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Vallisneria americana to evaluate its potential as a biomonitor of
organic contaminants**

Maciej Biernacki
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**The use of modular demography in the aquatic macrophyte
Vallisneria americana to evaluate its potential as a biomonitor
of organic contaminants**

by

Maciej Biernacki

A Dissertation
Submitted to the Faculty of Graduate Studies and Research
through the Department of Biological Sciences
in Partial Fulfilment of the Requirements for
the Degree of Doctor of Philosophy at the
University of Windsor

Windsor, Ontario, Canada

1996

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ABSTRACT

Macrophytes form the base of the food web in aquatic ecosystems, and are consumed as fresh biomass or as detritus. They also provide oxygen, mineral nutrients, substrate, habitat, and breeding and nursery areas for numerous biota. In North America, one of the most common and ecologically significant submersed macrophytes is *Vallisneria americana* Michx. *Vallisneria* was studied in two, interrelated ways. In the field of population ecology, the objective of several experiments was to identify factors affecting the performance of this plant in situations involving organic contamination. In the area of applied ecology, the objective was to assess the potential of *V. americana* as a biomonitor of environmental quality using parameters of population biology to measure performance.

In a number of laboratory and field experiments, I observed the growth and development of *Vallisneria americana* in terms of ramet density, rate of clonal growth, number of leaves and turions per ramet, rate of flowering, biomass per ramet, biomass allocation patterns, and surface areas of leaf and root. Plant performance was significantly affected by duration of exposure to contaminants, by seasonal differences and differences between years, sediment type and quality, water column characteristics, water temperature, light levels, and organic contaminant concentrations in the sediment pore-water and water column. The magnitude of plants responses may be modified by their genotype and the history of their earlier exposure to contaminants. Some *Vallisneria* clones proved to be tolerant to high levels of contamination by the organic solvent trichloroethylene (TCE). Plants exposed in the greenhouse to TCE accumulated it within their tissues, and particularly high concentrations were found in root tissue. In the field study, the concentration of organochlorine contaminants (PCBs) increased with time of exposure over the growing season. A significant correlation was observed in the laboratory experiments, and also in the field studies, between contaminant concentration and plant performance. Thus, *Vallisneria americana* may be an effective monitor of

environmental quality. In a long-term field study, the relative ranking of site quality did not change over four years of exposure to local environmental factors. The most useful measure of environmental quality was the ratio of leaf-to-root surface area. The ratio had greater values at sites more contaminated by organochlorine compounds and lower values at relatively less contaminated sites. The leaf-to-root surface area ratio appears to be independent of plant genotype and of differences in meteorological characteristics.

Performance of *V. americana* was significantly affected by and correlated with contaminant concentrations, and this suggests great potential as a biomonitor. The leaf-to-root surface area ratio may be particularly useful for objective biomonitoring of both short-term and long-term changes in environmental quality at highly contaminated areas.

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Chapter 1

GENERAL INTRODUCTION

In the past, lakes and rivers were used as a source of water for consumption, fishing, and transportation. However, with increased industrialization new uses emerged that frequently were in conflict with each other. Rivers and lakes remain the major sources of water for consumption, but they are also used for irrigation and industrial purposes. Increased aquatic pollution has been a consequence. Furthermore, watercourses are used for shipping, waste disposal, recreation, commercial fisheries, and aquaculture. Innovative management strategies are urgently needed for maintenance of conflict-free uses of water resources. Successful management programs should be supported by detailed study of anthropogenic effects on aquatic resources, and also any possible effects these may have on the integrity of aquatic ecosystems.

Significance of submersed macrophytes

Plants form the base of terrestrial and aquatic ecosystems. Emergent and submersed vascular plants provide numerous benefits to other biota present in aquatic ecosystems. Submersed rooted plants transform radiant energy into chemical energy, while absorbing carbon dioxide and releasing oxygen needed for respiration by other organisms. Plants underpin the aquatic food web, with high net primary production of up to $3 \text{ g C m}^{-2} \text{ day}^{-1}$ (Cebrian and Duarte 1995) and up to $1000 \text{ g C m}^{-2} \text{ year}^{-1}$ (Cyr and Pace, 1993). In the Huron-Erie corridor of the Great Lakes Basin of North America, aquatic plants were found to produce 83.1% of the 29,970 tons of ash-free dry mass produced annually in the St. Clair River, and submersed macrophytes contributed 9.1% of this. In Lake St. Clair, 49.3% of the 151,650 tons of biomass produced was by aquatic plants; of this 9.1% was from submersed macrophytes. Of the 28,210 tons produced in the Detroit River, 58.2% of primary production was carried out by aquatic plants, and of this 43.9% (25.6% of the total) was produced by submersed macrophytes (Edwards *et al.*

1989).

Submersed macrophytes provide a source of mineral nutrients, shade, substrate, shelter, breeding and nursing areas, and food for bacteria, fungi, protozoa, epiphytes, benthic nematodes and worms, mollusks, arthropods, amphibians, reptiles, fish, birds, and mammals (Barko *et al.*, 1986; Korschgen and Green, 1988; Lodge, 1991; Crowder and Painter, 1991; Catling *et al.*, 1994; Leslie and Timmins, 1995). Macrophytes may in larger lakes cover proportionally small littoral areas, and often spawning grounds of fish are limited to a few sites within the littoral zone; however, successful reproduction at these sites and subsequent dispersal determines the overall productivity and diversity of much larger areas of entire lake and river ecosystems (Manny *et al.*, 1988; Edsall *et al.*, 1988; Leslie and Timmins, 1995; Klinge *et al.*, 1995). Early life stages of a number of commercially important fish species were found to be significantly correlated with, and dependent upon emergent and submersed vegetation. However, later in their lives, fish may move in to the pelagic zone (Crowder and Painter, 1993; Leslie and Timmins, 1995). Aquatic macrophytes may be consumed directly (as fresh mass) by herbivores, or as plant-derived detritus by detritivores. Studies have shown that, on average, 30 % of fresh mass of net primary production by aquatic macrophytes gets removed by herbivores (Cyr and Pace, 1993). Flux in high-quality detritus produced by meadows of submersed macrophytes, may form a detrital resources exceeding 100 g C m^{-2} at some times in the year (Cebrian and Duarte, 1995). This is used in energy flow through benthic and pelagic food webs, buffering seasonal variation in available plankton, particularly in pelagic areas (Wetzel, 1995). Traditionally, particulate organic carbon is considered a sole route of energy flow in the aquatic food web (Hairston and Hairston, 1993), but dissolved organic carbon derived from detritus (of plant origin) may be found in concentrations from 6 to 10 greater than that of particulate organic carbon and dominate energy flow in aquatic ecosystems (Wetzel, 1995).

With their roots growing in sediment and leaves surrounded by the water column, submersed plants play a significant role in nutrient exchange between sediments and water column, and for macro- and micro-element uptake, storage, and cycling in aquatic

environments (Barko *et al.*, 1986, 1991). Meadows of submersed macrophytes slow down water currents, thereby increasing sedimentation of suspended particles; they stabilize sediments, impede wave action and decrease shoreline erosion; and they decrease water turbidity and increase water clarity, allowing deeper light penetration (Petticrew and Kalff 1992; Nichols 1991, Catling *et al.*, 1994).

Benefits provided by submersed macrophytes to surrounding ecosystems are even more significant and evident in anthropogenically altered areas, such as the designated Areas of Concern (AOC) of the Great Lakes of North America. In AOC, existing meadows of submersed macrophytes were found to support the greatest diversity and density of young-of-the-year of commercially important fish species (Crowder and Painter, 1991; Minns *et al.*, 1994; Leslie and Timmins, 1995). In particular, highly desired piscivorous species of fish were found to depend on submersed plants for reproduction, nursery area, refuge, and feeding habitat. Piscivorous fish were shown in a number of studies to be vital for the recovery and for the maintenance of productive, sustainable fisheries (Klinge *et al.*, 1995). In a field survey, an index of biotic integrity (IBI) for fish assemblages in three Canadian AOC was shown to be significantly and positively correlated with remaining beds of submersed plants in these impaired areas (Minns *et al.*, 1994). Diving ducks feeding preferentially on submersed plants in highly contaminated AOC were found to be less contaminated than other ducks living in the same areas but feeding mostly on zebra mussels (*Dreissena polymorpha*). Recent evidence suggests that ducks feeding exclusively on submersed rooted plants had low contaminant burden and, according to Ontario government guidelines, were acceptable for human consumption (Mazak, 1995). Furthermore, in highly eutrophic areas submersed macrophytes may significantly reduce blooms of nuisance algae by storing nutrient in their tissues over growing season (Barko *et al.*, 1986; Crowder and Painter, 1993).

Submersed aquatic plants are capable of sequestering metals and nutrients, from both the sediment and the water in which they grow (Barko *et al.*, 1986, 1991; Guilizzoni, 1991). However, few studies have addressed the presence of organic contaminants in

plants and their effects. Plants may accumulate, from the sediment and surrounding water column, high concentrations of organic chemicals including organochlorine pesticides and herbicides, PCBs, solvents, surfactants, and PAHs and byproducts of their decay. For example, PCB levels may be 3 - 4 times higher in plants than in sediment, and 6000 - 9000 times higher (on a dry mass basis) in plants than in the surrounding water (Lovett Doust *et al.*, 1994a). Contaminants present in plant tissues may enter the aquatic food web through herbivorous or detrital food chains. Thus, the quality of submersed macrophytes may be of critical value to the health of aquatic ecosystems.

Recent studies indicate that changes in plant tissue contaminant concentrations are significantly correlated with parameters of plant growth and development, suggesting that the measurement of plant performance may be useful as a tool for environmental monitoring. Such biomonitoring would have high environmental relevance and low relative cost, compared to direct chemical monitoring of environmental samples (Lovett Doust *et al.*, 1994a,b; Biernacki *et al.*, 1995a,b, 1996).

At present, aquatic plants are increasingly used to remediate wastewater in artificial wetlands (Brix, 1994). Other, largely unexplored potential uses of submersed rooted plants may include *in situ* phytoremediation of sediments contaminated with toxic metals and/or organic compounds (Cunningham *et al.*, 1996; Anderson *et al.*, 1995). Cousteau (1975) described the use by aboriginal people of emergent and submersed macrophytes for human and animal consumption, as a source of fibre and dyes, and also for medical applications. Unfortunately, most of this knowledge is likely to disappear with increased industrialization.

Environmental factors affecting submersed vascular plants

A variety of environmental factors interact and affect plant growth, development and reproduction, as well as the distribution and abundance of submersed macrophytes.

Light availability has been considered as one of the most important factors limiting growth of macrophytes. This is particularly evident in the patterns of macrophyte distributions at different water depths (Barko *et al.*, 1986). Light compensation points for

different species of submersed macrophytes vary, depending on their abilities to tolerate shade (Adams *et al.*, 1974; Catling *et al.*, 1994). Increased water turbidity and algal blooms that limit light available to macrophytes decrease plant abundance and biomass (Barko *et al.*, 1991). In particular, morphological and physiological adaptations that optimize the capture of light may determine species success in low-light environments and could also influence the outcome of interspecific competition (Titus and Adams, 1979). Studies indicate that plant light responses are mediated by the pigment phytochrome, which is affected by red (660 nm) and far red (>700nm) wavelengths (Chamber and Spence, 1984).

Water temperature has been shown to interact with other factors, including light, in affecting the efficiency of photosynthesis and subsequent growth and development of submersed macrophytes (Barko *et al.*, 1986; Lovett Doust *et al.*, 1994a). Low temperature may decrease the rate of seed and turion germination, plant growth and the depth distribution of submersed macrophytes, as well as decrease overall clonal growth, flowering and biomass production over the growing season (Moller, 1980, Catling *et al.*, 1994). Water temperature was also found to be associated with changes in species composition of submersed macrophytes (Barko *et al.*, 1986).

Availability of mineral nutrients to submersed plants may also affect their performance. Submersed macrophytes often make use of both aqueous and sedimentary nutrient sources, though, sediments appear to be the dominant source for phosphorus, nitrogen, and most micronutrients needed by plants (Barko *et al.*, 1991; Guilizzoni, 1991). Uptake of nutrients from sediments may be associated with the activity of the microbial community in the rhizosphere of submersed rooted plants (Smith *et al.*, 1984; Guilizzoni, 1991). There is evidence of N-fixing bacteria and vesicular-arbuscular mycorrhizae associated with roots of some submersed macrophytes (Barko *et al.*, 1986, 1991). Other nutrient, like K, Na, Mg, S may be absorbed and utilized by macrophytes directly from the water column. Nutrient losses by submersed macrophytes are mostly associated with plant senescence and decay at the end of the growing season (Guilizzoni, 1991).

Plant performance has been frequently found to be related to sediment quality. Plant growth, rooting depth, root biomass, and plant distribution were correlated with changes in sediment texture, ion exchange properties, contamination, nutrient content, and organic matter content (Barko *et al.*, 1991; Guilizzoni, 1991). Also, allelopathic substances released by some macrophytes may for a long period be associated with sediments and affect other macrophytes growing there (Barko *et al.*, 1986). Submersed macrophytes are capable of directly affecting the quality of sediments. They can release oxygen into the rhizosphere, excrete enzymes, affect root associated microbial communities, change sediment redox potential, and increase concentrations of bioavailable nutrients in the rhizosphere (Barko and Smart, 1980; Barko *et al.*, 1991).

Inorganic carbon as a nutrient may limit photosynthesis, growth and reproduction of submersed plants. Together with sufficient light, plants require free carbon dioxide or bicarbonate ions for photosynthesis. Concentrations of bioavailable inorganic carbon may be significantly affected by water temperature, pH, mineral composition of sediments, etc. (Titus, 1992; Titus and Hoover, 1993). Response to changes in inorganic carbon concentrations may vary between species and for some species may be adaptive (Barko *et al.*, 1986).

Effects of biotic factors such as grazing, herbivory, and pathogenicity on the performance of submersed macrophytes remain largely unexplored. However, some studies report herbivory on submersed macrophytes to be as high as 30%, which was on average twice as much as on terrestrial plants (Cyr and Pace, 1993).

Effects of anthropogenic disturbance on submersed macrophytes

Conflicting uses of aquatic ecosystems by humans have many detrimental effects on communities of submersed macrophytes.

Dredging of waterways may remove large volumes of sediments otherwise suitable for plant establishment. Such activity may affect local water currents and increase water turbidity, and subsequently decrease the light available to plants. Increased water depth in dredged areas may be not suitable for growth of many species

(Manny and Kenaga, 1991).

Shore protection programs and engineering activities may significantly decrease the shallow littoral areas suitable for macrophytes. This process is associated with reclamation of wetlands surrounding aquatic systems for residential, agricultural and industrial purposes. In some areas, such as the Detroit River, over 90% of wetlands inhabited by submersed vegetation has been reclaimed in the past 200 years (Manny *et al.*, 1988).

However, the greatest effects on remaining beds of submersed plants has been contamination from anthropogenic sources. Point sources from industry and urban areas, and non-point sources from agriculture, forestry and urban areas discharge large quantities of contaminants including, nutrients, solids, toxic metals, surfactants, and organic compounds (Edsall *et al.*, 1988; Manny *et al.*, 1988; Manny and Kenaga, 1991). Such discharges affect plants directly in the water column but also through contaminated sediment, when contaminants are adsorbed by sediments within beds of macrophytes. Contaminated sediments may be the primary route of macrophyte exposure to contaminants (Lovett Doust *et al.*, 1994a). Increased contamination of aquatic ecosystems is often coincident with a decline in abundance and distribution of submersed macrophytes. For example, in the Detroit River, Schloesser and Manny (1990) reported a 72% decline in the most common plant *Vallisneria americana*. Similar patterns have been observed in other aquatic ecosystems and for other species of aquatic macrophytes (Manny *et al.*, 1988; Edsall *et al.*, 1988; Crowder and Painter, 1991).

Need for including submersed rooted macrophytes in the repertoire of biomonitoring agents

As indicated above, submersed macrophytes provide many important benefits to aquatic ecosystems. They provide not only food and habitat for numerous biota but also maintain spatial heterogeneity in aquatic ecosystems. As shown by numerous studies, their quality and quantity is critically important to the welfare of aquatic biota. Thus, there is significant need for protection of submersed plants and a need for better

understanding of the effects of toxic compounds on submersed macrophytes (Crowder and Painter, 1991; Lovett Doust *et al.* 1994b; Lewis, 1993, 1995; Boutin *et al.*, 1995). Furthermore, there is an urgent need to develop biomonitoring programs focused on submersed macrophytes, to be used together with other, animal-oriented biomonitoring programs in aquatic ecosystems (Lovett Doust *et al.*, 1994b).

Metapopulation biology of plants

The two major features, shared by plants and different from animals, are plants' ability to grow and develop from meristems and that plants are sessile. The number and distribution of meristems on a plant determine pattern of plant growth and overall structure. A meristem that develops into a new shoot may multiply the number of meristems on a plant. This produces the modular structure of plants and has consequences on life history of plants. Meristems allow plants to grow vertically and also spread horizontally. Individual plant comprises the genet. Clonally produced by meristems parts of plants may develop roots and potentially initiate independent existence as ramets. The branching patterns of plant growth results in hierarchical structure: a genet is composed of ramets, ramets are composed of branches, branches or shoots bear leaves and inflorescences, inflorescences are composed of flowers that contain ovules and pollen. This hierarchical structure of plants affects their life history because the performance of a genet depends upon the survival and reproduction of ramets, and ramets depend upon the behaviour of their parts (Silvertown, 1989).

It has proved widely useful to investigate plant populations by studying the demography of their metapopulations at various levels of organization, particularly the individual ramets of clonal species, but also the turnover of individual leaves, flowers and fruits (e.g. Lovett Doust and Eaton, 1982; Lovett Doust *et al.*, 1994a). Plants may respond to environmental factors (e.g. contaminants) through changes in growth patterns, morphology and fecundity, as well as mortality. The modular structure of plants seen as changes in the rate of birth, death and turnover of parts; these measures may give an "early warning" of the cumulative detrimental effects of environmental insults.

Vallisneria americana

One of the most common submersed macrophytes in the Huron-Erie Corridor is *Vallisneria americana* (Hydrocharitaceae) (e.g., Lovett Doust and LaPorte, 1991; Catling *et al.*, 1994). Whereas the species suffered a decline in the Detroit River in the past 20 years (Schloesser and Manny, 1990; Manny and Kenaga, 1991), remaining populations of this species continue to support a rich biota. In particular, the over-wintering organs (turions) of *Vallisneria* are of importance as a food for migrating waterfowl in the Detroit River (Manny and Kenaga, 1991). In a field survey in 1995, *Vallisneria* was also found in all of the other Canadian AOC, with the exception of Wheatley Harbour in Lake Erie and Port Hope in Lake Ontario (Biernacki, personal observation).

Vallisneria americana (American wildcelery, Figure 1.1) has a cosmopolitan distribution (Lowden, 1992) and is a dioecious perennial, submersed aquatic of fresh or slightly brackish water. It has unbranched, fibrous roots, 20 - 200 in number. Leaves are ribbon-like, 1 - 25 in number and typically 8 - 100 (but may be up to 300) cm long and 3 - 25 mm wide with serrated margins, a darkened central stripe and transverse lightly-pigmented striations, borne on short vertical stems (caudex) arising from stolons 3 - 40 cm long. *Vallisneria* produce solitary pistillate flowers, 4 - 8 mm across, sessile, contained in a tubular spathe and extending on an elongate and eventually coiling scape 19 - 300 cm long to the surface of the water (Catling *et al.*, 1994; Titus and Hoover 1991; Schloesser and Manny 1990), with 3 sepal lobes, 2.5 - 3(5) mm long, 3 staminodia, and 3 petals. Staminate flowers are numerous (up to several hundred), 1 - 1.5 mm across; enclosed in an ovoid, 3-valved spathe 11 - 25 mm long and 7 - 15 mm wide with a stalk - 1.5 - 7.8 cm long and 1.5 - 3 mm wide. Male flowers separate from their pedicels and float to the surface where they mature. *Vallisneria* has elongate fruits, cylindrical, 1-locular, 2 - 15 cm long, 3 - 7 mm wide, containing 0 - 400 seeds. Seeds are cylindrical or ellipsoid, ranging in colour from white to dark brown, 1.8 - 2.6 mm long, 0.6 - 1 mm wide, and shed in a mass of gelatinous material.

