

THE EFFECTS OF ABIOTIC FACTORS ON PLANT HEALTH AND BIOMECHANICS: A MESOCOSM STUDY ON *POTAMOGETON CRISPUS*

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Interactions between flow and vegetation are widely investigated because vegetation is a primary factor controlling channel ecohydraulics, nearshore hydraulics and flood risk. Laboratory experiments are a critical tool in this research area and, to adequately represent the complexity of natural ecosystems, live plants, rather than artificial surrogates, are often used. In the present work, we expose a freshwater macrophyte (*Potamogeton crispus*) to a range of environmental conditions commonly found in ecohydraulic laboratories to investigate how these affect the level of plant health and associated variations in plant biomechanical properties. This is motivated by a need to understand how deterioration in live plants that are used in flume facilities affects their hydraulic performance and therefore the verisimilitude of the data they provide on flow interactions. Results show that short-medium term exposition to tap water or low irradiance levels is stressful for plants and can induce modifications in their biomechanics, with a potential effect on their hydrodynamic performance.

1 INTRODUCTION

Laboratory experiments are established tools in hydraulic and ecological studies that can link field observation and numerical/mathematical modelling. Most laboratory research on flow-vegetation interactions has been conducted using plant surrogates [1], because this allows a complete control over experimental conditions, facilitates experimental replication and provides major flexibility in experimental design. In contrast, use of live organisms in flume facilities allows full representation of natural systems, but careful husbandry is necessary to maintain plant health [2]. Indeed, our ability to maximise the benefits of using live specimens may be limited by the behavioral integrity of those plants in flume settings. It is therefore important to assess how vegetation interactions with flows are affected by any physiological and biomechanical changes that occur when plants are placed in the laboratory. While it is usually straightforward to determine whether a plant has died, its biomechanical properties and therefore its interactions with the flow may be affected by non-lethal deterioration and stress. In the present work, we start addressing this issue by focusing on plant health status and biomechanical properties and how these are affected by the characteristics of a laboratory setting.

Techniques to evaluate mechanical properties of biological materials are well established [see 3], even though their applications to ecohydraulics remains very limited and, consequently, biomechanical data of aquatic plants is scarce [e.g. 4]. A few methods for assessing plant health status are routinely employed in plant physiology and horticulture but we are not aware that they have been applied to ecohydraulic studies. A robust technique to monitor plant health status is chlorophyll fluorescence analysis, which provides an indirect measurement of the photosynthetic activity by measuring the fluorescence re-emitted by chlorophyll pigments. Since higher plants primarily react to environmental stresses via changes in photosynthesis [5], chlorophyll fluorescence is extremely sensitive to health stress [e.g. 6]. For this reason, and because it is non-intrusive and non-destructive, chlorophyll fluorescence analysis is a promising tool to monitor plant health status in ecohydraulic applications.

In the present study, we investigate how the most important abiotic factors causing stress in freshwater macrophytes affect the health status and the biomechanical properties of curled pondweed (i.e. *Potamogeton crispus*). Experiments were conducted in mesocosms using six treatments, which were designed to replicate a range of typical laboratory conditions. The health stress associated with each treatment was monitored with a chlorophyll fluorometer and plant biomechanical properties were measured using a benchtop testing machine. Specifically, the following research questions are addressed:

- (1) Is *P. crispus* stressed when exposed to environmental conditions typical of flume facilities?
- (2) Do biomechanical properties of *P. crispus* vary as a consequence of the exposure to these conditions?

2 METHODOLOGY

2.1 Experimental design

Table 1 Description of environmental conditions of each treatment.

Treatment identifier	Type of water	Temperature (°C)	PAR ($\mu\text{mol}_{\text{photon}}\text{m}^{-2}\text{s}^{-1}$)	
			z = 0 m	z = 0.24 m
Pond Water	Pond water	18-22	150±30	50±5
Tap Water	Tap water	18-22	150±30	50±5
Low Temperature	Pond water	12-13	150±30	50±5
High Temperature	Pond water	28-32	150±30	50±5
Low Irradiance	Pond water	20-23	2±1	0.5±0.1
High Irradiance	Pond water	22-28	350±25	85±5

The species *P. crispus* was selected for the experiments as it is widely distributed across Europe and is known to create water management concerns [7]. The most significant abiotic factors that cause stress in freshwater macrophytes are light, temperature, water-quality, characteristics of substrate, and water movement [8]. In the present study, we focused on the first three factors and designed six treatments (see Table 1) to cover a range of conditions representative of standard flume facilities, as determined by a survey of 26 researchers across Europe. Pond water for experiments was collected from a pond at Loughborough University campus, before use it was left to settle for 24 hours and subsequently filtered with a 53 μ sieve. For the ‘Tap Water’ treatment, unconditioned tap water was used, this was left in an aerated tank for 24 hours prior to starting the treatment. Compared to pond water, tap water had a lower concentration of non-purgeable organic carbon (2.59 ppm vs 4.20 ppm), and a higher concentration of phosphate (3.35 mg/l vs 0 mg/l) and chloride (100.86 mg/l vs 48.35 mg/l). Each treatment mesocosm consisted of an 80 l plastic container aerated and filled with water up to 0.28 m depth. Fluorescent lighting units were used to maintain the Photosynthetically Active Radiation (PAR) levels under a 14h day: 10h night cycle. Each treatment lasted 5 days and plant health status was monitored daily.

