Effects of heat stress on growth, photosynthetic pigments, oxidative damage and competitive capacity of three submerged macrophytes

Hendadura Chandani Chalanika De Silva and Takashi Asaeda

Department of Environmental Science, Saitama University, Saitama, Japan

ABSTRACT
There is an information gap regarding heat stress-induced oxidative damage and the species-specific behavior of plants under stress conditions. The present study was designed with the hypothesis that heat stress may induce species-specific oxidative damage that determines the competitive capacity of common submerged macrophytes. We conducted two laboratory experiments to simulate mono- and mixed cultures of three submerged macrophytes with the application of two heat shock treatments. The results showed that both heat shocks had significant effects on growth, photosynthetic pigments and the ability to induce strong oxidative damage for all three species. The comparative results of mono- and mixed cultures showed that P. crispus had an advantage in both the control and high-temperature treatments over the other two species as a strong competitor in the mixed culture. Further, the competitive capacity of P. crispus increased in the moderately high-temperature condition compared to the control.

Introduction
The elevation of temperature over an optimal level, which can cause non-reversible damage to plant growth and development, is termed heat stress. Elevated water temperature can severely affect submerged macrophytes; the magnitude and intensity of temperature fluctuations and the tolerability of plant species determine the severity of these effects (Wahid et al. 2007).

The effects of the deviation of abiotic factors on the growth and development of aquatic macrophytes have been discussed in the literature (Santamaria & van Viersen 1997; James et al. 2004; Declerck et al. 2005; Smolders et al. 2006; Ellawala et al. 2011b; Zaman & Asaeda 2013). Further, biotic and abiotic deviation-induced oxidative stress under elevated concentrations of reactive oxygen species (ROS) has been described in relation to water flow, turbulence, salinity, UV radiation, drought, heavy metals, nutrient deficiency, air pollutants, herbicides and pathogen attacks (Halliwell & Gutteridge 1985; Mittler 2002; Shin et al. 2005; Cruz de Carvalho 2008; Rucińska-Sobkowiak 2008; Ellawala et al. 2011a; Nawkar et al. 2013).

However, survival against oxidative stress depends on the equilibrium between the production and destruction of ROS by a series of enzymatic and non-enzymatic detoxification mechanisms. Enzymatic detoxification mechanisms that principally minimize cellular levels of superoxide radicals (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) include the production of antioxidant enzymes such as catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) (Sairam & Tyagi 2004). The non-enzymatic defense mechanism includes the activity of ROS-scavenging proteins such as thioredoxins and metallothioneins (Steffens et al. 2013). The overproduction of ROS beyond equilibrium may cause severe damage to lipids, cellular proteins, nucleic acids and enzymes, leading to the death of cells induced by programmed cell death (PCD) (Shah et al. 2001; Gill & Tuteja 2010).

Each plant has an optimum set of environmental temperatures for proper growth and development; the temperature optimum for one species may be highly stressful for another species. High-temperature-induced changes in morphology and biomass allocation may lead to increased growth and competitive capacity (Pilon & Santamaria 2002; Riis et al. 2012). For the structure and proper functioning of freshwater ecosystems, competition can be considered a paramount factor determining the species distribution in aquatic macrophyte communities (Moss et al. 2003, Doyle et al. 2007).

Furthermore, heat stress-induced oxidative stress has been widely discussed for terrestrial vegetation throughout the literature (Anderson 2002; Chaitanya et al. 2002; Mazorra et al. 2002; Mei & Song 2010, Kipp & Boyle 2013). Although dynamics in morphology, growth performance, photosynthesis and cellular respiration under the influence of heat stress are commonly cited, less attention has been paid to evaluating oxidative stress and the performance of antioxidant enzymes governed by heat stress. The full mechanism of the thermotolerance of aquatic macrophytes is largely unknown.

Further, there are few cases where the competitive interactions between different species of aquatic macrophytes have been measured (Herb & Stefan 2006), although competitive interactions between different macrophyte species are customary phenomena in natural environments. Most of these studies focused on the competition of various macrophyte species under different light regimes (Spencer & Rejmánek 2010). There are fewer experimental studies on the warming-stimulated competitive capacity of submerged macrophytes. Additionally, most studies on competition...
between aquatic macrophytes are based on variations in the basic morphology and morphometry of plants. The oxidative damage induced by the interactive effects of abiotic stresses, such as temperature, and competition is largely unknown for submerged macrophytes.

