*Pak. J. Bot.*, 55(5): 1599-1603, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-5(2)

# EFFECTS OF ELEVATED TEMPERATURE ON THE VEGETATIVE GROWTH AND FLOWERING OF LACE PLANT (*APONOGETON MADAGASCARIENSIS*)

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**Abstract**

Lace plant (*Aponogeton madagascariensis*) an aquatic plant, belongs to the family Aponogetonaceae. Under controlled experimental conditions, the effects of temperature on morphological and physiological parameters of lace plants were studied. Lace plants were grown under two temperature treatments (24°C and 30°C) in aquariums, and their morphological and physiological characteristics, such as leaf number and flowering percentage, plastid density and photosynthetic pigments, were measured. Results indicated that leaf number was negatively affected by the high temperature condition. The percentage of flowering was higher in lace plants grown under high temperature compared with plants grown under low temperature. On the other hand, chlorophyll content and plastid density was increased grown under high temperature. This study indicated that high temperature stimulated flowering process and chlorophyll synthesis, but reduced leaf number. This is an unprecedented finding on the effect of high temperature on flowering in lace plants. Our findings demonstrated a phenotypic plasticity in response to high temperature, suggesting evolved adaptations in lace plants to high temperature in the aquatic environment.

**Key words:** *Aponogeton Madagascariensis*, Flowering, Morphological and physiological, Temperature.

## Introduction

Global warming has severe effects on aquatic ecosystems by increasing temperature of surface water and reducing ice cover (Woolway *et al*., 2017). Macrophytes, including aquatic angiosperms, play an important role in aquatic ecosystems since they are used as food, habitats and/or protection places for a wide group of aquatic species, such as microorganisms, waterfowl, zooplankton and vertebrates, such as fish and frogs (Bornette & Puijalon, 2009).

Distribution of aquatic plants, productivity and community are often affected by changes in sea level, light intensity, salinity, temperature, pH, atmospheric $CO_2$ and UV radiation (Gillard *et al*., 2020). The reproduction in aquatic plants mostly occurs via vegetative methods, such as production of rhizomes and stolons or turions (Sculthorpe, 1967). Other aquatic plants, such as water chestnut (*Trapa natans* L.), also depend heavily on seed production in their reproduction (Monacelli & Wilcox, 2021). The best flowering time of the aquatic plants is, in general, during the monsoon, but some have extraordinary flowering out of the season, while others flower throughout the year (Rahman *et al*., 2007).

The flowering time in plants could be altered by multiple stress factors that also affect the process of seed production (Miller-Rushing & Primack, 2008; Kazan & Lyons, 2016). The flowering process should occur early enough during the growth season in order to obtain successful pollination and better fitness (Kehrberger & Holzschuh, 2019). There is an increasing concern regarding the rise of water temperature in aquatic ecosystems. This concern is particularly about epiphytes and rooted aquatic plants, which may be sensitive to high temperature and unable to move to new areas (Miller-Rushing & Primack, 2008). Water temperature affects the flowering process and productivity in aquatic plants in different ways (Madsen & Morgan, 2021). For example, it affects their productivity by regulating the rate of various chemical reactions, which are involved in this process (Simpson & Eaton, 1986). Most aquatic plants exhibit higher photosynthetic rate at relatively higher temperatures; between 25°C and 32°C (Pedersen *et al*., 2013), and between 28°C and 32°C, (Barko *et al*., 1986).

Ruiz *et al.,* (2018) reported that increased temperature induced flowering and negatively affected the leaf growth rate in the Mediterranean seagrass *Posidonia oceanica*. However, the effect of water temperature on the flowering process of aquatic plants is essentially unknown.