Vallisneria produces buds at the tip of horizontal stolons which develop into new plants. Due to extensive clonal growth, many stolon buds develop into daughter rosettes

during the same season. Buds produced at the end of the growing season on vertical stolons remain dormant through the winter and resume growth the following spring (Catling *et al.*, 1994). These overwintering buds, called turions, germinate the following spring. Clonal growth is extensive in this species. Within a single growing season, one overwintering bud may produce 20-40 new rosettes. Individual plant of *V. americana* or genet (genetic individual) may have many physiologically independent shoots (ramets).

In southern Ontario, (Huron-Erie corridor), plants emerge in May, flowering begins in July and fruits develop until late October. Greatest clonal growth and maximum biomass are found at the end of August. In mid-September, plants begin to form turions; these mature and are ready for overwintering by the time of plant senescence, in late October.

The optimum temperature for photosynthesis in *V. americana* was found to be 32.6 °C (Titus and Adams, 1979). American wildcelery may be found in water at depths from 0.3 to 6 m. In shallow areas (1 m) in the St. Clair and Detroit Rivers, *Vallisneria* densities may range from 80 to 200 shoots/m² (Lovett Doust and LaPorte, 1991). At greater depths (3-4 m), natural densities may be greater than 1000 plants/m².

Wildcelery may grow in a variety of substrates, including sand or mud, sand/gravel, clay sediment and coarse silt (Korschgen and Green 1988; Lovett Doust and LaPorte, 1991). Slow currents were more suitable for *Vallisneria* than either stagnant or rapidly moving water. Optimal water column pH may range from 5.1-7.2; higher or lower values may have detrimental effects on *Vallisneria* growth and reproduction (Titus, 1992). In the St. Clair River, Lake St. Clair and Detroit River, *V. americana* is essentially the dominant macrophyte. However, it may be found growing in communities with other submersed macrophytes (Schloesser and Manny 1986, 1989, 1990; Schloesser *et al.* 1985). In some communities, *Vallisneria* was found to be a strong competitor with other submersed macrophytes, especially at lower light levels (Titus and Adams, 1979; Smart and Barko 1989).

Vallisneria plant nutritive values were found to be above-average compared to other aquatic species (Boyd and McGinty 1981). In leaves, the ash content was from 25-

28%, crude protein 11-16%, crude fibre 14-36%, ether extract >1%, N-free extract >36% (Schuette and Alder, 1927; Gortner, 1934). Winter buds and fruits frequently eaten by waterfowl had a mean crude protein level of 11 %, ash 4.6 %, crude fibre 2.8 %, crude fat 0.8 % and nitrogen-free extract 80.8 % (Korschgen & Green 1988). Fruits and winter buds were high in dry matter compared to leaves, low in ash and fibre, and also had greater caloric values. *Vallisneria* is involved in the uptake, movement (from water and sediment) and redistribution of heavy metals and organic contaminants (Manny and Kenaga 1991; Lovett Doust *et al.*, 1994a,b; Biernacki *et al.*, 1996). Thus, it may be source of contaminants to herbivores or detritivores.

Wildcelery is a valuable component of the aquatic ecosystem, providing shade, shelter and food for numerous aquatic biota (Korschgen and Green, 1988; Catling *et al.*, 1994). Some studies found greater numbers of species and higher densities of birds and fish supported by *Vallisneria* than by other macrophytes (Sculthorpe, 1967; Keast, 1984). It has been found to yield high-quality detritus important to energy flow through benthic and pelagic food webs (Bianchi *et al.*, 1991). American wildcelery is an important source of food for many species of game birds. Leaves, fruits and underground parts have been identified in the stomachs of ducks (Mazak, 1995). It is estimated that approximately 75% of North American canvasback ducks (*Aythya valisineria*) feed primarily on turions of *Vallisneria* on the upper Mississippi river while in migration (Korschgen and Green 1988). A total of 24 duck species, 2 geese, 2 swan, 3 shorebirds, 3 rails, and other wildfowl were found to feed on all parts of the plant, but especially favouring winter buds and rootstocks (Fassett, 1957; Korschgen and Green, 1988). Phytophagous fishes, such as the Grass Carp (*Ctenopharyngodon idella* Val.) also feed on wildcelery (Catling *et al.*, 1994). Some other herbivores, like red-head ducks, muskrats and the red-bellied turtle may decrease the standing biomass of plants. As a result, grazed plants may become denser and shorter, develop new leaves and decrease the rate of flowering (Carter and Rybicki, 1985). Crayfish (genus *Orconectes*) is also capable of herbivory on *Vallisneria* (Lodge, 1991), and there are also reports of herbivory by insects (Catling *et al.*, 1994).

In some studies, wildcelery has been found to be resistant to most of the available

herbicides, including 2,4-D, diquat, and Aquatol-K, so aquatic harvesting methods were advised for *Vallisneria* control (Nichols, 1991). However, runoff of agricultural herbicide (e.g. atrazine) from cropland in the Chesapeake Bay area has been suggested as the most likely cause of the loss of submersed vegetation, including extensive beds of wildcelery (Correll and Pierce 1978; Stevenson *et al.* 1979). A significant decline in populations of *Vallisneria americana* was recently reported in the Huron-Erie corridor of the Great Lakes, and pollution was suggested as the most likely cause of this decline (Schloesser and Manny, 1990; Manny and Kenaga, 1991). However, there have been no field and/or laboratory studies of effects of organic contaminants on the population biology of *Vallisneria* to directly test these assumptions.

The objectives of the experiments included in the present study were to determine the effects of selected biotic factors (plant genotype, site of plant origin, plant sex) and abiotic factors (sediment type and source, light quantity, time of exposure, depth of the water column) on growth, development and reproduction in *V. americana*, with special focus on the effects of organic contamination. The potential of *V. americana* as a biomonitor of pollution in aquatic ecosystems is assessed.

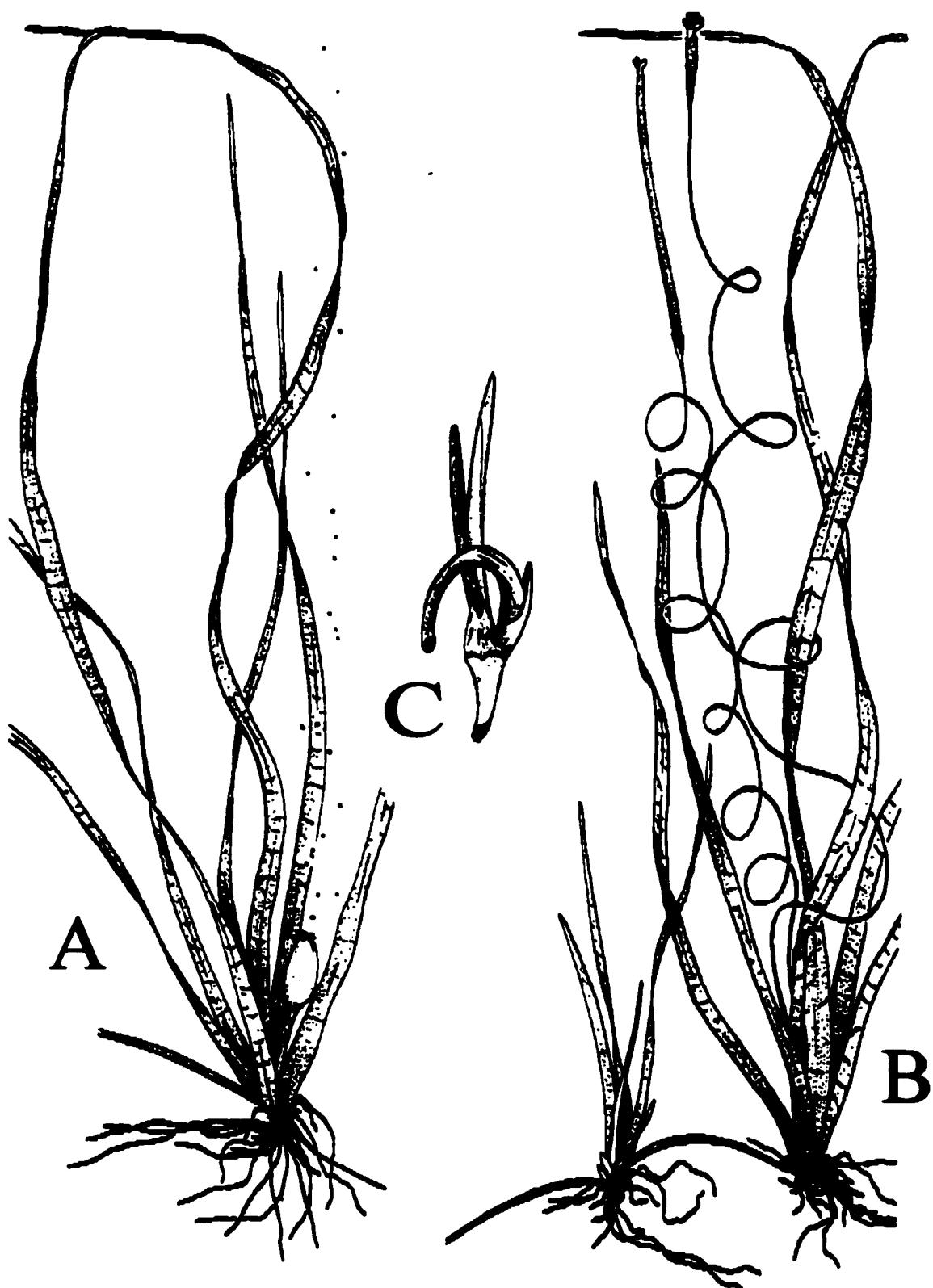


Figure 1.1. *Vallisneria americana*: A, male and B, female plants, C, overwintering turion (after Catling *et al.*, 1994).

Chapter 2

THE EFFECTS OF TRICHLOROETHYLENE, PLANT SEX AND SITE OF ORIGIN ON MODULAR DEMOGRAPHY IN *Vallisneria americana*¹

ABSTRACT

Aquatic macrophytes form the base of the aquatic food web. They provide cover, and breeding and nursery areas for numerous biota. Thus, detrimental effects of contaminants on macrophytes may have consequences for organisms, higher in the food chain.

The demographic effects of an organochlorine contaminant on *Vallisneria americana* were studied. Plants collected nondestructively from two natural populations in the Huron-Erie corridor of the Great Lakes Basin, were exposed to four treatments of trichloroethylene (TCE). Plant responses were determined based on genet mortality, ramet production and mortality, leaf birth- and death rates, leaf area and sexual reproduction. TCE caused significant ramet and genet mortality, and reduced the growth of surviving plants. Control plants produced significantly more leaves than contaminated plants. Contaminant-exposed plants continued producing their leaves for 4-6 wk longer than controls, and showed greater rates of leaf death. The total leaf area of genets exposed to TCE was significantly lower than that of unexposed plants. TCE caused a decrease in flower production. Plants that survived the TCE treatment appeared to be resistant to the chemical; they were as likely to flower as surviving untreated plants.

Levels of TCE were greatest in the sediment. Sediment from various sites

¹ The major results of this chapter have been published as follows:
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adsorbed TCE to differing degrees. TCE was more concentrated in the underground tissues than it was in leaves. Root and turion tissues of plants from one site contained significantly less TCE than those of plants from the other site, despite an identical concentration of TCE in the water. TCE bioaccumulated, particularly in roots. This appeared to be attributable to the fact that TCE accumulated, in the first instance, in sediment. The \log_{10} of TCE concentrations in sediment was linearly correlated with TCE concentrations in roots, suggesting that TCE uptake occurs through roots rather than leaves, and that uptake is driven by the concentration gradient between sediment and plant.

INTRODUCTION

Responses to catastrophic oil spills often include heroic efforts to reduce mortality. However, the less dramatic effects of persistent background exposure at sub-lethal levels, that is, chronic toxicity, can have similar, or even more serious ecological repercussions. If "risk-averse" (Fischhoff *et al.*, 1981) strategies and policies are being followed, that is, if management policy "errs on the side of caution", it is essential to evaluate the effects of long-term, low-to-moderate level exposures through careful and sophisticated monitoring procedures (Depledge, 1989). To evaluate the health of natural populations, records of reproductive success, and quality and survival of offspring are likely to provide important measures of environmental quality (International Joint Commission, 1987). Several studies have assessed the mean response to environmental factors of a population of test organisms, but this disregards the information provided by measures of variability between individuals (Bennett, 1987). It is important to develop an understanding of ecotoxicology at the ecosystem and the population level (e.g. Rosemarin, 1988; Depledge, 1990). Trendall and Prescott (1989) have proposed that in many cases the wellbeing of a population can best be assessed by determining the fraction of individuals affected to differing degrees in response to an environmental insult (such as, for example, pollution).

The North American Great Lakes are contaminated by many organochlorine compounds (e.g., International Joint Commission, 1987). Very little work has been done on plant exposure, despite the fact that the food web is seen as a major route of contaminant transfer for animals that are used as food by humans (IJC, 1992). Since both the herbivorous and the detritus food webs are based on these plants, their contaminant content and the effects of contaminants on their growth are of broad importance to the health of the ecosystem.

Vallisneria americana is the most abundant submersed macrophytes in the Huron-Erie region (Schloesser and Manny, 1982). Over the past 20-30 years there have been many reports of a regional decline in macrophytes, particularly *Vallisneria*. Many of the

studies concluded that pollution was the likely cause of the decline (Schloesser *et al.*, 1985; Schloesser and Manny, 1986; Government of Ontario 1986). Oetting (1985) reported a decline of macrophyte beds over the period 1950-1985, and a 73% decline in turion densities in existing beds was reported by Mills *et al.* (1966) and Trauger and Serie (1974) (turions are the storage organs that overwinter in this herbaceous species).

Trichloroethylene is a common pollutant of aquatic ecosystems (Moore *et al.*, 1991). It is present in up to 34% of water supplies tested in the USA. Concentrations in water are usually in the order of 10 - 100 µg/L (Rook *et al.*, 1975; Ewing *et al.*, 1977), but chemical industry discharges may contain up to 200 µg/L (Commission of the European Communities, 1976), and some well waters in Milan, Italy have been found to contain even higher levels (Cavallo and Grassi, 1976; Ziglio, 1983). Rain-water may contain concentrations in the µg/L range (McConnell *et al.*, 1975). The US EPA found that the lowest observable acute effect level (LOEL) for TCE in freshwater was 45 mg/L, and the chronic LOEL was 21.9 mg/L (US EPA, 1986). There are some data about carcinogenicity of TCE; it is a suspected carcinogen (see Moore *et al.*, 1991 and Eder, 1991). A tentative guideline of 30 µg/L in drinking-water has been recommended by the World Health Organization (WHO, 1984). Increased human exposure to TCE was associated with an increased incidence of cancers of lymphatic systems, skin, colon, lung, urogenital tract, pancreas and liver (Eder, 1991). At the present level of knowledge, the "threshold value", the highest concentration not affecting human health, was found to be 10 µg/L (WHO, 1984). At the cellular level TCE interacts with lipid membranes, induce lipid peroxidation, and produces radicals and unsaturated compounds that may interact with biological macromolecules like DNA (see Eder, 1991).

Trichloroethylene has a light, but pungent chloroform-like odour. It is denser than water (specific gravity = 1.47) and unless well mixed, it tends to form extensive globules on the bottoms of lakes and rivers (IJC, 1987). Thus it is likely that sediments will contain elevated levels of TCE relative to the water column, and that rooted plants will be particularly subject to contaminant exposure via underground organs. Solubility of TCE in water is moderate (1100 mg per litre at 25 °C, Pearson and McConnell, 1975), and

dependent on temperature. The logarithm of the partition coefficient of TCE between water/air at 20 °C is 2.74, and between 1-octanol and water it is 2.29 (US EPA, 1979). Trichloroethylene is moderately stable; it is decomposed slowly by light in the presence of moisture and higher temperatures. Degradation occurs less readily in water bodies than on land because in water, sunlight attenuates with depth, the concentration of oxygen is lower, and temperature is lower, particularly at the sediment-water interface where TCE accumulates (Wetzel, 1983). Trichloroethylene may be degraded by microorganisms (Bouwer and McCarty, 1983; Parsons *et al.*, 1984), but little is presently known about its possible metabolism in biota (Moore *et al.*, 1991). However, it is known that its oxidative metabolism is influenced by cytochrome P₄₅₀ with subsequent glutathione conjugation (Eder, 1991). In addition, TCE can react with other dissolved substances, such as bases, to produce more hazardous toxicants such as dichloroacetylene (Eder, 1991). Through the use of TCE as an industrial solvent, 60% of world production to date has been released to the environment (US EPA, 1979). Although recovery and re-use of 60-90% of spent solvent is practiced worldwide, evaporative losses and "spills" are considerable (WHO, 1984; Kaiser and Valdmanis, 1979; Kaiser *et al.*, 1983). Trichloroethylene is the chemical most often detected at "Superfund" (highly contaminated) sites in the USA (Abelson, 1990), and it commonly contaminates the Huron-Erie corridor of the Great Lakes. In surface waters of the St. Lawrence River, Lum and Kaiser (1986) found TCE levels of up to 90 µg/L. In the St. Clair River, levels of up to 42 µg/L were reported by the Government of Ontario (1986) and Kaiser and Comba (1986).

It has proved widely useful to investigate plant populations by studying the demography of their metapopulations at various levels of organization, particularly the individual ramets of clonal species, but also the turnover of individual leaves, flowers and fruits (e.g. Lovett Doust and Eaton, 1982; Lovett Doust *et al.*, 1994). Leaf demography provides a particularly sensitive measure of plant performance and response to environmental insults. For example, in a natural population of *Trapa natans*, the chinese water-chestnut (an example of a rare annual clonal aquatic plant), population dynamics over the growing season were dominated by the effects of clonal growth, and leaf and

ramet demography were very sensitive to initial plant density (Groth *et al.*, 1994). In the present study, the techniques of modular demography have been applied to the assessment of effects of organic contaminants in *Vallisneria americana*. Plants may respond to the presence of contaminants through changes in growth, morphology and fecundity, as well as mortality. The modular structure of plants is seen as changes in the rate of birth, death and turnover of parts; these measures may give an "early warning" of the cumulative detrimental effects of environmental insults.

This study investigates the impact on *Vallisneria americana* of different concentrations of trichloroethylene, a dry-cleaning and degreasing solvent that is locally abundant (Kaiser and Valdmanis 1979; Kaiser *et al.*, 1983; Kaiser and Comba 1986), particularly in the Sarnia region. It continues to be released as a result of recurring industrial "spills" in Chemical Valley, the petrochemical processing region along the shores of the St. Clair river (Government of Ontario 1986).

The objectives of this study were; to quantify demographic effects of TCE on plant growth, in terms of leaf and ramet demography, growth and reproduction; to establish any direct relationship between such effects and contaminant concentrations, both in the external environment (water/sediment) and in plant tissues; and to discover whether bioconcentration was occurring.

