments and antioxidant activity in *E. canadensis* shoots. Moderate alterations in temperature may favorably affect the physiology and growth of the invasive macrophyte *E. canadensis*. It is reasonable to expect that warming could lead to a gradual change in *E. canadensis* distribution and to changes in composition of freshwater ecosystems.

Key words: *Elodea canadensis*; antioxidants; moderate heat stress; malondialdehyde; electrolyte leakage

INTRODUCTION

Global warming can promote the growth of invasive aquatic macrophytes [1,2]. Macrophytes play an important role in aquatic ecosystems (as primary producers, sources of habitats and refuges) and it is essential to study them in the context of the effects of global warming [3]. Elevated water temperature can severely affect submerged macrophytes, as the magnitude and intensity of temperature fluctuations and the tolerability of plant species determine the severity of these effects [4]. Plant responses to high temperature vary with the degree and duration of temperature stress and plant type because the increase in temperature affects plant growth and development. Plant distribution varies with the temperature range in which they can grow. For example, the growth temperature ranges from 15 to 25°C for *Egeria densa* Planch., from 10 to 25°C for *Elodea canadensis*, from 10 to 25°C for *Lagarosiphon major* (Ridl.) Moss ex Wager, and from 8 to 36°C for *Hydrilla verticillata* (L. f.) Royle [3,5]. A temperature increase will be favorable for an aquatic plant species that has a high temperature threshold value, as observed in invasive submerged macrophytes such as *Hydrilla verticillata*, *Myriophyllum spicatum* L. [6]. However, the temperature range for different aquatic macrophytes tends to be narrower than that for terrestrial plants, although many aquatic macrophytes can exist in shallow waters (<1 m depth) where seasonal and diurnal temperature fluctuations are much larger [7,8].

Plant growth and development involve numerous biochemical reactions that are sensitive to temperature. Elevated temperatures induce oxidative stress in plants and the formation of reactive oxygen species (ROS). Survival in oxidative stress depends on the equilibrium between production and scavenging of ROS by a se-

How to cite this article: Savicka M, Petjukevičs A, Batjuka A, Škute N. Impact of moderate heat stress on the biochemical and physiological responses of the invasive waterweed *Elodea canadensis* (Michx. 1803). Arch Biol Sci. 2018;70(3):551-7.

ries of enzymatic and non-enzymatic detoxification mechanisms. ROS in plant tissues can initiate lipid peroxidation (LP) that damages cell membranes and is considered to be the most important mechanism of tissue damage [9]. Enzymatic detoxification mechanisms that principally minimize cellular levels of superoxide radicals (O^{2-}) and hydrogen peroxide (H_2O_2) include the production of antioxidant enzymes such as catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) [10]. The non-enzymatic antioxidant system includes vitamins (ascorbic acid and tocopherols), glutathione, carotenoids, phenols, etc., which are among the key antioxidants involved in scavenging toxic ROS [11]. Carotenoids are pigments found in plants and microorganisms. Carotenoids play a key role in the photosynthetic reaction center where they are involved in mechanisms regulating photoprotection against auto-oxidation [12]. Carotenoids are non-enzymatic lipophilic antioxidants that at sufficiently high concentrations protect lipids from peroxidative damage [13].

Heat-induced oxidative stress has been widely discussed for terrestrial vegetation [14-16]. However, less attention has been paid to heat-induced oxidative stress and antioxidant system activity in aquatic macrophytes. There are a few cases where the stress-induced oxidative reactions in aquatic macrophytes have been measured, and most of these studies have focused on the biochemical and physiological changes under different stressors such as hypoxia, salinity, heavy metals [17-21].