This plant is a true aquatic monocot belongs to the family Aponogetonaceae, and it is native to Madagascar rivers (Dauphinee *et al*., 2017). The plant forms a rosette leaves emerging from a spherical corm that is fixed in substrate by roots arising at random from it (Rowarth *et al*., 2021). Furthermore, it reproduces either by bulb separation, or produces seeds after flowering, which enable the new plant to form a root system and immature leaves before separation from the mother plant (Rowarth *et al*., 2021). One of the most distinctive features of lace plant is a having perforations in its leaves via programmed cell death (Rowarth *et al*., 2020). This process provides the plant with its most remarkable characteristic "skeletonized "or fenestrated leaves (Rantong & Gunawardena, 2018). Because of this key feature, it is essential to get suitable aquatic ecosystems for growth and reproduction of the lace plant plants, and to know how this plant is affected by the various abiotic factors in order to preserve such important plant in its natural habitat and scientific labs also.

For the best of our knowledge, this is the first study to investigate the effect of temperature on growth and flowering of lace plant. The overall aim of this study was to investigate the morphological and physiological changes of lace plant in response to two different temperatures.

## Materials and Methods

**Plant material and heat treatment:** In this study, lace plant (*Aponogeton madagascariensis*) supplied by AquaEssentials (Meadow Industrial Estate Crediton, England) were used. Plants were grown in four equal-size aquariums of 40 cm depth, 45 cm width, and 30 cm height (each aquarium contained three plants), containing 2-3 inches of substrate (Dustin's Fishtanks, 180 Louisa Dr., Nicholasville KY 40356) and 5 gallons of tap water under control conditions (room temperature of 24ºC; light/dark; photoperiod of 12 h; and photosynthetic photon flux density (PPFD) of 125 µmol $m^{-2}$ $s^{-1}$ at the water surface) for 10 days. Photosynthetic photon flux density (PPFD) was provided by daylight simulating fluorescent bulbs (Philips, Daylight Deluxe, F40T12/DX, Markham, Ontario) and measured with digital light meter (Sunfleck Ceptometer, Decagon Devices, Pullman, WA, USA). Then, the two control aquariums were randomly assigned and maintained under room temperature (24ºC), while heaters (Aqueon, Franklin, Wisconsin, USA) were set to the desired temperature (approximately 30ºC) and placed in the other two aquariums. Plants were grown under the experimental conditions inside the aquariums for 44 days, and in each aquarium PPFD and photoperiod were similar to the initial growth conditions. Data were taken after 44 days of growing plants in the aquariums and the experiments were conducted three times and each time the aquariums were reversed.

**Measurement of growth parameters:** Three plants from each aquarium were used for measuring the leaves and flower numbers, fortnightly.

**Measurement of photosynthetic pigments:** At the end of the experiment, three fully developed leaves from every treatment were used to measure the concentrations of chlorophyll (Chl) *a*, Chl *b*, carotenoids and total Chl according to Abo Gamar *et al*., (2019) with some modifications. From each treatment, three samples of 0.1 g leaf tissue were harvested from three different plants and placed into vials containing 10 mL of dimethyl sulfoxide. For complete chlorophyll extraction the solutions containing samples of leaves were incubated under dark condition for 48 h at room temperature. The absorbance at 664 nm, 648 nm and 470 nm was measured for Chl *a*, Chl *b*, and carotenoids, respectively, using a UV/visible spectrophotometer (Bausch and Lomb, Rochester, NY, USA). Pigment concentrations were expressed as µg $mg^{-1}$ FM (Chappelle *et al*., 1992).

**Light microscopy:** Fully-developed leaves from lace plants, from each treatment, were sampled and photographed (at 400×) to examine plastid density and color using a Leica Macroscope Z16 APO equipped with a digital camera DFC 500. Leaves were mounted in distilled water and 5 randomly selected microscopic fields at surface of 5 leaves from different plants were examined. Plastid density (number $mm^{-2}$) as the number of plastid cells per unit epidermal area was calculated.

### Statistical analysis

The data were presented as mean ± S.E. Two-tailed Student's t-test was used to determine significant group differences and means were considered as statistically significant if $p<0.05$.

