3rd International Conference - Water resources and wetlands. 8-10 September, 2014 Tulcea (Romania);
Available online at http://www.limnology.ro/wrw2016/proceedings.html
Editors: Petre Gâştescu, Petre Bretcan; ISSN: 2285-7923; Pages: 54-64; Open access under CC BY-NC-ND license

THE EFFECT OF MEAN FLOW AND TURBULENCE STRESSES ON THE HABITAT SELECTION OF SUBMERGED PLANTS WITH DIFFERENT MORPHOLOGIES

Kelum Sanjaya[1], Takashi Asaeda[1,2*], Y. Kaneko[3]
[1] Department of Environmental Science, Saitama University, 255 Shimo-okubo, Sakura, Saitama 338-8570 Japan
[2]Research Institute of Chuo University, Kasuga, Bunkyo, Tokyo 112-8551, Japan
[3] Biology Section in the Faculty of Education; Graduate School of Science and Engineering, Saitama University, 255 Shimo-okubo, Sakura, Saitama 338-8570 Japan
hlksanjaya@yahoo.com, asaeda@mail.saitama-u.ac.jp, yakaneko@mail.saitama-u.ac.jp

ABSTRACT

Mean flow and turbulence are two different patterns of mechanical stressors that simultaneously affect aquatic plants in natural waters. The mean flow creates a unidirectional tension, whereas loads from turbulence fluctuate with the forces and pressures of different magnitude and direction. However, whether the turbulence or mean flow has the most significant effect on habitat selection by submerged plants has not been determined. Therefore, our aim was to determine which water movement factor was most significant for habitat selection by submerged plants with different morphologies. The effects of mean flow and turbulence were tested with the whorled *Elodea nuttallii, Potamogeton crispus* and the leafy *Vallisneria asiatica*, with other factors controlled. A gradually constricted transparent pipe with plants in the middle was used in a recirculating tank to test the effects of the mean flow, and the effects of turbulence were examined by generating different turbulence levels in tanks with vertically oscillating grids. To determine plant response to these stressors, H_2O_2, IAA and pigment concentrations were measured. In addition structural changes of plant tissues were examined with transmission electron microscopy. At high turbulence levels H_2O_2 concentrations were significantly higher than in plants in mean flow or in stagnant water; thus, the physiological stress from turbulence was significantly higher regardless of morphology. *V. asiatica* benefited from mean flow, whereas whorled *E. nuttallii* and *P. crispus* were more stressed than plants in stagnant water. The IAA concentration which is responsible for the plant elongation was decreased with the increase of turbulence levels. Cell wall thickness in epidermal cells of the leaves was significantly high in plants those exposed to high turbulence levels and this may be a result of high accumulation of lignin and cellulose. Moreover, thylakoid destruction was observed in chloroplasts of the *Vallisneria* sp. that exposed to high turbulence levels. Therefore, the physiology and cell ultra-structure of submerged plants were affected more by turbulent motion than by mean flow.

Keywords: Mean flow, plant morphology, cell utltr-structure, reactive oxygen species, turbulence, submerged plants

1 INTRODUCTION

The diverse functions of aquatic plants are essential to maintain the ecological balance of aquatic ecosystems because aquatic plants contribute to primary production, provide food for consumers, provide habitat and refuge for a variety of aquatic fauna, function in biogeochemical cycles, and regulate the transport of sediment, among other roles. In natural waters, the distribution of submerged plants is dependent on various biotic and abiotic factors, and the abiotic factors include water movement (Asaeda & Rashid 2016), substrate characteristics, nutrient availability in sediments and the water column, climate, anoxic conditions, pH and temperature, among other factors (Bornette & Puijalon 2011). Abiotic factors such as these play a crucial role in determining the habitat for survival within a particular environment, and aquatic plants can become highly adapted to overcome the challenges of harsh environments (Heilmeier et al. 2005). Among the abiotic factors in aquatic environments, water movement is a ubiquitous factor for aquatic plants because depending on the magnitude, flow driven drag and lift forces have either positive or negative effects (Puijalon et al. 2005).

Mean flow and turbulence are the two primary components of water movement. Mean flow is the velocity averaged for some period that is relatively steady and unidirectional in streams and rivers. Turbulence is the deviation of instantaneous velocity from the average that changes in magnitude and direction through time. Some of the energy of mean flow generates turbulence when the Reynolds number exceeds some critical value (R_e < 2100 Laminar flow, 2100 < R_e < 4000 transitional flow, R_e > 4000 turbulent flow Peixinho et al. (2005)), and therefore, turbulence is always associated with high velocity mean flows in natural waters. However, the mechanical effects of these two flow components may differ on aquatic plants; whereas mean flow exerts tension stress on the plant body parallel to the direction of flow, turbulence loads mechanical forces and pressures in changing directions on the plant body (Atapaththu et al. 2015b).

