American Journal of Botany 84(12): 1743–1751. 1997.

VALLISNERIA AMERICANA (HYDROCHARITACEAE) AS A BIOMONITOR OF AQUATIC ECOSYSTEMS: COMPARISON OF CLONED GENOTYPES[1]

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We assessed the effects of local environment on survival, growth, and development in six clones (genotypes) of *Vallisneria americana* grown at five sites in the Huron-Erie Corridor. Detrimental effects of local environment on plant performance (rate of clonal growth, leaf and root production, surface area of leaves and roots, plant biomass, rate of flowering, and turion production) were correlated with sediment toxicity and levels of organic contamination determined in independent studies, and differed among plant genotypes. All surviving clones used in the study ranked environmental quality of the five sites in the same order. Two genotypes, which were tolerant of contaminants, survived the 2 yr of exposure at all sites, while other nontolerant clones died within the 1st yr of study, at the two most contaminated sites. The leaf-to-root surface area ratio was highly indicative of site quality, and was not affected either by year-to-year variation, or by differences between genotypes. The use of cloned plants in this biomonitoring study reduced variance, and increased precision and accuracy of site assessment compared to biomonitoring with genetically variable plants. Clones of *V. americana* tolerant of contaminants were particularly useful in assessing the most contaminated sites. An approach that uses an array of both tolerant and nontolerant clones is recommended.

Key words: aquatic rooted macrophyte; biomonitors; contaminants; genotypes; Hydrocharitaceae; leaf-to-root surface area ratio; tolerance; *Vallisneria.*

Submersed aquatic vascular plants are an important component of aquatic ecosystems, affecting biotic, physical, and chemical interactions. They provide oxygen, mineral nutrients, shade, substrate, shelter, breeding and nursery areas, and food for an array of organisms (see Catling et al., 1994). Macrophytes may also affect the physical characteristics of the aquatic environment. Beds of submersed plants impede wave action, influence currents, shade sediments and understory organisms, increase sedimentation, decrease turbidity, and increase water clarity. Furthermore, they affect physical and chemical processes that may alter sediment redox potentials, and local water and sediment pH (St-Cyr, Campbell, and Guertin, 1994). Plants are involved in nutrient cycling and nutrient exchange between water and sediment, thus significantly affecting water and sediment quality (Barko, Gunnison, and Carpenter, 1991; Nichols, 1991; Petticrew and Kalff, 1992; Catling et al., 1994).

Vallisneria americana is one of the most common submersed macrophytes in the Huron-Erie Corridor of the Great Lakes (Schloesser and Manny, 1990; Lovett-Doust and LaPorte, 1991; Catling et al., 1994). The species suffered a significant regional decline in abundance over the past two decades (Schloesser and Manny, 1990; Manny and Kenaga, 1991), but the surviving populations of this species are now expanding. *Vallisneria* is capable of extensive clonal growth; a single individual can produce up to 30 genetically identical new ramets (units of clonal growth) within a growing season (Catling et al., 1994; Lokker et al., 1994).

In the past, methods have been developed to assess overall quality and toxicity of sediments or water using bacteria, insects, and crustaceans (Giesy et al., 1988). However, there is a need to develop similar procedures using plants, which are located at the base of most food chains. Macrophyte growth and reproduction are directly dependent on the quality of sediments and water around them. Submersed aquatic plants are capable of absorbing metals and organic chemicals and breakdown products of organochlorine pesticides, herbicides, polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), octachlorostyrene (OCS), solvents, surfactants, and polyaromatic hydrocarbons (PAHs), from both the sediment and the water (Crowder and Painter, 1991; Guilizzoni, 1991; Lovett-Doust, Lovett-Doust, and Biernacki, 1994; Lovett-Doust, Schmidt, and Lovett-Doust, 1994; Lewis, 1995). Changes in contaminant concentrations in plant tissues have been found to be significantly correlated with measures of plant growth and development, and assessment of plant performance has been used as a tool for inexpensive environmental monitoring (Lovett-Doust, Lovett-Doust, and Biernacki, 1994; Lovett-Doust, Schmidt, and Lovett-Doust, 1994; Biernacki, Lovett-Doust, and Lovett-Doust, 1995a, b, 1996; Biernacki, 1996). The use of genetically identical individuals for biomonitoring may have additional advantages. Baird (1992) and Lovett-Doust, Schmidt, and Lovett-Doust (1994) suggested that the use of genetically identical individuals for biomonitoring may allow for precise, accurate, repeatable, and reliable results compared to results based on exposures of genetically mixed populations. On the other hand, a limited selection of clones may underrepresent the range of responses of organisms within a

[1] Manuscript received 12 June 1996; revision accepted 13 May 1997.

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This research was part of the Ph.D. work of MB; we are grateful for the support of an Environmental Research Program Grant provided by the Ontario Ministry of the Environment and Energy under the direction of Mr. D. Harper, for NSERC Research grant support to JLD, and an Environment Canada/NSERC Great Lakes University Research Fund award.