MATERIALS AND METHODS

The experiment began in May 1990 and was completed by September 1991. This time period covered two seasons' of growth. Plants of *Vallisneria* were collected in the field from depths of 0.6 -1.2 m as over-wintering turions in the first week of May 1990, before spring growth was initiated, from two sites, one adjacent to Turkey Island in the Detroit River, and the other in the Chenal Ecarte (hereafter referred to as the Ecarte site) in the delta of St. Clair River (see Lovett Doust and LaPorte, 1991 for further details on the field sites and source populations). There was a significant difference in the mean mass of the original 200 turions collected from each site: the average for Turkey Island was 975

mg (\pm 15.6 mg), while that for plants from Ecarte was 258 mg (\pm 10 mg). Turions were selected from microsites >1 m apart in order to increase the probability that their genotypes were different. In addition, turions from labelled plants of known sex were collected for use in the experiment. These had larger turions, 1100 mg (\pm 17.3 mg) and did not differ in mass between the two sites.

Sediments from the field were collected in the first week of May 1990. Sediments were placed in marked jars (0.5 L), and these were placed in glass tanks containing dechlorinated tap water (pH 7.1, no additional nutrients added). One turion was planted in each jar filled with sediment from its home site, and arranged at random in tanks such that plants would be exposed to high, medium or low levels of TCE or the control treatment, where no TCE was added. Each glass tank (31 cm x 62 cm x 91 cm) held 175 L of water and contained 24 jars. The water temperature did not exceed 27 °C during the summer, and ranged from 10 - 15 °C during the winter.

Each tank therefore contained:

- 12 jars with plants and sediment from Turkey Island site including:

- 2 female plants
- 2 male plants
- 8 plants of undetermined sex

- 12 jars with plants and sediment from St. Clair River site including:

- 2 female plants
- 2 male plants
- 8 plants of undetermined sex

A total of 384 plants in 16 aquaria was used, resulting in four replicates and four levels of contaminant concentration under static (regularly recharged) conditions. Aquaria were arranged randomly in the greenhouse, under banks of fluorescent lights supplemented with gro-lights to add wavelengths from the red end of the spectrum. These lights were kept on for the full day length experienced outdoors, throughout the period of the experiment.

Experimental TCE concentrations

TCE concentrations were selected on the basis of the "theoretical lethal concentration", which is estimated using a formula to describe the partitioning of the chemical between the lipid portion of the plant (represented by 1-octanol) and water (Gobas and Mackay, 1989; Gobas *et al.*, 1991):

$$C_{\text{plant}} = L * K_{\text{ow}} * C_{\text{water}}$$

where C_{plant} = concentration of chemical in the plant tissue

C_{water} = concentration of chemical in the water

L = lipid content (proportion of biomass that is lipid) of the plant tissue
(approximately 0.006 for *Vallisneria*)

K_{ow} = 1-octanol-water partition coefficient of TCE
($\log_{10} K_{\text{ow}} = 2.29$, so $K_{\text{ow}} = 195$).

The theoretical lethal concentration of TCE is estimated to be between 2000 - 6000 µmoles per kilogram of plant tissue (McCarty *et al.*, 1991). Thus, for the selection of appropriate levels of TCE in the water, C_{water} could range from 1709 µmoles TCE per litre of H₂O to 5130 µmoles TCE per litre of H₂O. Since sublethal effects, that might represent possible levels of exposure in the field, were particularly important, selected treatments corresponded to 0, 500, 1000 and 3000 µmoles TCE per litre of H₂O. Therefore, the experimental concentrations applied were: 0 TCE as control, 66 mg/L (low), 132 mg/L (medium), and 396 mg/L (high concentration). All of these values are below the solubility limit of 1100 mg per litre for TCE at 25 °C, and, for comparison with the experimental conditions, the present limit proposed by US EPA is 45 mg per litre (US EPA, 1986). To maintain treatment conditions, water was replaced on a weekly basis and TCE replenished. To prevent cross-contamination, water from each tank was removed using a separate siphon hose for each concentration of TCE. Hoses were connected to carbon filters to trap unabsorbed TCE. In each tank the water was stirred once each day to

maintain the desired uniform experimental concentrations of TCE, since it tended to accumulate as droplets at the bottom of the tank. To minimize evaporation of water and/or trichloroethylene, tanks were covered with plexiglass lids.

Measures of plant performance

Plant modular demography and growth analysis were used to determine the effect of the contaminant upon growth characteristics and the survival of plants. Data were collected for each plant separately. This allowed separate analysis of the data for male and female plants, plants from the Turkey Island site and the Ecarte site, and their responses to different levels of TCE exposure. A complete census of leaf demography was made once a week from May-September 1990; thereafter, records were made every two weeks after active growth had ceased (September-December). Information about the number of leaves, number of new-born leaves, number of leaves that had died, number of ramets and their leaves, type and number of flowers, time of flowering, and date of leaf, ramet and plant death were collected at each observation period. To distinguish leaf cohorts, new leaves > 10 mm long were labelled on each occasion with colour-coded rings made from plastic-coated telephone wire. Different colours were given to each cohort. In this way, it was possible to determine the age, lifespan, and birth and death rates for leaves.

On three occasions, data on leaf lengths and area were also collected. Overall, the data allowed us to assess the influence of trichloroethylene upon the growth, development and reproduction of *Vallisneria americana*.

Trichloroethylene analyses

Assessment of the relationship between the concentration of chemical in water and in the plant provided information whether bioaccumulation was occurring. Aquatic plants can reflect local exposure levels, and because they are continuously immersed in the local water they can provide a good estimate of exposure via water column (see Lovett Doust *et al.*, 1994a). Samples of plant tissues from leaves, roots and stolons were

collected at the end of the experiment for each tank and treatment, for each site and sex separately. Each plant was dissected, leaves, roots and stolons were packed separately and labelled. All material for analysis was stored in hexane-washed aluminum foil in an ultracold freezer at -80 °C.

The concentration of trichloroethylene in plant tissues and in water was measured by using the purge and trap technique (where volatile organic compounds are purged in a closed system from the medium that is being analysed) followed by gas chromatography/mass spectroscopy. The methodology for GC/MS of plant tissue samples is an adaptation of the standard EPA procedure for soils (Analytical method US EPA #624, in USEPA 1987); procedures were carried out by Clayton Environmental Consultants, Windsor.

Statistical analyses

Data were analysed with SYSTAT version 5.02 (1991), through ANOVA, and where appropriate, differences between means were tested for significance using Tukey's HSD test.

RESULTS

Table 2.1 summarizes the results of analyses of variance in terms of fractional survival of genets and plant modules according to treatment. Only significant effects or interactions are listed. There are significant differences in the average leaf duration per cohort, leaf area per genet, and the number of flowering plants in each group. Survival changed over time, and was significantly affected by TCE treatment. Figure 2.1 shows the fraction of original genets surviving in each treatment. The most severe genet mortality is seen in the high TCE treatment, reaching LD₅₀ after about 6.5 weeks. Untreated plants had a classic type I survival curve (Pearl, 1928) with over 95% of dieback occurring at the natural end of the growing season. Of course, overwintering

turions were still alive, and new ramets arose from these in the following season. For the low and mid-level treatments, survival was biphasic with a period of initial high mortality (in the first 10 weeks) that was more severe for the medium TCE treatment, followed by a survival trajectory that was similar to that for uncontaminated plants. The following spring, when plants grew back from overwintering turions, the greatest recovery (in terms of number of genets) was seen in untreated plants, followed by low, medium and high TCE treatments.

Over the whole study, the relative survival rates were 18% for high, 56% for medium, 65% for low, and 90% for untreated plants. In Figure 2.2, the various survival curves for males, females and vegetative plants are shown, with a separate plot for each treatment. In the control tanks, males last longer than females or vegetative genets, and vegetative plants suffer a period of early mortality between weeks 3-5. In low TCE males, females and vegetative plants show similar survival with an early period of mortality, followed by low risk, then mortality at the end of the season. In medium TCE males are no more affected in terms of ramet mortality than they were in low TCE, but females and vegetative genets suffer an initial period of high mortality (40% by week 9), low risk of death from weeks 9-21, followed by senescence in fall. In high TCE vegetative and female plants suffer a high rate of mortality for the first 10 weeks, followed by a reduced mortality rate from weeks 11-26. Males follow a similar pattern, but with lower mortality rates than females and vegetative plants from week 8.

The relative amount of clonal growth (ramet production) in each treatment follows a pattern of relative impact similar to that seen for genet survival (Figure 2.3). Clonal growth was greatest in the untreated plants, and least in the high contaminant treatment. A similar low level of clonal growth is seen in both the medium and low TCE treatment. The untreated plants produced on average 3 ramets per surviving genet in the first season, and five each per genet in the second. Surviving plants in all three contaminant treatments are able to produce approximately three ramets a piece in the second season. However, it must be remembered that a large number of (presumably susceptible) genets have already been eliminated in each of the contaminated treatments.

In terms of clonal growth, there are significant differences between males and females, males showing more clonal growth, with non-flowering plants generally falling between the two (Figure 2.4, for males, females and vegetative plants). In the untreated plants, the greatest peak in clonal growth is seen in the vegetative individuals; however their survival is lower than that for males.

In Figure 2.5, the mean number of leaves per genet is shown, for each treatment, for the first season of growth. Control plants produced the most leaves. Leaf production peaked earliest in the high TCE treatment, followed by the medium and low TCE treatments. Interestingly, the medium TCE plants have a higher peak, of about 12 leaves each, than the low TCE plants (about 8 leaves each), although from weeks 9-25 their leaf numbers are very similar.

Figure 2.6 show changes in leaf number separately for males, females and non-flowering plants. Under each set of conditions, treatment by treatment, males had the most leaves. For both sexes, the control plants had the greatest number of leaves, while the high TCE plants had fewest. Males and females differed in their relative response to medium and low TCE treatments; males in low TCE had fewer leaves than males in medium TCE, whereas females had more leaves in low TCE than they did in medium TCE.

A *per capita* death rate (number of leaf deaths per leaf present at the beginning of each time interval) is plotted for leaves in each treatment (Figure 2.7). Greater death rates are observed in high TCE, followed by medium and low TCE treatments. All plants, whatever the treatment, suffer the same *per capita* death rate at the end of the season. In Figure 2.8, the average lifespan of leaves in each cohort is shown. Leaves of control plants lived longest, whichever cohort they belonged to. Control plants stopped producing new cohorts earlier than contaminated plants. Plants in the high TCE treatment had leaf cohorts with the shortest average lifespans. The pattern for plants exposed to low and mid levels of TCE were similar, and intermediate.

Total leaf surface area of genets at weeks 1, 5 and 11 are shown in Figure 2.9. After one week the high TCE plants showed some effects, although the other treatments

did not differ significantly at that time. By week 5, control and medium TCE plants had significantly more leaf area than low ($p<0.05$) and high ($p<0.01$) TCE plants. By week 11, control plants had twice the leaf area of plants growing in medium and low TCE, while the total area of high TCE plants had declined to a mere 22 cm^2 (about 1/10 of the control plants' leaf area). Figure 2.10 show each cohort's proportionate contribution to the leaf area present at weeks 5 and 11. The relative contributions of each cohort are not significantly different between treatments at week 5; however, by week 11, it is clear that the plants in the high TCE treatment had lost all leaves belonging to the first two cohorts of leaves.

Almost 30% of the initial plants in the control treatment flowered, but far fewer (<8% in all cases) plants flowered in the contaminated treatments. The proportion of the surviving plants that flowered in each treatment is also greatest for the control plants, but, while flowering is reduced among the surviving plants in contaminated treatments, almost 20% of the survivors in the high TCE treatment flowered, with rather lower representation (<11%) of medium and low TCE plants. Of the plants that flowered in the high TCE treatment, the majority originated from the Ecarte site.

Figure 2.11 shows the effect of treatment on leaf number for genets from each of the two sites. The number of leaves per genet increased at the same rate for both populations at first in the control treatment, but Ecarte plants peaked first, at about 11.5 leaves per genet, while Turkey Island plants peaked a little later, at 16 leaves per genet. With TCE treatment, leaf number for genets from Turkey Island was considerably reduced, to a level similar to that of Ecarte plants. In each of the TCE treatments, the Ecarte genets showed an earlier peak, exceeding Turkey Island plants in leaf number at that time, then falling behind by week 10.

Table 2.2 shows the results of TCE analyses from leaves and roots of plants from the Ecarte and Turkey Island sites, by treatment. For the medium and high concentrations of TCE, roots always had greater concentrations of TCE than leaves. For control and low TCE groups, levels of TCE in plant tissues were below the detection limits, and there were no differences between root and leaf tissue. In the medium TCE treatment, the root

tissues of the plants from Ecarte contained about 3 times as much TCE as the corresponding roots of plants from Turkey Island. In the high TCE treatment, plants from Ecarte had twice as much TCE in their below-ground tissues as did plants from Turkey Island, and the leaves of Ecarte plants also contained more TCE than leaves from Turkey Island in high TCE.

Table 2.3 shows results of TCE analyses of sediments from Ecarte and Turkey Island for the three treatments and control together with analyses of water samples to determine the actual (measured) TCE concentration in each tank. In all treatment groups, the sediment from Ecarte accumulated significantly greater concentrations of TCE than sediment from Turkey Island, despite the fact that the jars of sediment had been placed in the same tanks. Note that the actual TCE concentration measured in the water was much lower than that applied. This may be due to evaporative loss or adsorbance of TCE to sediments.

DISCUSSION

There is at present little published information about the effects of TCE on aquatic macrophytes. This is despite the fact that TCE is now a common contaminant of surface waters and sediments (Kaiser and Valdmanis, 1979; Kaiser *et al.*, 1983; Kaiser and Comba, 1986), and despite the fact that aquatic plants are a major pathway whereby contaminants may enter the food chain (Moore *et al.*, 1991). There is a limited amount of information on the toxicity of TCE for some other aquatic organisms. Various LC₅₀ values have been established for algae including 63 mg/L for *Microcystis aeruginosa* (Verschueren, 1977); but a concentration of 1000 mg/L had no observable effects on *Scenedesmus quadricauda* (Bringmann and Kuhn, 1980). A short-term photosynthesis efficiency test gave an 50% inhibition of ¹⁴C uptake during photosynthesis at exposures of 8 mg/L in the unicellular marine alga, *Phaeodactylum tricornutum* (Pearson and McConnell, 1975). However, in tests carried out on *Thalassiosira pseudonana* and

Dunaliella tertiolecta, there were no observed effects on their relative abundance when they were exposed to 50 and 100 µg/L, in a mixed culture (Biggs *et al.*, 1979).

Survival

The results of the present study demonstrate clearly that TCE is able to alter, simultaneously, plant survival, growth and reproduction. The degree of impact was correlated with the TCE concentration in the water (Figure 2.12). Individual plants differed in the severity of their response, and some plants were TCE-resistant. In the high TCE treatment, most of the survivors simply maintained the number of leaves that they started out over the study period with, neither increasing nor decreasing in number. However, a few plants in the high TCE treatment were able to produce new flowers and ramets with a vigour equal to that of the control plants! Most of these resistant plants were from the Ecarte population, where higher background levels of TCE are present. Such differences indicate genetic variation within populations for contaminant resistance, and suggest that such variation can be selected under conditions where there is persistent exposure to a contaminant. In a natural environment, the extinction of a large fraction of genotypes, as seen in the high TCE treatment (82%), would significantly decrease population size, genetic variability, and the potential for future reproduction; it would also represent a strong selection pressure favouring contaminant resistance.

There were clear differences in the probability of survival for males, females and vegetative plants (Figure 2.2), with males showing superior survival; indeed the only reproductive plants that survived the high TCE treatment were males. One hypothesis that has been proposed is that females may suffer higher mortality because of the greater costs of flowering and fruiting in females compared to males (Lovett Doust and Lovett Doust, 1988; Lovett Doust and LaPorte, 1991). However, vegetative plants also suffered high mortality, despite the fact that they expend no energy on sexual reproduction. Reproductive costs do not, therefore, account for the differential mortality rates. In natural populations, one consequence of differential mortality of the sexes will be a skewed sex ratio, and a concomitant reduction in N_e , the effective population size (Crow,

1954, and see Lokker *et al.*, 1994). A decrease in variation within and between populations will produce lower evolutionary diversification (Wright, 1982) which in turn may decrease the versatility of the species' response to subsequent natural and cultural perturbations.

Clonal growth

Clonal growth is more frequent among aquatic vascular plants than among terrestrial plants (Sculthorpe, 1967; Hutchinson, 1975). This often allows aquatic plants to colonize and proliferate in areas where sexual reproduction and/or seed set is low (Arber, 1920; Sculthorpe, 1967; Hutchinson, 1975; Les 1988). In *Vallisneria* populations in the Huron-Erie corridor, the fraction of sexual ramets is low (28-42%), and much population growth is achieved through clonal growth rather than the recruitment of new genetic individuals (genets) (Lovett Doust and LaPorte, 1991; Lokker *et al.*, 1994). In the present study, clonal growth was greatest in the untreated plants (Figure 2.3), and least in the high contaminant treatment. A similar low level of clonal growth was seen in both the medium and low TCE treatment. Therefore, broadly speaking, TCE in the environment is liable to reduce the rate of clonal growth among the plants that survive.

Females, males and vegetative plants show different characteristic patterns of clonal growth (Figure 2.5). Vegetative plants produced the greatest number of ramets in control treatments, followed by males, then females. However, in the TCE treatments, males were able to produce more ramets, and were more likely to survive. Over time, in the field situation, this would lead to a decline in the abundance of females, reduction in N_e , and reduced seed production overall.

Flowering

For greenhouse-grown *Vallisneria americana*, plant biomass was an important determinant of the likelihood of flowering: in a study of 425 plants, 12% flowered. Those that failed to flower were below a threshold dry mass of 0.75 g (Titus and Hoover, 1991). In the present experiment 30% of the surviving control plants, and about 10% of the

surviving low- and medium- TCE plants, flowered (Figure 2.12). It is interesting to note that, of the few survivors in high TCE, a larger portion (20%) flowered. However, the duration of flowering was significantly shorter in high TCE, and no fruits were produced (the flowering plants were males). TCE is, therefore, associated with reduced flowering and reduced seed set in *Vallisneria*. Since seeds of *Vallisneria americana* are always outcrossed, reduced seed set would reduce the level of genetic variability within *Vallisneria* populations (Hamrick and Godt, 1990; Les, 1988, 1991; Laushman, 1991).

In addition to the demographic consequences for *Vallisneria*, there are possible community-level consequences of elevated exposure to TCE; a decrease in clonal growth and sexual reproduction of this dominant plant will reduce the primary production of the system, which may have energetic consequences in the aquatic food web.

Leaves

The leaves of *Vallisneria* are long and ribbon-like; the deeper the water, the longer they tend to grow. The large surface area and absence of a thick waxy cuticle could facilitate uptake of organochlorines such as TCE. As a submerged plant, *Vallisneria americana* is widely believed to absorb nutrients primarily through its leaves and stems, rather than through the roots (Sculthorpe, 1967; Painter, 1990), and it would be reasonable to postulate that contaminants may follow routes that are similar to those for nutrients. More recent work contradicts the assumption that uptake occurs primarily via leaves (see Biernacki *et al.*, 1994 and Barko *et al.*, 1991). In the present experiment, chemical analyses revealed that concentrations in roots were much higher than those in leaves. Plants from each site were growing in the same water column, and therefore were exposed to the same concentration of TCE as far as their leaves were concerned, but the sediments of each site had different TCE content: The Ecarte sediment contained 9 times as much TCE as the Turkey Island sediment (Table 2.2). This contradicts the assertion of Moore *et al.* (1991) that "little selective adsorption of TCE to the suspended or bottom sediments occurs". The observed adsorption may be a function of the contrasting particle-size distribution or organic matter content of each sediment type (Ball and Roberts,

1991). The contrasting sediment concentrations of TCE may have caused greater uptake of TCE by the roots of Ecarte plants resulting in higher TCE concentrations in these tissues. The Turkey Island plants had slightly greater total leaf area. In theory, therefore, if uptake through the leaves was predominant, the plants from Turkey Island should have shown the higher TCE concentrations, but the reverse was the case. It therefore seems reasonable to conclude that TCE uptake is occurring primarily through the roots. In Figure 2.13, the relationship between the \log_{10} concentration of TCE in sediment and the concentration in below-ground tissues is shown for each site. There is a distinct linear relationship for each population and its sediment.