Submerged macrophytes play a key role in biodiversity and water transparency in shallow freshwater ecosystems and they can be exposed to pronounced temperature fluctuations [22]. *E. canadensis* is a perennial invasive plant in natural aquatic ecosystems in Eurasia and is common in eutrophic waters [1]. *E. canadensis* forms thick stands, outcompeting native biodiversity. If the climate gets warmer, it is likely that *E. canadensis* will spread further [23]. *E. canadensis* is well-studied from various aspects, including invasion success and competitive ability, its impact on native aquatic communities [24] and on responses to environmental variables [25], and on morphological changes in response to temperature changes [26]. However, it is important to mention that there is almost no information about the influence of temperature on oxidative changes and activity of the antioxidant system in *E. canadensis* exposed to moderate HS. In this study we examined the influence of increases in water temperature on the physiological and biochemical changes in the submerged invasive macrophyte *E. canadensis*.

MATERIALS AND METHODS

High temperature treatments under controlled conditions

The experimental material (*E. canadensis* plants) was collected from the nearest lake. The plants were rinsed with clean water to remove debris and the attached algae were separated using forceps. The cleaned plants were cultured in glass aquaria under laboratory conditions. The experiment was conducted under controlled temperature in climate chambers (POL-EKO, Poland). All the treatments were subjected to a photoperiod regime of 8 h dark and 16 h light with a light intensity of 50 μmol photons $m^{-2}s^{-1}$. The air temperature in the climate chamber was maintained at 18±1.0°C/15±1.0°C (day/night) for the control plants, and at 30±1.0°C/25±1.0°C (day/night) in order to simulate moderate HS. The short-term effects of moderate HS were determined 24 h after exposure to HS for 7 days. *E. canadensis* samples from the control and heat-treated plants were collected 2, 3, 4 and 7 days after the initiation of high temperature treatment, and one day after the high temperature treatment was finished.

Plant tissue preparation for protein assay

Fresh plant samples (~300 mg) were extracted with ice cold 50 mM phosphate buffer (pH 7.8). Polyvinylpyrrolidone (PVP) was added to the extraction to mask the effects of phenolic compounds in the plant tissues. The extractions were centrifuged at 5 000 ×g at 4°C for 15 min, and the supernatant was kept at -80°C until further analysis.

Protein determination

The total protein content was detected by the "Invitrogen" protocol using the Quant-iT™ protein detection kit and Qubit™ fluorometer (Invitrogen™, USA).

Determination of catalase activity

The method of Aebi [27] was used to measure catalase (CAT) (EC 1.11.1.6) activity. The decomposition of H_2O_2 was measured as the decrease in absorbance at 240 nm. Fifty mM phosphate buffer (pH 7.8) and 10 mM H_2O_2 were used in the reaction solution.

Determination of electrolyte leakage

The electrolyte leakage (EL) in *E. canadensis* shoots was determined as described [28]. The EL was calculated from the following formula: $EL(\%)=(C_{before\ boiling}-C_{H2O}))/(C_{after\ boiling}-C_{H2O})\times100$, with C the conductivity of the solution before and after boiling.

Measurement of lipid peroxidation

The malondialdehyde (MDA) content was determined by the thiobarbituric acid reaction (TBARS) as described [29]. The concentration of MDA was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm using a molar extinction coefficient 155 $mM^{-1}cm^{-1}$.

Determination of photosynthetic pigments

The concentrations of chlorophyll (Chl*a*, Chl*b*, Chl*a*+*b*) and total carotenoids were determined spectrophotometrically in 200 mg of the plant material ground in a pre-chilled mortar in the presence of 5 mL of 80% acetone (v/v). After complete extraction, the mixture was centrifuged twice, and the volume adjusted to 10 mL with cold acetone. The absorbance of the extract was read at 663, 646, and 470 nm (Varian, USA) and pigment concentrations were calculated according to Lichtenthaler [30].

Statistical analysis

The data were expressed as the mean±standard deviation and were analyzed statistically using Microsoft Excel 2010. The data are presented after taking into consideration the standard deviation (SD) of three replicates. The results were analyzed by one-way ANOVA to identify significant differences between the groups, and their significance levels ($p<0.05$) were determined.

