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Application of Hydrogen Peroxide as an Environmental Stress Indicator for Vegetation Management

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ABSTRACT

Adaptive vegetation management is time-consuming and requires long-term colony monitoring to obtain reliable results. Although vegetation management has been widely adopted, the only method existing at present for evaluating the habitat conditions under management involves observations over a long period of time. The presence of reactive oxygen species (ROS) has long been used as an indicator of environmental stress in plants, and has recently been intensely studied. Among such ROS, hydrogen peroxide (H_2O_2) is relatively stable, and can be conveniently and accurately quantified. Thus, the quantification of plant H_2O_2 could be applied as a stress indicator for riparian and aquatic vegetation management approaches while evaluating the conditions of a plant species within a habitat. This study presents an approach for elucidating the applicability of H_2O_2 as a quantitative indicator of environmental stresses on plants, particularly for vegetation management. Submerged macrophytes and riparian species were studied under laboratory and field conditions (Lake Shinji, Saba River, Eno River, and Hii River in Japan) for H_2O_2 formation under various stress conditions. The results suggest that H_2O_2 can be conveniently applied as a stress indicator in environmental management.

1. Introduction

Vegetation management is an important practice around the world for the restoration of vegetation, the protection of endangered species, and weed control. Except for instances of mechanical weed control, adaptive management-based methods are used; after treatments are iteratively implemented, such methods require long-term monitoring of the conditions of a colony. Although this is the most fundamental method of identifying plant conditions, several years and large budgets are required to obtain reliable results. The prosperity or shrinkage of a plant community depends on the relationship between its environmental stresses and its tolerance. If the relative intensity of the overall environment stress causes the tolerance of a plant community to change, this indicates the preferred habitat condition level. Thus, evaluating the environmental stresses imposed on a plant community is essential in order to understand the current condition of the plants and predict their future prosperity.

The effect of environmental stress on plants has recently been intensely studied, resulting in reports of several plant body features being subjected to stresses [1,2]. The accumulation of reactive oxygen species (ROS) provides clear evidence for the identification of environmental stresses on plants, although it is rarely elucidated in a realistic environment [3–5]. The ROS hydrogen peroxide (H_2O_2) is generated at various sites within the plant cells, such as chloroplasts, mitochondria, peroxisomes, cell membranes, and so forth. Moreover, it is relatively stable and can be transported through biological membranes [2]. Compared with other ROS, such as the superoxide radical ($O_2^{\cdot-}$) and the hydroxyl radical ($^{\cdot}OH$), H_2O_2 can be conveniently quantified with minimum losses; therefore, it has been widely used to quantify levels of ROS damage or stress in many plant studies.

The objective of this study was to elucidate the applicability of H_2O_2 as a quantitative indicator of environmental stresses in plants, and to directly apply it to understanding the level of environmental stress. Therefore, the generation of H_2O_2 in submerged macrophytes and riparian vegetation under laboratory and natural conditions was studied. The submerged macrophytes *Egeria densa*, *Vallisneria asiatica* (*V. asiatica*), *Potamogeton crispus* (*P. crispus*), and *Elodea nuttallii* (*E. nuttallii*) were studied for turbulence responses,

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and *Myriophyllum spicatum* (*M. spicatum*) was subjected to hydrogen sulfide (H_2S) stress under laboratory conditions. *P. anguillanus* and *Egeria densa* were subjected to light-response testing in natural conditions at Lake Shinji, Saba River, and Eno River in Japan. The riparian vegetative conditions of *Phragmites australis*, *Phragmites karka*, *Miscanthus sacchariflorus*, *Salix pierotii*, and *Juglans mandshurica* var. *sieboldiana* were observed along the Hii River in Japan.

2. Materials and methods

2.1. Plant stock culture

Egeria densa, *V. asiatica*, *P. crispus*, *E. nuttallii* and *M. spicatum* were collected from nearby rivers and washed with dechlorinated tap water to remove attached debris. When algae were attached, the plants were carefully cleaned with the aid of forceps. The plants were then planted and cultured in glass tanks for several months under laboratory conditions ((25 ± 2) °C and 12/12 light duration with a photosynthetic photon flux density (PPFD) of 100–120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using fluorescent lamps). The glass tanks were thoroughly washed, layered with commercial sand (90% particles lower than 1 mm diameter) as a substrate, and provided with 5% Hoagland solution as the nutrient source. Prior to transplantation, the plants were observed for the presence of algae, and algae-free cultures were selected for experiments.

2.2. Turbulence stress

V. asiatica, *P. crispus*, and *E. nuttallii* were used in experiments. The experiments were conducted in glass containers (5.7 cm × 15.7 cm, 24.5 cm height), with a 4 cm layer of thoroughly washed commercial sand. Similarly sized apical tips (3–5 cm long) were cut from the culture tanks and planted six per experimental tank. The water level (nutrient medium: 5% Hoagland solution) of each tank was maintained at 17 cm above the substrate.