TCE-exposed plants seemed to respond to the chemical by producing new leaves at a greater rate than control early in the season (Figure 2.5). Control plants peaked later, at week 10. Per capita death rate of leaves for all TCE treatments showed a steeper slope than that for control plants (Figure 2.7). For plants in high TCE treatment, this curve reached values > 0.65 , but after 15 weeks, when the non-tolerant plants had died, the curve became similar to that for control plants.

Under TCE treatment, female and vegetative plants suffered greater depression of leaf number than male plants (Figure 2.6). The fact that male plants are able to maintain higher leaf numbers (and hence a greater cumulative leaf area, Figure 2.9) in contaminated conditions may explain their greater clonal growth, as well as their greater rate of flowering, and, ultimately, the observation that male plants show superior survival to female and vegetative plants. Mean lifespan of leaves is a sensitive indicator of environmental contamination (Figure 2.8). The average lifespan of leaves is reduced in higher concentrations of TCE.

Leaves belonging to different cohorts contribute differentially to the total leaf area of the genet. At week 5 (Figure 2.10), all cohorts (each corresponding to an 7-day interval) in all treatments make some contribution to the total leaf surface area. By week 11, the first and second cohorts had died out in the high TCE treatment (Figure 2.10). In all of the TCE treatments, younger cohorts contributed less to the total leaf area than older cohorts. In addition, the older leaves were smaller and narrower than their

predecessors. This indicates that exposed plants were suffering gradual depletion of their resources, suffering a net negative balance between photosynthesis and respiration with eventual death for the majority of high TCE plants.

A comparison of the performance of plants from the two sites suggests that plants from the Ecarte site suffer a proportionately smaller decrease of leaf number than plants from the Turkey Island site when they are exposed to TCE. Note that, because of the properties of the sediment from Ecarte, the Ecarte plants were actually exposed to higher TCE levels in sediment, and accumulated higher TCE levels in their tissues. The smaller effect of TCE on leaf demography, combined with the superior survival of plants, from Ecarte suggests that this population is inherently more tolerant of TCE than is the Turkey Island population. This is in accord with the observation that the most highly TCE-contaminated sediments in Canada are to be found at Ecarte (Government of Ontario, 1986). Thus, the population from Ecarte had a history of previous exposure to TCE, and may therefore have experienced previous selection pressure for TCE resistance.

Uptake of TCE by Vallisneria

Ecarte plants in the high TCE treatment contained 2 times more TCE in their leaves, roots, stolons and turions than did plants from the Turkey Island site in the same tank. However, the demographic data do not reflect this greater degree of contamination. If there is a biochemical mechanism that detoxifies or degrades TCE in exposed plants, it would appear that roots of the Ecarte plants have been able to reduce their TCE content to 1/700 of that in the sediment that surrounds them. Roots of the plants from Turkey Island have only been able to reduce their TCE content to 1/167 of that in the surrounding sediment. This is another indication that Ecarte plants may have a superior mechanism for excluding or possibly degrading TCE; the nature of resistance will be the subject of further studies.

Ramets of TCE-resistant clones (those that survived medium and high TCE treatment) were in turn been cloned and were studied in order to explore the genetic and physiological basis of contaminant resistance. Thus, TCE exposure had a significant

impact on vegetative vigour, sex expression, sexual reproduction and clonal growth in *Vallisneria*. Leaf demography and measures of clonal growth provided good indicators of contaminant burden (determined independently by chemical analysis).

Bioaccumulation and bioconcentration

Bioaccumulation of trichloroethylene has been studied in freshwater and marine environments. Geyer *et al.* (1984) found that *Chlorella fusca* var. *vacuolata*, exposed to TCE at 50 µg per litre for 1 day had a bioconcentration factor of 1,160. The greatest increase in concentration in the tissues of animals (birds' eggs, fish liver, and seal fat) that are relatively high in the food chain ranged from a few to nearly 100 times the level found in ambient water, but, because of its moderate solubility in water, TCE can also depurate quickly (Pearson and McConnell, 1975; Barrows *et al.*, 1980). In the present study, TCE was bioaccumulated, but not bioconcentrated; the levels in plant tissue were lower than those in the surrounding sediment and water columns. However, plants in higher TCE treatments contained more TCE and showed more mortality and impairment of growth and reproduction.

In conclusion, TCE had significant effects on measures of performance in *Vallisneria americana*. Contaminant concentrations were found to be greater in underground tissues than in leaf tissues. Contamination levels in plants were significantly correlated with sediment contamination.

Table 2.1. Summary of ANOVA results of *Vallisneria* growth and reproduction according to time of exposure, site of plant origin, plant sex and trichloroethylene treatment, and interactions among these factors. Significance is indicated as follows: *=p<0.001; **=p<0.01; *=p<0.05; NS = not significant.**

VARIABLE	TIME (A)	SITE (B)	SEX (D)	TREATMENT	BxC	BxD	CxD	BxCxD	AxB	AxC	AxD	AxBxC	AxBxD	AxCxD	AxBxCxD
survival of genets	***	NS	NS	***	NS	NS	NS	NS	*	***	NS	NS	NS	NS	NS
no. ramets/genet	***	NS	NS	***	NS	NS	NS	NS	**	**	NS	NS	NS	NS	NS
no. leaves/genet	***	NS	NS	***	NS	NS	NS	NS	***	**	NS	NS	NS	NS	NS
lvs duration/cohort	***	***	NS	***	NS	NS	NS	NS	***	**	NS	NS	NS	NS	NS
leaf area/genet	***	*	***	***	NS	NS	NS	NS	***	***	NS	NS	**	NS	NS
no. flowering plants	***	NS	***	**	NS	NS	*	NS	NS	***	**	**	NS	***	NS

Table 2.2. Trichloroethylene analyses of samples of above-ground and below-ground plant parts from *Vallisneria americana*, in the trichloroethylene experiment. LOD = limit of detection for a given set of samples. Values are reported as µg per kg dry mass. Lipid content was 0.16% for the plant tissues.

Site	Plant	Treatment	[TCE]	LOD
			(µg/kg)	
Turkey	leaves	control	<40	40
Turkey	roots	control	<40	30
Ecarte	leaves	control	<40	40
Ecarte	roots	control	<40	40
Turkey	leaves	low	<30	30
Turkey	roots	low	<30	30
Ecarte	leaves	low	<40	40
Ecarte	roots	low	<40	40
Turkey	leaves	medium	60	40
Turkey	roots	medium	70	30
Ecarte	leaves	medium	<40	40
Ecarte	roots	medium	220	30
Turkey	leaves	high	<50	50
Turkey	roots	high	150	50
Ecarte	leaves	high	80	40
Ecarte	roots	high	300	100

Table 2.3. Trichloroethylene analyses of samples of sediment and water in the trichloroethylene experiment. LOD = limit of detection for a given set of samples. Values for sediment are reported as µg per kg dry mass; for water as µg per litre.

Site	TCE Treatment	Measured [TCE] (µg kg ⁻¹ or µg L ⁻¹)	LOD
Sediment			
Turkey	control	<4	4
Ecarte	control	<5	5
Turkey	low	160	4
Ecarte	low	730	5
Turkey	medium	1000	4
Ecarte	medium	15,000	5
Turkey	high	25,000	4
Ecarte	high	210,000	5
Water			
0	control	<1	1
53,000	low	1000	1
106,000	medium	4,900	1
318,000	high	27,000	1

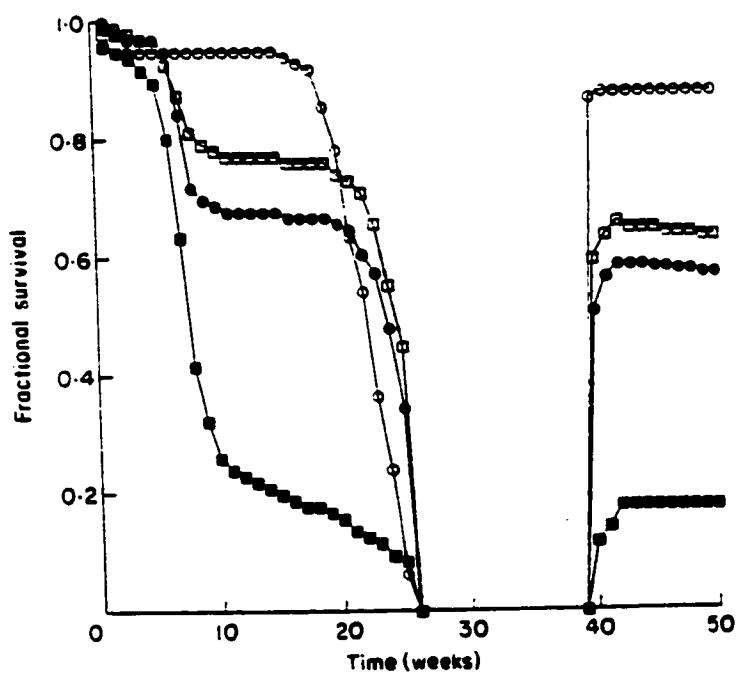


Figure. 2.1. Fractional survival (linear plot) of genets of *Vallisneria americana* exposed to different levels of trichloroethylene (TCE); high (■) medium (●) low (□) and control (○).

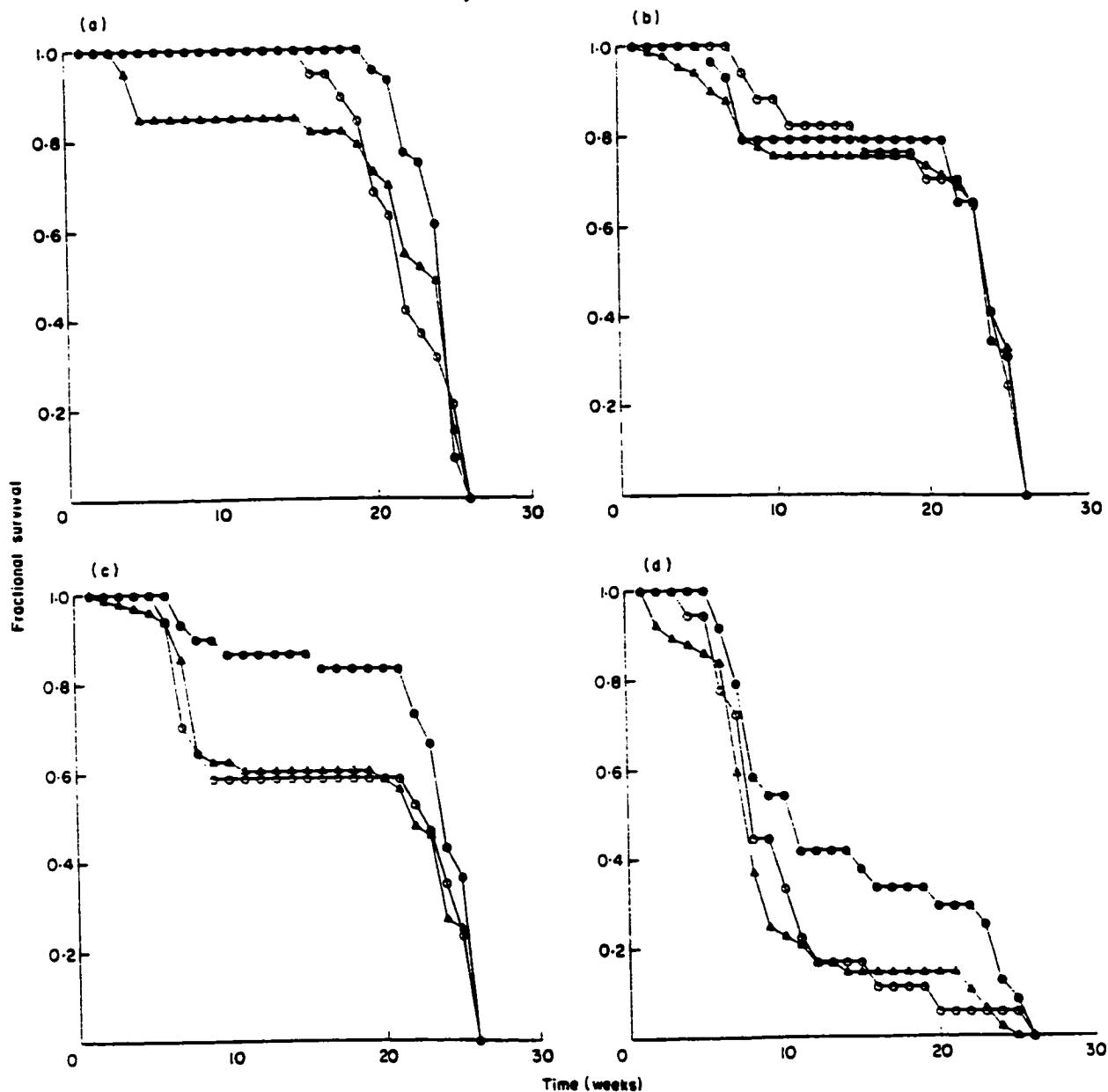


Figure 2.2. Fractional survival of all genets in the first year of the study, according to whether they are male (●), female (○), or vegetative (△). Results are shown separately for a) control; b) low; c) medium and d) high TCE treatments.

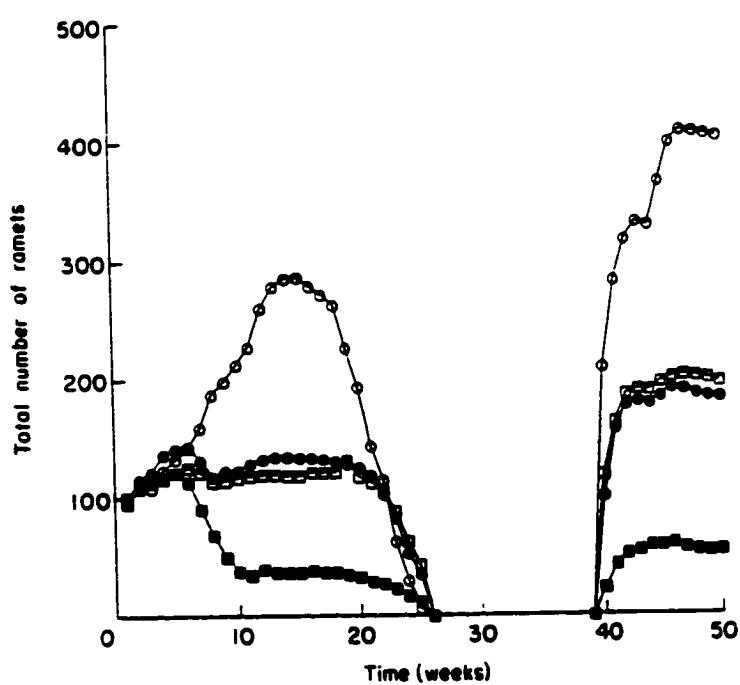


Figure 2.3. Total number of ramets of *Vallisneria americana* produced in the first season of growth, according to TCE treatment: high (■) medium (●) low (□) and control (○).

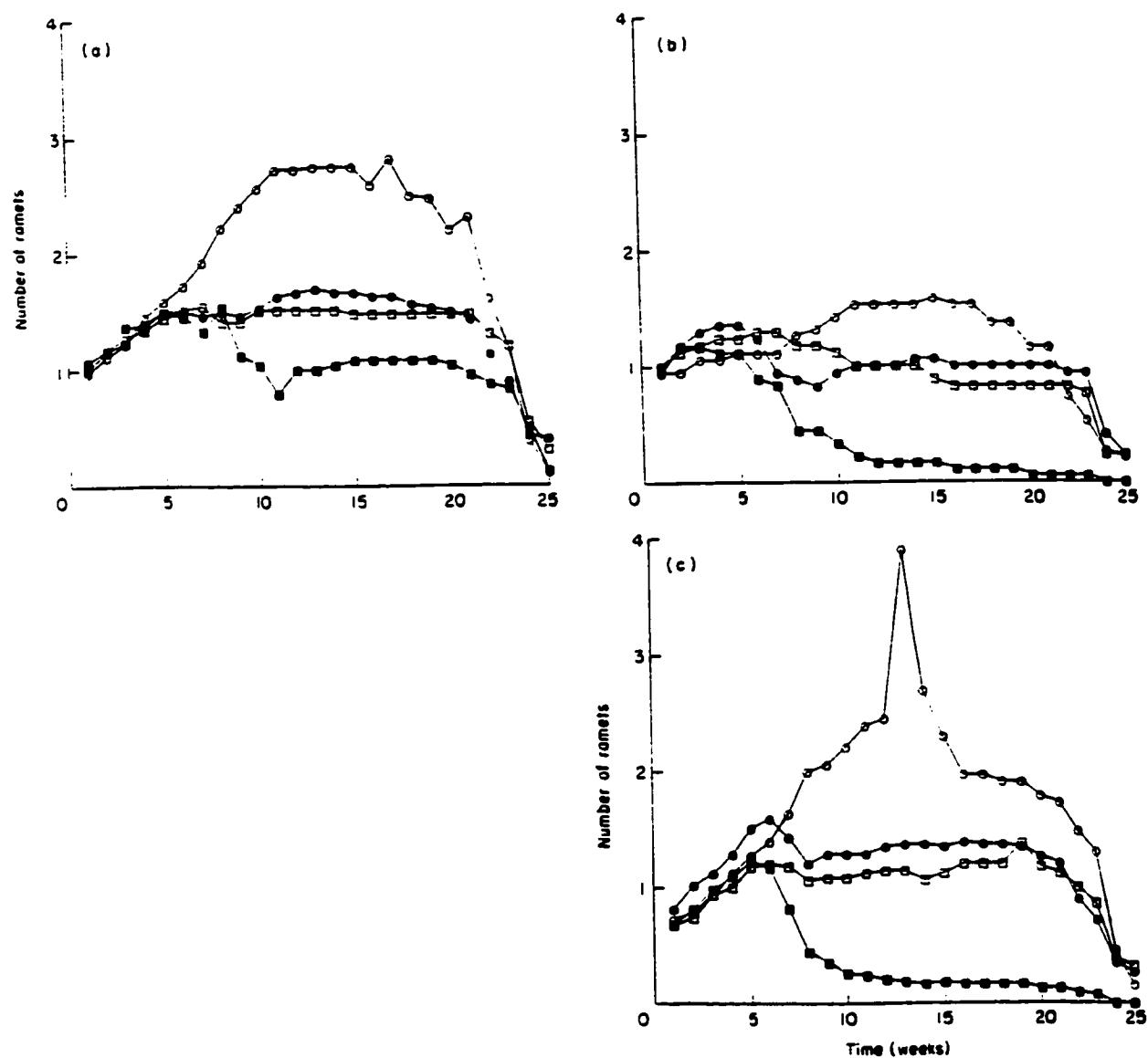


Figure 2.4. Mean number of ramets per genet (*per capita*) produced in the first season of growth, according to TCE treatment: high (■) medium (●) low (□) and control (○), for a) males, b) females and c) vegetative plants.