However, the degree of stress response and tolerance to water movements may differ depending on the species of submerged macrophyte. Moreover, the morphological differences between submerged macrophyte species may result in different responses to the mechanical stressors of water movement and contribute to the selection of preferred habitats. But, whether the turbulence or mean flow has the most significant effect on habitat selection by submerged plants has not been determined. Therefore, in this study, we addressed the following two research questions using *Elodea nuttallii*, *Vallisneria asiatica* and *Potamogeton crispus* as the experimental plants: (1) which is the most stressful to submerged plants from mean flow and turbulence? (2) Do the mechanical stressors in flowing water contribute to habitat selection?

2 METHODOLOGY

The closed circulation system used by Atapaththu and Asaeda (2015) was modified to increase the mean flow velocity. In figure 1 (a), the schematic of the modified closed circulation system used to generate mean flow (~14cms^{-1}) with negligible turbulence is shown. To expose plants to mean flow, the closed-flow system was composed of six, 45 cm long, transparent PVC pipes (P1 - P6; internal diameter 5 cm) with opaque PVC pots (C) filled with well-washed commercial sand substrate. The turbulence inside the pipes were reduced by installing diffusers at the inlet and the outlet using straws (Mc Donald-type) (Atapaththu & Asaeda 2015). The transparent pipes were submersed 10 cm below the water surface in a glass tank (45 × 45 × 120 cm^3), and the water circulation was maintained by pumping water back to the overhead tank using a submersible pump (S), maintaining a constant water level (30 cm). The P1 - P3 pipes were exposed to mean flow, whereas the P4 - P6 pipes were not attached to the overhead tank to maintain a stagnant condition as control.

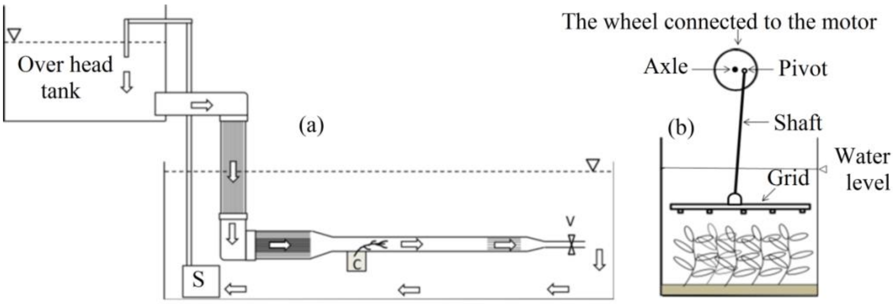

Figure 1. Diagrammatic representations of the (a) mean flow setup and (b) turbulence setup

The velocity inside the transparent pipes was measured at 10 cm intervals distant from the PVC pot, along the transparent pipe with a two-dimensional electromagnetic current meter (EMCM) (SF-5712; Tokyo-Keisoku Corporation, Tokyo, Japan). A depth-velocity profile was constructed at each 10cm point by measuring the velocity at 5 mm depth intervals inside the pipe. For each time, velocity fluctuations were recorded for 2 min. Velocity readings were transmitted to a data logger (GL200A; Graphtec Corporation, Yokohama, Japan) at 100 ms intervals and were analyzed using GL200-820-APS software, Version 1.01 to calculate the average velocity and turbulence inside the pipe.