2.2 Instrumentation

Two main devices were used during the experiments: a chlorophyll fluorometer and a benchtop testing machine. The chlorophyll fluorometer used was a Classic Fluorometer by Aquation (Aquation Pty Ltd, Umina Beach, Australia). A light pipe extension was applied on the sensor to convey measuring and saturating lights from the sensor to the testing sample (i.e. a leaf). During measurements, the testing sample was held using a leaf clip. An Instron Single Column 3343 benchtop testing machine (Instron, High Wycombe, UK) equipped with a 50N load cell was used for conducting mechanical tests. According to the manufacturer, the accuracy of the force readings is 1% and that of displacement readings exceeds 1%. Two types of mechanical tests were conducted with the testing machine: uniaxial tensile tests at breakage, and 3-point flexural tests.

2.3 Experimental protocol

Plants were collected on the 14th August 2017 by cutting the main stem above the roots, stored for up to 48 h in moisturized bags during transport and then placed in two 300 l storage tanks in the same conditions as the ‘Pond Water’ group. Within three days from collection, eight plants were randomly selected from the storage tanks and their mechanical properties were obtained as described below; these plants are the ‘Control’ group. The remaining plants were maintained in the storage tanks for up to 72 h prior to starting all treatments (for logistic reasons treatments were started on different days). Eight plants were randomly selected from the storage tanks and located in each treatment mesocosm. Every day the health status of each plant was monitored before dawn, as recommended by Murchie and Lawson [6], by measuring chlorophyll fluorescence on the youngest mature leaf (the same leaf was monitored throughout the experiments). This way, the minimum level of fluorescence F_0 and the maximum level of fluorescence F_m were measured in the Dark Adapted State (DAS). From these measurements the most robust proxy of plant health status, the maximum quantum yield of photosystem II $F_v/F_m = 1 - F_0/F_m$ [e.g., 6], was calculated. This parameter theoretically ranges from 0 to 1, and is consistently close to 0.83 in unstressed plants [e.g., 6]. After five days of treatment, plants were removed from the mesocosm and specimens for mechanical tests were prepared from their stems. A total of four specimens were cut from each plant, two from the top part and two from the bottom, with one for tensile testing and one for flexural testing in each case. To minimize end-wall effects specimens for tensile tests were prepared so that their diameter to length ratio was lower than 1:10

[3], while specimens for flexural tests were prepared with a diameter to span ratio lower than 1:15. From the tests, the following mechanical properties were obtained: tensile Young's modulus E_t , as the slope of the initial, linear part of the nominal stress-strain curve; flexural rigidity $E_b I = \delta s^3 / 48$, where δ is the slope of the initial part of the force-deflection curve, and s is the horizontal span of the specimen; and bending Young's modulus E_b as the ratio of flexural rigidity to the second moment of area of the specimen I .

3 RESULTS

3.1 Plant health status

Even though the 'Pond Water' treatment was designed to minimize plant health stress, the reduction of F_v/F_m throughout the experiment was significant (t-test, mean slope = -0.01 d^{-1} , $df=7$, $p \ll 0.01$), indicating that plant health status was negatively affected. From 48 hours after the beginning of the treatments, several plants in 'Tap Water' and 'Low Irradiance' treatments were so deteriorated that the number of valid measurements of chlorophyll fluorescence were considerably reduced. When chlorophyll fluorescence was measured on extremely deteriorated leaves, F_0 was below instrument accuracy and/or the resultant F_v/F_m was outside the theoretical range (i.e. 0, 1). In such cases, the measurement was invalid and F_v/F_m was assumed to be equal to 0, because this corresponds to the poorest health status. Since linear regression of F_v/F_m in time would not fit the data adequately for those plants whose leaves deteriorated during the experiments, the mean daily value of F_v/F_m for each treatment $\overline{F_v/F_m}$ was calculated. The trend of $\overline{F_v/F_m}$ for 'Tap Water' and 'Low Irradiance' treatments is considerably different (slope = -0.12 - 0.13 d^{-1}) from that of the remaining treatments (slope = -0.01 - 0.02 d^{-1}), which are indistinguishable from one another (Figure 1a) but significantly different from 0. These results suggest that the use of unconditioned tap water or low PAR level are most stressful for *P. crispus*. Because the number of valid measurements available for these treatments is limited compared to the remaining treatments, they are not considered in further analysis of plant health status.

For 'Pond Water', 'Low Temperature', 'High Temperature', and 'High Irradiance' treatments, the linear regression of F_v/F_m against time for each plant throughout the experiment was computed using the Least Square Difference method. The slopes of the linear regressions were then compared across groups using Tukey's Honest Differences test corrected with the Bonferroni technique. Results show that the slope for 'Low Temperature' treatment is different from that for the three remaining treatments ($p = 0.06$ - 0.13), which are statistically indistinguishable from one another (Figure 1b). Hence, low temperature conditions more negatively affect *P. crispus* health status compared to the remaining cases. However, bear in mind that in all cases a deterioration in plant health status is identified.