Therefore, the present study was designed with the hypothesis that heat stress may induce species-specific oxidative damage, which determines the competitive capacity of common submerged macrophytes.

Materials and methods
The experimental plant species *Elodea nuttallii*, *Potamogeton crispus* and *Vallisneria asiatica*, collected from the Moto-Arakawa River in Japan, 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 for approximately one month.

To determine the competitive capacity of the three selected species, two separate experiments, a mono- and a mixed culture, were conducted with the same temperature treatments. For the monoculture experiment, plant apical tips (*E. nuttallii* and *P. crispus*) and small plants (*V. asiatica*) approximately 5 cm in height were separately planted in PVC pots with well-washed river sand as the substrate. For the mixed culture experiment, three apical tips or small plants from the three species were planted in the same PVC pot. Several PVC pots were prepared following the same design, and the prepared PVC pots were separately acclimatized under controlled conditions until the plants became rooted. All treatments were conducted in glass microcosms (15.7 × 15.7 × 24.5 cm), and 5% Hoagland nutrient solution (HNS), as recommended by Atapaththu and Asaeda (2015) for better growth of submerged macrophytes, was used as the nutrient media in the microcosms. To stimulate heat shock, the PVC pots with planted apical tips were directly transferred from the acclimatization tank to the pre-prepared shock, the PVC pots with planted apical tips were directly subjected to a photoperiod regime of 12 h dark and 12 h light with a light intensity of 100 µmol m⁻² s⁻¹ for better growth of submerged macrophytes, was used as a light source. All treatments were conducted in glass microcosms set to temperatures of 25°C (as a control), heat treatments (30°C and 35°C) and the control treatment (25°C).

The oxidative damage induced by the interactive effects of abiotic stresses, such as temperature, and competition is largely unknown for submerged macrophytes.

**Plant tissue preparation for stress assay**
Stress assays, which included H₂O₂, CAT, APX and POD, were extracted by grinding the frozen (with liquid nitrogen) fresh plant samples (~500 mg) with ice cold pH 6.0, 50 mM phosphate buffer. Polyvinylpyrrolidone (PVP) was added to the extraction to mask the effects of phenolic compounds in the plant tissues. The extractions were centrifuged at 5000 × g for 15 min, and the supernatant was separated and incubated at ~80°C until further analysis. For each analysis, every extract was evaluated in triplicate, and the results were used to determine concentrations by fresh weight (FW).

**H₂O₂ concentration**
The concentration of H₂O₂ was determined using a pre-prepared standard curve for a known concentration series. The reaction mixture contained 750 µL of enzyme extract and 2.5 mL of 0.1% TiSO₄ in 20% H₂SO₄ (v/v); the mixture was centrifuged at 5000 × g for 15 min at room temperature. The intensity of the yellow color that developed in the reaction mixture was determined spectrophotometrically at 410 nm (Jana & Choudhuri 1982).

The H₂O₂ concentrations produced at each temperature treatment were determined for each category as H₂O₂ produced by species-specific competition and H₂O₂ produced by heat stress. The concentrations of H₂O₂ produced by competition were derived from the differences in H₂O₂ concentrations between the mono- and mixed culture experiments at each temperature treatment. Further, the concentrations of H₂O₂ produced by heat stress were determined from the differences in H₂O₂ produced under the heat treatments (30°C and 35°C) and the control treatment (25°C).

**CAT activity**
The CAT activity was measured according to Aebi (1984): 100 µL of 10 mM H₂O₂ and 2.0 mL of 100 mM potassium phosphate buffer (pH 7.0) were added to a cuvette before adding 500 µL of the enzyme extract to initiate the reaction. The absorbance reduction at 240 nm was recorded every 10 s for 3 min. Finally, the CAT activity was calculated using an extinction coefficient of 40 µmol−1 cm⁻¹ and results were expressed as µmol/min/mg protein (µmol/min/mg pr.).
**APX activity**

The APX activity was determined according to Nakano and Asada (1981). The reaction mixture contained 100 µL of enzyme extract, 200 µL of 0.5 mM ascorbic acid in 50 mM potassium phosphate buffer (pH 7.0) and 2.0 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was started by adding 60 µL of 1 mM H₂O₂. The decrease in absorbance at 290 nm was recorded every 10 s. The APX activity was calculated using an extinction coefficient of 2.8 mM⁻¹ cm⁻¹ and results were expressed as µmol/min/mg protein (µmol/min/mg pr.).