Turbulence conditions were generated using a vertically oscillating horizontal grid with an oscillating frequency of 2 Hz and an amplitude of 3 cm, according to published literature [6,7]. The microcosm employed for generating turbulence was relatively small; however, work at this scale was necessary in order to operate the DC motors under specifications. The horizontal velocity profile of the microcosm was measured using a two-dimensional electromagnetic current meter (SF-5712, Tokyo Keisoku Co., Ltd., Japan) from six different points symmetrically distributed over the area. At each point, velocity fluctuations were monitored at three depths (5, 10, and 15 cm from the water surface), and the turbulence velocity was calculated as described previously for each depth by averaging all six measurements.

The plants were acclimatized to the experimental conditions ((25 ± 2) °C, 12/12 light duration with a PPFD of 100–120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using fluorescent lamps) for 1 week before turbulence conditions were implemented. After acclimatization, the plants were continuously exposed to turbulence treatments for 21 d. Upon treatment completion, both control and treatment samples were immediately subjected to chemical analyses.

2.3. Hydrogen sulfide stress

Two *M. spicatum* apical tips (~6 cm long) were clipped, plugged into silicone sponge clumps, and placed in 500 mL glass beakers. The culture medium was 5% Hoagland solution without additional substrate. The plants growing in beakers were subjected to 0 (control), 0.1, 0.2, 0.5, and 1.0 $mmol \cdot L^{-1}$ H_2S exposure in triplicates. For the H_2S treatment, sodium hydrogen sulfide (NaHS) was applied as a H_2S donor.

Dissolved H_2S levels were colorimetrically determined by the methylene blue method described in Ref. [8] using a diamine reagent: 4 mL of mixed diamine reagent were reacted with 50 mL water samples, and the amount of absorbance was measured spectrophotometrically at 670 nm after 20 min. The NaHS calibration standard was used to obtain H_2S concentrations, and the results were expressed in $mmol \cdot L^{-1}$. The culture medium for each treatment was renewed at 24 h intervals, accounting for the short half-life of H_2S [9]. The pH of the solution was maintained in a 5.0–5.5 range by adding 1 $mol \cdot L^{-1}$ NaOH or HCl as required [10,11]. The initial and final shoot lengths were measured and the shoot growth rate (SGR) was calculated by dividing the difference between the final shoot length and the initial shoot length by the duration of the experiment.

2.4. Field observations

2.4.1. Sampling sites

Lake Shinji is a brackish water lake in western Japan that is famous for its high yield of *Corbicula* bivalves. Recently, *P. anguillanus* macrophytes have grown thickly across the lake; this disturbs the *Corbicula* harvests, which are taken by scratching the bottom sediment [12]. Thus, the cause of this intensive macrophyte growth and distribution must be determined for vegetative management. Observations were conducted on 22 August 2017, and macrophytes were harvested along two transects from the shoreline to a depth of 2.5 m.

Recently, thick stands of an invasive macrophyte, *Egeria densa*, have developed yearly in several rivers in western Japan where no macrophytes grew previously [13]. This has hindered the spawning of ayu fish, one of the most favored freshwater fish in Japan, and has significantly decreased catches. Field observations were conducted at several sections of the Saba and Eno Rivers. Sampling and observations were conducted at these locations on 11–15 June and 16–17 September 2016. Hii River samplings were taken on 11–13 October 2016. Sampling sections were selected in each river where either *Egeria densa* created a thick and homogeneous stand, or the velocity was relatively high and the amount of biomass was small. At each of the sampling sections, sampling and measurements were conducted every 2–5 m. Stream velocities were measured and recorded using an ultrasonic current meter at 20% (above the colony) and 80% (inside the colony) of the total water depth.

2.4.2. Sampling procedures for submerged macrophytes

In the field, *P. anguillanus* plant samples were collected from two transects (Tr1 and Tr2) at different depths and stored in a cool box containing dry ice. Collected samples were transported to the laboratory in the shortest possible time for chemical analyses. In addition, biomass was collected in triplicate for each quadrat (0.5 m × 0.5 m). The light intensity or PPFD in the water was measured using a portable quantum flux meter (MQ-200, Apogee Instruments, Inc., Logan, USA) at 10 cm depth increments from the bottom to the top of the water column. Furthermore, the pH, turbidity, temperature, and dissolved oxygen (DO) were measured using a portable water quality meter (U-53, Horiba, Ltd., Japan).

A substantial amount of ROS is generated in the photosynthesis process. To remove the fraction of H_2O_2 generated by photosynthesis, darkness-treated samples were collected. In the darkness treatment, a black plastic sheet (2 m × 2 m) was placed over the submerged plant colonies for 30 min. In flowing water, the black plastic sheet was fixed using iron poles to float it at the water surface without disturbing the macrophyte stand or the water velocity field around the colony. Plant samples were collected at 80% of the

water depth without exposure to light. Similarly, light-exposed samples were collected from uncovered areas of the same macrophyte stand.