## Results

**Leaf number:** Lace plants grown under high temperature (30°C) showed significant reduction in leaf number, compared to those grown under low temperature (24°C) (Figs. 1 and 2 (A-B)).

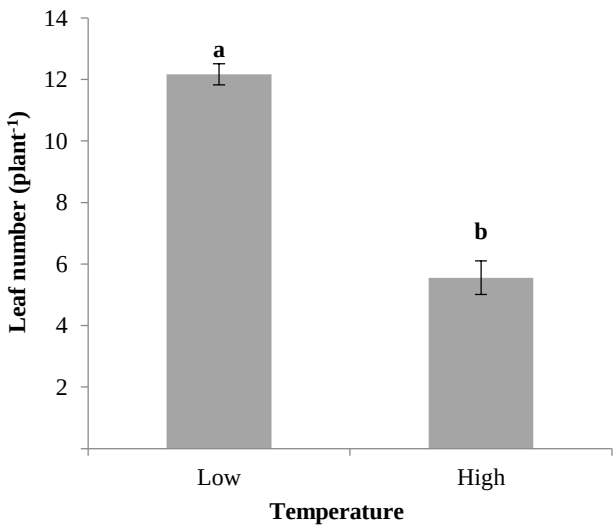


Fig. 1. Effects of temperature on leaf number of 54-day-old lace plants (*Aponogeton madagascariensis*). Plants were grown under 2 temperature regimes (24°C and 30°C) in aquariums for 44 days, after initial growth of 10 days under 24°C and 12 h light/12 h dark. Data are means ± SE of 18 samples from 3 trials. Different surmounted letters indicate statistical significance ($p< 0.05$).

**Flowering percentage:** Plants grown under high temperature had significant higher percentage of flowering, which was about 84% while plants grown under low temperature had about 44% flowering (Fig. 3). It was observed that under high temperature the inflorescences had noticeable thin peduncles as well as small flowers.

**Photosynthetic pigments**: Chl *a*, Chl *b*, carotenoid, and the total chlorophyll were significantly increased in lace plant leaves grown under high temperature (Fig. 4A-C).

**Plastid density:** Leaf pieces were mounted in distilled water and examined for their cell's plastid density and color. Leaf pieces from lace plant grown under higher temperature, showed significantly more plastid density and darker green color than the plants grown under low temperature condition (Figs. 5 and 6A-B).