**POD activity**

The POD activity was spectrophotometrically measured based on the oxidation of guaiacol in the presence of H₂O₂ (MacAdam et al. 1992). The reaction mixture contained 3.0 mL of pH 6, 50 mM potassium phosphate buffer, 40 µL of 30 mM H₂O₂ and 50 µL of 0.2 M guaiacol. The reaction was started by the addition of 100 µL of crude enzyme extract, and the increase in absorbance at 420 nm was recorded every 10 s for 3 min. The rate of change in absorbance was calculated, and the POD activity was determined using an extinction coefficient of 26.6 mM⁻¹ cm⁻¹ and results were expressed as µmol/min/mg protein (µmol/min/mg pr.).

**Statistical analysis**

The collected data were tested for normality using the Shapiro-Wilk test before statistical analysis. The percentage variations (increment or reduction) of the growth rate, total chlorophyll and H₂O₂ concentrations were calculated from the differences between the values of the prescribed parameters in the mono- and mixed cultures, with reference to the monoculture. The statistical analyses were conducted using SPSS 16v, and all results were presented as the mean ± SD of three replicates. The measured parameters in the mono- and mixed cultures were tested separately per species with one-way analysis of variance (ANOVA) using temperature as an independent variable. Two-way ANOVA tests were performed with temperature and culture condition combinations as independent variables per species.

**Results**

The variations in the RGR of the three species under the two different heat shocks compared to the control condition are shown separately in Figure 1 for the mono- and mixed cultures.

Among the three considered species, the lowest height increment rate, which ranged from 0.042 ± 0.007 to 0.098 ± 0.008 cm/day, was recorded for *V. asiatica* in both the mono- and mixed cultures. In both the mono- and mixed culture experiments, the height increments decreased with applied temperature for *P. crispus* and *V. asiatica*; these variations were statistically significant (*F* = 15.850, *p* < .05 and *F* = 27.043, *p* < .05 for *P. crispus* and *V. asiatica*, respectively) with applied temperature. A different kind of variation in the RGR was obtained for *E. nuttallii*, increasing in the moderate temperature treatment (30°C) and decreasing in the highest temperature treatment (35°C). A similar trend was observed in both the mono- and mixed cultures, but this variation was not significant (*F* = 2.585, *p* > .05) in the mixed culture. The height increment rates of *E. nuttallii* and *V. asiatica* were lower in the mixed cultures compared to the monocultures at each temperature treatment, and the variables were statistically significant for both species (*F* = 7.89, *p* < .05 and *F* = 34.89, *p* < .05 for *E. nuttallii* and *V. asiatica*, respectively). For *P. crispus*, the significant, highest RGR values (*F* = 32.66, *p* < .05) were recorded in the mixed culture compared to the monoculture in each temperature treatment (Table 1).

The variations in chlorophyll a (chl *a*), chlorophyll b (chl *b*) and total carotenoids in the three species under the two different heat shocks compared to the control condition are shown in Figure 2 separately for the mono- and mixed cultures.

The lowest values of chl *a*, chl *b* and total carotenoids were obtained for *V. asiatica* among the considered plant species. A similar trend of decrease was obtained for chl *a* and chl *b* between the control treatment and high-temperature treatments for *P. crispus* and *V. asiatica*. This same trend could be observed in both the mono- and mixed cultures. Different trends were observed in the variations of chl *a* and chl *b* for

![Figure 1. Variations in the RGR of *E. nuttallii*, *P. crispus* and *V. asiatica* with heat shock treatments (*n* = 3). The results are shown separately for the monoculture (A) and the mixed culture (B).](image-url)
E. nuttallii, for which chl a and chl b significantly increased in the 30°C heat shock treatment and decreased in the 35°C heat shock treatment. For E. nuttallii and V. asiatica, the chl a and chl b concentrations decreased in the mixed cultures compared to the monocultures, while they increased in the mixed cultures for P. crispus.