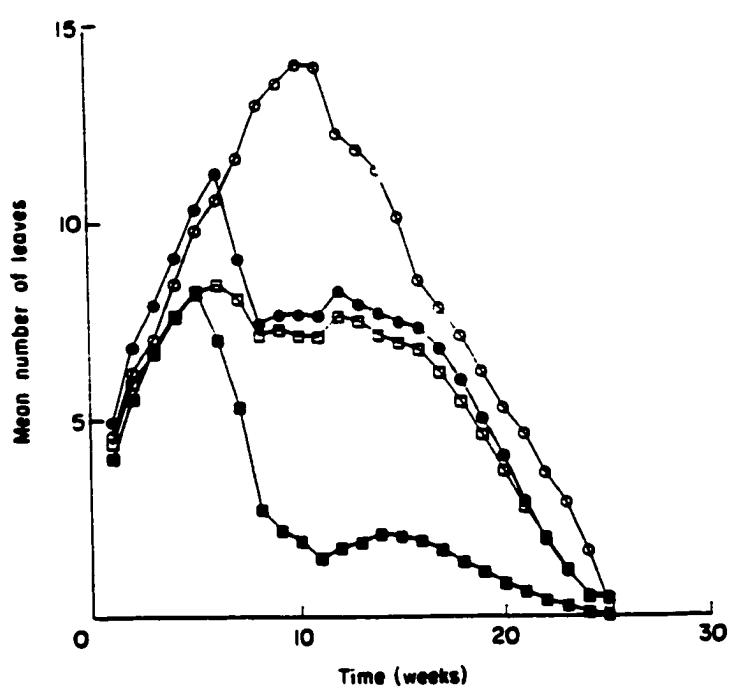


Figure 2.5. Mean number of leaves per plant over the first year of study in different TCE treatments: high (■) medium (●) low (□) and control (○).

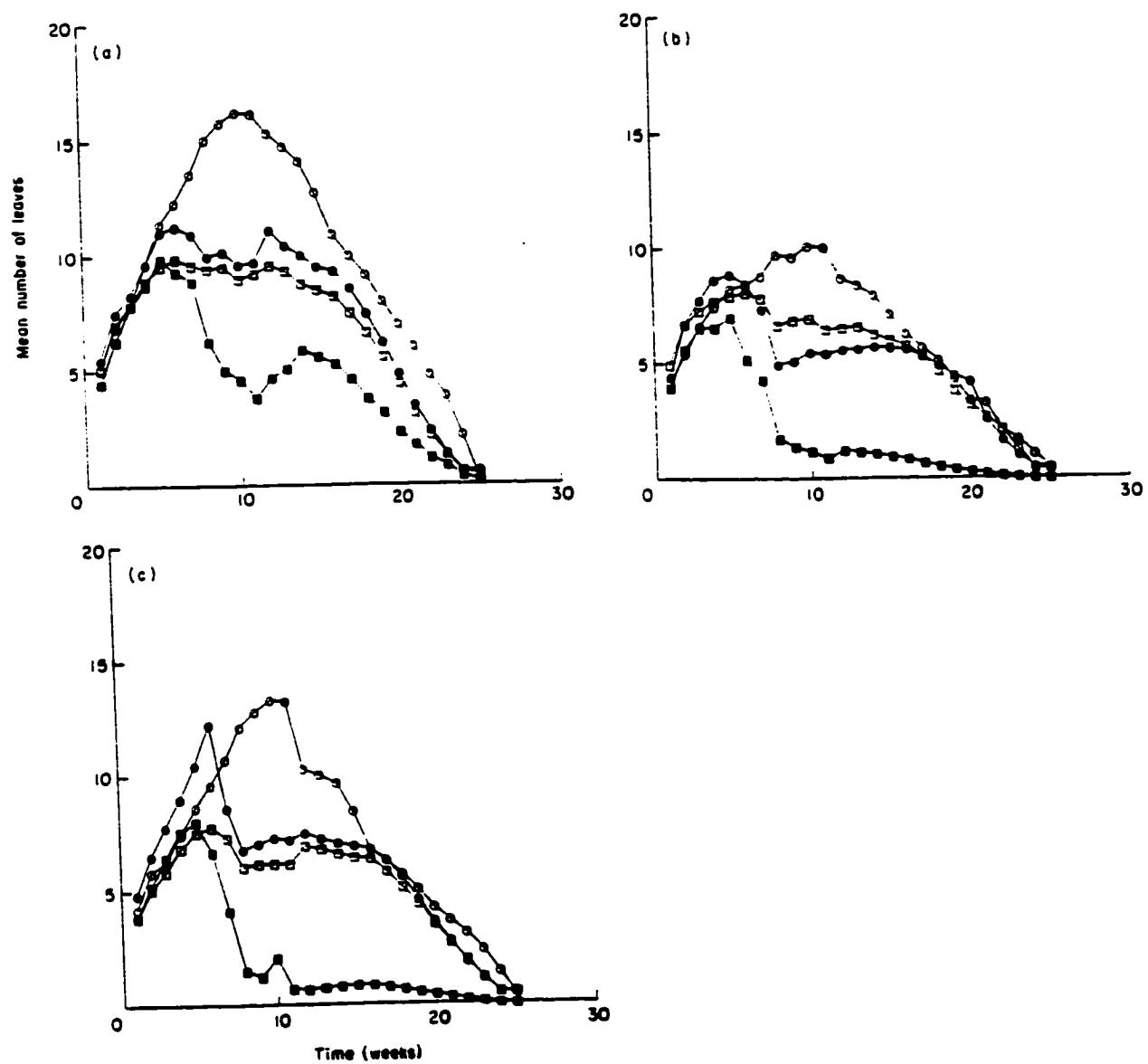


Figure 2.6. Mean number of leaves per plant over the first year of study, according to the level of TCE: high (■) medium (●) low (□) and control (○), shown separately for a) males, b) females and c) vegetative plants.

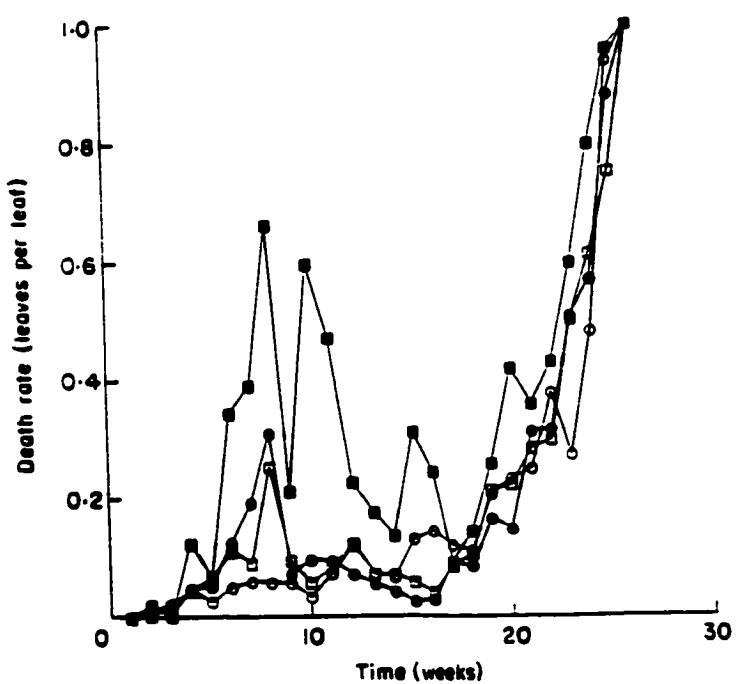


Figure 2.7. *Per capita* death rate of leaves (leaf mortality per leaf present at the beginning of each census period) over the first year of study, according to the TCE treatment: high (■) medium (●) low (□) and control (○).

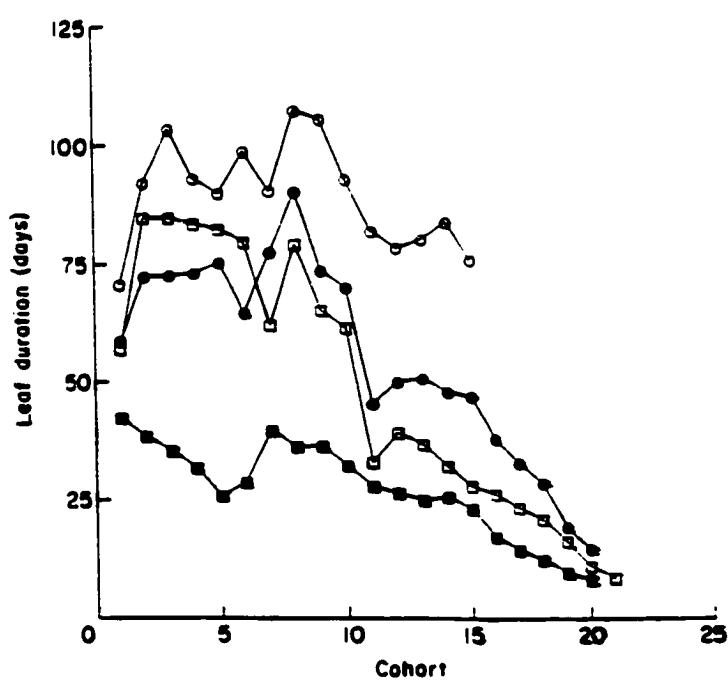


Figure 2.8. Average leaf duration (days) of leaves in each cohort on plants exposed to different TCE levels: high (■) medium (●) low (□) and control (○).

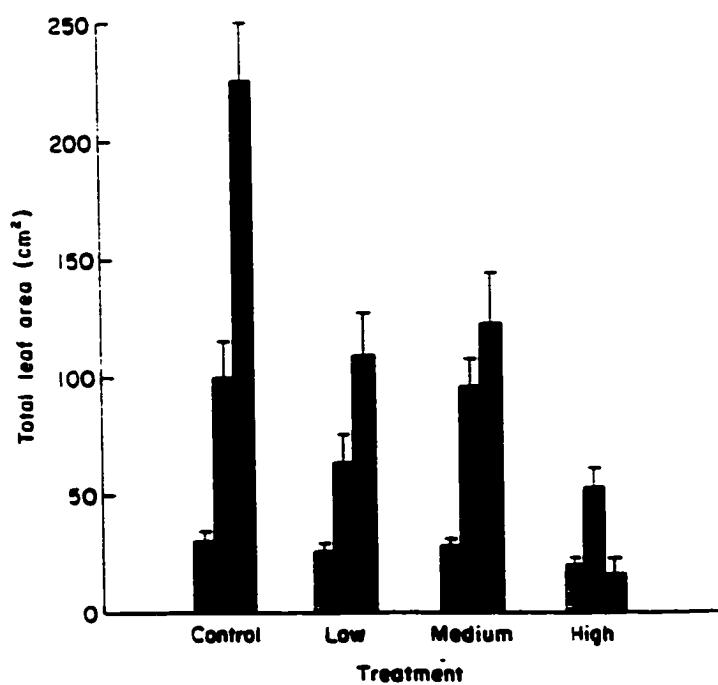


Figure 2.9. Total leaf area per plant present on plants exposed to each of the levels of TCE (control, low, medium and high), at 1, 5 and 11 weeks from initiation of the experiment.

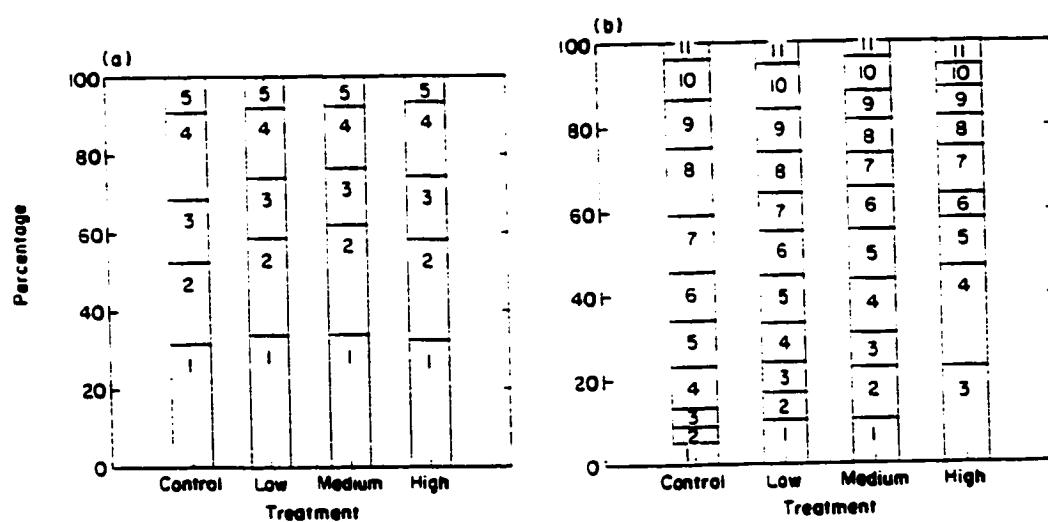


Figure 2.10. Fraction of all leaf area made up of leaves from each of the cohorts that were alive at a) 5 weeks, and b) 11 weeks from initiation of the experiment. Cohorts are numbered sequentially.

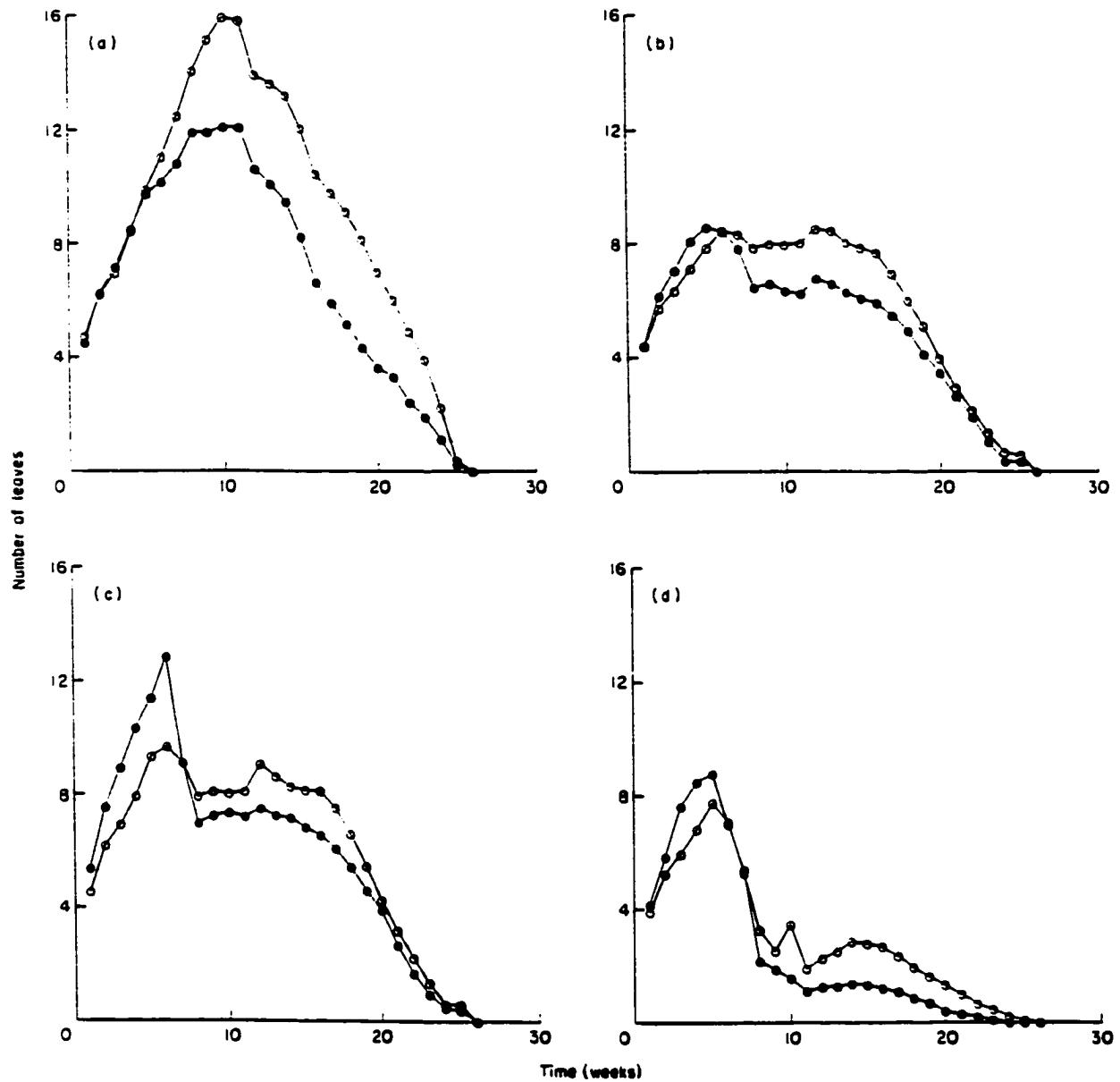


Figure 2.11. Number of leaves per plant on plants from Ecarte (●) and Turkey Island (○), for a) control, b) low, c) medium and d) high TCE levels.

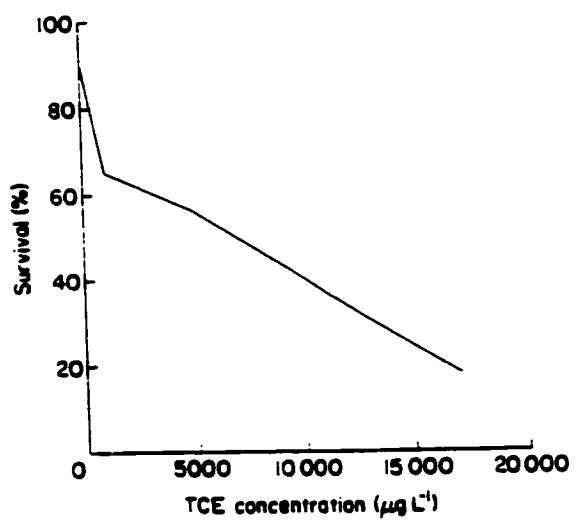


Figure 2.12. Fractional survival of plants according to the measured level of TCE in the surrounding water.

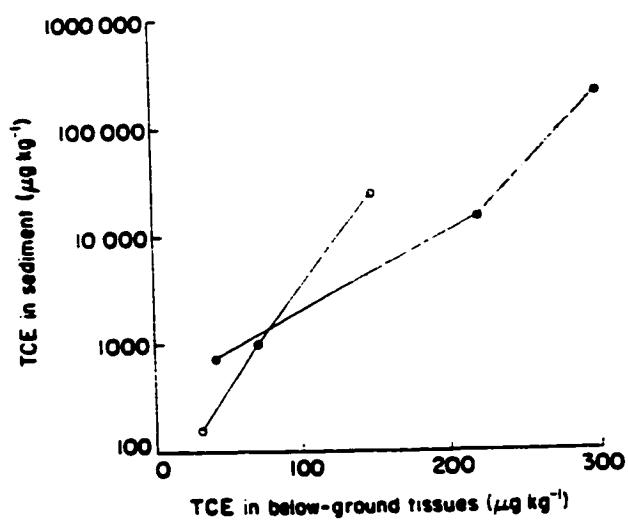


Figure 2.13. Relationship between the \log_{10} concentration of TCE in the sediment and the concentration of TCE in below-ground tissues of plants from Turkey Island (○) and Ecarte (●).

Chapter 3

RELATION BETWEEN UPTAKE OF CONTAMINANTS BY ROOTS AND BY LEAVES IN *Vallisneria americana*¹

ABSTRACT

Aquatic macrophytes can serve as useful early-indicators of local environmental conditions and can be used to monitor sub-lethal effects of organochlorine contaminants on growth and reproduction. A controlled greenhouse experiment was carried out using three concentrations of trichloroethylene (TCE) and four types of sediment to assess the influence of sediment composition on TCE uptake. Growth and survival of *Vallisneria americana* plants, leaf number and leaf duration were monitored. In addition, the surface areas of leaves and roots were recorded, over a period of six weeks. At the end of the experiment, TCE concentrations in sediment and water were determined using GC analysis. Leaf- and root-surface areas were significantly affected by both TCE treatment and sediment type. "Dilution" of natural sediment with sand reduced the TCE content of the sediment. The ratio of leaf-to-root surface area increased with increasing TCE concentration, but the addition of sand to the sediment reduced the effect. Changes in the leaf-to-root surface area ratio were evident within a week of application of the treatment. The leaf-to-root surface ratio may be a useful indicator of sublethal levels of organic pollution in aquatic environments.

