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Adaptations to increasing hydraulic stress: morphology, hydrodynamics and fitness of two higher aquatic plant species

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Abstract

Sessile organisms often exhibit morphological changes in response to permanent exposure to mechanical stimulation (wind or water movements). The adaptive value of these morphological changes (hydrodynamic performance and consequences on fitness) has not been studied extensively, particularly for higher plants submitted to flow stress. The aim was to determine the adaptive value of morphological patterns observed within two higher aquatic plant species, *Berula erecta* and *Mentha aquatica*, growing along a natural flow stress gradient. The hydrodynamic ability of each ramet was investigated through quantitative variables (drag coefficient and *E*-value). Fitness-related traits based on vegetative growth and clonal multiplication were assessed for each individual. For both species, the drag coefficient and the *E*-value were explained only to a limited extent by the morphological traits used. *B. erecta* exhibited a reduction in size and low overall plant drag at higher flow velocities, despite high drag values relative to leaf area, due to a low flexibility. The plants maintained their fitness, at least in part, through biomass reallocation: one tall ramet at low velocity, but shorter individuals with many interconnected stolons when flow velocity increased. For *M. aquatica*, morphological differences along the velocity gradient did not lead to greater hydrodynamic performance. Plant size increased with increasing velocities, suggesting the indirect effects of current favouring growth in high velocities. The fitness-related traits did not demonstrate lower plant fitness for high velocities. Different developmental constraints linked to plant morphology and trade-offs between major plant func-tions probably lead to different plant responses to flow stress.

Key words: Drag, ecomorphology, fitness, hydraulic ecology, hydrodynamic performance, mechanical constraint, morphological adaptation, submerged aquatic vegetation.

Introduction

A general paradigm in ecomorphology (Arnold, 1983; Wainwright, 1994; Koehl, 1996) consists of focusing on the link between the morphology of organisms and perform-ance in a given environment. Analysis of the performance of a given structure through mechanistic relationships contributes to elucidating the adaptive value of a phenotype in a given environment (Ricklefs and Miles, 1994). 'Morph-ology' refers to the way an organism is built, at any level of organization (Koehl, 1996). 'Performance' refers to the relative efficiency of an organism confronted with a given environmental pressure (Wainwright, 1994; Koehl, 1996). In the case of exposure to flow stress, the hydrodynamic performance of a plant is its ability to withstand hydrody-namic forces induced by water movement (e.g. through morphological adjustments). Sultan (1987) considered fit-ness as 'the adaptedness of an organism to its environment, which results in its relative success in survival and re-productive output'. Like other sessile organisms, aquatic plants exposed to moving fluids encounter mechanical forces: a drag force (parallel to flow) and a lift force (perpendicular to flow) (Koehl, 1982). To avoid uprooting, minimization of these forces is a major functional consid-eration for such organisms. Therefore, any morphological adjustment that leads to decreased plant drag, without

negative consequences to fitness (long-term survival and reproduction), could be interpreted as an adaptation to flow stress. Adaptation is considered here as a 'benefit on the organism with regard to its present relationship with its environment' (Sultan, 1987).

Wind, water movements, and mechanical stimulation are environmental constraints that shape the morphologies of sessile organisms, and which have been widely studied in the case of herbaceous terrestrial plants (Jaffe, 1973), trees (Telewski and Jaffe, 1986), macroalgae (Koehl and Alberte, 1988; Armstrong, 1989; Blanchette, 1997), aquatic mosses (Biehle *et al.*, 1998), and macrophytes (Idestam-Almquist and Kautsky, 1995; Boeger and Poulson, 2003). In moving water, morphological differences observed for exposed individuals of a given species have been associated with (i) resistance to breakage and uprooting through sturdier structures, (ii) avoidance of stress through reduced size or prostrated growth-forms, or (iii) reduction of hydrodynamic resistance through increased flexibility or reconfiguration abilities (Koehl and Alberte, 1988; Blanchette, 1997; Pratt and Johnson, 2002). Some causal relationships between morphology and hydrodynamic performance have been established for macroalgae, through theoretical studies and hydrodynamic comparisons of differently exposed thalli (Armstrong, 1989; Gaylord *et al.*, 1994; Blanchette, 1997). Surprisingly, this question has hardly been addressed for higher aquatic plants, despite their extensive morphological variability in response to water movement (Idestam-Almquist and Kautsky, 1995; Madsen *et al.*, 2001).

Plants exposed to flow generally present growth modifications and morphological changes (e.g. size reduction, changes in biomass allocation) (Idestam-Almquist and Kautsky, 1995; Coops and Van der Velde, 1996). Opposite morphological adjustments can be observed for different species submitted to the same hydraulic constraint (e.g. opposite size variations; Puijalon and Bornette, 2004). The hydrodynamic efficiency of these morphological changes has rarely been assessed in higher plant species (Madsen *et al.*, 2001). For this purpose, it is necessary to assess differences in hydrodynamic performance (and fitness-related differences) of forms that have grown in contrasting hydraulic conditions (Wainwright, 1994; Koehl, 1996). The mechanical advantage of a given morphology (e.g. gain in terms of drag or lift) determines the adaptive value of morphological changes (Sultan, 2000; Debat and David, 2001). As this gain can be negatively selected through a lower fitness, ecomorphological studies should also consider some ecological or biological traits that give information on relative plant fitness.