In the turbulence experiment, turbulence was generated inside glass microcosms (15.7 × 15.7 × 24.5 cm^3) by a vertically oscillating grid attached to a DC motor as shown in figure 1 (b), which minimized the mean flow (Ellawala et al. 2011). Therefore, relatively isotropic turbulence was generated without a mean flow, and the flow-induced stretch of plants was avoided in this system. The turbulence velocity was selected based on a comparison of the velocity fluctuations in macrophytes stands in the natural environment. A total of 10 setups with two replicates (n = 2) in each treatment (Stagnant condition as the control (T0), Low turbulence (T1), Medium turbulence (T2), High turbulence low amplitude (T3), High turbulence high amplitude (T4)) were randomly allocated in a complete randomized design. The different intensities of turbulence were generated by DC motors with different frequencies (0.3Hz for low turbulence, 1Hz for medium turbulence, 4Hz for high turbulence), with the identical turbulence energy theoretically, to investigate plant responses. The water level was maintained at 20 cm above the substrate, and each microcosm had a layer of commercial sand (~3 cm) and 5% Hoagland nutrient solution was used as the nutrient media (Atapaththu & Asaeda 2015). Water in the control microcosm was gently mixed with a glass rod to avoid the possible stratification. Six symmetrically distributed points over the water surface of the microcosm were selected for measuring the velocity profile. The velocity fluctuations were recorded at 5, 10 and 15 cm depths from the water surface at each point with the same two-dimensional EMCM. The measured average turbulence velocities (root mean square velocities) in T1-T4 were 0.29±0.11, 0.60±0.20, 1.28±0.16, 2.96±0.89 cm/s respectively.

The pH and water temperature of the microcosms were maintained within the ranges of 7.08-7.44 and 23-24 °C, respectively, for the experiments. The range of dissolved oxygen concentration was 8.2-8.6 mg/L among the treatments. All treatments were maintained with a photoperiod of 12 h dark and 12 h light with a light intensity of 110-120 µmol $m^{-2} s^{-1}$ using white fluorescent lamps.

The experimental plants were *Elodea nuttallii*, *Potamogeton crispus* and *Vallisneria asiatica*. The three species were collected from the identical stand of the Moto Arakawa River, Japan (36°7′30.1″ N, 139°24′20″ E). After collection, the plants were acclimated in aquaria for more than one month. Similar-sized apical tips of *E. nuttallii, P. crispus* and young plants of *V. asiatica* with initial lengths (IL) of ~5 - 8 cm were used for the experiments. For the turbulence experiments, three plants of each species were planted in each glass microcosm. Four plants of each species were grown inside each transparent pipe to expose the mean flow as a monoculture because the fluctuation in velocity created by the different morphology of one species can influence the other in a limited space, which is unlikely in the turbulence microcosm. Therefore, one individual plant was considered as one replicate (Cox & Reid 2000). The plants were acclimated to the experimental for one week and then exposed either to stagnant water (control), mean flow or turbulence. Mean flow and turbulence experiments were continued for 8 weeks and terminated when the apical tips of some plants were longer than the experimental tube in the mean flow setup or were close to touching the oscillating grid in the turbulence setup. Anatomical variations, the concentrations of H_2O_2, the indole acetic acid (IAA) and pigment concentrations were analyzed after the experimental period. The chemical analyses and observations of ultrastructural changes in cells were obtained for the apical and basal sections of the plants by separating the leaves and stems to examine differences in the localization of stress responses.

2.1 Determination of pigment concentrations

Plant shoots (~5 mg) were extracted with N-N dimethylformamide by maintaining them for 24 h in the dark. The concentrations of Chl.*a*, Chl.*b* and total carotenoids were determined spectrophotometrically (UV mini 1210; Shimadzu). Published coefficients (Wellburn 1994) were used for quantification. Triplicates were analyzed, and the results are presented based on the fresh weight (FW).

2.2 Plant tissue preparation for stress assays

Following freeze-drying with liquid nitrogen, approximately 100 mg of fresh plant tissue was weighed and extracted with 5 mL of ice-cold phosphate buffer (50 mM at pH 6.0). PVP (polyvinylpyrrolidone) was used to mask the phenolic compounds in the extract. The extracts were then centrifuged at 5000 × g for 15 min at 4℃, and the supernatant was stored at kept under -80℃ until analysis. Triplicates of each extract were analyzed, and the results are expressed based on the fresh weights (FW).

2.3 H_2O_2 assay in plant tissue

Enzyme extract (750 µL) was added to 2.5 mL of 1% $TiSO_4$ in 20% H_2SO_4 (v/v). After mixing, the solution was centrifuged at 5000 × g for 15 min at room temperature. The developed color of the reaction was measured with a spectrophotometer at 410 nm. The concentration was determined using a pre-prepared standard curve for known hydrogen peroxide concentrations (Jana & Choudhuri 1982).

2.4 Plant extract for IAA determination

Approximately 100 mg of freeze-dried plant tissue was ground in a mortar. The ground plant tissue was extracted with 5 mL of distilled water. The extracts were centrifuged at 5000 × g for 15 min at 4℃, and the supernatant was stored at kept under -80℃ until analysis. The IAA concentration was measured spectrophotometrically at 530 nm by adding 1.00 mL of the extract into 2.00 mL of modified Salowski reagent (1.00 mL of 0.5 M ferric chloride in 35% of $HClO_4$) and incubating the mixture for one hour to develop the resulting pink color. The concentration was determined using a pre-prepared standard curve for known IAA concentrations.