2.4.3. Procedures for the riparian zone sampling

Hii River samplings were performed in selected areas where *Phragmites australis*, *Phragmites karka*, and *Miscanthus sacchariflorus* stands were clearly separated. Herbaceous plant samples were collected at different elevations (up to 7 m) from the usual water level along a perpendicular line to the river. The riparian zone of the river is rich in *Salix pierotii* and *Juglans mandshurica*, which are common in Japanese rivers. Riparian tree species have individual preferred elevations for colonization. The numbers of *Salix pierotii* and *Juglans mandshurica* individuals were therefore counted along a 2 km distance of riparian area at 14 km downstream from Lake Shinji, with respect to their elevation from the typical water level. Solar-radiation-exposed leaves and darkness-treated leaves from these plants were collected for chemical analysis. All plant samples were immediately placed in a cool box containing dry ice and transported to the laboratory for chemical analyses.

2.5. Chemical analyses

The plant tissue samples described in Sections 2.2–2.4 were analyzed for biochemical stress indicators. Chlorophyll-a (Chl-a) and Chlorophyll-b (Chl-b) and carotenoid contents were determined spectrophotometrically by extracting the pigments of fresh shoots into 5 mL of *N*,*N*-dimethylformamide; the pigment content was calculated using the methods and equations described in Ref. [14]. Chl-a and Chl-b and carotenoid contents were expressed in $\mu g \cdot g^{-1}$ fresh weight (FW).

H_2O_2 assays were performed by extracting enzymes from ~100 mg of fresh plant shoots into ice-cold phosphate buffers (50 $mmol \cdot L^{-1}$, pH 6.0) containing polyvinylpyrrolidone (PVP). These extractions were centrifuged at 5000*g* ($g = 9.8\ m \cdot s^{-2}$) for 15 min at 4 °C. The supernatant was collected and immediately stored at −80 °C until analysis. The endogenous H_2O_2 concentration was assayed spectrophotometrically using the method modifications described in Ref. [15]. An aliquot of 750 μL was mixed with 2.5 mL of 0.1% titanium sulfate in 20% (v/v) H_2SO_4, and the mixture was centrifuged at 5000*g* for 15 min at 20 °C. The intensity of the resulting yellow color was measured spectrophotometrically at a wavelength of 410 nm. H_2O_2 concentrations were estimated using a standard curve for H_2O_2, and the results were presented in $\mu mol \cdot g^{-1}$ FW.

The indole acetic acid (IAA) concentration was also measured spectrophotometrically using the method described in Ref. [16] with modifications. Fresh plant tissue (~100 mg) from the apical tip was ground in 2.5 mL of distilled water and centrifuged at 5000*g* at 20 °C for 15 min, and the supernatant was collected. Then 1.0 mL of enzyme extract was mixed with 2.0 mL of modified Salkowski reagent; after an hour, the formation of pink color was measured at a wavelength of 530 nm. The IAA concentration was obtained from a standard curve and presented as $\mu g \cdot g^{-1}$ FW.

3. Results

3.1. Laboratory experiments

Fig. 1 presents the relationship between the turbulence intensity and the concentration of H_2O_2 in plant leaves and stems. Extremely high species-specific positive correlations were observed between turbulence intensity and H_2O_2 concentration. The *V. asiatica* has a leafy form, thus differing from *P. crispus* and *E. nuttallii*, and displays slightly different trends with higher H_2O_2 concentration than the others. More similar trends were observed in *P. crispus* and *E. nuttallii*. The associated relationships demonstrated more fluctuation with leaves than stems, as the H_2O_2 concentration was higher in the leaves. The relationships of the Chl-a content, IAA content, and elongation rate with the H_2O_2 concentration are shown in Figs. 2(a), 2(b), and 2(c), respectively. These relationships always registered as negative and were extremely depressed by H_2O_2 with regard to the elongation rate. This depression in elongation was evident when even a small amount of H_2O_2 was present in the tissues.

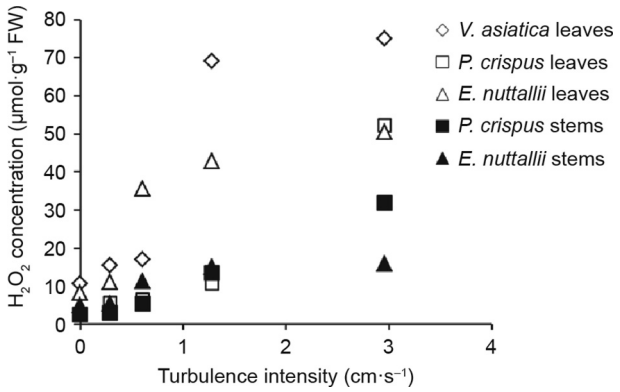

Fig. 1. The change in H_2O_2 concentration with the turbulence intensity of *V. asiatica*, *P. crispus*, and *E. nuttallii*. *P. crispus* and *E. nuttallii* data are presented for both leaves and stems, while *V. asiatica* data are presented for leaves.