In this study, two higher aquatic plant species were considered, *Berula erecta* (Hudson) Coville and *Mentha aquatica* L., co-occurring along a gradient of increasing flow velocity. Both species have contrasting morphologies. *M. aquatica* forms long, horizontal runners above ground and relatively rigid erect stems. Its leaves are entire and

opposite. *B. erecta* is a stoloniferous species, consisting of a rosette of petiolated dissected leaves. These contrasting morphologies (erect stems versus rosette) probably require different functional adjustments in the adaptation to flow. This study investigates the functional implications of morphological differences between individuals of these two species colonizing different current velocities. The objectives were (Fig. 1) (i) to determine if morphological changes identified along the increasing stress (i.e. hydraulic) gradient correspond to increasing hydrodynamic abilities of the plants, and (ii) to demonstrate the adaptive value of these morphological changes through the conservation of plant fitness.

In this study, morphology was documented at the whole plant level, through morphological measurements on plants collected *in situ*. Plant performance was assessed through quantitative variables (drag coefficient and *E*-value, both calculated from drag measurements) allowing a comparison of shape to be made without size-effect. As virtually no sexual reproduction occurs in riffles, fitness was assessed from traits based on vegetative growth and clonal multiplication (Liao *et al.*, 2003; Santamaria *et al.*, 2003).

Materials and methods

Study sites and plant collection

B. erecta (Apiaceae) and *M. aquatica* (Lamiaceae) colonize calcareous nutrient-poor flowing habitats (Carbiener *et al.*, 1990). Plants were collected in a riverine wetland located in the Rhône River floodplain. Five flow-patches of about 1 m² were selected along a gradient of increasing velocity. Four out of the five flow patches were common to both species and only patch 1 (flow velocity=0) differed for the two species. Water-depth was 0.2 m on average in all patches, except for patch 1 of *B. erecta*, which was deeper (0.7 m on average). Flow-patches were separated from each other by only a few metres. All patches were characterized by similar substrate grain size and water physico-chemistry.

As the wetland drains seepage water from the river, its discharge is highly correlated with the river discharge. Therefore, the flow velocity encountered by plants was extrapolated for the four months preceding plant sampling. For this purpose, flow velocity measured on patches at several dates was regressed against daily Rhône River discharge (Table 1). Four flow velocity measurements were made at random on each patch, avoiding hydraulic shelters (big cobbles, tall plants), on 17 dates. Flow velocity was measured with a propeller (C2 current meter, OTT Messtechnik GmbH & Co. KG, Kempten, Germany) at a water depth of 40% above the substrate, which gives a good estimation of the average flow velocity in the water column (Dingman, 1984). No flood scouring occurred during the 4 months preceding plant sampling.

All the experiments were carried out during the growing season, from 1 to 11 July 2002 for *M. aquatica* and from 1 to 14 April 2003 for *B. erecta*.

Eight submerged individual plants (ramets) were collected from distinct clones in each flow patch, for each species. A ramet of *M. aquatica* was defined as a shoot end comprising four rooted nodes. An individual ramet of *B. erecta* was defined as a single rooted rosette. Any horizontal stolon growing from the ramet was removed. After collection, the plants were stored in aerated tap water at 16 °C, for a maximum of 2 d until measurements were made.
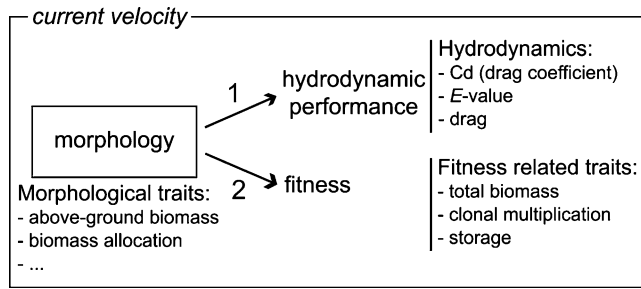
Fig. 1. The study of the relationships between morphology, performance and fitness within the framework of organisms exposed to hydraulic stress should (1) determine if morphological changes identified along the stress gradient correspond to a gain in plant hydrodynamics and (2) demonstrate their adaptive value through the conservation of plant fitness.

Table 1. *Median flow velocities (m s^{-1}) on patches where plants were sampled, calculated from Rhône discharges during the four months preceding sampling*

	Flow-patch				
	1	2	3	4	5
B. erecta	0	0.15	0.35	0.52	0.55
M. aquatica	0.004	0.13	0.29	0.46	0.52

Drag and morphological trait measurements were made on the same individuals.

Measurements of drag forces

Plant drag was measured under controlled conditions in an open water flume (Sagnes *et al.*, 2000). Two different hydrodynamic balances were used to measure plant drag, allowing drag measurements along two ranges of drag values. The first, used for *M. aquatica,* was adapted for measuring high drag values (Statzner *et al.*, 1999; Barrat-Segretain *et al.*, 2002) (Fig. 2a). Plants were fixed by a flexible ribbon to a hollow base (15 cm long, 9.5 cm wide, 4.5 cm high), in the natural growth position. The second balance (modified from the device used by Sagnes *et al.*, 2000), used for *B. erecta,* was adapted for measuring lower drag values. Plants were tied to a profiled stainless steel rod fixed to the balance (Fig. 2b). The rod formed an L-shaped right-angle with a 5 cm long horizontal segment parallel to the flow, close to the flume bottom, on the downstream side of the vertical segment. Plants were fixed to the downstream end of the horizontal part of the rod.

Roots and other below-ground parts of the plants were removed before measurements to avoid additional drag.

During drag measurements, flow velocity was measured in the water flume at a water depth of 40% of total depth (from the bottom), with the propeller used in the field, placed approximately 1 m upstream of the drag measuring device to avoid flow disturbance.

Flow velocity and drag were recorded simultaneously over 30 s. Drag was calculated as the mean of 300 values (10 values s^{-1}).