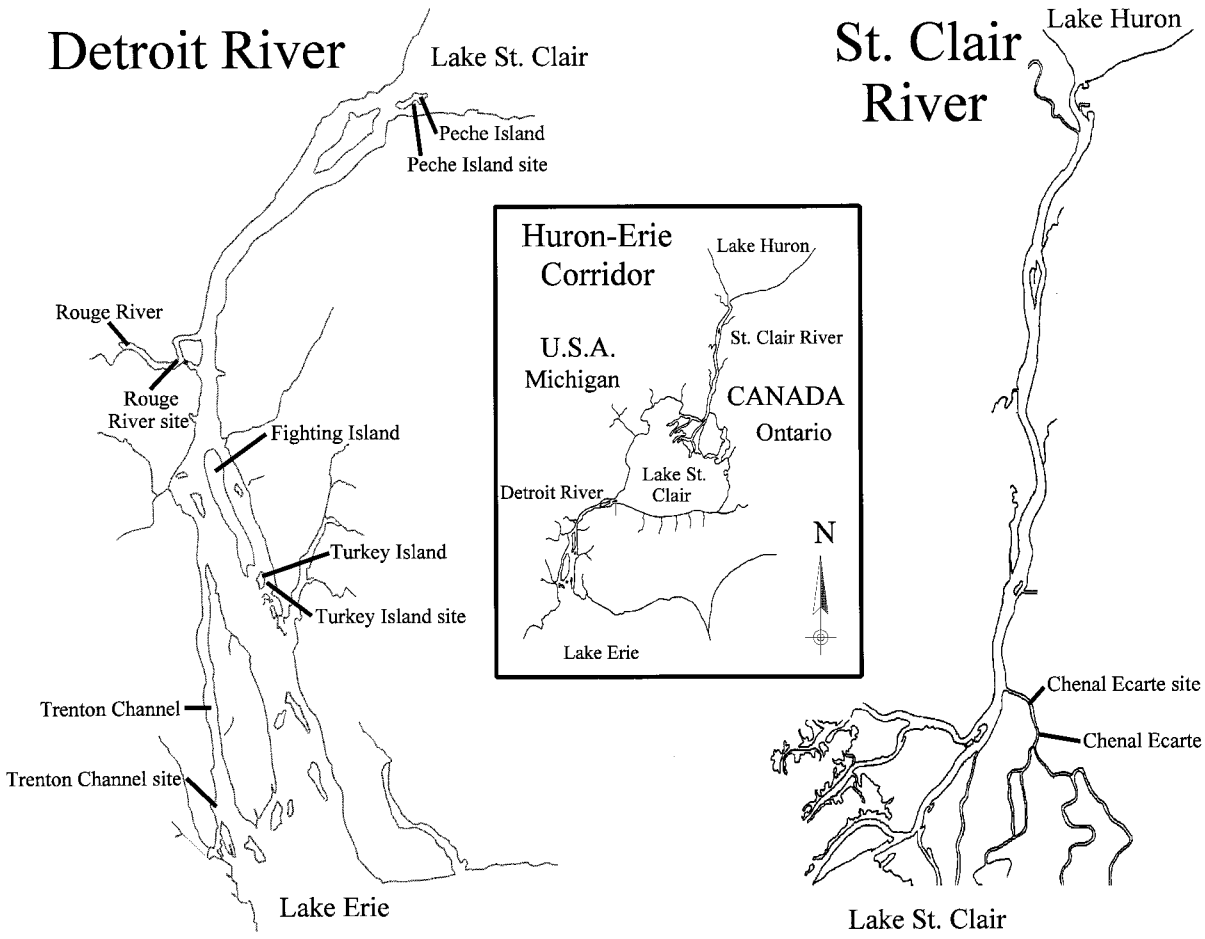

Fig. 1. Map of Huron-Erie corridor with location of experimental sites.

population, particularly if the clones of experimental organisms that are used are more tolerant to contaminants than most organisms (Guilizzoni, 1991; Forbes and Depledge, 1992).

In our earlier field and greenhouse studies we determined that some individuals of *Vallisneria americana* are particularly tolerant of elevated concentrations of contaminants (Biernacki, Lovett-Doust, and Lovett-Doust, 1995b, 1996). Some clones of *Vallisneria americana* survived the 2-yr exposure to high concentration (396 mg/L) of trichloroethylene (TCE), while most (98%) of the other individuals collected from the same field sites did not. It is likely that surviving plants in highly polluted areas may have evolved tolerance to contaminants (Biernacki, Lovett-Doust, and Lovett-Doust, 1995a). One concern is that use of such tolerant organisms in biomonitoring studies could produce a false sense of security, and would significantly underestimate environmental impairment due to contamination.

The present study was designed to estimate the utility of selected genotypes (specifically, genotypes known to be tolerant and nontolerant of contaminants) of *V. americana* for biomonitoring and assessing environmental quality. Our primary objective was to compare performance of selected genotypes at five experimental sites representing a gradient in environmental contamination, over 2 yr of exposure, and to evaluate their utility for biomonitoring.

MATERIALS AND METHODS

Five sites were studied in the Huron-Erie corridor of the Great Lakes, the section of connecting channels between the upper and lower Great Lakes. Contamination of the corridor by organochlorines and heavy metals [for example: PCBs (polychlorinated biphenyls), PAHs (polyaromatic hydrocarbons), OCS (octachlorostyrene), HCB (hexachlorobenzene), mercury, cadmium, lead, zinc, chromium], often exceeding Canadian and American guidelines, is well documented (e.g., Pugsley et al., 1985; Giesy et al., 1988; Manny and Kenaga, 1991). High levels of organic contaminants and heavy metals have been found in the Detroit River (including its tributary, the Rouge River) and the St. Clair River. Both rivers are recognized as Areas of Concern by the International Joint Commission (International Joint Commission, 1992). One site was located on the Chenal Ecarte in the delta of the St. Clair River, east of Walpole Island between Port Lambton and Wallaceburg, Ontario (Fig. 1). The other four sites were located in the Detroit River: one on the southern shore of Peche Island, in the mouth of the Detroit River; a second on the south-eastern shore of Turkey Island, downstream of Fighting Island; a third on the shore of the Trenton Channel, ~1 km upstream of Lake Erie; and a fourth in the delta of the Rouge River (Fig. 1). At all locations, natural populations of *V. americana* var. *americana* were present. Sites were selected to represent a gradient in environmental quality based on sediment toxicity and site contamination

reported in the following studies. Giesy et al. (1988) ranked toxicity of sediments in the Detroit River (using results of the *Daphnia magna* 48-h lethality test, the *Chironomus tentans* 10-d growth-reduction test, and the *Photobacterium phosphoreum* 15-min bioluminescence test), including three sites in the present study. According to Giesy et al. (1988), Turkey Island site sediment was less toxic than Trenton Channel or Rouge River sediments, and Rouge River sediment was more toxic than Trenton Channel sediment, collected at locations also used in our study. Lovett-Doust, Lovett-Doust, and Biernacki (1994) showed that Chenal Ecarte sediment was more toxic to submersed plants than Turkey Island sediment. Finally, Pugsley et al. (1985) found that both sediment and clams collected at Peche Island were least contaminated with PCBs and OCS compared to other Detroit River or St. Clair River samples. On the basis of these studies we ranked toxicity of sediments at our experimental locations as: Peche Island site was least contaminated, followed by the Turkey Island site, Chenal Ecarte site, Trenton Channel site, and the Rouge River site.

Since the summer of 1991, we have cultivated selected genotypes of dioecious *V. americana* var. *americana* (see Catling et al., 1994 for complete species description and Lowden, 1982 for taxonomic details) in an area surrounding Turkey Island in the Detroit River (Fig. 1). Over 2 yr, plants were allowed to propagate clonally, and ramets were confined separately in large plastic tubs (36 cm width × 46 cm length × 12 cm height). In early August 1993, genetically identical ramets were collected from each tub. The following genotypes were used in the study: (1) female plant originating from the Rouge River area that had already demonstrated tolerance to increased organochlorine contamination (see Biernacki, Lovett-Doust, and Lovett-Doust, 1996); (2) male plant originating from Chenal Ecarte, that had shown tolerance to increased TCE concentrations (see Biernacki, Lovett-Doust, and Lovett-Doust, 1995b); (3) female plant originating from Chenal Ecarte, that had shown tolerance to increased TCE concentrations (see Biernacki, Lovett-Doust, and Lovett-Doust, 1995b); (4) randomly sampled female plant originating from Chenal Ecarte, presumed to be nontolerant; (5) randomly sampled male plant originating from Chenal Ecarte, presumed to be nontolerant; (6) randomly sampled male plant originating from Turkey Island, presumed to be nontolerant.