RESULTS

The results revealed that the biochemical and physiological changes in *E. canadensis* shoots were significantly affected depending on HS duration. The measurement of MDA and EL as parameters of oxidative stress showed significant differences between the heat-treated and control plants. The concentration of MDA was significantly higher ($p<0.05$) in the heat-treated plants (Fig. 1A), while a significant decrease ($p<0.05$) in EL was observed in the heat-treated plants compared to the control (Fig. 1B); on day 2 after exposure to stress it was decreased by 26% and it reached 29% at the end of the investigated period compared to the control.

The change in the total protein content in *E. canadensis* shoots during moderate HS and on the day after the stress treatment as compared to the control are shown in Fig. 2A. A statistically significant de-

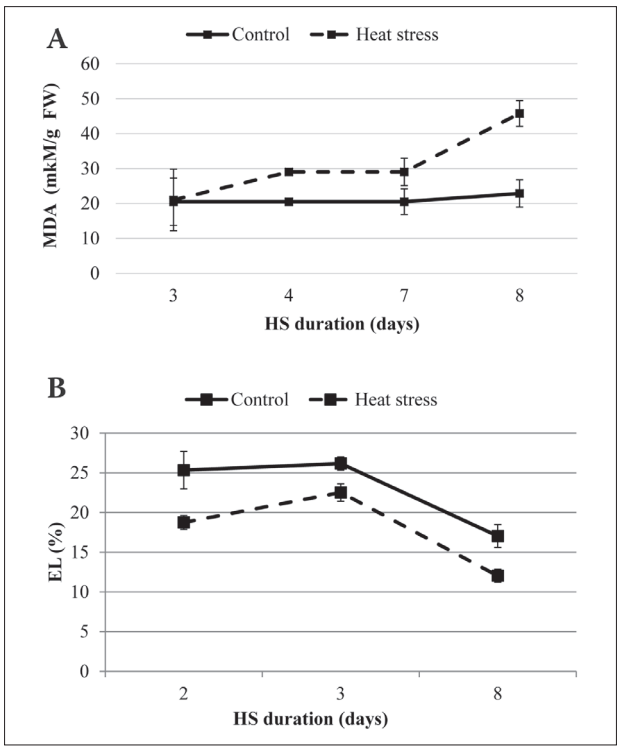

Fig. 1. The effect of duration of moderate HS (30±1.0°C/25±1.0°C, day/night) on the level of MDA (mkM/g fresh weight (FW)) (**A**) and electrolyte leakage (EL) (**B**) in *E. canadensis* (solid line – control, dashed line – heat stress (HS)). The bars indicate the standard deviations (n=3); missing deviation bars indicate that they are smaller than the symbol. Days 2, 3, 4, and 7 – HS treatment; day 8 – the day after the HS treatment.