The response of *M. spicatum* to increasing H_2S concentrations in the growth medium is illustrated in Fig. 3. The foliar H_2O_2 concentration decreased slightly by as much as 0.2 $mmol \cdot L^{-1}$ H_2S, and then increased with higher H_2S concentrations. The Chl-a and carotenoid concentrations of *M. spicatum* are shown in Fig. 4(a), which demonstrates the negative correlation between the Chl-a content and the foliar concentration of H_2O_2. The carotenoid content exhibited a relatively scattered response to increasing H_2O_2 in tissues. The specific growth rate of *M. spicatum* was negatively affected by tissue H_2O_2 content, and particularly by content greater than 8.0 $\mu g \cdot g^{-1}$ FW (Fig. 4(b)).

3.2. Lake Shinji observation

Fig. 5 presents the foliar H_2O_2 concentration with respect to the PPFD of the *P. anguillanus* top canopy, which was measured at a depth of approximately 20 cm deep. The H_2O_2 concentration was low until 200–300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD, after which it increased as the PPFD increased. Fig. 6 gives the biomass distribution of *P. anguillanus* with respect to water depth. Its biomass was greatest at a depth of 1.2 m and decreased at both shallower and deeper sites; it was particularly noted that there was no biomass at depths of less than 0.3 m.

The figure includes the midday PPFD at different depths and at 50 cm above the bottom of the lake. Light intensity was estimated using a light extinction coefficient of 0.025 per centimeter, which was obtained in the lake. The PPFD just below the water surface was assumed to be 1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which is approximately the maximum value during the growth season.

3.3. River observations

In the Eno and Saba Rivers, the water temperatures were recorded as (19 ± 1) °C in June 2016 and as (24 ± 0.5) °C in September 2016. The total nitrogen (TN) and total phosphorus (TP) content were approximately 0.3–0.8 $mg \cdot L^{-1}$ and 0.01–0.10 $mg \cdot L^{-1}$, respectively; the turbidity and pH of the water were respectively less

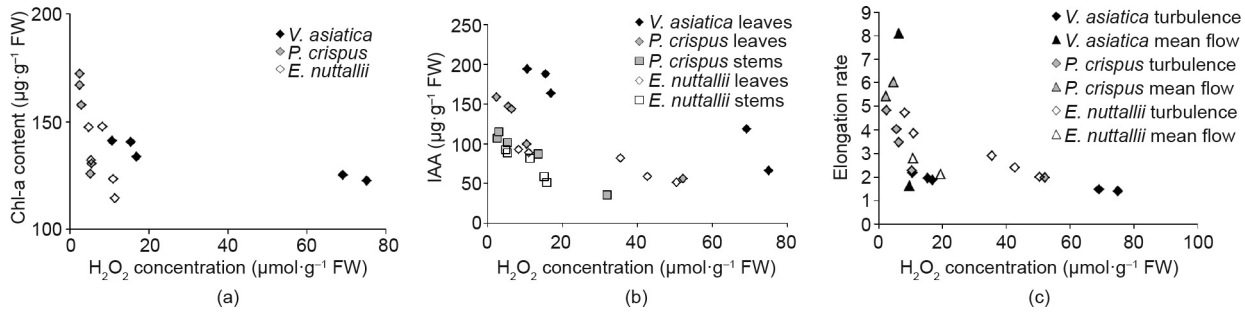

Fig. 2. (a) Chl-a content versus plant H_2O_2 concentration of *V. asiatica, P. crispus*, and *E. nuttallii*; (b) IAA content versus H_2O_2 concentration of leaves and stems of *V. asiatica, P. crispus*, and *E. nuttallii* (for the *V. asiatica*, only the data for leaves are presented); (c) plant elongation rate (final plant length/initial plant length) versus H_2O_2 concentration of *V. asiatica*, *P. crispus*, and *E. nuttallii* under turbulence and mean flow conditions.

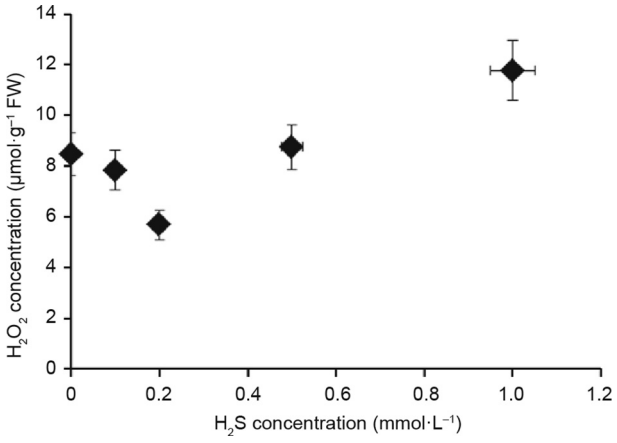

Fig. 3. Foliar H_2O_2 concentration of *M. spicatum* under the presence of different H_2S concentrations in the growth medium. Error bars represent standard deviation.

than 100 nephelometric turbidity unit (NTU) and 6.5–7.5, except during floods; and the DO was nearly saturated. These parameters indicate a suitable condition for *Egeria densa* growth [17]. Typical views of these rivers are shown in Fig. 7. Macrophyte stands were not formed in shallow sites less than 40 cm deep; the biomass became rich at approximately 1 m deep, and then decreased again at sites deeper than 1.5 m.