In addition to field cultivation, the above genotypes of *V. americana* were grown in a greenhouse at the University of Windsor over a 3-yr period (1992–1995) to compare their performance in common sediment and environmental conditions. Plants were rooted in sediment and grown in aquaria (31 cm width × 92 cm length × 62 cm height) filled with water, with no additional nutrients in the water or sediment. There were three replicate aquaria for each genotype. Nondestructive data were collected monthly over the growing cycle, and destructive data were recorded once at the end of each growing cycle. Plants grown in the greenhouse completed two growing cycles per year.

Sediment at each site had been collected from a water depth of 0.5–0.8 m, and manually sieved to remove any plants or plant parts. Processed sediment was placed in plastic tubs and stored in shallow water (0.6 m deep). *Vallisneria* was planted in the tubs after 2 d acclimation.

In August 1993, ramets were carefully removed from their culturing tubs and intact, undamaged plants were stored in labelled, large plastic containers fully submerged in water and placed overnight in a coldroom at 6°C. The following day, ten ramets were planted in each of 30 plastic tubs (36 cm width × 46 cm length × 12 cm height), filled with local sediment, at each of the five locations. A total of 1500 *Vallisneria* ramets (250 ramets of each of six genotypes) were therefore deployed at the five sites as follows: there were five replicate tubs of each of six genotypes, at each site. Every tub contained, initially, ten identical (but physiologically separate) ramets. Tubs were set into the sediment in water that was 0.8–1.2 m deep, depending upon site. Plant leaves therefore extended into the local water column, and the roots were growing in local sediment in the tubs. All tubs were permanently marked.

Data concerning plant survival, clonal growth (production of new ramets), leaf number per ramet, and leaf number per unit area were collected once a month throughout the growing season (May–October) for 2 yr. Data were collected separately for each replicate tub at each location. During flowering, information about the number of male and female flowers per shoot and per tub was recorded. On several occasions, water temperature at the surface of the sediment in tubs was determined.

In mid-September, 1994 and 1995, two tubs representing each plant genotype were harvested at each location. Plants were carefully removed from the tubs, and sediment was gently washed away to minimize damage to roots, leaves, and other plant parts. Plants were immersed in cold water until they were processed. For each plant, in each tub, the number of leaves and roots, their individual lengths, leaf width, root diameters, and number of flowers were determined. Leaf and root surface areas were estimated according to the procedure described in Biernacki, Lovett-Doust, and Lovett-Doust (1996). Fresh biomass of leaf, root, stolon, and turion (overwintering organ) was determined in order to characterize the pattern of biomass distribution for ramets at each location. For analyses and figures that follow, all nondestructive and destructive measurements have been converted to performance per square metre (measurement per tub was divided by 0.1656 m^2 surface area of a tub).

Coefficients of variation for a range of measures of plant performance, for each of the genotypes of *Vallisneria* used, were compared with those in another study carried out in 1994 at two of the sites studied here (Turkey Island and Chenal Ecarte, described in Lovett-Doust, Lovett-Doust, and Biernacki, 1994 and Biernacki, 1996). In that study, a mixture of ten different genotypes of *Vallisneria* was initially planted in each tub. Plants compared between the studies had grown over the 1994 growing season in the same sediment, the same local water column, and following the same research protocol. Significance of differences between coefficients of variation in the two studies (i.e., using either single genotypes, or multiple genotypes of *Vallisneria*) was tested using the Kruskal-Wallis test. Coefficients of variation associated with various measures of plant performance in both studies were ranked in decreasing order.

Data were analyzed with SYSTAT for Windows version 7 (1997), using the Kruskal-Wallis nonparametric procedure and the Scheirer-Ray-Hare extension of the Kruskal-Wallis procedure (Sokal and Rohlf, 1995). Differences among sites were tested using the Kolmogorov-Smirnov two-sample test (Sokal and Rohlf, 1995). Nonparametric procedures have been used because data were not normally distributed, and/or variances were not homogeneous and transformations were not able to correct these problems. Nonparametric Spearman rank correlation analysis was used to test for correlations between the relative ranks of site sediment toxicity based on published literature (sites were ranked increasingly with increased toxicity), and the relative rank of plant performance at experimental sites based on results of the present study.

RESULTS

Selected genotypes of *Vallisneria americana* raised at the University of Windsor greenhouse in a common sediment type and the same general conditions differed significantly from each other in at least one of the basic measures of plant morphology, growth, or reproduction observed over six growing cycles (Table 1). There were no significant differences in plant growth and reproduction between replicate aquaria of the same genotype.

Weather patterns did not differ significantly between the 1994 and 1995 growing seasons (Environment Canada, 1994, 1995). Also, water temperature (mean 23.4°C and SD = 3.1°C), measured at the end of July, August, and September, was not significantly different among the sites ($P > 0.05$, $F = 1.377$ with df = 4, 70). However, the water temperature was significantly lower ($P < 0.001$,

TABLE 1. Summary of measures of growth and reproduction in different genotypes of *Vallisneria americana* grown in common sediment in glass aquaria (31 cm width × 91.5 cm length × 61 cm height) in six growing cycles (two cycles per year) over 1992–1995 at the greenhouse of the Department of Biological Sciences, University of Windsor. *F* values (and degrees of freedom), significance (*** indicates $P \leq 0.001$), mean values (and standard deviation) per growing cycle, for selected measures of plant performance are shown. Means marked with different superscript letter differed significantly at $P \leq 0.05$.