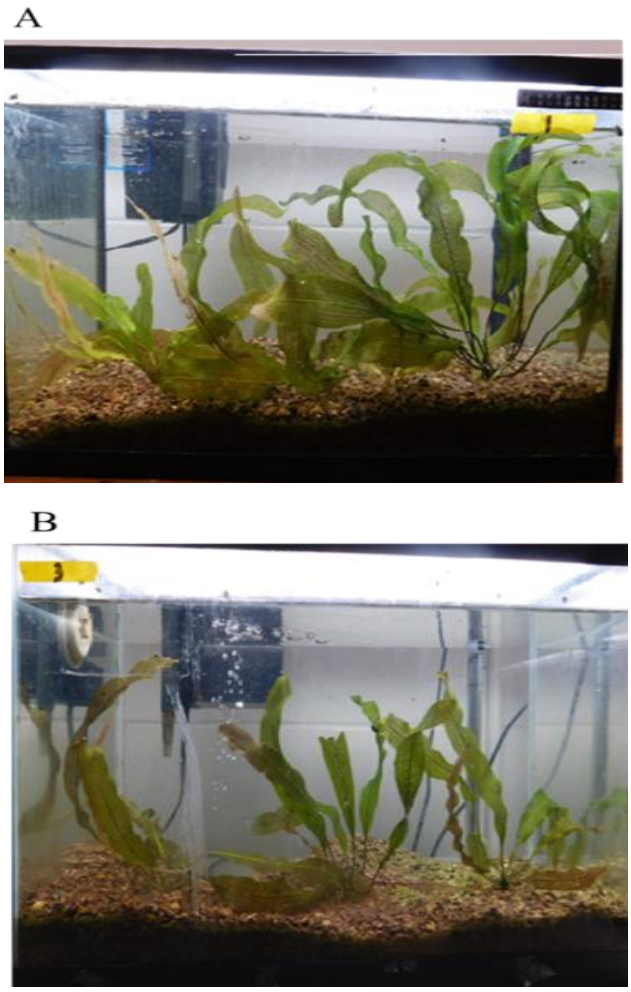


Fig. 2. Lace plants (*Aponogeton madagascariensis*) grown under 2 temperature regimes (24°C and 30°C) in aquariums for 44 days, after initial growth of 10 days under 24°C and 12 h light/12 h dark. (A) Plants grown under lower temperature, (B) Plants grown under higher temperature.

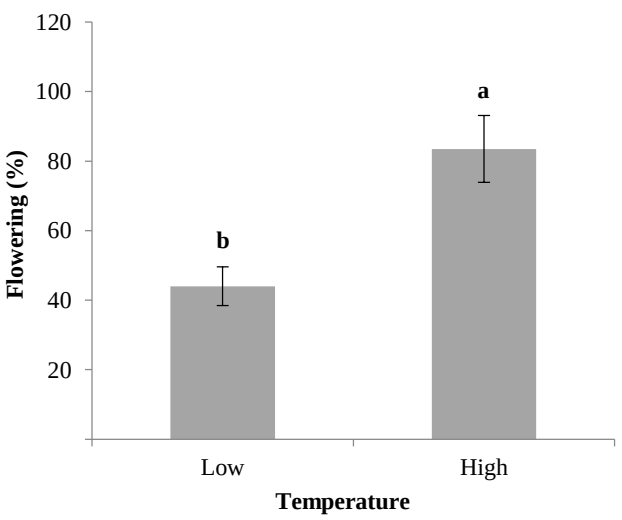


Fig. 3. Effect of temperature on flowering (%) of 54-day-old lace plants (*Aponogeton madagascariensis*). Plants were grown under 2 temperature regimes (24°C and 30°C) in aquariums for 44 days, after initial growth of 10 days under 24°C and 12 h light/12 h dark. Data are means ± SE of 18 samples from three trials. Different surmounted letters indicate statistical significance ($p < 0.05$).