Total carotenoids increased from the control treatment (25°C) to the high-temperature treatment for all three species. For E. nuttallii and V. asiatica, the carotenoid concentrations increased from the monocultures to the mixed cultures, and this difference was statistically significant (\( F = 171.589, p < .05 \)) for V. asiatica but not statistically significant (\( F = 0.921, p > .05 \)) for E. nuttallii. Further, the carotenoid concentrations of P. crispus at each temperature treatment significantly decreased (\( F = 50.676, p < .05 \)) in the mixed cultures compared to the monocultures (Table 2).

The variations in H2O2 concentrations generated by the inter-species competition and by the heat shock treatments of E. nuttallii and V. asiatica are illustrated in Figure 3.

The H2O2 concentrations of E. nuttallii, P. crispus and V. asiatica increased with applied heat shock treatments in both the mono- and mixed cultures. Further, for E. nuttallii and V. asiatica, the highest values for H2O2 were obtained in the mixed cultures compared to the monocultures at every temperature treatment. The H2O2 concentration decreased in the mixed culture compared to the monoculture for P. crispus. When considering the % H2O2 formation, the H2O2 formed by heat shock decreased while that formed by competition among plants increased with increased temperature for E. nuttallii. In contrast, the H2O2 formed by heat shock increased and that formed by competition among plants decreased with increased temperature for V. asiatica.

The variations in H2O2 concentrations with applied temperature in each treatment of each plant species are shown in Figure 4.

Figure 4 illustrates that there were significant positive correlations between the H2O2 concentrations and applied temperatures for all species under both the mono- and mixed cultures.

Varying patterns of growth, photosynthetic pigments and primary ROS were observed; H2O2 provided certain clues for the stress induced by inter-specific competition among the considered species in the mixed culture experimental setup at each temperature treatment. Figure 5 illustrates the variations in percentage increments or reductions in the growth rate, total chlorophyll and H2O2 concentration in the mixed cultures compared to the monocultures of the three plant species with applied temperature treatments.

According to Figure 5, there were reductions in the RGR and total chlorophyll in the mixed cultures compared

![Figure 2](image-url)  
**Figure 2.** Variations in the chl a, chl b and total carotenoids of E. nuttallii, P. crispus and V. asiatica with heat shock treatments. The results are shown separately for the mono- and mixed cultures.
Species Variable Source were increments of total chlorophyll (18–35%) for *P. crispus*, which significantly increased ($F = 24.137$, $p < .05$) with applied temperature.

A completely different trend was obtained for the $\text{H}_2\text{O}_2$ concentrations. There were increments of $\text{H}_2\text{O}_2$ in the mixed cultures relative to the monocultures of *E. nuttallii* and *V. asiatica*, demonstrating that oxidative stress was induced by inter-specific competition. However, the percentage increment of *E. nuttallii*, which ranged from 39% to 43%, increased but was not significantly different ($F = 0.135$, $p > .05$) with applied temperature. In the case of *V. asiatica*, the percentage increment (22–58%) significantly decreased ($F = 19.189$, $p < .05$) with applied temperature. In contrast, there was a reduction of $\text{H}_2\text{O}_2$ in *P. crispus* in the mixed culture with applied temperature, but these values were not statistically significant ($F = 7.763$, $p > .05$).

Variations in the activity of the primary antioxidant enzymes, CAT and APX, with applied temperature treatments are separately presented for the mono- and mixed cultures in Figure 6 for the three considered species.

According to Figure 6, the activities of CAT and APX increased with the applied temperature treatments, both in the monocultures and mixed cultures, for all considered species. The CAT and APX activities in the mixed cultures increased compared to the monocultures for *E. nuttallii* and *V. asiatica*. The mean CAT activity in the monoculture was significantly different ($F = 6.50$, $p < .05$) from that in the mixed culture, while the mean APX activity in the monoculture was not significantly different ($F = 1.269$, $p > .05$) from that in the mixed culture for *E. nuttallii*. In the case of *V. asiatica*, the mean values of both the CAT and APX activities in the monocultures were not significantly different from those in the mixed cultures ($F = 1.64$, $p > .05$ and $F = 1.24$, $p > .05$ for the CAT and APX activities, respectively). In contrast, for *P. crispus*, the CAT and APX activities decreased in mixed cultures compared to the monocultures, but the mean values were not significantly different ($F = 2.32$, $p > .05$ and $F = 1.27$, $p > .05$ for the CAT and APX activities, respectively). Additionally, significant positive correlations were obtained for the $\text{H}_2\text{O}_2$ concentrations and CAT or APX activities for all three species.
Discussion