Trait	*F* (df)	Genotype 1	2	3	4	5	6
Plant sex		Pistillate	Staminate	Pistillate	Pistillate	Staminate	Staminate
Site of plant origin		Rouge River	Ecarte	Ecarte	Ecarte	Ecarte	Turkey Is.
Tolerance to TCE		Yes	Yes	Yes	No	No	No
Rate of clonal growth	1096.3*** (5, 1494)	9.3^{a}(3.1)	2.1^{b}(0.4)	1.9^{b}(0.5)	2.9^{c}(0.7)	3.1^{c}(0.6)	2.2^{b}(0.5)
Number of leaves per ramet	147.3*** (5, 1494)	18.4^{a}(4.4)	12.4^{b}(2.1)	11.3^{b}(2.2)	13.6^{c}(3.4)	15.1^{d}(2.9)	16.6^{e}(3.6)
Number of flowers per ramet	1017.4*** (5, 1494)	6.2^{a}(1.6)	1.6^{b}(0.3)	3.4^{c}(0.6)	1.4^{b}(0.5)	2.9^{d}(0.6)	3.5^{c}(0.8)
Number of roots per ramet	339.3*** (5, 1494)	84^{a}(26)	47^{b}(14)	61^{c}(12)	36^{d}(12)	29^{e}(14)	51^{f}(19)
Biomass per ramet (g)	3119.6*** (5, 1494)	9.9^{a}(1.2)	1.9^{b}(0.5)	3.2^{c}(0.6)	2.4^{d}(0.7)	2.3^{d}(0.6)	4.7^{e}(1.3)
Number of turions per ramet	545.9*** (5, 1494)	5.7^{a}(2.1)	2.3^{b}(0.3)	1.7^{c}(0.2)	2.7^{d}(0.5)	3.3^{e}(0.6)	2.2^{b}(0.4)
Length of a leaf (cm)	118.4*** (5, 1494)	67^{a}(36)	27^{b}(14)	41^{c}(19)	38^{cd}(17)	34^{d}(19)	62^{a}(28)
Biomass of a turion (g)	4200.0*** (5, 1494)	1.69^{a}(0.31)	0.29^{b}(0.05)	0.32^{b}(0.07)	0.19^{c}(0.03)	0.21^{c}(0.04)	0.82^{d}(0.12)

$F = 7.383$ with df = 4, 41) at Chenal Ecarte (mean 12.8°C and SD = 2.3°C) than at the other sites (mean 18.6 and SD = 2.9°C) at the end of June; this may explain why plants at Chenal Ecarte were phenologically 1–2 wk behind plants at the other sites at that time. At all of the experimental sites the Secchi disk was visible to the bottom (0.8–1.2 m).

Results of the Kruskal-Wallis test revealed significant effects of site of exposure and plant genotype on plant performance (Table 2). Plant phenology (seen as a significant ''month'' effect) also influenced plant growth and development. Differences in growing seasons (year effects) significantly affected plant flowering. Performance of the three tolerant genotypes (genotypes 1, 2, and 3) was significantly different from that of the three nontolerant clones ($P < 0.001$).

The number of ramets produced per square metre was significantly greater ($P < 0.001$) for all genotypes at Peche and Turkey Island sites, and least at Trenton Channel and Rouge River sites (Table 3, Fig. 2). Genotype 1 had significantly greater densities of ramets per square metre at Trenton Channel and Rouge River than any other genotype at these sites. Genotypes 4 and 5 did not survive the first growing season at Trenton Channel, nor did genotypes 4, 5, and 6 at the Rouge River site. Furthermore, all replicates of genotype 2 died at the Rouge River site in the 1995 growing season.

There were significant differences in the rate of clonal growth (i.e., number of new ramets produced by the parent ramet over a growing season) between different sites (Table 3); plants at Peche and Turkey Island sites had the greatest rates of clonal growth; while plants at Trenton Channel and Rouge River had the lowest (see Appendix, also Biernacki, 1996). The rate of clonal growth was greatest for genotype 1, and least for genotype 6 at all sites. However, while there was variation in the rate of clonal growth within growing seasons, there was no significant difference in clonal growth at sites between the 2 yr (Table 2).

The number of leaves per square metre at each site showed a similar pattern to that for ramet density (see Biernacki, 1996). Plants growing at the Peche and Turkey

TABLE 2. Summary of probabilities following Kruskal-Wallis test (and the Scheirer-Ray-Hare extension of Kruskal-Wallis procedure) for effects of main factors on measures of growth and reproduction in *Vallisneria americana.*

Trait	Factors: Year	Month	Site	Genotype
(df)	(1)	(4)	(4)	(5)
Nondestructive monitoring				
Number of ramets per m^2	0.853	0.0001	0.0001	0.012
Number of leaves per m^2	0.876	0.0001	0.0001	0.0001
Number of leaves per ramet	0.947	0.0001	0.063	0.0001
Number of flowers per m^2	0.012	0.0001	0.021	0.001
Rate of clonal growth	0.286	0.0001	0.0001	0.017
Destructive monitoring				
Number of turions per m^2	0.976		0.0001	0.035
Biomass of ramets per m^2	0.988		0.0001	0.0001
Biomass per ramet	0.970		0.0001	0.0001
Turion-to-ramet biomass ratio	0.733		0.0001	0.0001
Biomass of a turion	0.894		0.0001	0.0001
Leaf-to-root biomass ratio	0.870		0.008	0.012
Leaf-to-root surface area ratio	0.912		0.0001	0.979

TABLE 3. Summary of Kolmogorov-Smirnov two-sample test for differences among sites, based on measures of growth and reproduction in *Vallisneria americana.* Sites marked with different letters differed significantly ($P < 0.05$).

Trait	Site: Peche Island	Turkey Island	Chenal Ecarte	Trenton Channel	Rouge River
Nondestructive monitoring					
Number of ramets per m^2	a	a	b	c	c
Number of leaves per m^2	a	a	b	b	b
Number of leaves per ramet	a	a	b	b, c	c
Number of flowers per m^2	a	a	b	c	c
Rate of clonal growth	a	b	c	d	d
Destructive monitoring					
Number of turions per m^2	a	b	c	d	d
Biomass of ramets per m^2	a	b	c	d	d
Biomass per ramet	a	b	c	c, d	d
Turion-to-ramet biomass ratio	a	a	b	c	c
Biomass of a turion	a	b	c	d	d
Leaf-to-root biomass ratio	a	a	b	c	c
Leaf-to-root surface area ratio	a	b	c	d	e

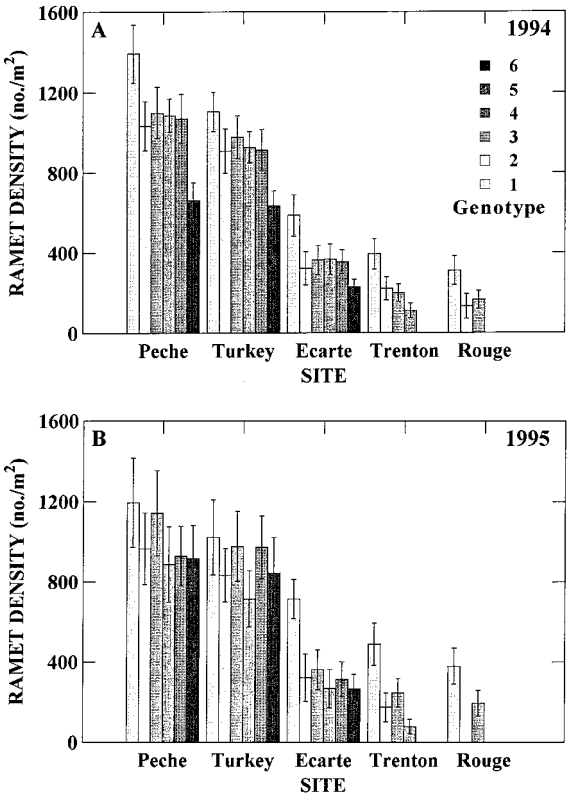

Fig. 2. Mean density (±1 SE) of *Vallisneria* genotypes raised at different sites in (A) 1994 and (B) 1995. Individual genotypes are indicated by numbers from 1 to 6.