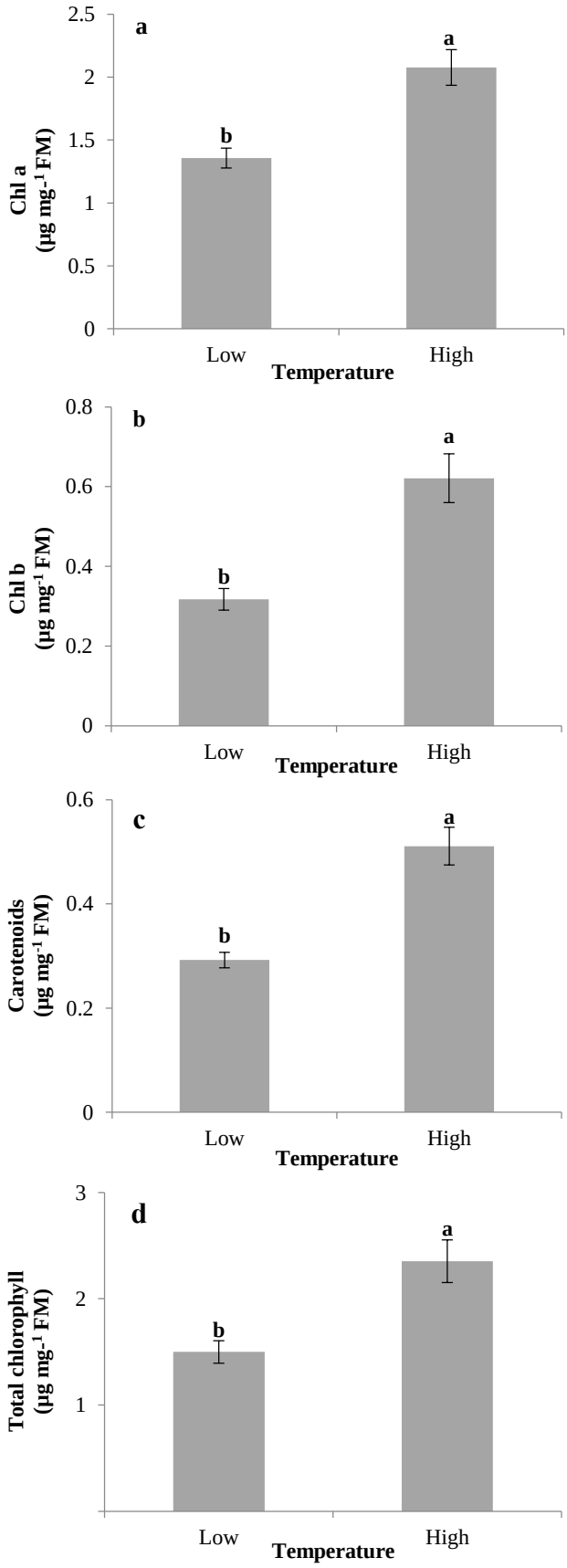


Fig. 4. Effect of temperature on photosynthetic pigments' contents of 54-day-old lace plants (*Aponogeton madagascariensis*). Plants were grown under 2 temperature regimes (24°C and 30°C) in aquariums for 44 days, after initial growth of 10 days under 24°C and 12 h light/12 h dark. Data are means ± SE of 9 samples from 3 trials. Different surmounted letters indicate statistical significance ($p<0.05$).

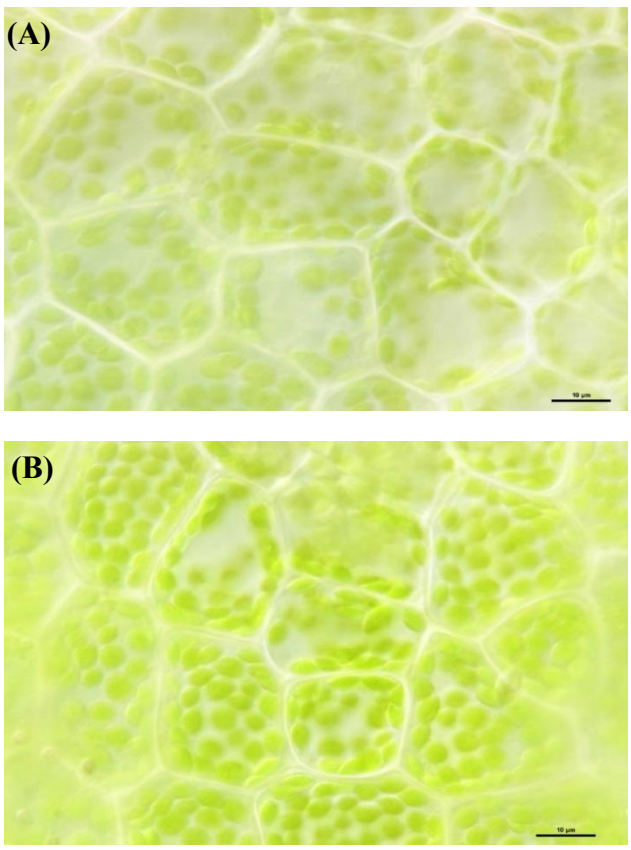


Fig. 6. Plastid abundance in lace plant cells (*Aponogeton madagascariensis*) grown under 2 temperature regimes (24°C and 30°C) in aquariums for 44 days, after initial growth of 10 days under 24°C and 12 h light/12 h dark. (A) Plastids of leaves from lace plants grown under lower temperature, (B) Plastids of leaves from lace plants grown under high temperature had more abundance and darker green color. Scale bars: A-B = 10 µm.