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INTRODUCTION

Many studies of toxicity in the past were based on the identification of a standardized mortality, such as the LD₅₀ or LC₅₀, the dose or concentration that caused 50% of individuals to die. However, measurements of plant growth and reproduction are more sensitive, and can reflect the subtle and gradual damage done by persistent low-level exposure to contaminants (Lovett Doust *et al.*, 1994a, 1994b; Depledge, 1990).

Vallisneria americana was used in this study because it is one of the most abundant submersed macrophytes in the Huron-Erie Corridor (Schloesser *et al.*, 1985; Lovett Doust and LaPorte, 1991), and it has shown potential as a biomonitor of organic contamination in the field (Lovett Doust *et al.*, 1994a).

Very little is known about the relative importance of water and sediment as sources of uptake of contamination (Barko *et al.*, 1991; Guilizzoni, 1991, Everard and Denny, 1985). The situation for species that have both a well-developed root-rhizome system, and that have totally submersed foliage (e.g. *Potamogeton*, *Myriophyllum* and *Vallisneria*) is very complex (Jones *et al.*, 1986) and not well understood (Guilizzoni, 1991; Nicholson and Best, 1974).

In preliminary field surveys, it was found that macrophytes accumulate significant amounts of organochlorines *in situ*, and that these concentrations and total body burdens increased over the growing season (Lovett Doust *et al.*, 1994a). Contamination of *Vallisneria americana* was comparable to contamination of other aquatic plants in the Huron-Erie Corridor, including species of *Potamogeton*, *Najas*, *Myriophyllum* and *Elodea* (Biernacki, unpublished). In a survey of contaminants in aquatic macrophytes, including *Vallisneria americana*, it has been confirmed that these plants accumulate contaminants within their tissues, particularly in roots. In addition, plants collected from different sites contained different concentrations of contaminants (Lovett Doust *et al.*, 1994a).

Some previous studies have used changes in root length to assess environmental quality and to detect toxic chemicals. Klaine *et al.* (1990) reported cumulative root length

in *Hydrilla verticillata* exposed to metals and synthetic organics in the sediment. Etzion and Neumann (1993) used changes in root and leaf length of cultivated plants to monitor the toxicity of industrial mineral nutrients. Fiskesjo (1993) used measures of onion root growth to assess water and soil quality. Przymusinski and Gwozdz (1994) observed decreased root length in lupin exposed to elevated concentrations of lead, copper and sodium nitrite. Ryan *et al.* (1993) described toxic effects on roots (inhibition of root growth) in *Zea mays* when their meristems were exposed to elevated concentration of aluminium. In the present study in addition to leaf and root length, also cumulative leaf length, leaf width, cumulative root length and root diameter were measured.

In an earlier study, where *Vallisneria* plants were exposed to different concentrations of trichloroethylene (Chapter 2; Biernacki *et al.*, 1995a), a degreasing solvent and common pollutant of the Great Lakes (Moore *et al.*, 1991; Kaiser and Comba, 1986; Kaiser *et al.*, 1983; Kaiser and Valdamanis, 1979) it was found that, despite equal concentrations of chemical (TCE) in the water, plants had different chemical burdens within their tissues depending on the sediment type they were growing in (Chapter 2 and Biernacki *et al.*, 1995a). In that experiment, plants, despite being equally exposed to the chemical in the water column, were being exposed to different chemical loads in the sediment. Sediment composition influences uptake of TCE from the water column by the sediment (Moore *et al.*, 1991), thus plants growing in sediments of differing physical composition are in fact being exposed to different concentrations of contaminants as far as their roots are concerned (Chapter 2; Biernacki *et al.*, 1995a). Exposure to sediment-borne organochlorine contaminants is particularly important for submerged rooted macrophytes --- sediment frequently has higher concentrations of contaminants than the water column does, thus plants that grow in contaminated environments have much higher concentrations of pollutants in below-ground tissues than they do in foliage (Lovett Doust *et al.*, 1994a; Biernacki *et al.*, 1995a).

To better understand the finding that sediments from different sites absorbed different amounts of TCE from the water column, which indirectly influenced plant

exposure to the chemical, a short-term greenhouse experiment was set up. The objectives of this study were to establish the relationship between contaminant concentrations in the water and the sediment, and demographic effects upon the plant.

MATERIALS AND METHODS

The experiment was set up in the greenhouse of the University of Windsor and ran for six weeks, from August 1992 to October 1992. Plants of *Vallisneria americana* were exposed to three TCE concentrations in the water column and four distinct sediment types. Three tank replicates were set up for each TCE treatment. During the experimental period, the water temperature ranged from 21 °C to 27 °C. Plants were exposed to the natural photoperiod and light flux of up to 2000 $\mu\text{mol s}^{-1} \text{m}^{-2}$ on sunny days and approximately 500 $\mu\text{mol s}^{-1} \text{m}^{-2}$ on cloudy days (measured at the bottom of the aquaria; LI-COR, light meter Model Li-189, sensor Model SPQA). Dechlorinated tap water (pH 7.1) was used and no additional nutrients were added to the sediment or the water.

Sediments

The sediment that was used in this experiment was collected from the Chenal Ecarte in the delta of the St. Clair River. It was 41% sand by volume (particle diameter from 0.05 mm to 2.0 mm), 38% silt (particle diameter from 0.002 mm to 0.05 mm), 21% clay (particle diameter less than 0.002 mm) and had a pH of 7.3.

Four sediment types were prepared by mixing the original, cleaned sediment (washed several times with distilled water) with pure silica sand (particle diameter from 0.05 to 1.0 mm) in various proportions by volume. These were:

- 1- control treatment of "undiluted" natural sediment;
- 2- natural sediment mixed with 5% silica sand;
- 3- natural sediment mixed with 10% silica sand and
- 4- natural sediment mixed with 20% silica sand by volume.

According to the literature, silica sand has no affinity for organochlorine contaminants including TCE (Moore *et al.*, 1991). The experimental sediment was placed in 500 mL glass jars (9.5 cm tall) and set up in the aquaria with water. Each aquarium (capacity 175 L; 92 cm (L) x 31 cm (W) x 62 cm (H)) contained twenty-four jars filled with sediment, comprising four sediment types, with six replicate jars of each type of sediment.

Experimental plants

Plants of *Vallisneria americana* were collected from a natural population at the Chenal Ecarte in the delta of the St. Clair River (for description of local population see Lovett Doust and LaPorte, 1991) in July of 1992. Individual ramets were selected from microsites at least one metre apart to ensure that a wide range of plant genotypes was used. *Vallisneria* plants were placed in plastic containers filled with water for storage and the day, after the collection, all plants were planted in glass jars filled with the previously-prepared four types of experimental sediment described above, and the jars were placed in the aquaria. One plant was planted in each jar. Plants were left for one month to establish before the experiment was initiated. Only well-established plants were used in the experiment. Altogether, 216 genetic individuals of *Vallisneria* were used in the experiment.

TCE treatments

TCE concentrations were applied following the protocol described in Chapter 2. TCE of analytical reagent quality was purchased from BDH Inc. (BDH Inc., Toronto, 1989). In the control treatment, no TCE was added to the water; in the medium TCE treatment 132 mg/L was applied; and in the high TCE treatment 396 mg/L was applied. The concentrations of TCE in all of the experimental treatments were below its limit of solubility (Pearson and McConnell, 1975). The water in the aquaria was changed once a week and a new dose of TCE was added. Water in the aquaria was kept well-mixed by an aquarium bubbler located at the bottom of the aquarium. All aquaria were covered with loosely-fitting plexiglass to minimize TCE evaporation.

Data collected

Over the 6 weeks of this experiment, information was collected about plant survival and clonal growth, leaf number, and leaf duration for each plant (in each jar) separately. Leaf and root dimensions of harvested ramets were measured using a digital micrometer (Mitutoyo Corporation, 1992). It was possible to calculate the projected surface area of leaves and roots knowing their dimensions and cumulative length. The final destructive harvests were made in the sixth week of exposure to TCE and samples of sediment and water were collected and stored for subsequent gas chromatography analyses. It was therefore possible to relate plant performance including changes in leaf and root areas to contaminant concentrations in water and sediment for each treatment, separately.

Analysis of the TCE concentrations in water and sediment

In the water, TCE concentration was determined using the headspace technique (Dietz and Singley, 1979) following the method described by Peng (1993). A Hewlett Packard 19395A head-space sampler coupled to a Hewlett Packard 5890A Gas Chromatograph with HP-1 (Methyl-Silicone Gum), 5 m x 0.53 mm x 2.65 µm capillary column and a FID detector was used. The gas chromatograph was calibrated with standard samples. The temperature for the oven with column was 50 °C, for FID detector was 250 °C, and for the injector port was 225 °C. The volume of water sample was one mL.

In the sediment, TCE concentration was determined using GC/MS with purge and trap unit following the standard EPA procedure for soils (Analytical Method US EPA #624, in Mueller and Smith, 1992). Analyses were carried out in the Department of Civil and Environmental Engineering, University of Windsor.

Statistical analyses

Data were analyzed using SYSTAT for Windows, version 5.02 (1992), through ANOVA, and where appropriate, differences between means were tested for significance

by Tukey HSD pairwise comparison tests.

RESULTS

As in the previous study (Chapter 2; Biernacki *et al.*, 1995a), despite uniform water concentrations of TCE, the four sediment types contained different concentrations of TCE (Figure 3.1). Higher water-TCE concentration yielded higher TCE concentration in the sediment. The highest concentration of TCE had accumulated in the undiluted original sediment from the Chenal Ecarte. The lowest concentration of TCE in the sediment was found in the sediment with the highest sand content.

A summary of the results of ANOVA of different aspects of *Vallisneria* growth after three weeks of exposure are shown in Table 3.1. At this stage, there were no significant effects on number of ramets per genet, number of leaves per genet, or leaf duration (the average lifespan of leaves). However, TCE concentration did have a significant effect on biomass, and both TCE treatment and sediment type, and the interaction between these treatments, had highly significant effects on leaf area and root surface area. After 3 weeks of exposure to TCE, the biomass of ramets was over 6 g in the control treatment, 4 g in the medium TCE treatment, and less than 2 g per ramet in the high TCE treatment (Figure 3.2). After three weeks, there were no significant differences in terms of biomass among sediment types in any of the three TCE treatments.

TCE concentrations

With greater concentrations of TCE in the water, leaf surface area per ramet decreased from over 200 cm² in clean water to less than 90 cm² in the high TCE concentration (Figure 3.3) after 3 weeks of exposure. The decrease in surface area was due to both decreased leaf length and decreased leaf width. However, the total number of leaves per ramet or genet did not change significantly over the six weeks from the beginning to the end of the experiment. Sediment exposed to TCE in the water column

adsorbed significant amounts of the contaminant. The concentration of TCE in the sediment was in all cases higher than in the surrounding water (Figure 3.1) and this was associated with a decrease in root surface area in ramets exposed to the contaminant. The surface area of roots declined from over 70 cm² in the control treatment to below 10 cm² in the high TCE treatment (Figure 3.4) after 3 weeks of exposure. The decrease in root surface area associated with TCE exposure was due to a significant ($p<0.001$) decrease in number of roots, root length, and diameter of roots (Figure 3.5). The diameter decreased from 320 µm in the control, to 110 µm in the high TCE treatment. There were also significant changes in root surface area per gram of root tissue - surface area per gram decreased with increased TCE concentration in the sediment (Figure 3.6).

Leaf-to-root surface area ratio

Manual measurements of leaf and root dimensions were found to be reliable. Initial, replicated measurements of the same leaves and roots did not differ significantly between measurements ($p>0.05$). Since the surface areas of leaves and roots were known, it was possible to calculate the ratio of leaf-to-root surface area. Values differed between TCE treatments, and among the different sediment types at higher TCE concentrations. The ratio of leaf-to-root surface area increased with increasing TCE concentration, but the addition of sand to the sediment reduced that effect (Figure 3.7). Leaf to root surface area ratio may, therefore, be used to assess environment quality in an integrated way that includes aspects of both water contamination and sediment composition. *Vallisneria* plants were able to detect small differences in the TCE concentration that accumulated in four sediment types, exposed to the same TCE levels in the water.

The response of *Vallisneria* (in terms of a shift in the leaf-to-root surface area ratio) to contaminants present in the water and in the sediment is strikingly rapid. Ramets exposed to TCE achieved their final leaf to root surface area ratio after less than a week of exposure (Figure 3.8).

DISCUSSION

As was described above, after three weeks, the strongest and highly significant effects in terms of both TCE concentration and sediment composition were on leaf and root surface areas. It is interesting that the ratio of leaf-to-root surface area is particularly sensitive and responsive to TCE and sediment structural composition. Plants of *Vallisneria* can clearly respond to differences in TCE concentration among different sediment types in each of the TCE treatments, although these differences were subtle and were detected only with the use of GC techniques (Figure 3.7 and 3.1). Thus, these results suggest that the leaf-to-root surface area ratio has great potential as a simple estimator of exposure to environmental contamination.

The concentrations of TCE in the sediment and water in this experiment were lower than some of the higher values found in the St. Clair River and Lake St. Clair (Government of Ontario, 1986; Moore *et al.*, 1991), so *Vallisneria* in this experiment was exposed to contaminant levels that are representative of values found in the Huron-Erie Corridor of the Great Lakes. TCE and some other organic contaminants accumulate in deeper areas of the lake and river and, as a result of the low temperatures, lack of sunlight and oxygen, and the fact that they are frequently adsorbed to sediments, their decay is much slower than it is under terrestrial conditions (IJC, 1992; Moore *et al.*, 1991; Government of Ontario, 1986). The invasion of the zebra mussel (*Dreissena polymorpha*) to the Great Lakes has affected water clarity, increasing the depth of light penetration (Nalepa and Schloesser, 1993), thereby increasing the abundance of aquatic macrophytes. Submersed plants like *Vallisneria* now are established in deeper waters than before the invasion of zebra mussel (Griffiths, 1993). The resulting increase in contact of submerged macrophytes with sediment-borne contaminants may cause increased movement of pollutants through the food web. Thus, a knowledge of the short-term responses of macrophytes to sediment-borne pollutants is of significant value.

A number of factors may influence the shift in leaf and root surface areas that was observed in *Vallisneria americana*. Marberly (1993) found that the surface area of leaves

of *Potamogeton obtusifolius* was responsive to water depth, which affects light quantity and quality available to submersed plants. Root surface area was responsive to sediment nutrient availability (Chambers and Kalf, 1987; Nicholson and Best, 1974) and sediment texture (Barko *et al.*, 1991). Both leaf and root surface areas are also affected by the concentration of inorganic carbon (Marberly, 1985), temperature (Moeller, 1980), pH of water and sediment (Titus, 1992), pressure of the water column (Titus and Stephens, 1983; Titus, 1983), plant density and phenology (Nicholson and Best, 1974), and current (Barko *et al.*, 1991). All of these factors that could modify leaf and root surface area were controlled for in this experiment: water depth, temperature, light, available nutrients, pH, plant phenology, plant density and water movement were uniform for all experimental plants. The only differences were in terms of sediment type and TCE treatment. Further evidence that these additional factors were not involved is the fact that a shift in leaf-to-root surface area ratio between sediment types was only seen if TCE was applied. In the control treatment, where no TCE was added, plants in different sediment types did not differ significantly in leaf-to-root surface area ratio (Figure 3.7). Results suggest that plants were primarily responsive to contaminant treatment and secondarily to sediment texture, which affects the ability to adsorb nutrient and contaminants from the surrounding environment (Barko *et al.*, 1991; Moore *et al.*, 1991). Nicholson and Best (1974), in a survey of *Vallisneria* and *Potamogeton* plants from a population in Lake Chautauqua, NY, found that plants had similar root-to-shoot mass ratios despite the fact that they were genetically different, and the sampled plants had a wide range of individual masses.

In earlier long-term experiments, where *Vallisneria* was exposed to TCE, it was found, that increased contamination decreased survival of plants, clonal growth, biomass of ramets, leaf lifespan, and the probability that a plant would flower (Chapter 2, Biernacki *et al.*, 1995a). Most of these effects became statistically significant after five to six weeks of exposure to TCE, but changes in reproductive parameters were not apparent until the end of the growing season. In the present experiment, it was surprising to see such rapid effects on the leaf-to-root surface area ratio (within a week, Figure 3.8). Thus

assessment of leaf-to-root surface area ratio appears to provide a more rapid assay of contamination than do measures of ramet demography.

Development of new, rapid and inexpensive methods for estimation of leaf and root surface areas of aquatic plants using photometric technique (Watala and Watala, 1994) or digital image analysis (Tagliavini *et al.*, 1993) may make surveys of leaf and root surface areas particularly useful for environmental managers, as a tool for rapid screening and accurate evaluation of environment quality from the perspective of the macrophytes, that form the basis of the aquatic food web (Lodge, 1991). This simple measure of leaf-to-root surface area ratio may provide an effective, convenient and inexpensive metric of site quality from the perspective of the macrophytes that are growing there.

Table 3.1. Summary of results of ANOVA of aspects of *Vallisneria* growth after three and six weeks of exposure to TCE in the greenhouse experiment. Significance is indicated as follow: NS = not significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, * = $p \leq 0.001$.**

Variable	TCE Treatment	Sediment Type	Interaction
<i>After three weeks:</i>			
# Ramets/Genet	NS	NS	NS
# Leaves/Genet	NS	NS	NS
Leaf Duration	NS	NS	NS
Mass/Ramet	*	NS	NS
Leaf Area	***	***	***
Root Area	***	***	***
<i>After six weeks:</i>			
# Ramets/Genet	NS	NS	NS
# Leaves/Genet	NS	NS	NS
Leaf Duration	NS	NS	NS
Mass/Ramets	**	NS	NS
Leaf Area	***	***	***
Root Area	***	***	***

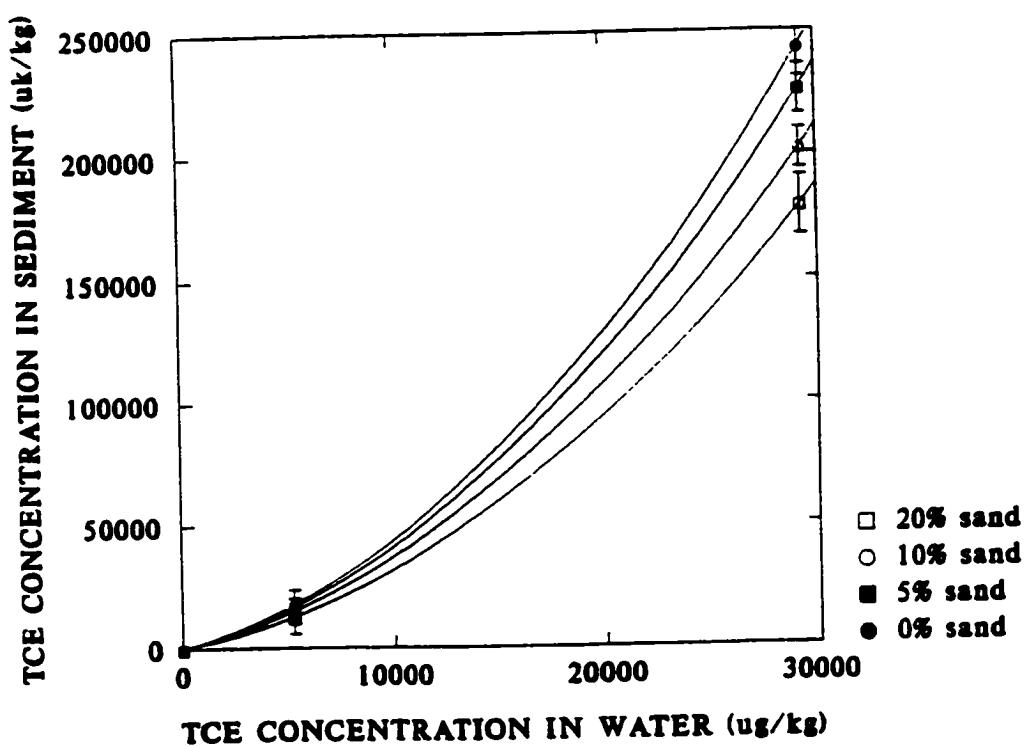


Figure 3.1. Relationship between water- and sediment-TCE concentrations in the four types of sediment.