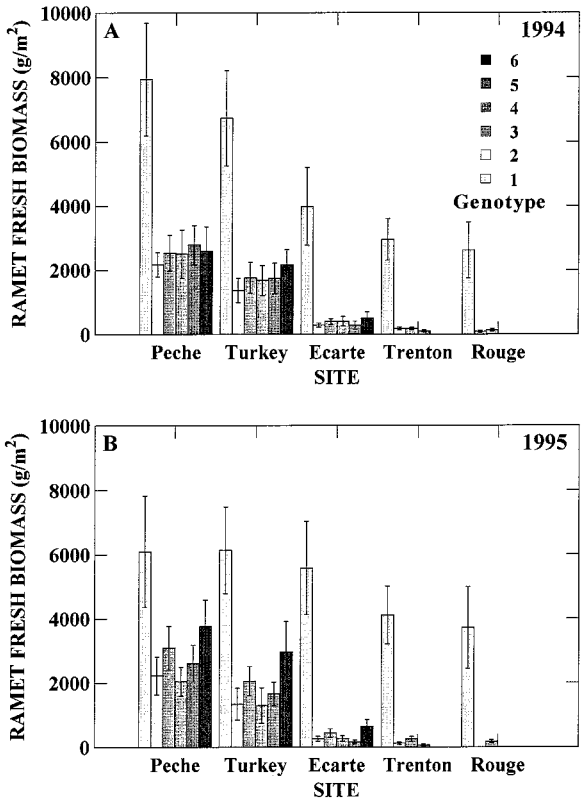

Fig. 3. Mean fresh biomass of ramets (±1 SE) of *Vallisneria* genotypes raised at different sites in (A) 1994 and (B) 1995. Individual genotypes are indicated by numbers from 1 to 6.

Island sites had significantly more leaves per square metre than plants grown for 2 yr at the Trenton Channel and Rouge River sites (Tables 3 and 2, respectively). The Chenal Ecarte site was intermediate between these values. At each site, genotype 1 typically had nearly three times as many leaves per square metre as any other genotype. The number of leaves per ramet differed significantly according to site (Table 3); plants had fewest leaves per ramet at the Peche and Turkey Island sites and most at Trenton Channel and Rouge River sites, over 2 yr (see Biernacki, 1996). Genotype 1 had nearly twice the number of leaves per ramet found in any other genotype.

Different growing seasons (years) and plant phenology (''monthly'' differences) both had significant effects upon flower production in *Vallisneria* (Table 2). At Chenal Ecarte, Trenton Channel, and Rouge River flowering was diminished compared to the Peche and Turkey Island sites (Table 3), where plants of both sexes flowered each year. At the Rouge River site, the only plant that flowered over the 2-yr study was female genotype 1.

The greatest mean biomass per square metre occurred in genotype 1 growing at the Peche and Turkey Island sites; other clones also achieved their maximum biomass at these two sites in each year of the study (Fig. 3). At the Trenton Channel and Rouge River sites, biomass per square metre of surviving genets was lowest and significantly different than at other sites (Table 3). Despite this, genotype 1 was still able to produce as much biomass per square metre at these sites as the other genotypes did at the Peche Island site. There were significant differences in fresh mass of individual ramets at each location (see Biernacki, 1996). At every site, the biomass of ramets of genotype 1 was greatest, and biomass per ramet typically declined in the order Peche > Turkey > Ecarte > Trenton > Rouge.

There were significant differences among sites in patterns of biomass allocation in ramets (see Biernacki, 1996). The leaf-to-root biomass ratio did not change significantly between the 2 yr of study (Table 2). Also, there were no significant differences in biomass between ramets grown at the Peche and Turkey Island sites, or between plants grown in the Trenton Channel and Rouge River sites (Table 3, also Appendix). However, there were significant differences between ramets grown at the Trenton Channel and Rouge River sites compared to ramets grown at the other sites (Table 3). The pattern of biomass allocation was different in the Chenal Ecarte site, compared to the Peche and Turkey Island sites (at Chenal Ecarte there was significantly more biomass allocated to above ground tissues). At the Rouge River site, plants had up to 80 times as much biomass (per ramet) in leaf compared to root, whereas at the Peche and Turkey Island sites this ratio did not exceed four (see Biernacki, 1996).

The number of turions per square metre was greatest at the Peche and Turkey Island sites, and least at Trenton Channel and Rouge River; the Chenal Ecarte site was intermediate (Fig. 4, Table 3). At the Trenton Channel and Rouge River sites the density of turions was <500 turions/m^2 in any genotype in both years, while at the

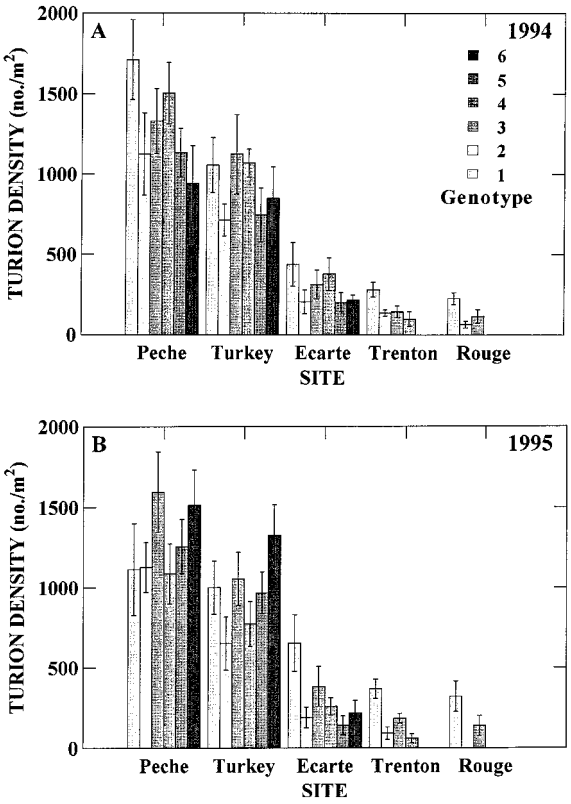

Fig. 4. Mean density of turions (±1 SE) of *Vallisneria* genotypes raised at different sites in (A) 1994 and (B) 1995. Individual genotypes are indicated by numbers from 1 to 6.