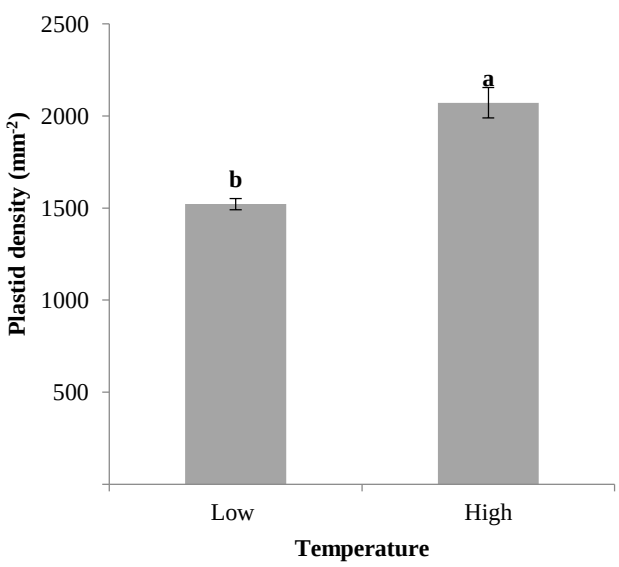


Fig. 5. Effect of temperature on plastid density of 54-day-old lace plants (*Aponogeton madagascariensis*). Plants were grown under 2 temperature regimes (24°C and 30°C) in aquariums for 44 days, after initial growth of 10 days under 24°C and 12 h light/12 h dark. Data are means ± SE of 5 microscopic fields on each surface of 5 leaves from different plants. Different surmounted letters indicate statistical significance ($p$<0.05).

## Discussion

In this study, we investigated the impact of different temperatures on lace plant morphology, physiology and growth. It was shown that high temperature (30°C) reduced leaf number (Figs. 1 and 2). Reduction of leaf number for plants under high temperature indicates that high temperature may cause redirecting nutrients, especially nitrogen away from photosynthetic apparatus to other processes, such as flowering, which could provide an explanation to the reduction in leaf number and, in turn, plant biomass (Halsey & Jones, 2015). Our results are inconsistent with Zhang *et al*., (2019) who reported that root and shoot biomasses as well as relative growth rate of the *Potamogeton lucens* L., *Vallisneria spiralis* L. and *Elodea nuttallii* L. plants increased significantly with high temperature. However, because of the significant reduction in the leaf number under the high temperature treatment (Figs. 1 and 2), our data indicate that the growth of lace plants are negatively affected by temperature 30°C, which implies that, in the future, Madagascar may not provide favorable thermal conditions for growth of lace plants if the temperature keeps rising.

High temperature induced flowering in lace plants (Fig. 3). The induction of flowering under high temperature (Fig. 3) is consistent with earlier studies on *Zostera marina* L. by De Cock (1981), who reported that the number of male flowers increased with rising temperature. Also, Rivero-Lepinckas *et al*., (2006) found that 23–25°C range was the best for lace plant growth. Higher flowering percentage for lace plants grown under high temperature could be explained as a heat stress escape mechanism by enhancing the biochemical reactions involved in flowering process (Durako & Moffler, 1987), thus, higher temperatures appear to positively affect this aspect of lace plant. Recent studies have also shown that flowering is now occurring earlier than in the past because of global warming and increasing water temperature (Fitter *et al*., 1995; Parmesan & Yohe, 2003).