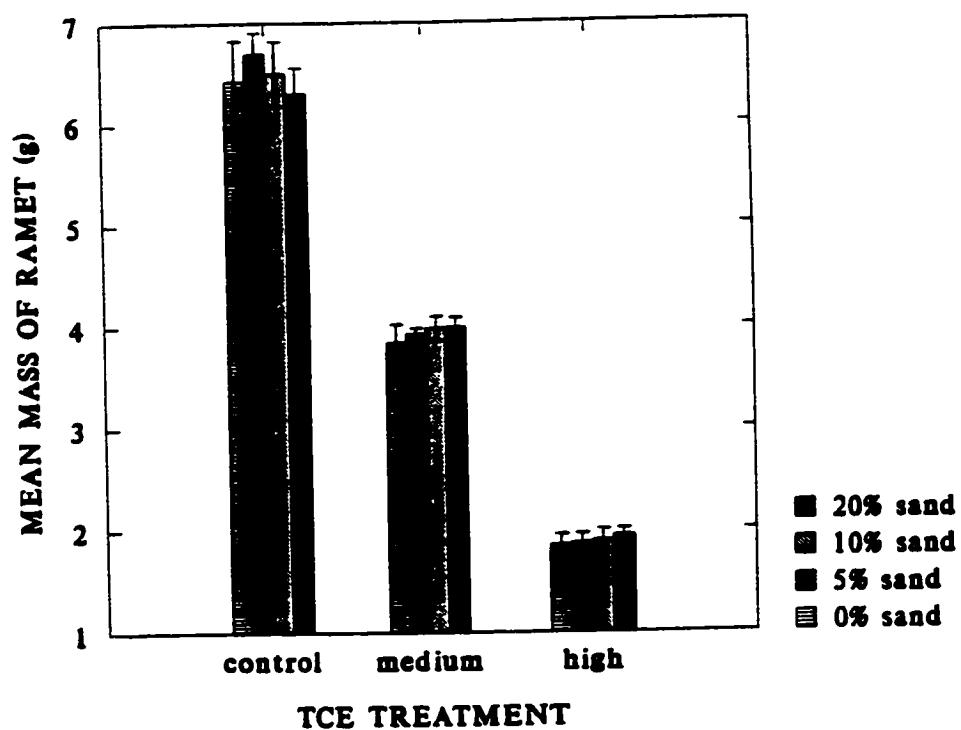


Figure 3.2. Mean mass of ramets in different TCE treatments and different sediment types.

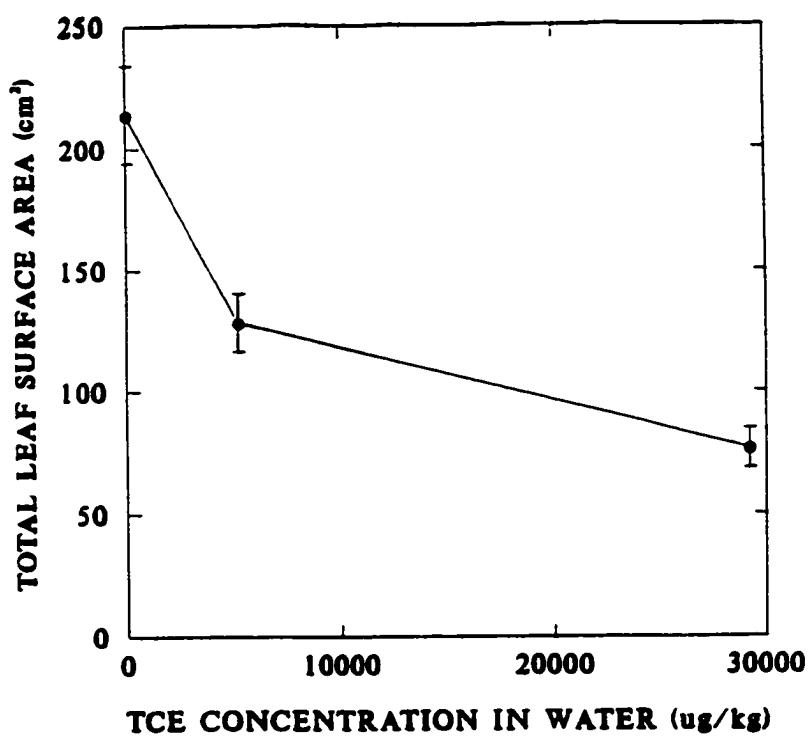


Figure 3.3. Total leaf surface area per ramet in different TCE concentrations.

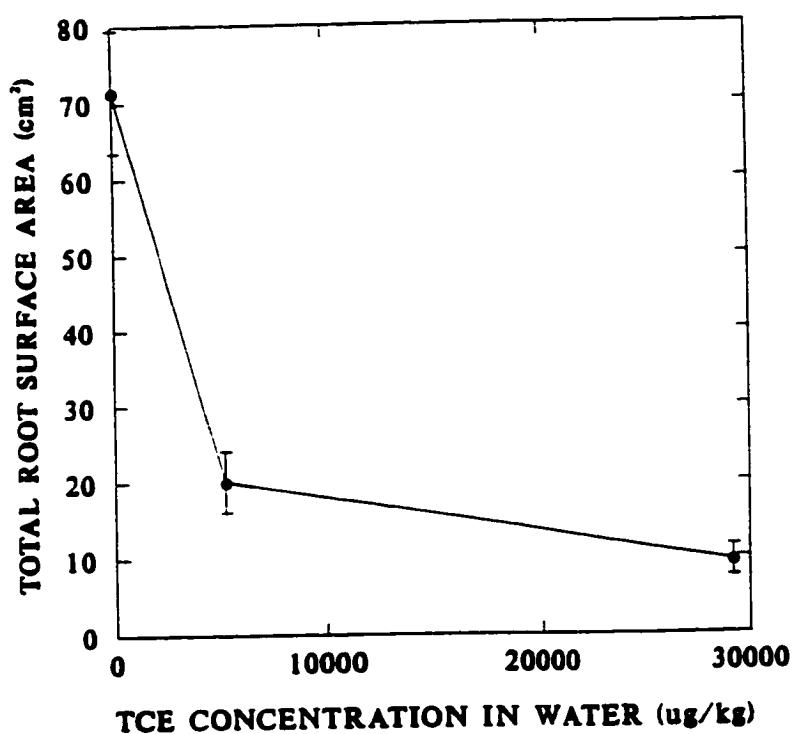


Figure 3.4. Total root surface area per ramet in different TCE concentrations.

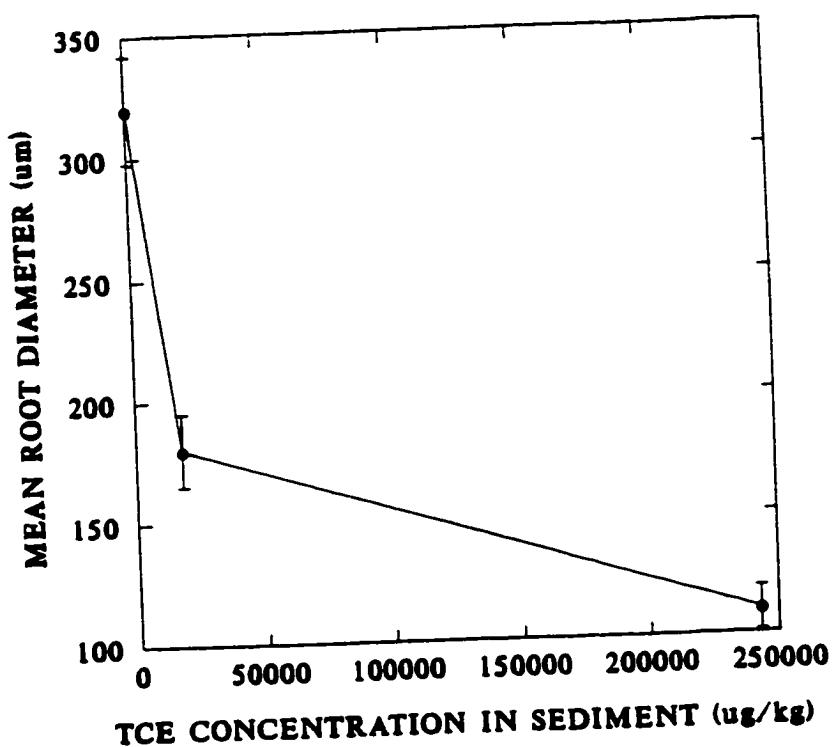


Figure 3.5. Mean root diameter in different sediment-TCE concentrations.

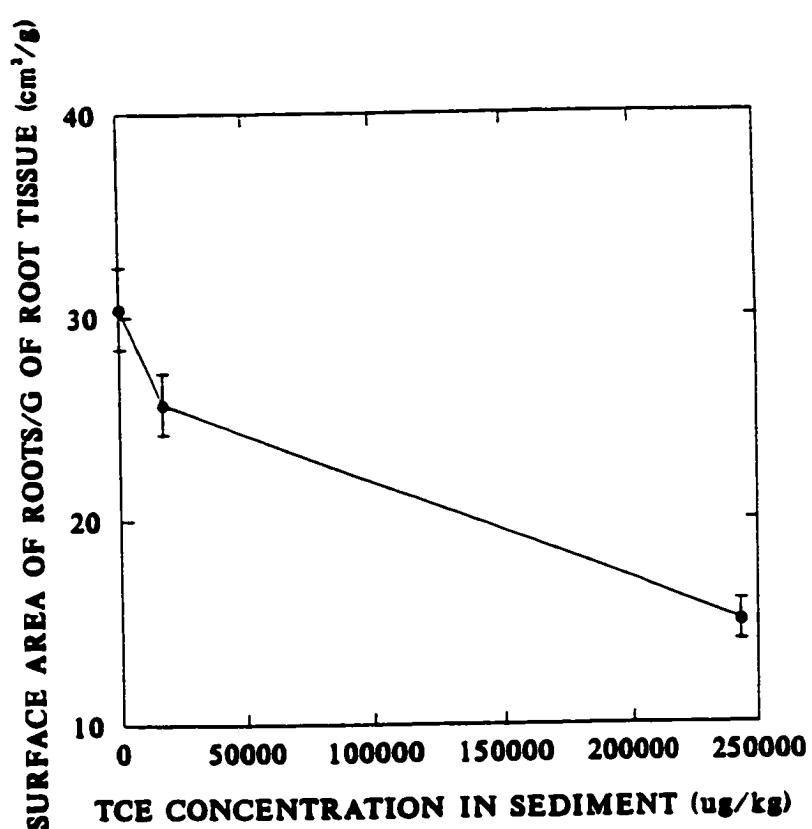


Figure 3.6. Root surface area per gram of root tissue in different sediment-TCE concentrations.

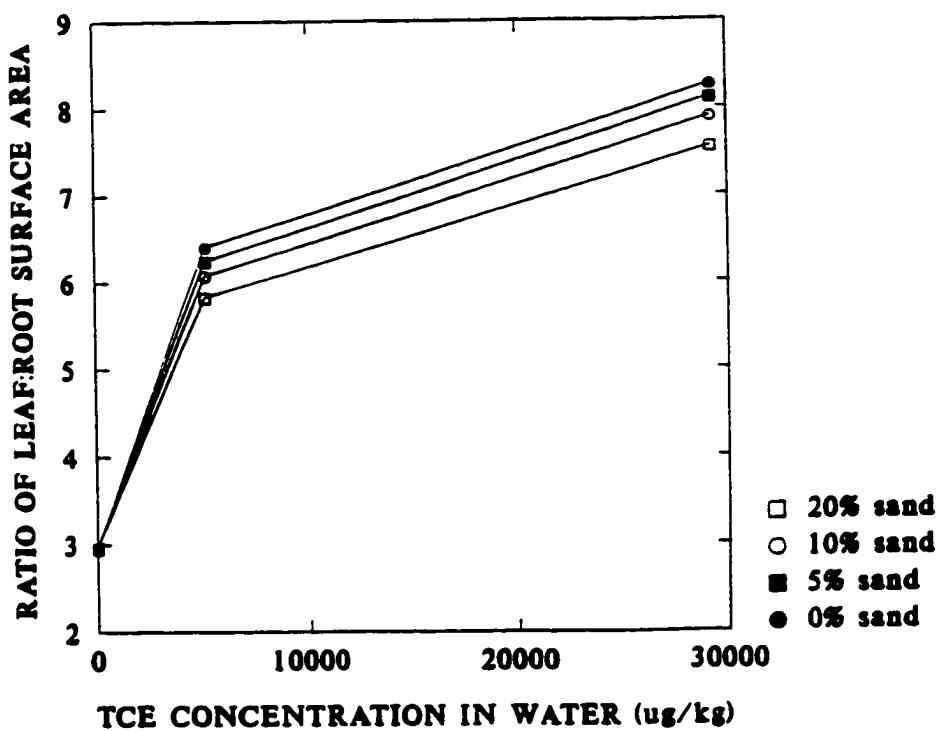


Figure 3.7. Leaf-to-root surface area ratio in different TCE concentrations and in different sediment types.

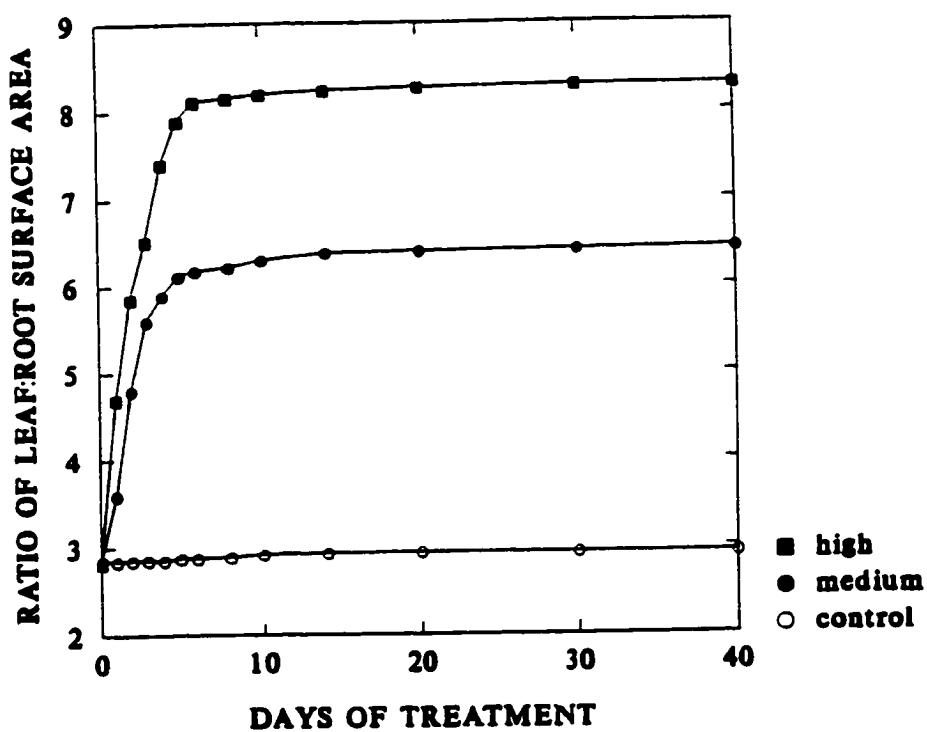


Figure 3.8. Changes in leaf-to-root surface area over time.

Chapter 4

EFFECTS OF SEDIMENT, WATER COLUMN AND SITE OF PLANT ORIGIN ON GROWTH AND REPRODUCTION OF *Vallisneria americana*: SHORT-TERM RECIPROCAL TRANSPLANT-REPLANT EXPERIMENT¹

ABSTRACT

Plant growth and reproduction can provide a means of assessing local environmental conditions. In a field survey, *Vallisneria* plants in the St. Clair and Detroit Rivers were found to accumulate significant amounts of organochlorine contaminants, and the concentration increased over the growing season. Root tissue contained the highest concentrations on each occasion, suggesting contaminant transfer occurred from sediments to the roots. A factorial experiment was set up at two stations in the channel connecting Lakes Huron and Erie to assess the separate effects of contaminant concentrations in the sediment, water column, and source population of the plants, on growth and reproduction of *Vallisneria* plants. Contaminant concentrations in plant tissues were significantly correlated with detrimental effects on plant growth, reproduction and demography. A clear relationship between exposure to contaminants and effect on plant was observed. Results indicated that exposure first to the water column, and secondarily to the sediments from the more contaminated site had detrimental effects on plant performance and survival. Plants at each site appeared to be locally adapted and differed in their apparent resistance to organochlorine contaminants. An "impairment index" (reflecting relative plant performance) was calculated and can be

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Lovett Doust, L., Lovett Doust, J. and Biernacki, M. 1994. American wildcelery,
Vallisneria americana as a biomonitor of organic contaminants in aquatic
ecosystems. *Journal of Great Lakes Research*, 20:333-354

used to calibrate the degree of contamination of different sites relative to a "clean" reference site. This may allow prioritization of remediation of contaminated sites, and should allow field managers to track and document the restoration of environmental quality in recognized Areas of Concern in the Great Lakes.

INTRODUCTION

Past evaluations of water quality in contaminated natural environments have focused on concentration measurements of toxic and carcinogenic chemicals. However, for environmental management and protection of human health, contaminant concentrations do not necessarily account for, or enable prediction of, the impairment of biota. If the objective is to monitor and improve environmental quality for the biota, and to assess potential risks of human exposure, biotic measures of contamination are frequently more useful than measures of ambient contaminant concentrations. Furthermore, the cost of measurement of contaminants, particularly persistent, toxic, organochlorines, is substantial, and is generally impractical for routine monitoring programs. Therefore, for biological and economic reasons, it is important to develop biological monitors of environmental quality that are useful and valuable.

The presence of indicator organisms provides a measure of cumulative exposure to contaminants over time and avoids the need for frequent sampling. Furthermore, the use of a biomonitor removes concerns about the bioavailability of contaminants that must be dealt with if only water or sediments are sampled.

Scientists model contaminant dynamics in aquatic ecosystems by measuring rates of change in contaminant concentrations in various plant and animal tissues. Plants form the basis of both the herbivore and detrital food webs, so they are a likely pathway for contaminant movement between trophic levels. Nevertheless, most models of contaminant trophic transfer have not yet explored in detail the role of plants (Gobas *et al.*, 1991).

Plants are good biomonitoring of environmental conditions in aquatic ecosystems. Many macrophytes are capable of extensive clonal growth, thus the genotype can be held constant. To evaluate environmental conditions, this means that genetically identical individuals can be deployed at numerous sites and the effect of environmental conditions clearly evaluated. In addition, genetically identical replicates can be deployed at a single site, and harvested regularly over the period of study to measure both acute and chronic

exposure to contamination. Depending on the contaminant, rates of bioaccumulation in plants may be very high (Painter, 1990).

Most of the existing biomonitoring studies have involved caged animals that are recovered after some specific period of exposure. However, in addition to the difficulty of genetic variation among individuals, there is always a concern that cages may create unnatural conditions, particularly for filter feeders that need to maintain their own local water currents, and for animals that normally range freely. Plants, unlike animals, do not need to be caged in order to keep them in the same area, which obviates some of the problems that arise with animal biomonitoring. Indeed plants are likely to be superior biomonitoring of point source impact zones (PSIZ), as described in the Revised Great Lakes Water Quality Agreement (Government of the United States and Government of Canada, 1987). In the highly contaminated areas, identified by the International Joint Commission (IJC) as Areas of Concern, plants can monitor cumulative exposure at a particular location.