2.5 Transmission electron microscopy (TEM)

The changes in cell ultrastructure of the plants subjected to turbulence and mean flow were observed using a conventional electron microscopy (Seki et al. 2014). Before overnight fixation in a refrigerator, the stems and leaves were fixed with 2% glutaraldehyde in 0.05 M potassium phosphate buffer (pH 7.0) for 2 h at room temperature. The plant tissues were then post-fixed in 2% OsO_4 in 0.05 M potassium phosphate buffer (pH 7.0) for 2 h at room temperature. The plant tissues were dehydrated in an acetone series and then embedded in Spurr's resin. The prepared ultra-thin sections were observed under an electron microscope (H-7500; Hitachi) following the method described by Seki et al. (2014). The cell wall thickness was measured in enlarged TEM images as described by Atapaththu et al. (2015a).

2.6 Statistical analyses

Data were tested for normality using the Shapiro-Wilk test before statistical analyses. The results are presented as the mean ± SD (n = 2 for turbulence treatments, n = 4 for mean flow treatment). The data were subjected to one-way analysis of variance (one-way ANOVA) followed by Tukey's multiple comparison tests to evaluate differences among means at a 0.05 significance level. Statistical analyses were performed using SPSS for Windows (release 13; SPSS INC., Chicago, IL).

3 RESULTS AND DISCUSSION

The H_2O_2 concentrations in leaves and stems after exposure to different turbulence velocities (a) and mean flow (b) are shown in figure 2. Compared with the turbulence treatments, the H_2O_2 concentrations in the stems or leaves of plants exposed to mean flow were significantly lower ($F = 13.12$, $P = 0.001$). The H_2O_2 concentrations in the stem and leaves of all species increased with the turbulence velocity and mean flow, with the exception of the concentrations in *V. asiatica*, which decreased when plants were exposed to mean flow. Independent of the intensity or the type of mechanical stress, high concentrations of H_2O_2 were detected in the leaves of *E. nuttallii* and *P. crispus*. In all plants, the stem and leaf H_2O_2 concentrations increased significantly in response to medium and high turbulence velocities in comparison to stagnant conditions ($P < 0.01$).

H_2O_2 is a strong reactive oxygen species that is derived from O_2. Approximately 1% of the oxygen consumed by plants is converted into ROS in various subcellular loci. Whether ROS benefit the plant or cause deleterious effects depends on the concentration; high ROS concentrations damage biomolecules, whereas at low/moderate concentrations, ROS act as secondary messengers in an intracellular signaling cascade that can mediate different responses in plant cells.