Analysis of chlorophyll content has been found to be as an important method for assessing the photosynthetic capacity of plant leaves (Clark *et al*., 2000) and considered as a rapid method for evaluating plant abilities to tolerate different types of stress factors, such as temperature (Percival & Sheriffs, 2002). In this study, high temperature causes the lace plants to accumulate significantly more photosynthetic pigments than plants grown under low temperature condition (Fig. 4A-D). This result is further supported by the observation that lace plants grown under high temperature produced more and darker green-colored chloroplasts (Figs. 5 and 6A-B). These results revealed that plants grown under high temperature tried to evade impairment in photosynthetic machinery resulting from heat stress by accumulating more photosynthetic pigments. Similar results were reported by Araus *et al*., (1998), who found that plants reduce problems with over-heating by accumulating more chlorophyll pigments to help plants to dissipate the extra heat by performing more chlorophyll fluorescence. Lower leaf number and higher chlorophyll content under 30°C suggested that high temperature affected Rubisco and so reduced photosynthetic capacity, which might decrease growth rate (Dutta *et al*., 2009).

## Conclusion

It is reported for the first time that the flowering can be induced in lace plants by high temperature. Induction of flowering under high temperature clearly indicates that temperature rising related to the global warming seemed to be the chief control of the flowering in lace plant. Leaf number was reduced while plastid density and photosynthetic pigments' contents were increased under high temperature indicating that high temperature affects the vegetative growth of lace plant.

## Acknowledgments

We thank the deanship of research at Yarmouk University/Jordan for funding this project.

### References

Abo-Gamar, M.I.A., A. Kisiala, R.N. Emery, E.C. Yeung, S.L. Stone and M.M. Qaderi. 2019. Elevated carbon dioxide decreases the adverse effects of higher temperature and drought stress by mitigating oxidative stress and improving water status in *Arabidopsis thaliana*. *Planta,* 250: 1191-1214.

Araus, J., T. Amaro, J. Voltas, H. Nakkoul and M. Nachit. 1998. Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under mediterranean conditions. *Field Crops Res.*, 55(3): 209-223.

Barko, J.W. and R.M. Smart. 1986. Sediment-related mechanisms of growth limitation in\submersed macrophytes. *Ecology*, 67(5): 1328-1340.

Bornette, G. and S. Puijalon. 2009. Macrophytes: ecology of aquatic plants. E.L.S. 55:4 pp. 1–9. John Wiley & Sons, Chichester, UK.

Chappelle, E.W., M.S. Kim and J.E. McMurtrey. 1992. Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves. *Remote Sens. Environ.*, 39(3): 239-247.

Clark, A.J., W. Landolt, J. Bucher and R. Strasser. 2000. Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence performance index. *Environ. Pollut.*, 109(3): 501-507.

Dauphinee, A.N., J.I. Fletcher, G.L. Denbigh, C.R. Lacroix and A.H. Gunawardena. 2017. Remodeling of lace plant leaves: antioxidants and ROS are key regulators of programmed cell death. *Planta*, 246(1): 133-147.

De Cock, A.W.A.M. 1981. Influence of temperature and variations in temperature on flowering in *Zostera marina* L. under laboratory conditions. *Aquat. Bot.*, 10: 125-131.

Durako, M.J. and M.D. Moffler. 1987. Factors affecting the reproductive ecology of *Thalassia testudinum* (hydrocharitaceae). *Aquat. Bot.*, 27(1): 79-95.

Dutta, S., S. Mohanty and B.C. Tripathy. 2009. Role of temperature stress on chloroplast biogenesis and protein import in pea. *Plant Physiol.*, 150: 1050-1061.

Fitter, A., R. Fitter, I. Harris and M. Williamson. 1995. Relationships between first flowering date and temperature in the flora of a locality in central England. *Func. Ecol.*, 9: 55-60.

Gillard, M.B., J. Aroviita and J. Alahuhta. 2020. Same species, same habitat preferences? The distribution of aquatic plants is not explained by the same predictors in lakes and streams. *Freshwater Biol*., 65(5): 878-92.