For each plant, drag was measured for at least 10 different flow velocities (maximum=13) over the range 0.1–1.15 m s^{-1}. Under each of these velocity conditions, the projections of the height (y) of the plant above the horizontal plane of the anchoring point and of the maximal length of the plant (x) downstream from the anchoring point were measured. The bending angle of the plant was calculated from these measurements: $\varphi = \tan^{-1}(y \times x^{-1})$ (Sand-Jensen, 2003).

During drag measurements, water depth in the flume was maintained at approximately 18.0 cm (18.0±0.5 cm and 18.2±0.5 cm, mean ±SD, for *B. erecta* and *M. aquatica*, respectively, NS, $P < 0.001$, *t*-test).

Plant drag was calculated as the difference between the (plant+device) drag and the drag of the device at the same velocity (see rationale in Sagnes *et al.*, 2000). The drag of the device alone (base or rod) was measured in a comparable velocity range and fitted with polynomial curves to determine the drag values corresponding to each velocity condition used with plants.

The hydrodynamic performance of the plants was assessed through (i) quantitative variables describing relative drag and plant reconfiguration, and (ii) estimation of the absolute overall forces exerted on each plant for the five velocity conditions corresponding to the five patches.

Quantitative measures of hydrodynamic abilities of plants: drag coefficient and E-value: Drag coefficient (Cd) represents drag relative to flow velocity and leaf area exposed to flow. It is usually considered as a dimensionless standard variable allowing comparison of drag experienced by objects of different shapes (Vogel, 1984). Drag coefficients were calculated using the standard equation: Cd=2D/($\rho S U^2$), where D is drag (N), ρ is the density of water (kg m^{-3}), S is the total leaf area (m^2), and U the flow velocity (m s^{-1}). Cd was then plotted against flow velocity. Under similar flow conditions, Cd is lower for more hydrodynamically adapted shapes and typically decreases following an exponential model with increasing flow velocity (Vogel, 1984, 1994). The threshold value of Cd reached by plants at the highest velocities was used as a measure of the ability of a plant to withstand high current velocity. Because flow velocity regulation in the flume is inaccurate for high velocities, a model of the form Cd=$f(U)$=aU^b (a and b are constants) (Bell, 1999) was used for calculating Cd for the upper velocity limit of the water flume (i.e. 1.15 m s^{-1}). Cd (1.15 m s^{-1}) was called the minimum drag coefficient (Cd$_{min}$) and was used for comparing the hydrodynamic abilities of individuals sampled in the different flow patches.

For *B. erecta*, Cd always decreased as flow velocity increased, and the curve shape was consistent with theoretical curves (i.e. a decrease of Cd values with flow velocity). Consequently, all points were included in the model. By contrast, for *M. aquatica*, with increasing experimental velocities, some curves exhibited an initial increase of Cd up to a maximum value (reached for flow velocity around 0.3 m s^{-1}), followed by the expected decreasing trend. This shape for the Cd=f (velocity) curves has already been observed by Hawes and Smith (1995), and could be due to spatial reconfiguration of plant foliage at low velocities (Vogel, 1994). To avoid complex modelling of the initial rise of the curves that probably does not play any role in the Cd$_{min}$ value, the decreasing part of the curves only was used for Cd$_{min}$ estimation. To get homogeneous modelling for all individuals of *M. aquatica*, all curves were treated in the same way: the measurements corresponding to flow velocities below 0.35 m s^{-1} were excluded from the model. To test the validity of the Cd$_{min}$ estimation (i.e. the reality of a threshold value of Cd under the highest velocity conditions used here), for each individual the difference between the Cd values measured at the two highest experimental velocities were calculated. As this difference was, on average, below 5% (5.00% and 4.72% on average for *B. erecta* and *M. aquatica,* respectively), it was considered that the threshold value of Cd was almost attained and that the Cd$_{min}$ (estimated for a flow velocity of 1.15 m s^{-1}) was a reliable estimator of the Cd value reached by each plant at the highest velocity obtained in the water flume.

The *E*-value is a measurement of plant reconfiguration when water velocity increases (Vogel, 1984, 1989). It indicates how drag varies with flow velocity, but is independent of the absolute drag value. The lower the *E* value, the more the plant reconfigures. The *E*-value was
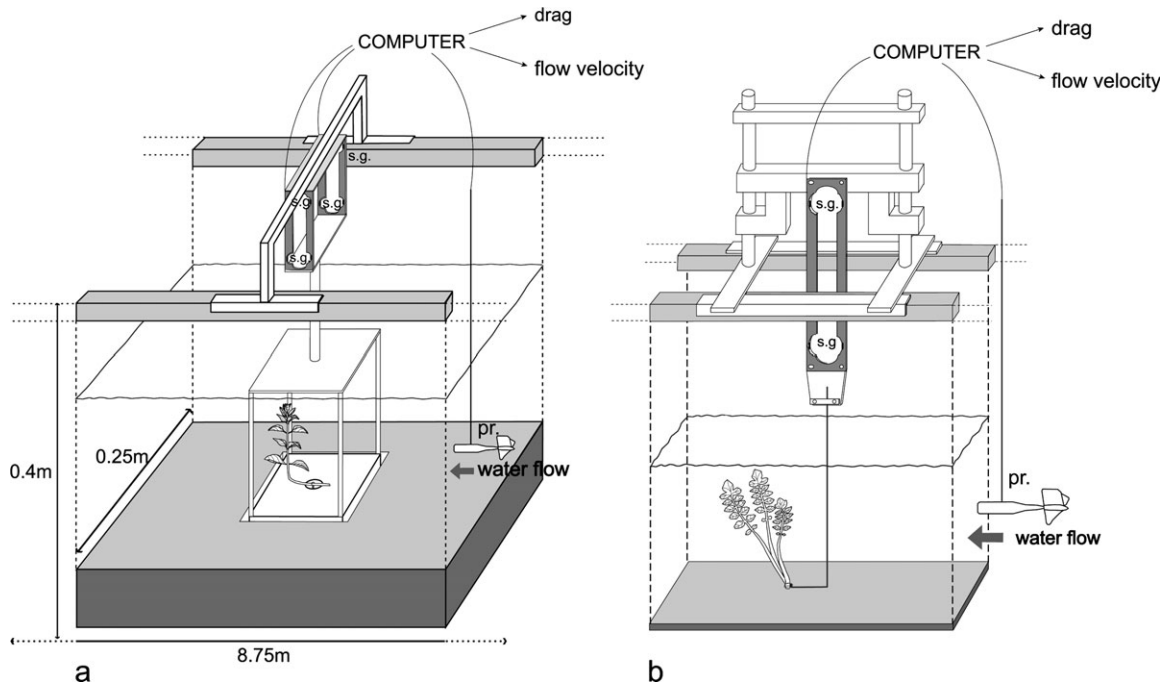
Fig. 2. Hydrodynamic devices used for drag (and associated velocity) measurements for (a) *M. aquatica* and (b) *B. erecta*, respectively. Pairs of strain gauges (s.g., fixed on the flexible part of the device) and propeller (pr) used for velocity measurement are labelled. For *M. aquatica*, the base fixed to the balance was placed inside a hole in the water flume base, in order to place the plant in near-bed flow conditions.