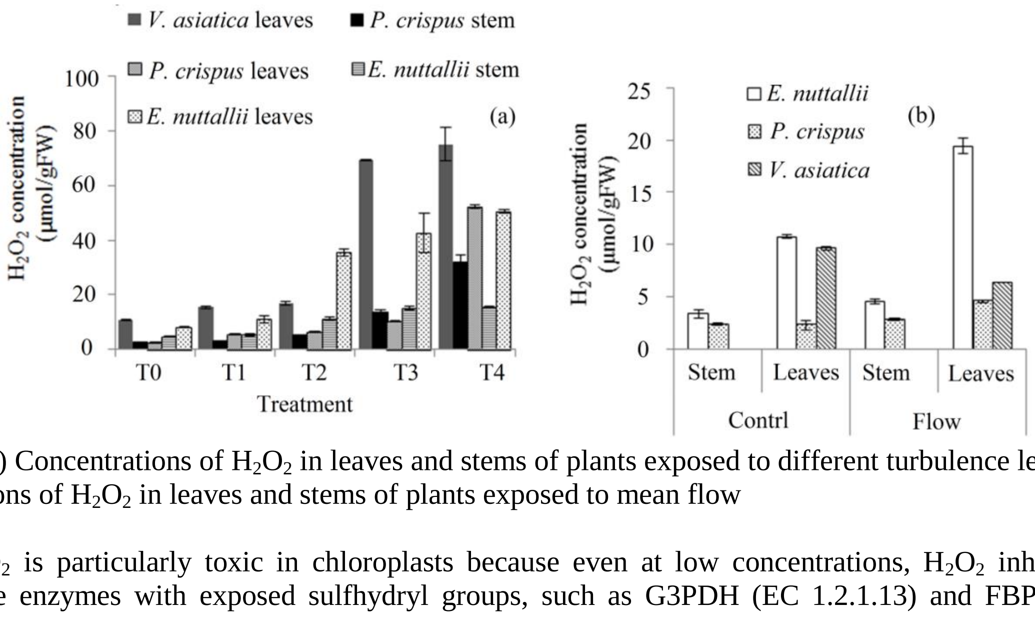

Figure 2. (a) Concentrations of H_2O_2 in leaves and stems of plants exposed to different turbulence levels. (b) Concentrations of H_2O_2 in leaves and stems of plants exposed to mean flow

H_2O_2 is particularly toxic in chloroplasts because even at low concentrations, H_2O_2 inhibits the Calvin cycle enzymes with exposed sulfhydryl groups, such as G3PDH (EC 1.2.1.13) and FBPase (EC

3.1.3.11), which reduces the photosynthetic assimilation of carbon dioxide (Takeda et al. 1995). The concentration of H_2O_2 is indicative of the plant stress level (Sharma & Dubey 2005); hence, excessive concentrations of H_2O_2 indicate high levels of stress on the plant. All plants exposed to high turbulence had higher concentrations of H_2O_2 and therefore higher stress levels.

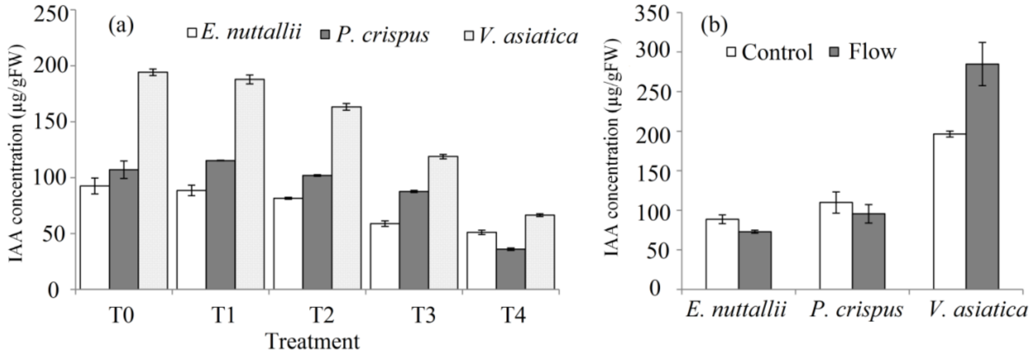

Figure 3. IAA concentration in plants exposed to turbulence and mean flow treatments

The IAA concentration in plants exposed to turbulence treatments and stagnant water (a) and mean flow (b) is shown in Figure 3. For all species, the IAA concentration decreased monotonically with increasing turbulence level. The IAA concentrations were higher in *V. asiatica* plants exposed to mean flow treatments, whereas the lowest concentrations were measured in *P. crispus* plants subjected to high turbulence treatments. IAA is a prime measure of stress-induced morphogenic responses of plants (Kawano et al. 2003, Potters et al. 2007). Furthermore, consistent with the results of our study, declines in IAA levels with reductions in plant growth were observed for terrestrial and aquatic plants after exposure to different mechanical stressors (Saidi et al. 2009, Ellawala et al. 2011).

The concentrations of photosynthetic pigments varied among the treatments with the intensity of the mechanical stress. In the stagnant water (control) used in both the turbulence and the flow experiments, the concentrations of Chl.*a*, Chl.*b* and carotenoids in plants were not significantly different. The Chl.a/Chl.b ratio was increased with increasing the turbulence velocity, whereas the Chl.a/carotenoids (Crt) ratio decreased when increased the turbulence velocity (Table 1). The highest Chl.a/Chl.b ratio and the lowest Chl.a/carotenoid ratios were observed in the high turbulence high amplitude treatment which generated highest turbulence velocity. High ratios of Chl.*a*/Chl.*b* and low ratios of Chl.*a*/carotenoids indicate a stress response in terrestrial plants (Mobin & Khan 2007). In our study, the pigment ratios were also an indication that the plants exposed to high turbulence treatments were highly stressed. In contrast, *V. asiatica* had a significantly lower accumulation of H_2O_2 as well as significantly higher Chl.a/carotenoid and significantly lower Chl.a/Chl.b ratios than in *E. nuttallii* and *P. crispus* plants exposed to mean flow. This indicated that the *V. asiatica* had a positive effect from the mean flow.