Fig. 8 shows the relationship between the turbulence intensity (root mean square velocity) at 80% depth and the H_2O_2 concentration of macrophytes separately for the two observed conditions: light exposure and 30 min of imposed darkness. The H_2O_2 concentrations in darkness-treated plants gradually increased at nearly the same rate as the increasing turbulence intensity. Compared with the darkness-treated samples, the macrophytes in light-exposed conditions had a nearly constantly higher concentration of H_2O_2 at equal turbulence levels. Although the deviation increased with increasing turbulence, the increasing rate of H_2O_2 content with respect to turbulence intensity was nearly equivalent between the darkness-treated and light-exposed samples.

The distributions of three major herbaceous species, *Phragmites australis*, *Miscanthus sacchariflorus*, and *Phragmites karka*, are depicted in Fig. 9. *Phragmites australis* was densely present in areas that were close to water at elevations below 1 m, whereas a gradual shift to *Miscanthus sacchariflorus* occurred at higher elevations. The distribution of *Phragmites karka* ranged from close to the water to high elevations up to 4 m. The H_2O_2 concentration of *Phragmites australis* was low at lower elevations under relatively high moisture conditions and rapidly increased with increasing elevation, which ranged from 0 to 1 m. Conversely, the H_2O_2 concentrations of *Miscanthus sacchariflorus* remained high until an elevation of 2 m, and then decreased at greater elevations. The H_2O_2 concentrations of *Phragmites karka* remained nearly constant regardless of elevation (Fig. 10). There was no difference in the H_2O_2 concentration of tissues between light and dark samples.

The riparian zone of the Hii River is primarily occupied with herbaceous species. Recently, however, tree species—particularly *Salix pierotii* and *Juglans mandshurica*—are increasing. *Salix pierotii* usually prefers to grow close to water while *Juglans mandshurica* grows in slightly elevated sites, approximately 2 m above the normal water level. The number of individuals and the foliar H_2O_2 concentrations of two species from the observed area are shown in Fig. 11(a) and (b), respectively. *Salix pierotii* exhibited a decreasing trend in the number of individuals as the elevation increased and appeared at almost zero recorded elevations higher than 3 m; however, its H_2O_2 concentration exhibited the opposite trend. *Juglans mandshurica* was not found until an elevation of 1 m from the water level; after 1 m elevation, the number of plants increased

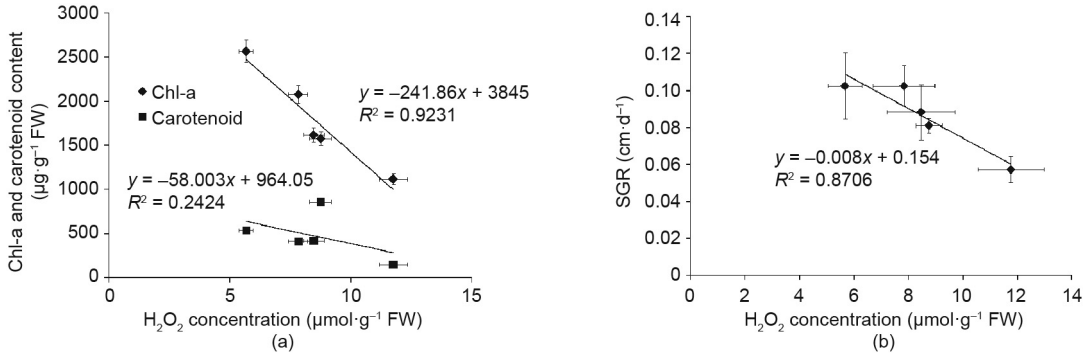

Fig. 4. (a) Chl-a content and carotenoid content of *M. spicatum* versus H_2O_2 concentration; (b) specific growth rate (SGR) versus H_2O_2 concentration of *M. spicatum*. Error bars represent standard deviation.

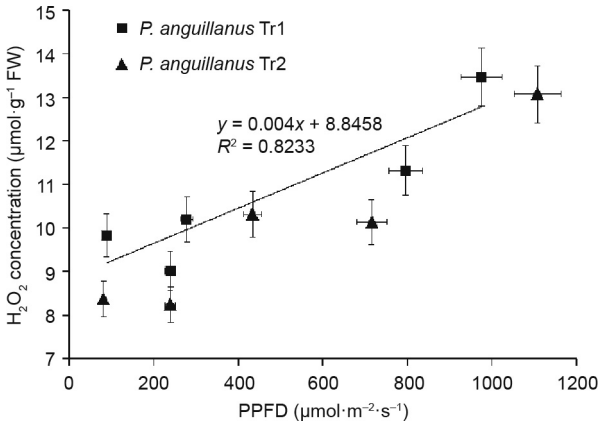

Fig. 5. H_2O_2 concentration of *P. anguillanus* top canopy versus PPFD.