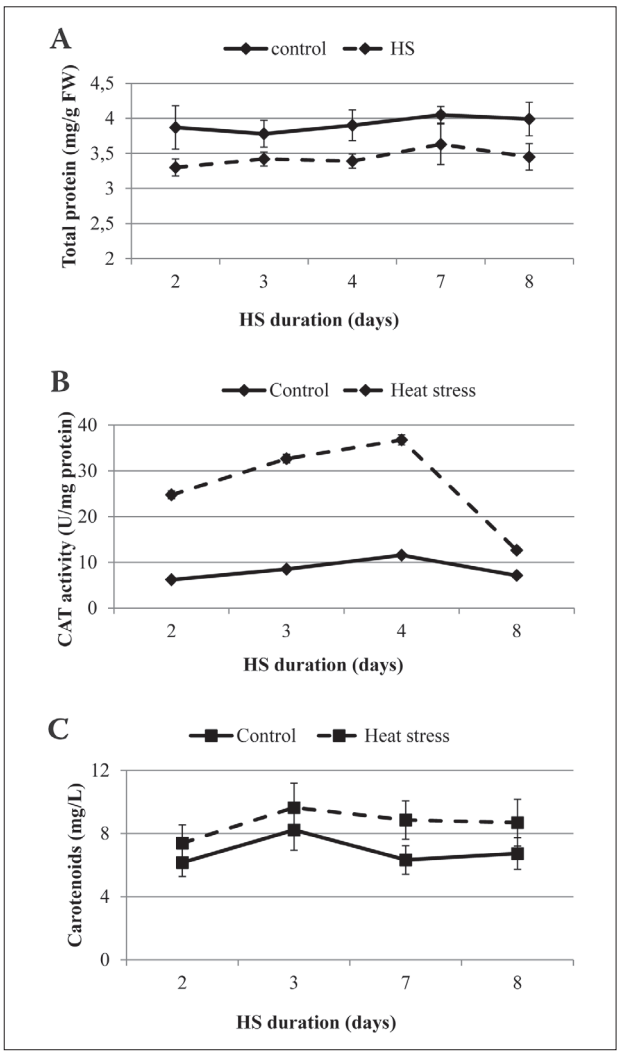

Fig. 2. The effect of duration of moderate HS (30±1.0°C/25±1.0°C, day/night) on the total protein content (mg/g fresh weight (FW)) (**A**), CAT activity (**B**), and carotenoid concentrations (**C**) in *E. canadensis* (solid line – control, dashed line – heat stress (HS)). The bars indicate standard deviations (n=3); missing deviation bars indicate that they are smaller than the symbol. Days 2, 3, 4, and 7 – HS treatment; day 8 – the day after the HS treatment.

crease (p≤0.001) in the total protein content by 14% was observed after the HS treatment. CAT under HS also exhibited significant differences when compared to the control (Fig. 2B). However, a 4-fold increase (p≤0.001) in CAT activity during the HS was observed during the investigated period compared to the control, despite the significant decrease in total protein. Lipid damage, expressed as the increase in MDA in the cell membrane, was increased two-fold a day after the HS treatment was finished (Fig. 1A, p<0.05), whereas the activity of CAT significantly decreased (Fig. 2B).

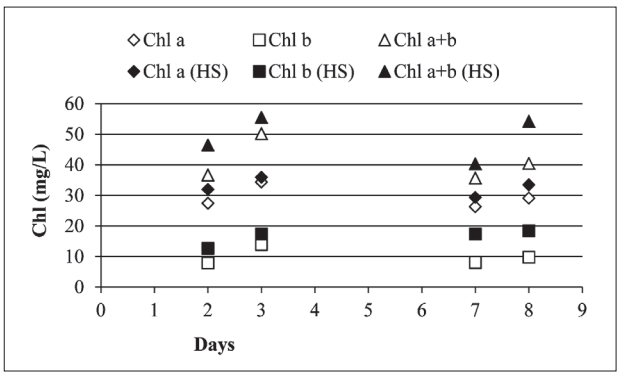

Fig. 3 The relationship between the duration of moderate heat stress (HS) and the concentrations of Chl *a* (-◆-), Chl *b* (-■-) and Chl *a+b* (- ▲-) in *E. canadensis* leaves (white markers – control (15/18°C), black markers – HS (25/30°C)), p<0.01. Days 2, 3, and 7 – HS treatment; day 8 – the day after the HS treatment.

Parallel with the high activity of CAT, the moderate heat stress altered the carotenoid content in *E. canadensis* leaves. The carotenoid content was significantly increased in plants exposed to HS. The changes in total carotenoids in *E. canadensis* leaves during and after exposure to HS as compared to the controls are shown in Fig. 2C. The total carotenoids increased during the investigated period: on day 2 after exposure to HS the carotenoid content increased by 20% and it reached 40% at the end of the investigated period when compared to the control (p<0.01) and remained at the same level for a day after the HS (Fig. 2C).

Fig. 3 shows the relationships between the duration of HS and Chl *a*, Chl *b* and Chl *a+b* in *E. canadensis* leaves (p<0.01). Chlorophyll *a* and *b* increased gradually under moderate HS (p<0.01). The concentration of chlorophyll *a* increased by 12% while a 2-fold increase (p≤0.01) in chlorophyll *b* was observed at the end of the investigated period in comparison to the control.