Table 1. Ratios of the concentrations of Chl.a/Chl.b and Chl.a/carotenoids in plants exposed to turbulence and mean flow (mean ± SD)

Species		Turbulence level					MF	*F*	*P*
		T0	T1	T2	T3	T4			
E. nuttallii	Chl.a/Chl.b	2.75 ± 0.06^a	2.78 ± 0.01^a	2.56 ± 0.08^b	4.02 ± 0.26^c	3.73 ± 0.24^c	2.48 ± 0.09^b	52.73	0.0001
	Chl.a/Crt	1.96 ± 0.01^a	1.96 ± 0.01^a	1.35 ± 0.02^b	0.96 ± 0.03^c	0.81 ± 0.01^d	1.42 ± 0.02^b	654.12	0.0001
P. crispus	Chl.a/Chl.b	3.39 ± 0.21^a	3.55 ± 0.04^a	5.65 ± 1.19^c	6.26 ± 0.11^c	8.56 ± 0.78^d	3.59 ± 0.04^e	39.54	0.0001
	Chl.a/Crt	1.45 ± 0.61^a	1.43 ± 0.04^a	0.97 ± 0.01^b	0.72 ± 0.01^c	0.66 ± 0.01^d	1.22 ± 0.01^e	1739.02	0.0001
V. asitaica	Chl.a/Chl.b	2.07 ± 0.05^a	2.07 ± 0.03^a	1.97 ± 0.04^b	2.23 ± 0.08^c	2.63 ± 0.08^d	1.72 ± 0.06^e	43.63	0.0001
	Chl.a/Crt	3.62 ± 0.06^a	2.72 ± 0.02^b	1.97 ± 0.04^c	1.71 ± 0.043^d	1.56 ± 0.04^e	4.94 ± 0.06^f	2018.94	0.0001

Different superscripts in the identical row indicate significant differences. Crt: carotenoids; MF: mean flow; T0: control; T1: Low turbulence; T2: medium turbulence; T3: high turbulence low amplitude; T4: high turbulence high amplitude.

Figure 4 shows the inside and outside flow velocity of the well grown plants stands exposed to mean flow. The initial velocities among the transparent pipes (P1, P2 and P3) before introducing the plants were not significant ($F = 0.389$, $P = 0.681$).

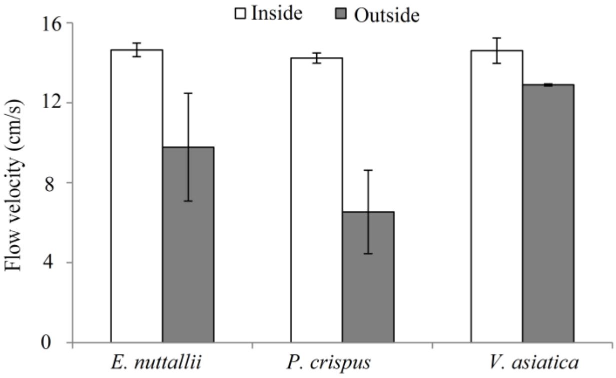

Figure 4. The inside and outside flow velocities of the plant stands exposed to mean flow

The morphology of plants strongly affected the velocity distribution in mean flow. Mean flow velocity inside and outside the plant stands was significantly different. Moreover, the velocities were lower in *E. nuttallii* and *P. crispus* stands than in *V. asiatica* stands. The difference between inside and outside velocities for *V. asiatica* was approximately 2 cm s^{-1}; whereas the differences between inside and outside for *E. nuttallii* and *P. crispus* were 6 to 8 cm s^{-1}, respectively. The leaves of *E. nuttallii* are typically whorled with serrated margins, *P. crispus* has undulating leaves in an alternate pattern and *V. asiatica* has long, streamlined leaves. Therefore, the leaf arrangement of *P. crispus* is intermediate between that of *E. nuttallii* and *V. asiatica*. The velocity differences are likely caused by the whorled leaves obstructing mean flow, creating a pressure difference between the front and the lee side of the leaves and a drag force toward the flow. The entire plant body follows the primary flow and is stretched downstream by the sum of these forces, which in mean flow with whorled leaves, is loaded to the stem. However, in leafy plants, the tension stress acts on the surface of plants through surface friction from water flow parallel to the direction of flow.