In the present study, two laboratory experiments were designed to investigate the effects of elevated water temperature or heat stress on three plant species with three different morphologies by means of their individual effects and competitive ability. In this experiment, the accumulation of ROS was significantly higher in plants grown under elevated temperature conditions compared to the control conditions (room temperature, 25°C) in both the mono- and mixed culture experiments. Similarly, the activities of the antioxidant enzymes (CAT and APX) were also significantly higher in plants grown under high-temperature treatments. Further, the accumulation of ROS and the prescribed antioxidant activities were significantly higher in the mixed cultures in comparison to the monocultures of certain plant species. However, the applied temperature values and H$_2$O$_2$ (a strong ROS) had a positive relationship in each treatment for each species.

It was clear that heat stress can lead to overproduction of ROS, as supported by earlier findings by Wahid et al. (2007). The ROS are generated in various sub-cellular loci by the partial reduction of oxygen to radical and non-radical oxygen species. Cellular levels of ROS can determine the plant’s survival ability under unfavorable conditions, as high concentrations of ROS can damage important biomolecules (including proteins and nucleic acids). Meanwhile, low or moderate concentrations can serve as secondary messengers in intracellular signaling cascades and regulate various responses (Takeda et al. 1995). Because it is the only ROS that can diffuse through aquaporins in membranes and over longer distances within cells, H$_2$O$_2$ has received prime attention as a signal ROS molecule that can regulate biological processes under environmental deviations in plants (Bienert et al. 2007). Additionally, H$_2$O$_2$ is a reasonably stable ROS compared to other ROS.

Excess accumulation of H$_2$O$_2$ in plant cells is mediated by the activities of various types of enzymatic and non-enzymatic compounds. The two major antioxidant enzymes that can efficiently scavenge stress-induced H$_2$O$_2$ generated in plants are CAT and APX. CAT was the first characterized enzyme that can catalyze the dismutation of two molecules of H$_2$O$_2$ into water and oxygen. APX is considered the most efficient scavenger of H$_2$O$_2$ due to its higher affinity for H$_2$O$_2$ compared to CAT. Carotenoids, which are non-enzymatic lipophilic antioxidants, have the ability to detoxify H$_2$O$_2$. In addition to their direct deactivation of H$_2$O$_2$, carotenoids also quench triplet sensitizers and excited chlorophyll (Sharma et al. 2012). When plants are exposed to a continuous stress, the equilibrium between H$_2$O$_2$ production and the activity of antioxidant mechanisms becomes unbalanced, leading to excessive accumulation of H$_2$O$_2$ in the plant body. This excess accumulation of H$_2$O$_2$ further increases the levels of CAT, APX and carotenoids, as they are continuously involved in rebalancing the equilibrium. Therefore, the activity of CAT and APX and the concentration of carotenoids exhibited positive relationships with the H$_2$O$_2$ concentration in the present study. A positive relationship between H$_2$O$_2$ concentration and temperature was also observed, indicating higher stress in plants due to excess accumulation of H$_2$O$_2$.