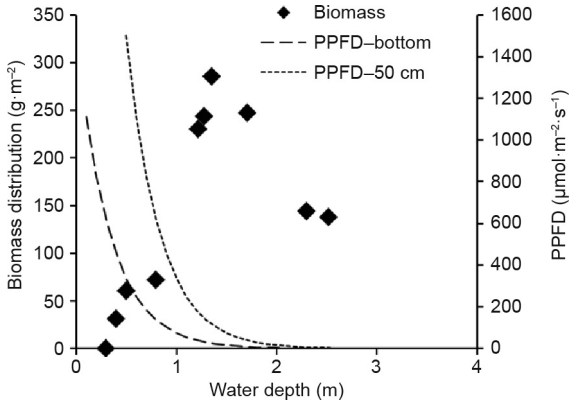

Fig. 6. Biomass (dry weight) of *P. anguillanus* and vertical profile of the PPFD distribution in the plant biomass of Lake Shinji. In the legend for biomass, PPFD–bottom and PPFD–50 cm represent the estimated PPFD in *P. anguillanus* biomass at the bottom of the lake and 50 cm above the lake bottom, respectively.

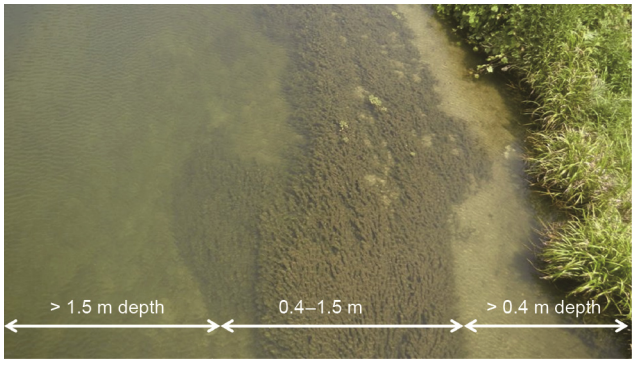

Fig. 7. Biomass distribution inside the rivers. Macrophytes are present at depths between 0.4–1.5 m, whereas shallower or deeper areas are free of macrophytes.

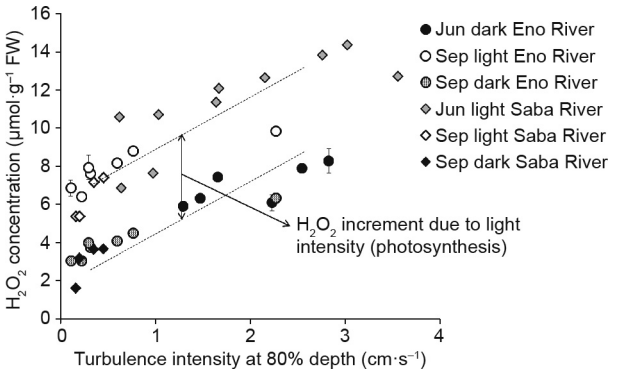

Fig. 8. H_2O_2 responses of submerged macrophytes to different turbulence intensities at 80% depth in the Eno and Saba Rivers during the months of June (Jun) and September (Sep). The terms "light" and "dark" stand for light-exposed samples and 30 min darkness-treated samples, respectively. The dash line triangle refers to the H_2O_2 increment associated with turbulence.

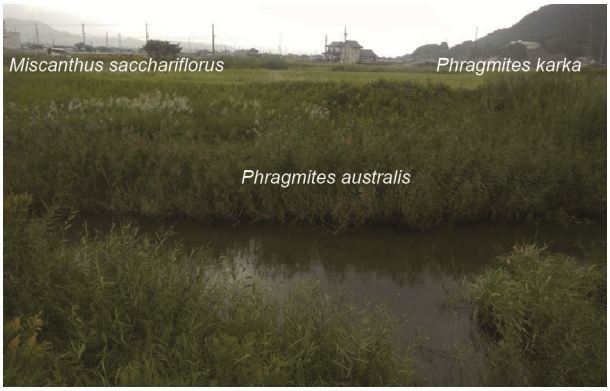

Fig. 9. Distribution of three herbaceous species found in Hii River, Japan.