The cell walls of *E. nuttallii*, *P. crispus* and *V. asiatica* leaves and *E. nuttallii* basal stems significantly thickened following exposure to high turbulence (Figure 5b).The ultrastructure of chloroplasts exhibited significant deformation in plants exposed to turbulence. In particular, the arrangement of thylakoid membranes with grana stacks was greatly distorted far from the ordinary in the epidermal cells of the leaves (Figure 5a-C). By contrast, well-developed chloroplasts were observed in the leaves of *V. asiatica* exposed to mean flow (Figure 5a-B) compared with those grown in stagnant condition (Figure 5a-A).

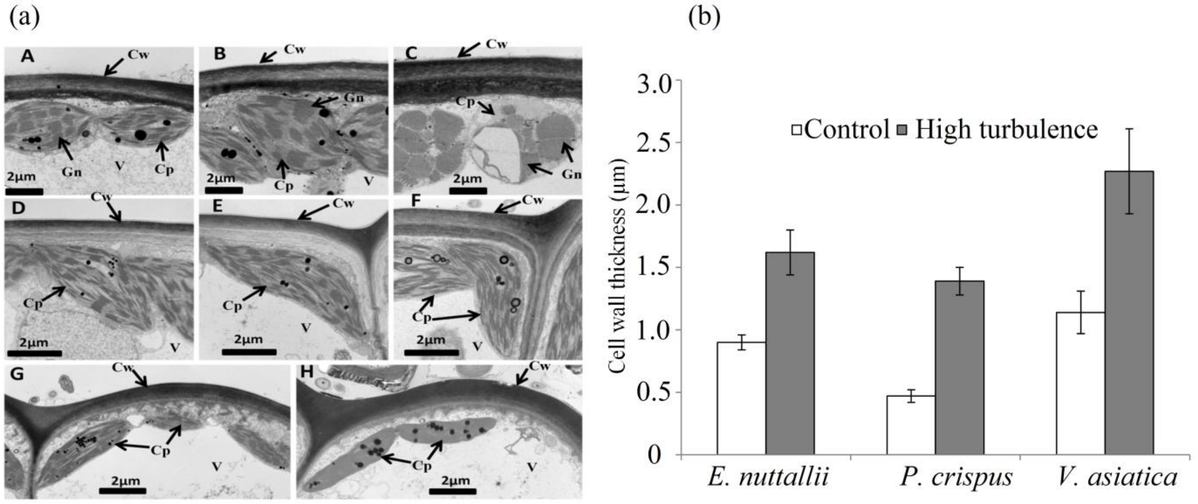

Figure 5. (a) Structural differences in the epidermal cells of leaves and stems exposed to different conditions. Adaxial epidermal leaf cells of *V. asiatica* (A-C), *P. crispus* (D -F), and *E. nuttallii* (G, H). A, D, and G were grown in stagnant water; B and E were exposed to mean flow; and C, F and H were subjected to high turbulence. (Cw: cell wall, Cp: chloroplast, Gn: grana, V: vacuole), (b) The cell wall thickness of the epidermal cells of the plants in control and high turbulence treatments (n = 10)

The thickened cell walls could be a result of excessive accumulation of cellulose and lignin in cell walls. Specially the lignification is an adaptive defense mechanism against mechanical stressors (Saidi et al. 2009, De Jaegher et al. 1985), the high accumulations of lignin might be an adaptation to prevent plants from breaking following exposure to vigorous fluctuating motions with high turbulence. Moreover the, the distortion of the thylakoid structure may be a result of vigorous motion created by high turbulence. Plants exposed to mean flow are loaded with static tension stress rather than fluctuating motion. The concentrations of H_2O_2 in plants exposed to mean flow were always lower than those in plants exposed to turbulence. Therefore, the fluctuating motion of plant tissues seemed to be more stressful to plants than static tension. However, with the exception of *V. asiatica*, the concentrations of H_2O_2 in plants exposed to mean flow were higher than those in plants in stagnant water. Static tension, therefore, also exerted stress.

4 CONCLUSIONS

The mean flow was affected differently by the different morphologies of the three species of plants. The leafy plant species (i.e., *V. asiatica*) received benefits with mean flow, but whorled plants (i.e., *E. nuttallii* and *P. crispus*) showed physiological stress with mean flow compared with plants in stagnant water. Considering all the results of this study, turbulent motion had a greater effect on the metabolism of plants than mean flow, and the growth of submerged plants was affected more by growth in turbulent water than in mean flow.