In the present study, the H$_2$O$_2$ concentration gradually increased with increased temperature for all species in both the mono- and mixed cultures, suggesting that heat stress-induced oxidative damage was significant at 30°C and 35°C. Further, the H$_2$O$_2$ concentration of P. crispus decreased in the mixed culture in comparison to the monoculture, while it increased for E. nuttallii and V. asiatica in the present study. The increased H$_2$O$_2$ concentration in the mixed cultures compared to the monocultures of E. nuttallii and P. crispus indicated that growing under mixed culture conditions placed extra stress on the plants. The H$_2$O$_2$ formation data (Figure 3) showed that the H$_2$O$_2$ formed due to competition among species and increased with increased temperature for E. nuttallii, suggesting that warming further suppressed the competitive ability of the plant, which triggered higher oxidative damage. Conversely, in the case of V. asiatica, the portion of H$_2$O$_2$ produced by competition among species decreased with increased temperature. Thus, although temperature itself could damage V. asiatica under mixed culture conditions, ROS production was low at high temperatures. In contrast, the lower production of H$_2$O$_2$ in the mixed culture in comparison to the monoculture of P. crispus indicated that growing under mixed culture supported P. crispus in both the control and high-temperature conditions. This finding led to P. crispus’s status as a strong competitor over E. nuttallii and V. asiatica, thereby creating a type of allelopathic effect on them. The previous literature has reported that allelopathic stress can induce oxidative damage mediated by the overproduction of ROS (Ding et al. 2007). Therefore, the excess accumulation of H$_2$O$_2$ in the mixed cultures of E. nuttallii and V. asiatica can be considered an indicator of allelopathic stress. Further, the percentage reduction of H$_2$O$_2$ for P. crispus under the mixed culture was significantly reduced for the 30°C heat shock compared to the control condition, suggesting the improvement of the competitive capability of P. crispus through a reduced stress level under moderately high temperature.

Reductions in chl $a$ and chl $b$ were observed in P. crispus and V. asiatica exposed to moderate and high temperatures, while E. nuttallii exposed to moderate temperatures had higher concentrations of chl $a$ and chl $b$ compared to the control under both the mono- and mixed cultures. Reductions in

![Figure 4. Variations in H$_2$O$_2$ concentrations with applied temperature for each treatment for E. nuttallii, P. crispus and V. asiatica (n = 3).](Image)
chlorophyll pigments under heat stress were suggested to be associated with the production of ROS and thereby indirectly represent the stress level of the plants. Additionally, the activity of PSII, which is highly thermolabile, is significantly reduced under high temperatures (Camejo et al. 2005). The high concentrations of chl \(a\) chl \(b\) for \(E.\ nattallii\) exposed to moderate (30°C) temperature indicate an improvement in the chlorophyll pigments. Any alterations in plant photosynthetic attributes under heat stress can be considered reliable indicators of thermotolerance in plants (Wahid et al. 2007). Therefore, it can be suggested that \(E.\ nattallii\) showed thermotolerance under moderate temperature (30°C), while \(P.\ crispus\) and \(V.\ Asiatica\) became highly stressed under moderate and high temperatures.

Moreover, the total chlorophyll of \(E.\ nattallii\) and \(V.\ asiatica\) showed significant reductions in the mixed cultures in comparison to the mono-cultures.
comparison to the monocultures but increased in the mixed culture for *P. crispus*. The percentage increment of total chlorophyll in *P. crispus* significantly increased under the moderately higher temperature treatment (30°C). Therefore, the competitive ability of *P. crispus* increased in the warming condition, with *P. crispus* having an advantage over *E. nuttallii* and *V. asiatica* by being a strong competitor under moderate warming conditions. It was clear that both *E. nuttallii* and *V. asiatica* were suppressed by *P. crispus* under both the control and high-temperature conditions. The results showed that this suppression was further increased with increased temperature for *E. nuttallii* in the mixed culture because the reduction of total chlorophyll further increased with increasing applied temperature. However, in the case of *V. asiatica*, the reduction of total chlorophyll was lower than that for *E. nuttallii* with increased applied temperature, suggesting that warming confers certain advantages to overcome suppression by competition under moderate and high temperatures.

As they showed good correlation with plant growth, these adverse effects on photosynthetic attributes could limit plant growth at high temperatures. The shoot elongation of *P. crispus* and *V. asiatica* in both the mono- and mixed cultures decreased with temperature. The growth of *V. asiatica* was severely affected at 35°C, showing more than a 50% reduction in the growth rate compared to the control plants. In contrast, variations in the growth rate of *E. nuttallii* followed a similar trend as chlorophyll pigments, showing thermotolerance under moderately high temperatures. These findings were highly compatible with previous findings (JianMin et al. 2009) in which 25–30°C was considered the optimum temperature range for the growth of *E. nuttallii*. Further, it was found that when the water temperature was greater than 30°C, plant growth was inhibited.