calculated as the slope of a double logarithmic plot of speed-specific drag (D/U^2) against speed (U), for regions of curves without inflection points (following Vogel, 1984). Note that E is equal to exponent b in the equation $Cd=aU^b$ applied to these regions of the curves.

Extrapolated plant drag along the velocity gradient: The drag values that a given individual would have if placed in the different patches can be extrapolated from the experimental drag measurements. For each individual, plots of drag values against velocity were fitted by second order polynomial regression. Fitted curves were next used to calculate, for each plant, the drag it would face if placed in the median velocity of each of the five flow-patches (cf Table 1). Such an estimation should allow the hydrodynamic (dis)advantage of the morphology found in a given patch to be compared to those of the alternative morphologies.

Morphometry

The morphological traits measured on each plant were as follows: (i) plant height (m), defined for *M. aquatica* as the maximum length of the shoot and for *B. erecta,* as the height of the plant above ground; (ii) number of branches for *M. aquatica* and of interconnected stolons for *B. erecta*; (iii) plant mass (g): plants were divided into roots, stems, leaves for *M. aquatica* and into roots, stems, petioles, and leaflets for *B. erecta*. The different parts were weighed to obtain fresh and dry mass (measured after 48 h at 85 °C). (iv) Leaf area (cm²): leaves were scanned (150 dpi, Epson Expression 1680 scanner) and the images were analysed with WinFolia 2001 image analysis software (Regent Instrument Inc., Quebec, Canada). These values were used to calculate two sets of traits.

The first set (five traits) was used to assess the morphological determinants of Cd_{min} and E-values. These traits were selected for their potential relevance in explaining plant hydrodynamics.

(i) Plant height, total leaf area, and above-ground biomass were highly correlated (for *B. erecta*, $r^2=0.59$, $P < 0.001$, and $r^2=0.84$,

$P < 0.001$ for correlations between above-ground biomass, plant height, and total leaf area, respectively; for *M. aquatica*, $r^2=0.55$, $P < 0.001$, and $r^2=0.86$, $P < 0.001$ for the same correlations). Consequently, above-ground biomass (stem and leaves for *M. aquatica* and petioles and leaflets for *B. erecta*) was used as a measurement of plant size and energetic investment in tissue production (Dudgeon and Johnson, 1992; Schutten and Davy, 2000).

(ii) Water content of 'axes' (i.e. stem for *M. aquatica* and petiole for *B. erecta*) and 'photosynthetic surfaces' (i.e. leaves for *M. aquatica* and leaflets for *B. erecta*) were measured for analogous structures that play the same functional role, rather than homologous structures less relevant for contrasting growth forms (rosette with reduced, buried stems for *B. erecta* and large, erect stem for *M. aquatica*). Water content (=1−(dry mass/fresh mass)) should correlate with the proportion of different types of tissues and tends to be negatively correlated with density (Garnier and Laurent, 1994; Pyankov *et al.*, 1999).

(iii) Specific leaf area, SLA=leaf area/leaf dry mass (cm² g⁻¹), correlates with leaf thickness (Garnier and Laurent, 1994; Pyankov *et al.*, 1999) and was used to assess anatomical variation unrelated to water content.

(iv) The bending angle (°) of the plant in flow is an important determinant of plant drag (Sand-Jensen, 2003). It represents the way the plant reconfigures and bends under increasing flow velocity and can be viewed as a synthetic variable expressing flexibility and bending capacity. Two different bending angles were used to explain Cd_{min} and E-value. As Cd_{min} corresponds to the lowest Cd under experimental flow conditions, the lowest angle (φ_{min}) reached by the plant (i.e. highest bending) was used as an explanatory variable of Cd_{min}. The E-value measures the change of drag with flow velocity. Consequently, the difference between bending angles at the lowest and highest flow velocities ($\Delta\varphi$) was used as an explanatory variable of the E-value. A mixed stepwise multiple regression between bending angles (φ_{min} and $\Delta\varphi$) and the four other morphological traits was performed to check that these angles were not redundant

when compared with other traits. Morphological traits explained 31.2% (above-ground biomass) and 27.9% (*SLA* and above-ground biomass) of the variability of φ_{min} for *B. erecta* and *M. aquatica*, respectively. No morphological trait was significantly correlated with $\Delta\varphi$, neither for *B. erecta* nor for *M. aquatica*.