ACKNOWLEDGMENTS

A generous grant-in-aid from Scientific Research (B) (15H04045), Japan Society for the Promotion of Science; and a Development Grant for River Management Technology from the Ministry of Land, Infrastructure, Transportation and Tourism, Japan; River Foundation of Japan supported this study.

REFERNCES

Asaeda T, Rashid MH (2016). Effects of turbulence motion on the growth and physiology of aquatic plants. *Limnologica- Ecology and Management of Inland Waters* **Accpeted**.

Atapaththu K, Miyagi A, Atsuzawa K, Kaneko Y, Kawai-Yamada M, Asaeda T (2015a). Effects of water turbulence on variations in cell ultrastructure and metabolism of amino acids in the submersed macrophyte, Elodea nuttallii (Planch.) H. St. John. *Plant Biology* **17,** 997-1004.

Atapaththu KSS, Asaeda T (2015). Growth and stress responses of Nuttall's waterweed Elodea nuttallii (Planch) St. John to water movements. *Hydrobiologia* **747,** 217-233.

Atapaththu KSS, Miyagi A, Atsuzawa K, Kaneko Y, Kawai-Yamada M, Asaeda T (2015b). The effects of water turbulence on variations in cell ultra-structure and metabolism of amino acids in the submersed macrophyte, Elodea nuttallii (Planch.) H. St. John. *Plant Biology* **17,** 997-1004.

Bornette G, Puijalon S (2011). Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences* **73,** 1-14.

Cox DR, Reid N (2000). *The theory of the design of experiments,* CRC Press.

De Jaegher G, Boyer N, Gaspar T (1985). Thigmomorphogenesis inBryonia dioica: Changes in soluble and wall peroxidases, phenylalanine ammonia-lyase activity, cellulose, lignin content and monomeric constituents. *Plant Growth Regulation* **3,** 133-148.

Ellawala C, Asaeda T, Kawamura K (2011). Influence of flow turbulence on growth and indole acetic acid and H2O2 metabolism of three aquatic macrophyte species. *Aquatic Ecology* **45,** 417-426.

Heilmeier H, Durka W, Woitke M, Hartung W (2005). Ephemeral pools as stressful and isolated habitats for the endemic aquatic resurrection plant Chamaegigas intrepidus. *Phytocoenologia* **35,** 449-468.

Jana S, Choudhuri MA (1982). Glycolate metabolism of three submersed aquatic angiosperms during ageing. *Aquatic Botany* **12,** 345-354.

Kawano N, Kawano T, Lapeyrie F (2003). Inhibition of the Indole-3-acetic acid-induced Epinastic Curvature in Tobacco Leaf Strips by 2, 4-Dichlorophenoxyacetic Acid. *Annals of Botany* **91,** 465-471.

Mobin M, Khan NA (2007). Photosynthetic activity, pigment composition and antioxidative response of two mustard (Brassica juncea) cultivars differing in photosynthetic capacity subjected to cadmium stress. *Journal of Plant Physiology* **164,** 601-610.

Peixinho J, Nouar C, Desaubry C, Théron B (2005). Laminar transitional and turbulent flow of yield stress fluid in a pipe. *Journal of Non-Newtonian Fluid Mechanics* **128,** 172-184.

Potters G, Pasternak TP, Guisez Y, Palme KJ, Jansen MA (2007). Stress-induced morphogenic responses: growing out of trouble? *Trends in Plant Science* **12,** 98-105.

Puijalon S, Bornette G, Sagnes P (2005). Adaptations to increasing hydraulic stress: morphology, hydrodynamics and fitness of two higher aquatic plant species. *Journal of Experimental Botany* **56,** 777-786.

Saidi I, Ammar S, Demont-Caulet N, Thévenin J, Lapierre C, Bouzid S, Jouanin L (2009). Thigmomorphogenesis in Solanum lycopersicum: morphological and biochemical responses in stem after mechanical stimulation. *Plant Science* **177,** 1-6.

Seki Y, Nitta K, Kaneko Y (2014). Observation of polyphosphate bodies and DNA during the cell division cycle of Synechococcus elongatus PCC 7942. *Plant Biology* **16,** 258-263.

Sharma P, Dubey RS (2005). Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant Growth Regulation* **46,** 209-221.

Takeda T, Yokota A, Shigeoka S (1995). Resistance of photosynthesis to hydrogen peroxide in algae. *Plant and Cell Physiology* **36,** 1089-1095.

Wellburn AR (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology* **144,** 307-313.