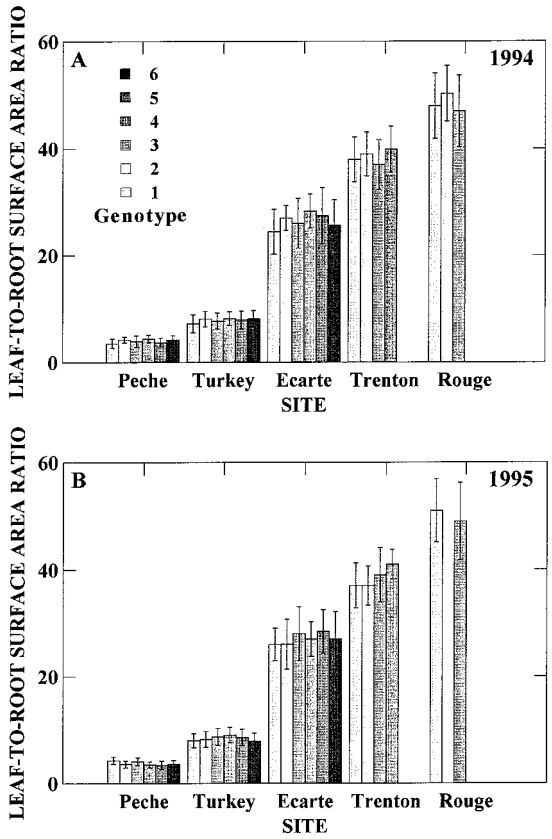

Fig. 5. Mean leaf-to-root surface area ratio (±1 SE) of *Vallisneria* genotypes raised at different sites in (A) 1994 and (B) 1995. Individual genotypes are indicated by numbers from 1 to 6.

Turkey Island site the density exceeded 1000 turions/m^2 and at Peche Island there were 1500 turions/m^2 in both seasons. Turion production by plants of genotype 1 was significantly greater than that of other genets at Chenal Ecarte, Trenton Channel, and Rouge River.

The leaf-to-root surface area ratio was significantly different among experimental sites (Tables 2 and 3), with the lowest values found at Peche Island, and the greatest values at Rouge River (Fig. 5). There was some variation in the ratio between ramets of different genotypes grown at the same location, but these differences were not statistically significant. Furthermore, the leaf-to-root surface area ratios at each location did not differ significantly between years (Table 2). Leaf-to-root surface area ratio was not significantly affected by differences among genotypes (Table 2).

Ranks of site quality based on ranks of various measures of plant performance were similar. However, only ranks based on the index of leaf-to-root area ratio were capable of detecting significant differences among the degree of impairment of sites (Table 3). The Spearman rank correlation coefficient between increasing ranks of relative site toxicity and increasing ranks of the leaf-to-root surface area ratio had the greatest value, $r_s = 0.981$ ($P < 0.001$). Other measurements of plant growth and development had much lower values of Spearman rank correlation coefficient for relative ranks of plant performance and ranks of relative site toxicity.

There were significant differences in the coefficient of variation for plant performance between single-genotype and multi-genotype test designs ($P < 0.001$; Kruskal-Wallis test). The coefficient of variation was from five to ten times greater in the multi-genotype experiments compared to the single-genotype tests (Table 4).

DISCUSSION

Genets grown in the greenhouse differed significantly in the patterns of growth and reproduction observed over six growing cycles for each genotype of *Vallisneria americana* used in the present study (Table 1). These differences indicate that plants are likely to be genetically different from each other. Lokker et al. (1994) used cellulose acetate gel electrophoresis to characterize allozyme phenotypes in *V. americana.* They observed high genetic

TABLE 4. Mean coefficients of variation for selected plant traits in single-genotype and multigenotype test designs at Turkey Island and Chenal Ecarte site.

Trait	Coefficient of variation in	
	Single-genotype test	Multigenotype test
Rate of clonal growth	0.16	0.65
Number of leaves per ramet	0.17	0.67
Biomass per ramet	0.29	1.03
Biomass per turion	0.07	1.46
Turion-to-ramet biomass ratio	0.09	0.63
Number of roots per ramet	0.31	0.95
Length of a leaf	0.48	1.63

diversity in this clonal aquatic plant sampled in the Huron-Erie corridor.

Most biomonitoring protocols assume that the experimental species is homogeneous and physiologically uniform. This approach ignores the fact that individuals used in such tests may be genetically distinct, and the reaction to a particular contaminant concentration or loading may vary significantly among individuals. The results of the present study indicate that the coefficient of variation of various measures of performance in *Vallisneria* may be five to ten times greater in experiments using randomly selected mixtures of genotypes, compared to when replicated, cloned genotypes are used.

Any natural population has a unique history of exposure to toxic metals, organic contaminants, and other pollutants. Local selection pressures due to such contamination will strongly affect the range of genotypes that have survived, so it is difficult to choose individuals that are truly ''representative'' of natural populations. Mixed cultures of experimental organisms kept in the laboratory may tend toward monoculture over time (Baird et al., 1991; Baird, 1992). Nevertheless it has been argued that mixtures of individual genotypes should be used to represent (at least some of) the genetic diversity extant in nature (Forbes and Depledge, 1992). There are some specific problems with using genetic ''mixtures'' of individuals in biomonitoring studies. Experimental organisms initially collected from a limited number of sites (often a single location) may carry inherent bias in genotype composition, and are unlikely to parallel the array of genotypes in populations at other sites (Baird et al., 1991; Baird, 1992). In particular, due to physical limitations, laboratory studies often use small numbers of individuals (e.g., for sediment toxicity testing) and this significantly limits the range of genetic variation studied. Generally, the number of individuals used decreases as the size of experimental organisms increases.

It is often assumed in ecotoxicological studies that less polluted or relatively pristine sites will contain greater genetic diversity, but this has rarely been confirmed experimentally. Some studies have shown that individuals highly sensitive to one contaminant are not necessarily the most sensitive to other contaminants (Baird et al., 1991). Therefore we cannot necessarily assume that organisms from a contaminated site are the best biomonitors of environmental contamination, because tests based on measurements of a few growth parameters using such (presumptive) tolerant individuals may underestimate the extent of environmental degradation and detrimental effects of contaminants to biota (Guilizzoni, 1991; Forbes and Depledge, 1992). Furthermore, the mixtures of chemicals to which they are tolerant may not occur at a particular test site. However, in highly polluted areas, locally evolved contaminant-tolerant biota may form the majority or even the entirety of the genotypes present there (e.g., see Antonovics, Bradshaw, and Turner, 1971), and ''the most sensitive'' genotypes are unlikely to be present where contaminants are present in nature. Tolerant genotypes should not be overlooked in toxicological studies because they form the basis for natural recovery of impaired ecosystems and may be of great value in remedial activities (see Salt et al., 1995).