The second set of traits (three traits) was used to compare the fitness of individuals growing in the different flow-patches.

(i) Total dry mass (g) of the ramet (Liao *et al.*, 2003; Pilon and Santamaria, 2002).

(ii) Clonal multiplication: as growth forms of both species differ, the number of stolons connected to the main ramet was used for *B. erecta* (stoloniferous growth) and the number of terminal buds of the ramet (i.e. number of branches potentially able to become an independent ramet) was used for *M. aquatica*.

(iii) Dry mass allocation to storage organs (root and stems) (Storage Organs Ratio=dry mass of storage organs/total dry mass) (Cornelissen *et al.*, 2003).

Finally, as anchorage strength affects the ability of the plant to resist the flow, but was considered as unrelated to the hydrodynamic ability of the above-ground part of the plant, it was felt necessary to measure an anchorage trait as well. Above-ground fresh mass/total fresh mass (above-ground ratio) measures the plant investment in its above-ground biomass, directly submitted to flow stress, compared with total plant biomass, including below-ground biomass participating in plant anchorage.

Statistical analysis

Within-species variations of morphological traits and hydrodynamic parameters along the gradient were assessed through a one-way ANOVA, conducted after tests of normality and homoscedasticity (Kolmogorov-Smirnov and Bartlett's tests) and where necessary, log-transformation of data (Sokal and Rohlf, 1995). The non-parametric Kruskal–Wallis test was used when data did not meet the assumption of normality or equality of variance after being transformed. Spearman's test was used to test the variation tendency of the different variables along the flow velocity gradient. A sequential Bonferroni correction was applied for multiple tests to control a type I error rate (Sokal and Rohlf, 1995).

To evaluate the causal relationship between morphology and hydrodynamic performance, the first set of morphological traits was used in a mixed stepwise multiple regression on both Cd_{min} and *E*-value. The criterion for entry of a trait and for keeping it in the final model was $P <0.05$.

R-Software (R development Core Team 2003) was used for all statistical calculations.

Results

Morphological variations along the flow velocity gradient

All morphological traits differed significantly between the five flow-patches, except $\Delta\varphi$ (for both species) and water content of axes and photosynthetic surface for *M. aquatica* (Tables 2, 3). The above-ground biomass (i.e. size) of *B. erecta,* decreased significantly and φ_{min} increased along the gradient (Spearman test, $P <0.01$ and $P <0.001$, respectively). In *M. aquatica*, *SLA* decreased significantly and above-ground biomass increased (Spearman test, $P <0.05$ and $P <0.001$, respectively).

Concerning fitness-related traits, total dry mass increased by a factor of 2.8 between flow-patches 1 and 5

Table 2. *Mean ($\pm sd$) and ANOVA (a) ($df=4, 35$) or Kruskal–Wallis (k) ($df=4$) tests for morphological traits, fitness-related traits and anchorage traits along the flow-velocity gradient for B. erecta (1=lowest velocity, 5=highest velocity)*

Bold type indicates *P*-values that remain significant after sequential Bonferroni correction.

		Flow-patches (mean ±sd)					ANOVA or Kruskal–Wallis test
		1 (n=8)	2 (n=8)	3 (n=8)	4 (n=8)	5 (n=8)	
Morphological traits	Above-ground biomass (g)	0.55±0.18	0.23±0.12	0.14±0.08	0.25±0.11	0.20±0.11	**P <0.001** (a)
	Water content of axes	0.92± 0.01	0.82±0.10	0.85±0.06	0.90±0.01	0.88±0.06	P=0.02 (k)
	Water content of photosynthetic surface	0.84±0.01	0.63±0.21	0.69±0.19	0.82±0.04	0.75±0.20	**P=0.003** (k)
	SLA (cm² g⁻¹)	554±39	398±76	409±43	436±31	450±53	**P <0.001** (a)
	φ_{min} (°)	17.3±2.7	24.1±5.7	26.8±5.8	29.4±3.1	29.8±4.1	**P <0.001** (a)
	$\Delta\varphi$ (°)	10.1±3.4	11.4±2.9	14.6±7.4	10.0±4.6	11.0±3.3	P=0.3 (a)
Fitness-related traits	Total dry mass (g)	$8.90\times10^{-2}\pm2.89\times10^{-2}$	$6.10\times10^{-2}\pm2.55\times10^{-2}$	$2.80\times10^{-2}\pm9.2\times10^{-3}$	$4.77\times 10^{-2}\pm1.61\times10^{-2}$	$4.16\times10^{-2}\pm2.00\times10^{-2}$	**P <0.001** (a)
	Number of stolons	0.13±0.34	0.91±0.68	1.10±0.85	0.78±1.09	1.44±1.63	**P <0.001** (k)
	Storage organ ratio	0.32±0.12	0.28±0.05	0.31±0.12	0.44±0.03	0.48±0.10	**P=0.001** (k)
Anchorage trait	Above-ground ratio	0.72±0.08	0.74±0.09	0.75±0.18	0.62±0.06	0.55±0.17	P=0.02 (k)

for *M. aquatica* (*P* <0.01, Spearman test) (Table 3). The other traits did not differ significantly between patches (number of terminal buds) or differed between patches but without significant tendency (storage organ ratio, Spearman test, *P*=0.07, Fig. 3). For *B. erecta*, fitness-related traits varied inversely (Table 2), since total dry mass decreased along the gradient by a factor of 2.8, while the number of stolons and the storage organ ratio increased (Spearman test, *P* <0.001, *P* <0.01, *P* <0.001 for these three traits, respectively).