Halsey, K.H. and B.M. Jones. 2015. Phytoplankton strategies for photosynthetic energy allocation. *Ann. Rev. Mar*., 7: 265-297.

Kazan, K. and R. Lyons. 2016. The link between flowering time and stress tolerance. *J. Exp. Bot.*, 67(1): 47-60.

Kehrberger, S. and A. Holzschuh. 2019. How does timing of flowering affect competition for pollinators, flower visitation and seed set in an early spring grassland plant?. *Sci. Rep.*, 9(1): 1-9.

Madsen, J.D. and C.M. Morgan. 2021. Water temperature controls the growth of waterhyacinth and South American sponge plant. *J. Aquat. Plant Manag.*, 59s: 28-32.

Miller-Rushing, A.J. and R.B. Primack. 2008. Global warming and flowering times in Thoreau's Concord: A community perspective. *Ecol*., 89(2): 332-341.

Monacelli, K. and D.A. Wilcox. 2021. Competition between two floating-leaved aquatic plants. *Aquat. Bot.*, 172: 103390.

Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421(6918): 37-42.

Pedersen, O., T.D. Colmer and K. Sand-Jensen. 2013. Underwater photosynthesis of submerged plants–recent advances and methods. *Front. Plant Sci.*, 4:140.

Percival, G.C. and C.N. Sheriffs. 2002. Identification of drought-tolerant woody perennials using chlorophyll fluorescence. *Arboric. J*., 28(5): 215-223.

Rahman, M.A., H. Hasegawa, K. Ueda, T. Maki, C. Okumura and M.M. Rahman. 2007. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): a good option for phytoremediation. *Chemosphere*, 69(3): 493-499.

Rantong, G. and A.H. Gunawardena. 2018. Vacuolar processing enzymes, AmVPE1 and AmVPE2, as potential executors of ethylene regulated programmed cell death in the lace plant (*Aponogeton madagascariensis*). *Botany*, 96(4): 235-247.

Rivero-Lepinckas, L., D. Crist and R. Scholl. 2006. Growth of plants and preservation of seeds. In: (Eds.): Salinas, J. and J.J. Sanchez-Serrano. Arabidopsis protocols. Methods in molecular biology™, Vol 323. Humana Press.

Rowarth, N.M., A.N. Dauphinee, G.L. Denbigh and A.H. Gunawardena. 2020. Hsp70 plays a role in programmed cell death during the remodeling of leaves of the lace plant (*Aponogeton madagascariensis*). *J. Exp. Bot.*, 71(3): 907-918.

Ruiz, J.M., L. Marín-Guirao, R. García-Muñoz, A. Ramos-Segura, J. Bernardeau-Esteller, M. Pérez, N. Sanmartí, Y. Ontoria, J. Romero, R. Arthur and T. Alcoverro. 2018. Experimental evidence of warming-induced flowering in the Mediterranean seagrass *Posidonia oceanica*. *Mar. Pollut. Bull*., 134: 49-54.

Sculthorpe, C.D. 1967. The biology of aquatic vascular plants. St. Martin's Press, New York.

Simpson, P. and J. Eaton. 1986. Comparative studies of the photosynthesis of the submerged macrophyte *Elodea canadensis* and the filamentous algae *Cladophora glomerata* and spirogyra sp. *Aquat. Bot.*, 24(1): 1-12.

Woolway, R.I., M.T. Dokulil, W. Marszelewski, M. Schmid, D. Bouffard and C.J. Merchant 2017. Warming of Central European lakes and their response to the 1980s climate regime shift. *Clim. Change*, 142(3-4): 505-20.

Zhang, P., B. Grutters, C.H. van Leeuwen, J. Xu, A. Petruzzella, R.F. van den Berg and E.S. Bakker. 2019. Effects of rising temperature on the growth, stoichiometry, and palatability of aquatic plants. *Front. Plant Sci.*, 9: 1947.

(Received for publication 7 March 2022)