**Conclusions**

In the present study, we observed that both *P. crispus* and *V. asiatica* exhibited reduced growth and photosynthetic pigment levels with increasing applied temperature due to increased physiological stress. High H$_2$O$_2$ production coupled with high antioxidant production was observed in plants exposed to high and moderate temperatures. *E. nuttallii* exhibited tolerance under moderately high temperatures and showed acquired thermotolerance, while it was adversely affected by the highest temperature treatment. Further, heat shock treatment-induced oxidative stress, which significantly impaired the plants’ growth and photosynthesis, was pronounced in all the species subjected to these treatments. The comparative results of the mono- and mixed cultures suggested that *P. crispus* was the strongest competitor in both the control and warmer conditions; its competitive capacity increased in moderately high-temperature treatments. Further, elevated levels of H$_2$O$_2$ can be seen as an indicator of allelopathic and temperature stress.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This research was financially supported by a Research Grant-in-Aid for the Scientific Research (B) 15H04045, for Japan Society for the promotion of Science, River Fund, for the River Foundation, Research Fund for River Ecology, for Ministry of Land, Infrastructure, Transport and Tourism in Japan.

**ORCID**

Hendadura Chandani Galanika De Silva [http://orcid.org/0000-0002-5897-0689](http://orcid.org/0000-0002-5897-0689)

**References**

Aebi H, editor. 1984. [13] Catalase in vitro. New York: Academic press. Anderson JA. 2002. Catalase activity, hydrogen peroxide content and thermodtolerance of pepper leaves. Sci Hort. 95:277–284.

Atapathi KSS, Asaeda T. 2015. Growth and stress responses of Nuttall’s waterweed Elodea nuttallii (Planch) St. John to water movement. Hydrobiologia. 747:217–233.

Bienert GP, Møller AL, Kristiansen KA, Schulz A, Møller IM, Bienert GP, Møller AL, Kristiansen KA, Schulz A, Møller IM, Bienert GP, Møller AL, Kristiansen KA, Schulz A, Møller IM, Bienert GP, Møller AL, Kristiansen KA, Schulz A, Møller IM. 2007. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem. 282:1183–1192.

Camejo D, Rodriguez P, Morales MA, Dell’Amico JM, Torrecillas A, Alarcón JI. 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J Plant Physiol. 162:281–289.

Chaitanya K, Sundar D, Masilamani S, Reddy AR. 2002. Variation in heat stress-induced antioxidant enzyme activities among three mulberry cultivars. Plant Growth Regul. 36:175–180.

Cruz de Carvalho MH. 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal Behav. 3:156–165.

Declerck S, Vandekerckhove J, Johansson L, Muylaert K, Conde-Porcuna J, Van Der Gucht K, Perez-Martinez C, Lauridsen T, Schwenk K, Zwart G, et al. 2005. Multi-group biodiversity in shallow lakes along gradients of phosphorus and water plant cover. Ecology. 86:1905–1915.

DeEll JR, Toivonen PM. 2003. In DeEll JR, Toivonen PM. Practical applications of chlorophyll fluorescence in plant biology. New York: Springer; p. 203–242.

Ding J, Sun Y, Xiao CL, Shi K, Zhou YH, Yu JQ. 2007. Physiological basis of different allelopathic reactions of cucumber and fiddle gourd plants to cinnamic acid. J Exp Bot. 58:3765–3773.

Doye R, Grodowitz M, Smart M, Owens C. 2007. Separate and interactive effects of competition and herbivory on the growth, expansion, and tuber formation of *Hydrilla verticillata*. Biol Control. 41:327–338.

Ellawala C, Asaeda T, Kawamura K. 2011a. Influence of flow turbulence on growth and indole acetic acid and H$_2$O$_2$ metabolism of three aquatic macrophyte species. Aquat Ecol. 45:417–426.

Ellawala KC, Asaeda T, Kawamura K. 2011b. The effect of flow turbulence on plant growth and several growth regulators in *Egeria densa* Planchon. Flora-Morphology, Distribution, Functional Ecology of Plants. 206:1085–1091.

Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 48:909–930.

Hallwell B, Gutteridge JM. 1985. Free radicals in biology and medicine. Oxford: Oxford University Press.

Herb WR, Stefan HG. 2006. Seasonal growth of submerged macrophytes in lakes: The effects of biomass density and light competition. Ecol Model. 193:560–574.