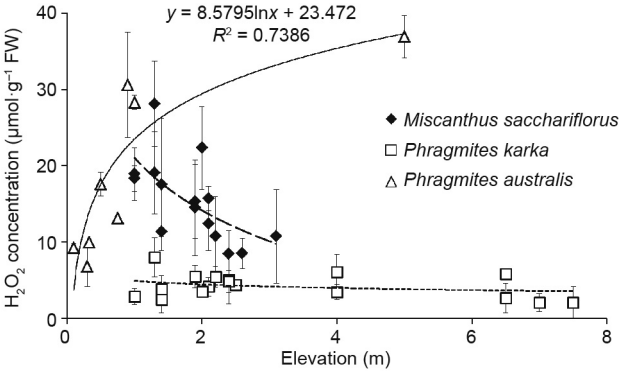

Fig. 10. H_2O_2 responses of three herbaceous species to different elevations from the normal water level of Hii River. The continuous, dashed, and dotted trend lines represent *Phragmites australis*, *Miscanthus sacchariflorus*, and *Phragmites karka*, respectively. Error bars represent standard deviation.

to a maximum at around 2 m elevation. The H_2O_2 concentration of *Juglans mandshurica* decreased with elevation, and thus exhibited an opposite trend to *Salix pierotii*.

4. Discussion

4.1. Hydrogen peroxide as an indicator of environmental stresses

Water movement, and specifically the mean flow and turbulence, is a ubiquitous factor affecting aquatic plants. Mean flow the former being relatively steady and unidirectional, whereas turbulence is due to deviations and fluctuations in the magnitude and direction of the instantaneous water velocity over time. The result of the mechanical stress experiment showed that at higher turbulence, H_2O_2 concentrations and antioxidant enzyme activities were significantly increased, compared with those in plants that were subjected to mean flow or that were in stagnant water. When macrophytes were exposed to oscillating motion, morphological changes occurred in the cells and cell walls [18]. The plants also

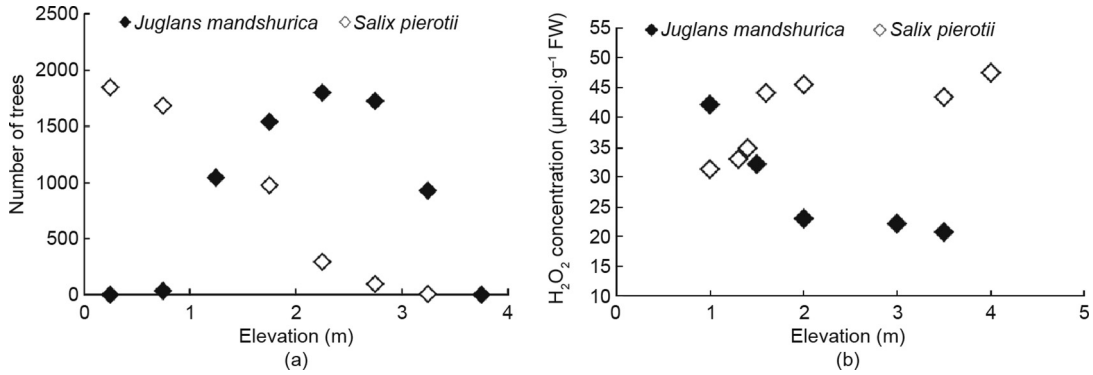

Fig. 11. (a) Distribution (number of trees) of two woody species with elevation from the normal water level of Hii River; (b) distribution of H_2O_2 content of two woody species with elevation from the normal water level of Hii River.

showed a reduction in growth and in cell plasmolysis, and an accumulation of starch granules, wherein the size of the granules increased with turbulence [18]. In addition, the mechanical stress triggered by turbulence led to the accumulation of ascorbate, γ-aminobutyric acid (GABA), and asparagine (Asn), thus altering several metabolic pathways in *E. nuttallii*—in particular, the isocitrate-oxalate, glutamic acid-histidine (Glu-His), Glu-GABA, ascorbate-oxalate and aspartic acid-asparagine (Asp-Asn) pathways. Turbulence is always associated with a mean flow greater than the critical flow velocity given by the Reynolds number, which makes it difficult to determine which creates stress: mean flow velocity or turbulence. However, the result of the microcosm setup clearly indicated that turbulence intensity is the dominant stressor for macrophytes, rather than mean flow.

Another environmental driver affecting aquatic organisms is H_2S. At its optimum level, H_2S plays an important role in the biological, physical, and chemical processes in an aquatic ecosystem. It has been reported that a low concentration of H_2S can promote plant growth [19]. However, biochemical processes such as microbial organic matter degradation and dissimilatory sulfate reduction waterlogged and hypoxic soil condition could generate an excessive amount of H_2S [20,21]. Higher levels of H_2S are toxic to many aquatic organisms including the macrophytes, affecting their physiology and consequently induced stress [20–23]. The experimental results on the effects of H_2S clearly demonstrated that higher H_2S treatment induced a higher order magnitude of oxidative stress to macrophytes compared with lower H_2S treatment, as reflected in the high H_2O_2 concentrations. High concentrations of H_2S imposed high stress on the submerged macrophytes, resulting in oxidative stress and subsequently reducing plant development [10]. In addition, a high concentration of H_2S in an aquatic environment is known to deplete the DO in water by increasing the oxygen demand rate; thus, it creates an anoxic condition. The anoxic condition consequently interferes with nutrient uptake and with the processes of photosynthesis and metabolism [24,25].