DISCUSSION

Elevated temperatures can affect the growth and development of aquatic macrophytes, leading to variations in their distribution patterns and competitive advantage in the natural environment [31]. HS can induce oxidative stress through peroxidation of membrane lipids and disruption of cell membrane stability by protein denaturation [32]. Therefore, under differ-

ent environmental stresses EL and the concentration of MDA in plants played a key role in oxidative damage [33]. The products of lipid peroxidation are commonly used to assess oxidative injury of membrane lipids [28]. In the present study, the concentration of MDA as an expression of lipid peroxidation, was increased by the high temperature treatment. Increased MDA due to HS in terrestrial plants has been reported [15,29,34]. There are no data available on the changes of MDA concentrations in aquatic macrophytes after HS. However, at the same time there was a constantly low level of EL in the heat-treated plants. It was observed [35] that moderate HS (below 45°C) decreased the EL of date palm, but a temperature above 53°C was lethal because EL was increased by more than 50%. The low level of EL might be due to the increased concentration of carotenoids and increased CAT activity in *E. canadensis* plants that reduced the generation of free radicals and lowered lipid peroxidation. Increased CAT activity was observed despite the reduced total protein content. Obviously, the plants exposed to mild or moderate HS exhibited an increase in activity of the enzymatic antioxidant systems. Similar results were observed in plants exposed to other moderate environmental stresses, such as water [36,37] and salt stress [20].

In stress conditions, the ROS concentration is elevated to damaging levels in mitochondria, chloroplasts and peroxisomes when compared to the level produced during normal growth conditions [38]. Elimination of ROS is mainly achieved by antioxidants such as CAT and carotenoids. Carotenoids influence many plant processes, and as antioxidants they can protect photosynthetic organisms against oxidative stresses and serve as modulators of membrane microviscosity [39]. These functions reduce the effects of changing temperatures and light intensity, thus maintaining plant development during environmental stress. The synthesis of carotenoids was increased in *E. canadensis* under moderate HS, perhaps because these compounds acted as antioxidants which minimized the oxidative damage induced by HS [12,13].

In general, moderate short-term HS might be favorable for the growth and development of *E. canadensis* because of increased antioxidant activity (CAT) and carotenoid levels, membrane stability (EL), and elevated concentrations of photosynthetic pigments ($p<0.01$). It was reported [3] that an increase in water temperature to 23°C significantly favored the growth *E. canadensis* and *Egeria densa*. Our results agree with those reported previously [40] which showed that moderate HS (30°C) led to increased antioxidant system activity and concentrations of photosynthetic pigments in *Elodea nuttallii* Planch. St. John. However, increased ROS accumulation was observed at a higher temperature (35°C) for *E. nuttallii*; as a result, it suppressed the competitive ability of the plant [40]. Similar results were observed after moderate salt stress [20] and exposure to low concentrations of urea [41] in *E. densa*.

Growth enhancement, measured as an increase in shoot length, plant height, leaf surface area and biomass production, has been reported in different submerged and emergent macrophytes exposed to temperature 3-7°C above the ambient temperature [2]. Enhanced growth could also be attributed to the increase in physiological activities such as respiration and photosynthesis [42]. Thus, the obtained results supplement the existing data on the effect of global warming on the growth and development of aquatic plants.

Our results show that the moderate HS leads to improved membrane stability and increased concentrations of photosynthetic pigments and antioxidant activity in *E. canadensis* shoots. Therefore, moderate alterations in temperature may favorably affect the physiology and growth of the invasive macrophyte *E. canadensis*. It is reasonable to expect that warming can gradually lead to a change in distribution of *E. canadensis,* which could substantially change ecosystem structure.

Acknowledgements: This study was supported by the National Research Program 2014-2017 "EVIDEnT" sub-project 1.4. "Functioning of food-webs".

Author contributions: Marina Savicka participated in all phases of the research and in writing the manuscript; Aleksandrs Petjukevičs and Anna Batjuka performed the laboratory measurements; Natalja Škute contributed to the drafting and writing of the manuscript.

Conflict of interest disclosure: The authors report no conflict of interest.

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