James WF, Barko JW, Eakin HL. 2004. Impacts of sediment dewatering and rehydration on sediment nitrogen concentration and macrophyte growth. Can J Fish Aquat Sci. 61:538–546.

Jana S, Choudhuri MA. 1982. Glycolate metabolism of three submersed aquatic angiosperms during ageing. Aquat Bot. 12:346–354.

JianMin M, TongXia J, Feng H, Juan W, ShuiPing C, ZhenBin W. 2009. Responses of *Elodea nuttalli* and *Ceratophyllum demersum* to high temperature. Fresen Environ Bull. 18:1588–1596.

Kipp E, Boyle M. 2013. The effects of heat stress on reactive oxygen species production and chlorophyll concentration in *Arabidopsis thaliana*. Res Plant Sci. 1:20–23.
MacAdam JW, Nelson CJ, Sharp RE. 1992. Peroxidase activity in the leaf elongation zone of tall fescue I. Spatial distribution of ionically bound peroxidase activity in genotypes differing in length of the elongation zone. Plant Physiol. 99:872–878.

Mazorra LM, Nunez M, Hechavarria M, Coll F, Sánchez-Blanco MJ. 2002. Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures. Biol Plantarum. 45:593–596.

Mei Y, Song S. 2010. Response to temperature stress of reactive oxygen species scavenging enzymes in the cross-tolerance of barley seed germination. J Zhejiang Univ Sci B. 11:965–972.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405–410.

Moss B, McKee D, Atkinson D, Collings S, Eaton J, Gill A, Harvey I, Hatton K, Heyes T, Wilson D. 2003. How important is climate? Effects of warming, nutrient addition and fish on phytoplankton in shallow lake microcosms. J Appl Ecol. 40:782–792.

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22:867–880.

Nawkar GM, Maibam P, Park JH, Sahi VP, Lee SY, Kang CH. 2013. UV-induced cell death in plants. Int J Mol Sci. 14:1608–1628.

Pilon J, Santamaría L. 2002. Clonal variation in the thermal response of the submerged aquatic macrophyte Potamogeton pectinatus. J Ecol. 90:141–152.

Riis T, Olesen B, Clayton JS, Lamberti C, Brix H, Sorrell BK. 2012. Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. Aquat Bot. 102:56–64.

Rucinska-Sobkowiak R. 2008. Oxidative stress in plants exposed to heavy metals. Postepy Biochemii. 52:191–200.

Sairam R, Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci-Bangalore. 86:407–421.

Satnamaría L, van Vierssen W. 1997. Photosynthetic temperature responses of fresh-and brackish-water macrophytes: a review. Aquat Bot. 58:135–150.

Shah K, Kumar RG, Verma S, Dubey R. 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Sci. 161:1135–1144.

Sharma P, Jha AB, Dubey RS, Pessarakli M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot. 1–26.

Shin R, Berg RH, Schachtman DP. 2005. Reactive oxygen species and root hairs in Arabidopsis root response to nitrogen, phosphorus and potassium deficiency. Plant Cell Physiol. 46:1350–1357.

Smolders A, Lamers L, Lucassen E, Van der Velde G, Roelofs J. 2006. Internal eutrophication: how it works and what to do about it – a review. Chem Ecol. 22:93–111.

Spencer DF, Rejmánek M. 2010. Competition between two submersed aquatic macrophytes, Potamogeton pectinatus and Potamogeton gramineus, across a light gradient. Aquat Bot. 92:239–244.

Steffens B, Steffen-Heins A, Sauter M. 2013. Reactive oxygen species mediate growth and death in submerged plants. Front Plant Sci. 4:1–7.

Takeda T, Yokota A, Shigeoka S. 1995. Resistance of photosynthesis to hydrogen peroxide in algae. Plant Cell Physiol. 36:1089–1095.

Wahid A, Gelani S, Ashraf M, Foolad MR. 2007. Heat tolerance in plants: an overview. Environ Exp Bot. 61:199–223.

Wellburn AR. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol. 144:307–313.

Zaman T, Asaeda T. 2013. Effects of NH4-N concentrations and gradient redox level on growth and allied biochemical parameters of Elodea nuttallii (Planch.). Flora-Morphology, Distribution, Functional Ecology of Plants. 208:211–219.