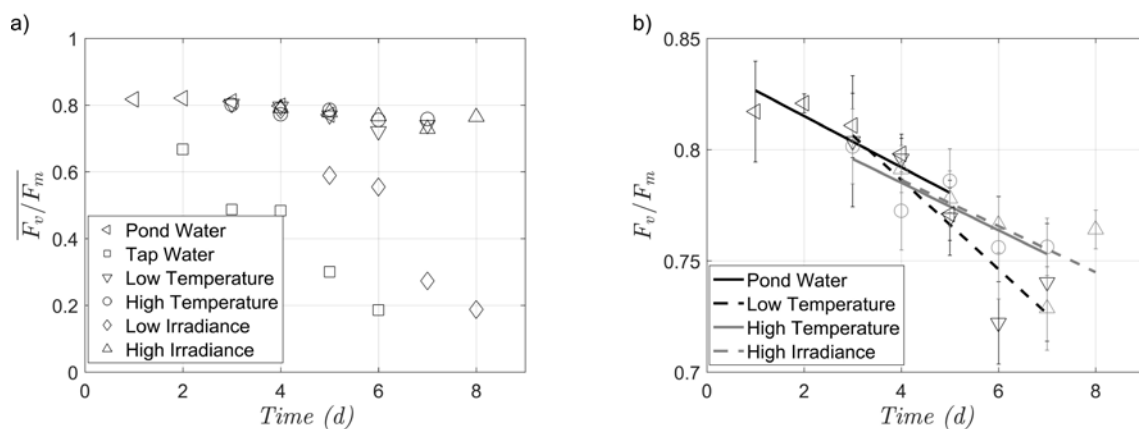


Figure 1. (a) Mean daily values of F_v/F_m across all treatments. (b) Linear regressions of F_v/F_m calculated using the mean intercept and slope for each treatment (markers represent mean values, bars are twice the standard deviation; note that markers correspond to those in (a) and that the scale is different between (a) and (b)).

3.2 Plant biomechanics

Biomechanical properties of freshwater macrophytes can vary depending on position along the stem [4], so in the present study we measured the distance of each testing specimen from the top of the plant by counting plant

internodes. We verified that the number of internodes from the top of the plant i_n is an important variable in defining the biomechanical properties of a specimen by using Analysis of Covariance with i_n as covariate and treatments as factor. The position of the specimen along the stem has a significant effect on estimates of E_t and E_bI ($p < 0.01$), but not on E_b . To remove the effect of i_n we analyzed specimens prepared from the top and the bottom of plants separately. By using Analysis of Variance, we compared the mechanical properties of specimens cut from plants in the ‘Control’ group with those from the remaining groups (i.e. five pairwise comparisons for top specimens and five pairwise comparisons for bottom specimens) and we corrected the significance level to account for the multiple non-independent tests conducted. Results show that E_t increases significantly for plants exposed to tap water or low temperature (bottom part), and marginally for those exposed to low PAR level (details of tests are reported in Table 2). Conversely, E_bI decreases significantly for plants exposed to low PAR levels. It is worth noting that the treatments inducing the highest effect on plant health status are associated with significant changes in plant biomechanical properties. Furthermore, it is important to note that in the cases investigated the variations in mechanical properties induced by the treatments appear to be potentially significant to flow-plant interactions, with changes between 24% and 68% of the reference values obtained for the ‘Control’ group.

Table 2. Most significant effects of treatments on biomechanical properties (note that three biomechanical properties, six treatments, and two plant parts are considered, for a total of 36 cases).

Mechanical property	Plant part	Treatment	F	p	η^2	Mean value, ‘Control’	Mean value, treatment
E_t	Bottom	Tap Water	19.41	5.98×10^{-4}	0.58	31.67 MPa	46.88 MPa
E_t	Bottom	Low Irradiance	5.04	0.042	0.26	31.67 MPa	39.39 MPa
E_t	Top	Low Temperature	8.57	0.011	0.38	32.16 MPa	21.04 MPa
E_bI	Top	Low Irradiance	7.71	0.015	0.36	1.75×10^{-5} MNm ²	5.66×10^{-6} MNm ²

4 CONCLUSIONS

This work reports on the effects of environmental conditions typically found in ecohydraulic laboratories on the health status and biomechanical properties of freshwater macrophyte *P. crispus*. Results show that the use of tap water and low irradiance levels cause a considerable stress to plants, which appear to deteriorate within a few days. Crucially, the two most stressful treatments for *P. crispus* are also typical of ecohydraulic laboratories. Importantly, these environmental conditions can also induce changes in biomechanical properties that suggest the potential for impacts on flow-plant interactions. Therefore, further research on this topic is required to understand if these modifications are recorded in more species and affect vegetation hydrodynamics. Our results are also valuable in beginning to define appropriate husbandry and monitoring protocols for live plants used in flumes.

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