Increasing H_2O_2 concentrations in tissues lead to cellular damage, thus altering plant development and physiology [2,26]. Although the plant activates its ROS scavenging systems as a natural defense mechanism [5], the continuous production and accumulation of H_2O_2 results in eventual damage to the organelles. As a result, H_2O_2 accumulation decreases the growth rate of plant species and weakens their ability to compete with other species in order to expand their territory [27].

Various environmental drivers such as turbulence, low oxygen, and H_2S are known physical and biochemical processes that adversely affect the development and distribution of aquatic plants. Inevitably, these factors elicit biochemical stress responses. The use of biochemical evidence in the form of ROS has been widely employed as an indicator to evaluate plant stress responses to different abiotic and biotic sources of stress. Among ROS, H_2O_2 is the most stable and is relatively easy to measure.

4.2. Effect of solar radiation

Field observations showed that the light-exposed samples had higher H_2O_2 concentrations than the darkness-treated samples. This is because a substantial amount of ROS is generated during photosynthesis at photosynthetic complexes. The accumulation of ROS creates high PPFD-induced damage to organelles in the system and reduces photosynthesis and growth rate. Unlike terrestrial plants, which evolved under strong solar radiation, submerged macrophytes have extremely low tolerance for strong solar radiation.

During the high season of macrophyte growth, a shortage of solar radiation is considered to be a restricting factor for macrophyte colonization in lakes and rivers [28]. However, the present results indicated that strong light is also an inhibiting factor for macrophyte growth in sufficiently transparent waters. In shallow waters, the high light intensity at the bottom appears to hamper macrophyte colonization. Deep sites (i.e., water over 1.5 m deep) with clear water, where the light intensity at the bottom is not too high, provide more suitable conditions for macrophyte growth. Furthermore, a light intensity of 200–300 μmol·m^{-2}·s^{-1} PPFD measured at the middle of the water column appears to strongly promote the increase of macrophyte biomass. In addition, the greater growth of macrophytes at medium depths (i.e., 1 m depths) in rivers rather than at shallow depths is explained by solar radiation, which imposes stresses on macrophytes that are comparable or even higher than flow velocity.

4.3. Effect on competitive species

Phragmites australis and *Miscanthus sacchariflorus* are two major species in Japanese river riparian zones. Although both grow widely in riparian sites, the sequence of comparisons performed in this study indicated that *Phragmites australis* occupies areas closer to the water whereas *Miscanthus sacchariflorus* stands develop in higher zones [29–31]. The H_2O_2 concentrations of the leaves obtained in the present study were exactly consistent with this trend: They were low close to the water and high at elevated sites for *Phragmites australis*, but high at low sites and low at high zones for *Miscanthus sacchariflorus*. Furthermore, *Phragmites karka* typically grows independently in habitats, given their large morphology, and the unclear relationship between H_2O_2 concentrations and elevation is consistent with their wide distribution along riversides. Conversely, both *Salix pierotii* and *Juglans mandshurica* are common species in the Hii River area [30]. However, their habitats are segregated, with *Salix pierotii* growing

near the water and *Juglans mandshurica* occupying elevated sites. The trend was clearly reflected by their H_2O_2 distributions.

These results consistently support the applicability of H_2O_2 concentration as an indicator of environmental stress gradient in identifying dominant colonization sites for competing plant species.

4.4. Application to vegetative management

Common approaches to vegetation management are based on adaptive methods. Several types of treatments are employed iteratively. During each treatment, the growth and prosperity or shrinkage of managed stands are monitored over a long period before a suitable treatment is fixed and implemented. These approaches require long-term commitments and large costs. During endangered species restoration, these approaches often take too long to successfully restore the target species.

Harvesting is the primary method of weed management, although it is extremely laborious. If conditions that are unfavorable to the target species are known, it is possible to employ non-laborious methods of weed removal. The present study proposes a quick monitoring system for plant conditions that utilizes relatively easy methods, including sampling and some chemical analyses, to evaluate the oxidative stress introduced by environmental stressors. Furthermore, this method quantifies the effect of the total environmental stress on the plants. This approach could also be applied to identify the dominant stressor among a practical number of candidates.

5. Conclusion

The results of the laboratory experiment and field data showed the existence of a relatively unique trend and relationship between H_2O_2 concentration and environmental stress gradients (particularly turbulence and H_2S). Based on the relationship between H_2O_2 and the stress gradient, the effects of different sources of stresses could be separated with higher accuracy. Thus, the use of biochemical stress responses holds potential as a means of providing evidence of environmental stressors and as an indicator for identifying the sources of plant stress. Future experiments performed under different laboratory conditions will provide useful data for adopting the proposed H_2O_2 monitoring method as a promising technique for understanding plant conditions in various environments.

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Compliance with ethics guidelines

Takashi Asaeda, Senavirathna Mudalige Don Hiranya Jayasanka, Li-Ping Xia, and Abner Barnuevo declare that they have no conflict of interest or financial conflicts to disclose.

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