The literature indicates clearly that degradation and contamination of our experimental sites differed significantly among sites (Pugsley et al., 1985; Edsall, Manny, and Raphael, 1988; Giesy et al., 1988; Manny, Edsall, and Jaworski, 1988; Manny and Kenaga, 1991; Biernacki, Lovett-Doust, and Lovett-Doust, 1996). On the basis of the published data described above (see Materials and Methods section), collected independently of this study, the study sites ranked in the same order for contamination level as for relative growth impairment of *Vallisneria* genotypes. The Peche Island site was the least contaminated site and plant performance at this site was best. More contaminants were present at the Turkey Island and Chenal Ecarte sites. Plants at the Trenton Channel and Rouge River sites were the most impaired and, according to Giesy et al. (1988) these sites are the most contaminated of those we studied. Ramets found in natural populations in the Rouge River had greater biomass (up to 35 g fresh mass) than any other highly contaminated area in the Huron-Erie corridor that we have surveyed (Biernacki, Lovett-Doust, and Lovett-Doust, 1996). Clearly, plant biomass alone may be misleading as a measure of site impairment.

Genets differed in the degree of their tolerance to contaminants. One male genet (genotype 2) tolerant to TCE did not survive in the field at the Rouge River site, although tolerant genotypes 1 and 3 (which were both female) did. Tolerant genets proved useful for biomonitoring, and at some sites even more useful than nontolerant genotypes, as they were able to survive in the most polluted sites and reflect relative impairment. In contrast, nontolerant genotypes died, preventing ranking of impairment for these genotypes.

Detailed records of growth for cloned plants allow more precise measurement of plant responses than does simple survival at a location. In the present study, the density of ramets (Fig. 2), the rate of clonal growth, density of leaves per unit area, biomass of ramets per unit area (Fig. 3), and density of turions produced per unit area (Fig. 4) were all excellent measures of relative environmental quality at our study sites.

The parameter of plant performance most indicative of environmental quality was the leaf-to-root surface area ratio (Fig. 5, Tables 2 and 3). It was highly responsive to site quality and independent of plant genotype and year. Indeed, the leaf-to-root surface area ratio in the present study did not differ from that found in our 1993 survey of *Vallisneria* ramets from a large sample of natural populations, including the five sites used here (Biernacki, Lovett-Doust, and Lovett-Doust, 1996). Thus, ramets grown in tubs did not differ in their response (and, presumably, exposure) to environmental factors, compared with ramets growing naturally at the study sites.

In natural populations, individuals are exposed to selection pressures that vary continuously in intensity and direction; this results in a dynamic balance in population genetic structure. Hence cloned plants are more likely to reveal true patterns of response to environmental quality than using uncontrolled, randomly assembled mixtures of individuals. Many plants (but also other organisms, such as *Daphnia*) can reproduce clonally, producing replicate copies of the same genotype. Macrophytes are relatively easy to cultivate. Through clonal growth, *Vallisneria* can produce a sufficient number of genetically identical ra-

mets for a large field or laboratory investigation. Tolerant genotypes of *Vallisneria* may survive in the most contaminated sites and allow for properly replicated experimental designs, even when nontolerant genotypes die out.

In summary, we conclude that the use of an array of cloned genotypes of *Vallisneria americana* in biomonitoring of environmental quality increased the precision, accuracy, and reliability of our assessment of environmental quality. Tolerant genotypes were particularly useful, indeed essential, in the most highly contaminated sites, where nontolerant genotypes died. However, nontolerant genotypes were useful in providing a graded assessment of sites with intermediate levels of contamination. The measurements of leaf-to-root surface area ratios in *Vallisneria* showed particular utility for measuring differences in environmental quality because they gave a consistent measure of site quality that was unaffected by genotype, time, or the somewhat artificial conditions of growth in a plastic tub.

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APPENDIX. Summary of measures of performance in different genotypes of *Vallisneria americana* grown at five sites in the Huron-Erie corridor over 1994 and 1995 growing seasons. The *F* values (and degrees of freedom), significance (*** indicates $P \leq 0.001$), and mean (and standard deviation) for selected measures of plant performance are shown.