The above-ground ratio differed significantly between flow-patches for both species (Tables 2, 3), but with opposite variation trends along the gradient (negative, *P* <0.001 for *B. erecta* and positive, *P* <0.001 for *M. aquatica*, Spearman test).

Hydrodynamic abilities

Drag coefficients and E-values: The Cd_{min} of *B. erecta* differed significantly between patches (*P* <0.01, ANOVA)

and consistently increased as the average velocity in the corresponding flow-patches increased (Spearman test, *P* <0.001, Fig. 4), indicating that plants experiencing high current speed had higher drag relative to leaf area under high velocity conditions. The Cd_{min} of *M. aquatica* did not differ significantly between the five flow-patches of the gradient (*P*=0.1, ANOVA), even though it decreased significantly between flow-patches 2 and 5 (Spearman test, *P* <0.05, Fig. 4).

E-values did not differ significantly along the gradient for *B. erecta* (*P*=0.3, ANOVA, Fig. 4). They differed significantly for *M. aquatica* (*P* <0.05, ANOVA), but without any apparent trend (Fig. 4).

Extrapolated plant drag along the velocity gradient: For both species, individual plant drag consistently increased with increasing flow velocity (Fig. 5), in accordance with theoretical expectations that higher flow velocity induces higher drag.

For both species (except the null velocity patch), the drag of plants originating from different flow-patches differed

Table 3. *Mean (±sd) and ANOVA (df=4, 35) or Kruskal Wallis (df=4) tests for morphological traits, fitness-related traits and anchorage traits along the flow-velocity gradient for* M. aquatica *(1=lowest velocity, 5=highest velocity)*

Bold type indicates p-values that are still significant with sequential Bonferroni correction.

		Flow-patches (mean ±sd)					ANOVA or Kruskal-Wallis test
		1 (*n*=8)	2 (*n*=8)	3 (*n*=8)	4 (*n*=8)	5 (*n*=8)	
Morphological traits	Above-ground biomass (g)	2.32±0.66	3.94±1.69	3.22±0.68	4.06±2.59	7.17±1.90	**P <0.001** (a)
	Water content of axes	0.85±0.04	0.85±0.02	0.84±0.02	0.86±0.02	0.85±0.03	*P*=0.7 (a)
	Water content of photosynthetic surface	0.88±0.02	0.86±0.01	0.86±0.01	0.87±0.01	0.87±0.01	*P*=0.1 (a)
	SLA (cm^2 g^{-1})	472±108	310±30	349±18	332±61	312±24	**P=0.001** (k)
	φ_{min} (°)	7.34±3.8	12.4±4.4	10.7±4.0	8.27±2.2	7.55±1.6	*P*=0.02 (a)
	$\Delta\varphi$ (°)	8.88±6.2	11.0±7.3	12.4±5.4	8.64±3.0	6.57±2.1	*P*=0.2 (k)
Fitness-related traits	Total dry mass (g)	0.40±0.20	0.65±0.30	0.54±0.12	0.59±0.44	1.11±0.28	**P <0.001** (a)
	Number of terminal buds	1.75±0.71	1.75±0.89	2.50±1.31	1.63±0.92	3.00±1.20	*P*=0.07 (k)
	Storage organ ratio	0.81±0.03	0.69±0.05	0.72±0.06	0.63±0.07	0.76±0.03	**P <0.001** (a)
Anchorage traits	Above-ground ratio	0.81±0.06	0.85±0.04	0.88±0.06	0.93±0.03	0.89±0.07	**P <0.001** (a)
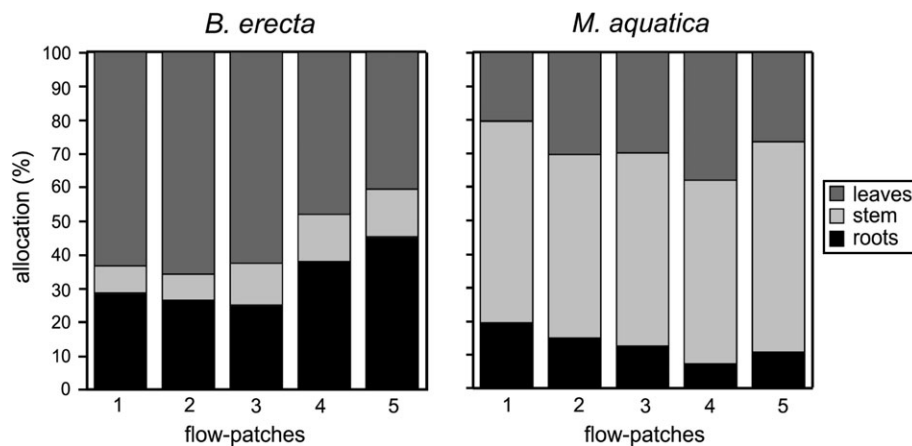
Fig. 3. Biomass allocations of *B. erecta* and *M. aquatica* in the five flow patches ranked along the flow-velocity gradient (1=lowest velocity, 5=highest velocity, Table 1). For the significance of the trends observed, see Tables 2 and 3.
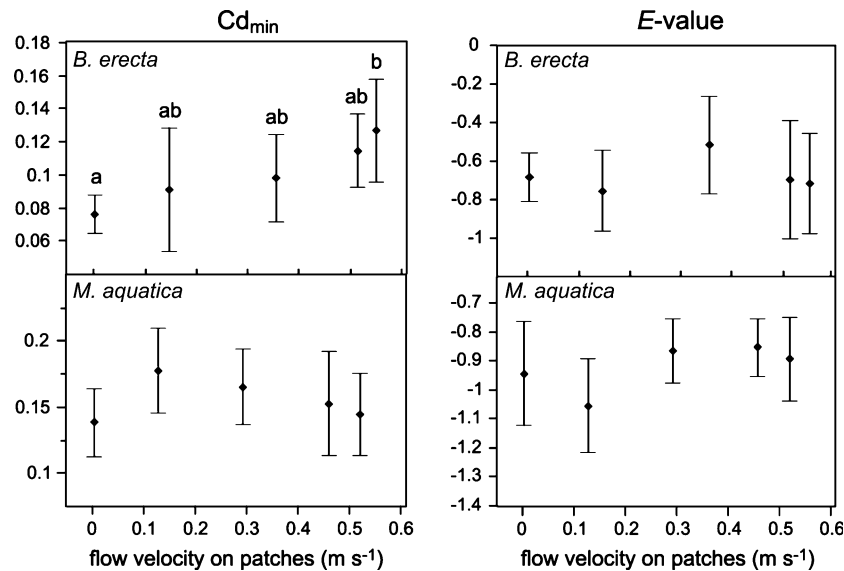
Fig. 4. Average ($\pm$sd) Cd_{min} and E-values of *B. erecta* and *M. aquatica* along the flow-velocity gradient. For Cd_{min} of *B. erecta*, different letters indicate significant differences in means ($P <0.05$, Tukey–Kramer test).