Trait	*F* (df)	Genotype 1	2	3	4	5	6
Peche Island							
1994							
Number of leaves per ramet	543.9***(5, 5244)	12.1(1.9)	5.1(1.1)	5.3(0.8)	5.5(0.9)	6.1(1.0)	5.1(1.6)
Rate of clonal growth	1033.1***(5, 5244)	13.8(2.1)	10.6(1.7)	11.1(1.8)	10.2(2.1)	10.6(1.8)	6.4(1.4)
Biomass per ramet (g)	1545.7***(5, 5244)	5.7(1.8)	2.1(0.6)	2.3(0.7)	2.3(0.6)	2.6(0.8)	3.9(0.8)
Biomass of a turion (g)	3197.2***(5, 5244)	1.11(0.14)	0.29(0.02)	0.32(0.02)	0.26(0.01)	0.29(0.03)	0.66(0.05)
Leaf-to-root biomass ratio	89.4***(5, 5244)	4.8(1.8)	2.4(1.7)	4.1(1.5)	3.1(1.4)	4.2(1.5)	3.3(1.9)
1995							
Number of leaves per ramet	327.3***(5, 2992)	10.8(2.5)	5.4(1.1)	5.2(1.1)	5.3(0.9)	5.9(1.4)	5.7(1.2)
Rate of clonal growth	1173.6***(5, 2992)	10.1(2.2)	9.1(1.7)	9.9(1.8)	8.8(2.1)	9.2(1.8)	5.6(1.4)
Biomass per ramet (g)	1222.2***(5, 2992)	5.1(2.5)	2.3(0.8)	2.7(0.5)	2.3(0.9)	2.8(0.9)	4.1(1.3)
Biomass of a turion (g)	2132.5***(5, 2992)	1.25(0.15)	0.27(0.02)	0.30(0.02)	0.28(0.02)	0.29(0.02)	0.65(0.04)
Leaf-to-root biomass ratio	133.1***(5, 2992)	4.9(1.1)	2.4(1.1)	4.7(2.1)	4.1(1.5)	3.6(1.2)	6.1(1.9)
Turkey Island							
1994							
Number of leaves per ramet	89.7***(5, 4520)	12.3(2.0)	5.4(1.1)	5.6(0.6)	5.8(0.9)	6.5(1.1)	5.3(1.1)
Rate of clonal growth	978.9***(5, 4520)	9.4(1.4)	7.8(1.3)	8.3(1.1)	8.4(1.6)	7.8(1.3)	5.2(0.9)
Biomass per ramet (g)	2006.5***(5, 4520)	6.1(1.3)	1.5(0.4)	1.8(0.4)	1.8(0.5)	1.9(0.7)	3.4(0.7)
Biomass of a turion (g)	2755.3***(5, 4520)	1.42(0.14)	0.25(0.02)	0.23(0.01)	0.19(0.02)	0.26(0.01)	0.53(0.04)
Leaf-to-root biomass ratio	74.9***(5, 4520)	5.1(1.9)	4.3(2.0)	4.1(2.2)	4.4(1.6)	5.1(1.8)	4.3(2.2)
1995							
Number of leaves per ramet	73.5***(5, 2658)	11.8(2.1)	5.5(1.0)	5.4(0.8)	5.9(1.0)	6.4(1.4)	6.1(1.2)
Rate of clonal growth	1172.8***(5, 2658)	8.1(1.4)	6.5(1.1)	7.8(1.1)	6.8(1.6)	6.3(1.3)	4.7(0.8)
Biomass per ramet (g)	1763.2***(5, 2658)	6.0(1.7)	1.6(0.6)	2.1(0.7)	1.8(0.4)	1.7(0.5)	3.5(1.1)
Biomass of a turion (g)	3244.6***(5, 2658)	1.34(0.16)	0.25(0.02)	0.26(0.02)	0.22(0.01)	0.22(0.01)	0.51(0.04)
Leaf-to-root biomass ratio	65.6***(5, 2658)	6.5(1.8)	3.2(1.5)	4.3(1.9)	5.9(2.2)	4.4(1.5)	5.2(1.7)
Chenal Ecarte							
1994							
Number of leaves per ramet	118.4***(5, 1831)	15.3(2.5)	6.6(1.2)	6.7(1.1)	7.1(1.1)	7.9(1.3)	6.8(1.2)
Rate of clonal growth	1452.8***(5, 1831)	8.1(1.3)	5.9(0.7)	5.7(1.2)	6.3(0.8)	5.9(1.0)	4.1(0.7)
Biomass per ramet (g)	2877.4***(5, 1831)	6.8(2.1)	0.9(0.3)	1.1(0.2)	1.1(0.2)	0.8(0.3)	2.2(0.4)
Biomass of a turion (g)	4103.9***(5, 1831)	1.95(0.11)	0.13(0.01)	0.14(0.02)	0.11(0.01)	0.12(0.01)	0.33(0.04)
Leaf-to-root biomass ratio	59.1***(5, 1831)	10.5(4.0)	6.7(3.0)	8.2(3.7)	8.1(3.6)	7.9(4.1)	9.3(4.8)
1995							
Number of leaves per ramet	89.8***(5, 1110)	14.1(2.4)	6.6(1.3)	6.4(1.1)	7.3(1.3)	7.7(1.4)	7.8(1.5)
Rate of clonal growth	1300.2***(5, 1110)	7.1(1.3)	5.5(0.7)	4.9(0.7)	4.8(0.8)	4.7(1.0)	2.8(0.7)
Biomass per ramet (g)	2427.7***(5, 1110)	7.8(1.3)	0.8(0.3)	1.2(0.5)	1.0(0.4)	0.5(0.3)	2.4(0.8)
Biomass of a turion (g)	3251.4***(5, 1110)	1.84(0.11)	0.14(0.01)	0.14(0.01)	0.12(0.01)	0.11(0.01)	0.35(0.04)
Leaf-to-root biomass ratio	88.8***(5, 1110)	8.9(3.4)	5.4(2.5)	6.8(3.0)	8.1(3.0)	6.6(2.3)	10.9(5.6)
Trenton Channel							
1994							
Number of leaves per ramet	146.2***(5, 759)	7.6(1.2)	7.4(1.3)	8.5(1.4)			
Rate of clonal growth	1111.7***(5, 759)	6.4(1.1)	4.3(0.8)	3.5(0.6)	2.1(0.5)		
Biomass per ramet (g)	1199.7***(5, 759)	7.5(1.9)	0.8(0.3)	0.9(0.3)	0.9(0.3)		
Biomass of a turion (g)	3587.8***(5, 759)	2.14(0.13)	0.10(0.01)	0.11(0.01)	0.09(0.01)		
Leaf-to-root biomass ratio	67.9***(5, 759)	45.0(17.1)	48.0(33.0)	47.0(27.0)	35.0(16.8)		
1995							
Number of leaves per ramet	175.7***(5, 486)	17.7(3.1)	8.1(1.4)	7.7(1.8)	9.2(1.7)		
Rate of clonal growth	989.9***(5, 486)	5.3(1.1)	3.6(0.8)	2.8(0.6)	2.2(0.7)		
Biomass per ramet (g)	1435.3***(5, 486)	8.4(2.3)	0.7(0.3)	1.0(0.3)	0.8(0.4)		
Biomass of a turion (g)	4312.7***(5, 486)	2.57(0.14)	0.12(0.01)	0.13(0.01)	0.10(0.01)		
Leaf-to-root biomass ratio	96.5***(5, 486)	36.0(13.7)	31.0(19.0)	53.0(22.7)	46.0(23.8)		
Rouge River							
1994							
Number of leaves per ramet	277.5***(5, 498)	17.3(2.8)	8.8(1.4)	8.4(1.2)			
Rate of clonal growth	783.6***(5, 498)	6.1(0.9)	2.4(0.5)	2.9(0.5)			
Biomass per ramet (g)	2121.5***(5, 498)	8.4(2.7)	0.6(0.2)	0.8(0.3)			
Biomass of a turion (g)	2998.7***(5, 498)	2.43(0.14)	0.08(0.01)	0.10(0.01)			
Leaf-to-root biomass ratio	142.8***(5, 498)	59.0(25.8)	78.0(32.0)	55.0(24.5)			
1995							
Number of leaves per ramet	354.7***(5, 279)	19.1(3.3)		8.9(1.5)			
Rate of clonal growth	243.8***(5, 279)	5.1(0.9)		2.1(0.5)			
Biomass per ramet (g)	1756.6***(5, 279)	9.8(2.1)		0.9(0.4)			
Biomass of turion (g)	2311.1***(5, 279)	2.68(0.15)		0.12(0.01)			
Leaf-to-root biomass ratio	93.9***(5, 279)	85.0(29.6)		55.0(31.8)			