significantly under the flow velocity conditions of a given patch ($P <0.001$, ANOVA) (Fig. 5). Plant drag rankings, according to the patch where they originated are preserved for the four non-null velocities of the gradient, but varied in the opposite way for the two species. For a given flow velocity (except 0), plant drag is significantly negatively correlated with the flow-patch of origin for *B. erecta* ($P <0.01$ for all non-null flow velocities, Spearman test): plants from high velocity patches have at least half the drag values of plants from low velocity patches (Fig. 5). For *M. aquatica*, at a given flow velocity (except 0), plant drag is significantly positively correlated with the flow-patch of origin: plants of more exposed flow-patches have higher drag values than plants of less exposed flow-patches ($P <0.001$ for all non-null flow velocities, Spearman test) (Fig. 5).

Relationships between morphology and hydrodynamics: morphological determinants of Cd_{min} and E-values

For both species, the minimal bending angle is the only significant trait explaining Cd_{min}. This morphological trait explained 49.1% of the variability of Cd_{min} for *B. erecta* ($P <0.001$) and 14% for *M. aquatica* ($P <0.05$).

For both species, no morphological traits correlated with the E-value.

Discussion

Morphological and hydrodynamic differences along the flow-velocity gradient

Both species exhibited significant morphological differences along the flow velocity gradient. The sharp size

reduction of *B. erecta* with increasing flow velocities is a very frequent response for individuals of a given species exposed to mechanical stresses (Jaffe, 1973; Gaylord *et al.*, 1994; Idestam-Almquist and Kautsky, 1995; Blanchette, 1997). The size increase of *M. aquatica* constitutes a rarely observed response for higher plants (Jaffe, 1973; Coops and Van der Velde, 1996).

Increased allocation to below-ground biomass observed for *B. erecta* has been demonstrated to increase anchorage strength (Crook and Ennos, 1996; Goodman and Ennos, 1996). Surprisingly, biomass root allocation decreased along the gradient for *M. aquatica*, suggesting lower anchorage strength for high flow velocity and therefore an increased uprooting risk. Such a strategy may increase the dispersal ability of this species in a high flow habitat.

For both species, the only trait explaining Cd_{min} is the minimal bending angle of the plant. This is consistent with previous studies demonstrating that flexible organisms have lower drag because they bend and reconfigure into more streamlined shapes (Koehl, 1977; Vogel, 1994, Usherwood *et al.*, 1997). Bending angle depends not only on flexibility but also on the force exerted on the plant, which causes bending (Sand-Jensen, 2003). Bending places the plant closer to the substrate in a zone of lower flow velocity, which decreases plant drag (Koehl, 1977; Vogel, 1994; Sand-Jensen, 2003).

Unexpectedly, morphological traits were not correlated with Cd_{min} and E-values (Schutten and Davy, 2000). The bending angle only explained less than half of the Cd_{min} variability. Carrington (1990) previously demonstrated that Cd mainly correlated with the algal surface exposed to flow and not to morphology *per se*. However, the anatomical complexity of higher plants suggests that tissues characteristics (proportion and deformation ability) should be
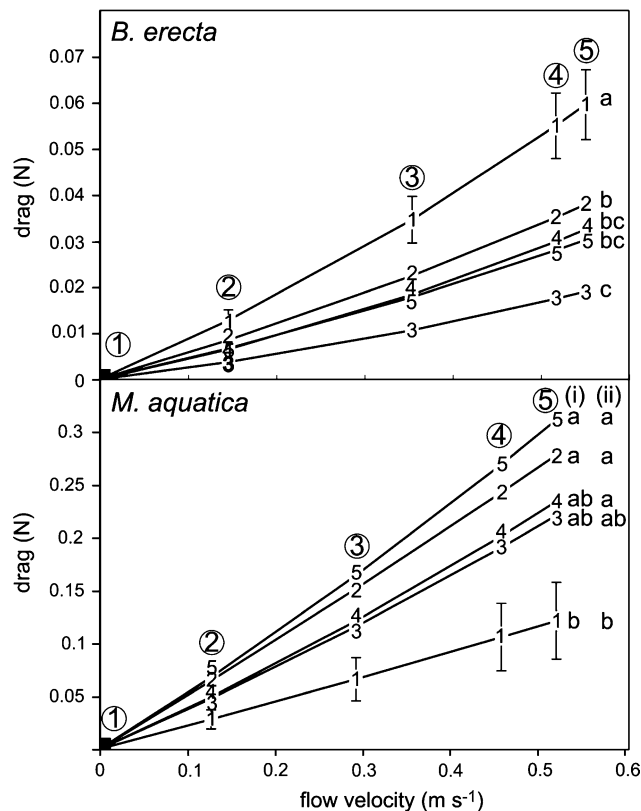
Fig. 5. Extrapolated plant drag for the median flow velocities (see Table 1) of the five patches sampled (circled numbers). Numbers of the curves indicate the flow patch of origin of the plants sampled. Examples of standard deviation are given for curves of the plant from flow-patch 1 for both species. On the right side of each plot, the different letters indicate significant differences between the average drag of plants by flow-patch ($P <0.05$, Tukey–Kramer HSD). For *B. erecta*, differences are consistent for the four non-null flow velocities. For *M. aquatica*, indicated differences relate to flow-patches 2 and 3 (labelled (i)) and 4 and 5 (labelled (ii)).

considered for an accurate description of plant biomechanics (Patterson, 1992; Usherwood *et al.*, 1997).

For *B. erecta,* minimization of plant drag results from a size reduction, without particular shape streamlining (higher Cd_{min}) probably because of the lower bending ability of small plants compared with larger ones. However, the sharp size-reduction leads to a reduction in overall drag forces on plants and places them partly within the boundary layer where flow velocity is reduced (Koehl, 1977, 1982; Sheath and Hambrook, 1988). This flow stress 'avoiding strategy' has already been described between species (animals, algae or macrophytes; Koehl, 1977; Denny *et al.*, 1985; Sheath and Hambrook, 1988), but also within species, with dwarfing of more exposed individuals (Idestam-Almquist and Kautsky, 1995; Blanchette, 1997). The apparent decrease in plant performance (drag increase and decrease of allocation to roots) observed for *M. aquatica* is an uncommon response to mechanical constraint. Taller plants are more likely to be damaged under high flow conditions (as demonstrated for macroalgae, Gaylord *et al.*, 1994; Blanchette, 1997).

However, the high stem breaking force (>8.8 N by pulling along the stem, preliminary results) probably preserves *M. aquatica* from breaking in high flow velocity patches (where it faces 0.3 N only, Fig. 5). Anchorage allocation decreased along the gradient suggesting an increased uprooting risk and subsequent increase of plant dispersal. However, a very high density of strongly anchored runners has been observed for individuals colonizing high velocity patches, suggesting a higher number of anchorage points relative to the number of erect ramets within the same individual. Changes in anchorage efficiency could be quantified more accurately through calculating, for each plant, the ratio of the uprooting force to the drag force (Crook and Ennos, 1996; Pratt and Johnson, 2002).

Adaptive value of morphological changes and maintenance strategies of both species

Size-limitation of the above-ground part of *B. erecta* could at first be considered as an important reduction in plant fitness. However, the close relationship between size and drag makes it necessary to consider other fitness-related traits. Plant investment in clonal reproduction and storage increased with current velocity (Table 2), suggesting that plants would maintain their fitness, at least in part, through a spatial biomass reallocation at the whole individual level, when velocity increases. Biomass did indeed appear reorganized, from a vertical organization in low flow-velocity patches (one tall ramet), to a horizontal organization with the biomass concentrated close to the substrate (shorter individual avoiding high flow velocities, divided into many interconnected ramets, with higher storage) in high velocity patches. Horizontal reorganization of biomass would, if this hypothesis is verified, be a way of partly reducing the detrimental effect of a reduction in ramet size.

Trends observed for *M. aquatica* (drag increase, decrease of root biomass) probably do not affect its survival ability, because of its very strong stem resistance and the probable underestimation of anchorage strength. The size increase combined with the decrease of drag coefficient (for high velocities) could indicate an increase of plant performance and fitness. However, the size increase could result from indirect effects: (i) increased current velocity could reduce boundary layer thickness at the plant surface (Westlake, 1967; Madsen and Sondergaard, 1983), thus favouring plant growth; (ii) under high flow velocities, foliated shoots of *M. aquatica* are brought closer to the substrate, where flow stress is lower, thus favouring their growth, and the growth of new anchorage points along the ramet; (iii) reconfiguration of leaves occurred below 20 cm s^{-1} in the flume (S Puijalon, personal observation). When a plant reconfigures, leaves stack up on the stem, inducing self-shading, and probably reducing their photosynthetic efficiency (as only the lower face of leaves remains exposed to light) (Koehl and Alberte, 1988). For patches 2 to 5, light stress could,

consequently, also promote stem elongation (Pilon and Santamaria, 2002). Finally, the fitness-related traits that were measured did not permit the conclusion that the morphological changes occurring along the gradient, even when they are not directed towards greater hydrodynamic performance, decrease plant fitness.

This study has emphasized the importance of size in mechanically stressful habitats, with both species exhibiting completely opposed responses. Changes in terms of morphology or size, exhibited by a given species exposed to flow, are probably restricted by: (i) developmental constraints linked to plant morphology and (ii) trade-offs between major plant functions (e.g. light capture, nutrient acquisition), which require the efficiency of the organs that carry out these functions. The response in terms of trait variations and hydrodynamic performance observed for *M. aquatica* could be due to the necessary trade-off between a morphology that maximizes light interception and one minimizing drag (Koehl and Alberte, 1988; Vogel, 1989). For *B. erecta*, rosette growth form does not allow the plant to adapt through stem morphological variation (e.g. stem length or flexibility), but enables the plant to produce a constant leaf number, whatever its size. On the other hand, an erect stem (in the case of *M. aquatica*) probably offers better opportunities for the reorganization of leaves along the axis.

Acknowledgements

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