In the field, plants are exposed to a complex array of chemicals and to the physical and biological conditions that exist at different locations. If a biomonitor experiment is to be useful, it has to discriminate between effects that are due to properties of hydrology and sediment, and the geographic location itself (which may encompass various factors, such as latitude and geology).

The present study evaluates plants as biomonitoring of organochlorine contamination, and correlates plant growth and both sexual and asexual reproduction with the degree of contamination of the water column and sediments. The major objective was to use plant growth to measure degree of contamination in different sites or treatments, and to correlate the amount of growth with the actual measured contaminant content of the plants.

MATERIALS AND METHODS

The present study used plants growing naturally, and planted experimentally at two sites in the Huron-Erie corridor of the Great Lakes, the section of connecting channels between the upper and lower Great Lakes. The investigation focused on conditions in the Detroit and St. Clair Rivers since these are both recognized as areas of concern by the IJC (Hartig and Thomas 1988). Specifically, plant were examined in the Chenal Ecarte in the delta of the St. Clair River, 37 km downstream of Sarnia and the petrochemical industrial region known locally as "Chemical Valley", and adjacent to Turkey Island in the Detroit River.

Vallisneria americana is a dioecious submersed macrophyte, common in the Huron-Erie corridor of the Great Lakes of North America (for details of plant biology see Catling *et al.*, 1994 and Lovett Doust and LaPorte, 1991). The plant grows clonally by producing connected daughter plants or "ramets", that can spread to occupy large areas.

Contaminant Distribution in two natural populations of Vallisneria americana

In spring (May), summer (July) and fall (October) of 1991, 20 samples of plants from each site (Turkey Island and Chenal Ecarte), were taken at random from the submerged macrophyte bed. The samples were kept in an iced cooler, and returned to the laboratory where they were stored in hexane-washed aluminum foil at -80°C until analysis for organochlorine contaminants. Each plant was divided up into the following three tissue categories: 1. turion (the overwintering storage organ) plus stolon (the organ of clonal growth); 2. root, and 3. leaf. Tissues from the 20 replicates were pooled for the purposes of organochlorine analysis.

The proportion of lipid contained in each plant tissue was measured separately for each sample. Percent lipid was determined gravimetrically, i.e. it was assessed in terms of the increment in mass of the extracting solution following extraction of the fraction of each plant sample (of known mass) that was DCM/hexane-extractable. Obviously, the organization of lipid distribution in plants is rather different from that found in animal

tissues. Rather than containing localized fatty deposits and having some tissues of high lipid content as in animals, plants contain relatively low, and evenly distributed lipid, much of it found in cellular membranes and leaf cuticles (the cuticle is very thin in *Vallisneria* as it is a submerged plant). It is therefore very important to ensure that the extracting solution has access to, and sufficient time to penetrate, all regions where lipids are present. Since the % lipid was known, it was possible, for each sample, to calculate lipid-corrected values for each contaminant. The average % lipid for these plant tissues was 0.16; this is 1-2 orders of magnitude lower than the lipid content of most animal tissues.

Contaminant distribution in experimental transplants and replants in two populations of *Vallisneria americana*

In addition to the study of plants growing naturally at each site, further samples of *Vallisneria* were taken from the experimental tubs at Turkey Island and Chenal Ecarte (see experimental design below). Plants were again partitioned into their respective tissues (turion, root, stolon and leaf), and sampled separately in terms of the following eight categories: plants originating from Turkey Island placed in Turkey Island sediment at the Ecarte site, placed in Turkey Island sediment at the Turkey Island site, in Ecarte sediment at the Ecarte site, and in Ecarte sediment at the Turkey Island site; similarly, plants originating from the Ecarte site were taken from tubs in which they were growing in Turkey Island sediment at the Ecarte site, Turkey Island sediment at the Turkey Island site, in Ecarte sediment at the Ecarte site, and in Ecarte sediment at the Turkey Island site. Tub samples were taken on two occasions, in July and September 1991. Organochlorine residues in plants from each treatment were analyzed to determine whether impairment of growth and reproduction was associated with contaminant content.

Analytical Procedure

All sample extracts from the field and experimental sites were analyzed using gas chromatography and electron capture detection (GC/ECD) for the presence and

concentrations of penta- and hexa-chlorobenzene (QCB, HCB), octachlorostyrene (OCS), trans-nonachlor, 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (pp'-DDE), and an array of polychlorinated biphenyls (PCBs [PCB numbering scheme from Ballschmiter and Zell 1980]). A Hewlett-Packard Model 5790A capillary column GC-ECD fitted with a 15 mm x 0.25 mm fused silica column containing a cross-linked DB-1 stationary phase (supplied by J & W Scientific, Rancho Cordova, CA), was used for the analysis, performed by the Great Lakes Institute, University of Windsor. A Hewlett-Packard auto-injector and a model 3390 A integrator completed the apparatus.

Each sample was extracted in a solvent mixture of DCM:hexane (1:1 by volume), then concentrated to an approximate volume of 2 mL, which was then passed to the florisil clean-up procedure, evaporated again, made up to a suitable volume with hexane, cleaned on activated copper powder (to remove sulfur-containing secondary plant substances), and analyzed by GC/ECD. Earlier macrophyte samples, in the first instance, contained two peaks that corresponded to organic sulphur-containing compounds. In the copper clean-up procedure, approximately 0.5 g of activated copper powder was added to the 5 mL volumetric flask. The solution was carefully vortexed for 1-2 min, and then stored in a refrigerator at 4 °C for 24 h before GC/ECD analysis.

Two experiments were performed simultaneously to assess the recovery of separated compounds using 10 g of "clean" macrophyte samples spiked with a standard spiking "cocktail" solution. The mixture was left for 1 h to ensure the contaminants were absorbed by the tissue, then the sample was prepared in the manner described above, for regular samples. All compounds were found to show 85-110% recovery efficiencies. The standard used for quantification of samples was a standard mixture solution containing 10 organochlorine compounds, and Arochlor mixture 1242:1254:1260 in the ratio 1:1:1, provided by the Canadian Wildlife Service (CWS) Laboratories.

Quantification of each compound was achieved by comparing the sample peak areas with the standard peak areas from the CWS-Standard Solution, which contained the same compounds at known concentrations. This calibration was repeated every month, and quality control verification was made independently every three months. The limit of

detection (LOD) for these compounds was determined in a concurrent study involving clam and fish analyses. Comparisons with the baseline study indicated that if concentrations were < 0.01 ng/g (0.01 µg/kg), they were below the detection limits of the procedure.

Design of the reciprocal-transplant-replant experiment

In the past, most studies of toxicity have been based on identification of lethal doses; the goal then was to measure how much contaminant corresponded to standardized mortality, for example the LD₅₀ (lethal dose) or LC₅₀ (lethal concentration), where 50% of individuals died. However, plants may:

1. Increase/decrease overall rate of growth (including clonal growth) as determined by biomass, stolon and ramet production
2. Increase/decrease rate of production and turnover of leaves
3. Change their relative rates of sexual reproduction and clonal growth
4. Die

The present field experiment was designed to evaluate sublethal effects of (a) sediment type; (b) water column (i.e. living at one site or an other); (c) the site of origin of a particular plant; and (d) time since setup of the experiment, on performance of *Vallisneria* using analysis of variance, and to assess the relative importance of each of the main factors (above), as well as their interactions.

In September 1990, a total of 800 *Vallisneria* plants, 400 originating from each of the two sites was deployed. Plants were contained in plastic tubs (51 cm X 38 cm X 12 cm; 10 plants/tub). Half of the tubs were placed at each of the two sites. The first site was on the Chenal Ecarte of the St Clair River, east of Walpole Island between Port Lambton and Wallaceburg, Ontario (42°37'N, 82°28'W). The second site was approximately 86 km downstream, on the western shore of Turkey Island (42°11'N, 83°28'W), downstream of Fighting Island in the Detroit River, and approximately 7 km upstream of Lake Erie. Tub containing plants were set on the sediment surface.

In a factorial design, plants from each site were grown in tubs, each containing

one of the two sediment types. Their leaves extended in the water column present at each site. Thus, at each site there were 40 tubs ---20 filled with sediment from the Turkey Island site and 20 with sediment from the Ecarte site, and each tub contained 10 plants (genetic individuals, or genets made up, initially, of a single ramet or rosette).

All of the tubs were permanently marked according to source of sediment and plant origin so they could be easily identified. Measurements were made of plant survival, clonal growth (production of new shoots via stolons) and leaf number regularly throughout the year, and on two occasions (July 22, and September 15, 1991) destructive harvests were made and patterns of biomass allocation and contaminant content were determined.

Correlation of plant performance with plant tissue contaminant burden

The nonparametric Spearman Rank correlation test was used to determine whether if any significant correlations exist between the relative ranks of contaminant concentration in plant tissues and the relative ranks of plant performance in experimental treatments (eight treatments; two sites of exposure X two sediment types X two sites of plant origin)(Lovett Doust *et al.*, 1994a).

Relative impairment

The "relative impairment" of plants is an index that can be calculated to represent performance at one site compared to another. Ideally, in applying the biomonitor approach used here, the standard of comparison should be with the comparatively "cleanest" site, where plants grow optimally. Relative impairment should be calculated from that baseline. In the present study, at the Turkey Island site plants from both locations produced more ramets per genet and more plants flowered, so it was chosen as the baseline for this preliminary study.

Relative Impairment (RI) =

$$\begin{aligned} & [(\text{performance at Turkey Is.} - \text{performance at Ecarte}) / \text{performance at Turkey Is.}] \times 100\% \\ & =[1 - (\text{performance at Ecarte} / \text{performance at Turkey Is.})] \times 100\% \end{aligned}$$

Statistical analyses

The replicated factorial experimental design allowed analysis of variance of all aspects of plant growth and performance, and where ANOVA indicated significant factors or interactions, follow-up tests of comparison of means were performed using statistical software SYSTAT ver. 5.03 (1992). The nonparametric Spearman Rank Correlation test was also used.

RESULTS

Contaminant content of *Vallisneria americana* in natural populations

Contaminant content of plants in the field was measured by GC/ECD scanning (Tables 4.1 and 4.2). The macrophytes accumulate significant amounts of organochlorines *in situ*, and these increase over the growing season. In May, (before leaves had been produced from the overwintering turion), most contaminants were concentrated in roots (Tables 4.1 and 4.2); the levels were higher for HCB, OCS, pp'-DDE and the Arochlor mix of PCBs in plants at Ecarte than at Turkey Island. By the end of June-early July, leaves contained measurable amounts of contaminants, but roots still contained the highest concentrations, and plants from Ecarte were more heavily contaminated. By October, when female plants were in fruit, again roots contained the highest levels of contaminants, although levels in leaves were also high, at both sites. Fruits held disproportionate amounts of PCBs 52, and 66/95. Roots contained most contaminant year-round. Overall, in comparing the two sites, plants at Ecarte were 3-4X more contaminated than those at Turkey Is.; the levels of HCB were about 6X as high at Ecarte, OCS about 50X as high as at Turkey Is. Roots did not contain more lipid than other plant organs. However, on a lipid-corrected basis, for the Turkey Is. plants, roots contained 4X as much contaminant, and for the Ecarte plants, roots had approximately 10X the concentration of contaminants found in other tissues.

Non-destructive monitoring in Reciprocal-Transplant-Replant experiment

Time, site, plant origin and sediment type all had significant effects on plant growth (Table 4.3). Significant interactions are also shown in Table 4.3. Clonal growth (assessed as number of ramets/genet) was most strongly affected by the various factors and their interactions, and therefore provides a particularly sensitive measure of relative plant vigour. The mean length of a leaf and the cumulative length of leaves on a ramet seem characteristic of plant's site of origin and responsive to environmental conditions on where plant was grown.

In general, the relatively adverse effect of having been grown at Ecarte was greatest, with sediment type and site of origin of the plant also showing statistically significant effects (Table 4.3). The number of ramets produced per m² was greater for any of the treatments at Turkey Island than at Ecarte (Figure 4.1). At both sites, by the end of the season, "locals" (i.e. plants originating at the site to which they were transplanted) produced more ramets than "aliens" after controlling for sediment type. A similar pattern is evident for the number of leaves per m² at each site (Figure 4.2). The number of leaves per ramet was comparable at the two sites, being in the range of 5-7 leaves per ramet (Figure 4.3). Plants originating from Turkey Island had more leaves per ramet at the outset, in tubs placed at both sites, but by the end of the season Turkey Island plants in Ecarte sediment, raised at the Ecarte site had the fewest leaves. At the Ecarte site, flowering did not occur in any plants grown in Ecarte sediment; at the Turkey Island site, flowering by plants from Ecarte was negligible, and plants from Turkey Island flowered significantly more if they were in Turkey Island than in Ecarte sediment.

Destructive harvests in Reciprocal-Transplant-Replant experiment

It should be noted that the biomass samples of 1991 represent the contents of a single tub from each treatment on each occasion, therefore plants, rather than tubs, are replicated. The results are therefore descriptive, and provide no assessment of between-tub variance.

In absolute terms, greatest biomass per m² was found in Turkey Island plants

growing at Turkey Island, whether they were grown in Ecarte sediment or sediment from Turkey Island (Figure 4.5). By September, this differential had increased; greater biomass per unit area was recorded for Turkey Island plants at their native site, with those in their natural sediment producing more biomass than those in Ecarte sediment (Figure 4.5). In terms of the biomass of individual ramets in each treatment, a similar pattern was seen (Figure 4.6). Ramets from the Turkey Island population were of greater biomass, and by September were bigger in the Turkey Island site, and in Turkey Island sediment.

In July, all plants (from each source or sediment type) at Turkey Island had proportionately more leaf tissue, and less root and stolon tissue than was the case for the corresponding treatment at Ecarte (Figure 4.7). By September, for each treatment, the plants growing at Turkey Island had proportionately less biomass in leaves and roots, and more in stolons (Figure 4.8).

Number of leaves per genet was also greatest for Turkey Island plants growing at Turkey Island in July (Figure 4.9); by September the Turkey Island plants in their natural sediment, but growing at Ecarte, had almost as many leaves (Figure 4.9). In natural populations, plants from Turkey Island typically have longer leaves. By September, it was apparent that Ecarte leaves did not become longer at Turkey Island, whereas the length of Turkey Island leaves was reduced when Turkey Island plants were grown in Ecarte sediment, or at the Ecarte site (Figure 4.10).

The effects of treatments by July 1991, and September 1991 respectively (i.e. 10 and 12 months after the experiment was set up) are shown in Tables 4.4 and 4.5.

Exposure to both water and sediment from the Ecarte site had the greatest depressant effect on plant growth, whatever the original source of the plants. The cumulative length of leaves per genet reflects both the size and number of leaves and ramets. By September (Table 4.5), cumulative leaf length at the Ecarte site was greatest for the plants in Turkey Island sediment. At the Turkey Island site, greater cumulative leaf length per genet was noted in plants originating from Turkey Island, whichever sediment they were grown. The number of ramets per genet at Ecarte was greater in Turkey sediment, but at Turkey Island, plants originating from Turkey Island had produced more ramets. Cumulative

length of leaves per ramet was greater for plants from Turkey Island at both sites, and again was greatest for plants from Turkey Island growing at Turkey Island in Turkey Island sediment.

At both sites the number of ramets per m² was greater for plants grown in sediment from Turkey Island, overall ramet production was greatest at the Turkey Island site. At both sites, flowering was most frequent for plants from Turkey Island growing in sediment from Turkey Island.

Relative impairments are shown in Table 4.6. Exposure to the Ecarte water column was the most damaging factor for plants from both populations. The Ecarte sediment was the next most detrimental factor.

In Table 4.7, coefficients of selection are presented. These measure the probable degree of adaptation of plants to their native site, and are calculated as:

$$\text{Selection coefficient (s)} = 1 - (\text{performance of alien plants} / \text{performance of native plants})$$

Contaminant distribution in *V. americana* plants sampled from Reciprocal-Transplant-Replant experiment

Analyses by GC/ECD confirmed that plants grown at Ecarte had become more contaminated over the year of the experiment, whatever was their original source (Tables 4.8 and 4.9). Organic contaminant concentrations in sediment, water and in plant tissues matched the relative impairment of growth that was observed under each set of circumstances. For most contaminants, particularly the PCBs, OCS, and HCB, the Ecarte sediment was more contaminated, and roots, in particular, bioaccumulated these materials most strongly.

Table 4.8 shows the concentration, in µg/kg, of each of the organochlorine contaminants that was tracked in the experimental plant tissues, water, and sediment. The level of HCB was greatest in roots (up to 125 µg/kg), when plants were raised in Ecarte sediment, both at the Turkey Is. site and the Ecarte site. Similar patterns of contaminant distribution are seen for OCS, and for PCBs (Table 4.9). Lipid-corrected values for the concentration of contaminants are shown in parentheses (Tables 4.8 and 4.9).

Spearman rank correlation analysis of ranks of contaminant concentrations in *Vallisneria* tissues and ranks of measures of plant performance in experimental treatments showed highly significant correlations (Table 4.10). Most of the contaminants detected in plant tissues were significantly correlated with measurable changes in plant growth, development, and reproduction. Significance of correlation between ranks of contaminations of *Vallisneria* tissues and ranks of plant performance decreased with increasing hydrophobicity of contaminants.

DISCUSSION

Submersed aquatic plants are well known to sequester metals, and nutrients from both the sediments and the water in which they grow (Hutchinson, 1975; Harding and Whitton, 1978; Forstner and Wittmann, 1979; Franzin and McFarlane, 1980; Schierup and Larson, 1981; Lovett Doust *et al.*, 1994b). However, very few workers have studied the presence of organic contaminants in plants. Stewart *et al.*, (1992) showed that the macrophyte *Potamogeton foliosus* and filamentous algae from a settling basin containing contaminated sediment became enriched with PCBs (as well as heavy metals). In the present study, plants responded to different levels of organochlorine contamination in term of leaf production, rates of clonal growth, sexual reproduction (flowering) and plant survival. Growth measurements are more sensitive, and are more likely to reflect the subtle and gradual damage done by persistent low-level exposure to contaminants than simple observations of mortality.

Pugsley *et al.* (1985, 1988) measured contaminant levels (PCBs, OCS, lead and cadmium) simultaneously in a unionid clam (*Lampsilis radiata siliquoidea*) and surrounding sediments from 102 sites in Lake St. Clair and the Canadian shoreline of the St. Clair and Detroit Rivers (including areas studied in the present study). Clams are long-lived filter-feeders that move little (Imlay, 1982) and so are to a degree comparable to plants in their utility as a biomonitor (though the analysis of the separate roles of

sediment and water column in contaminating the organism are not so tractable for the clams).

Measurements of contaminants in naturally growing plants at each site suggest that the two populations of *Vallisneria* differ in their exposure to, and uptake of, the suite of organochlorine contaminants examined. The pattern of greatest contaminant concentration in roots with progressive accumulation in all tissues over the growing season suggests an important role for uptake of these organochlorine contaminants via the sediment to roots, rather than to leaves from the water column. It also shows that plant tissues are not in equilibrium with each other or with contaminant concentrations in the surrounding water column. Furthermore, since lipid-corrected values do not show equilibrium between tissues, it appears that contaminant content in different plant tissues is not simply a function of relative solubility in aqueous and lipid phases, but rather demonstrates dynamic, and possibly regulated gradients between sediment, root, turion/stem and leaves.

The effects of both abiotic and biotic factors, characteristic for each site, are often of critical importance in determining contaminant burdens in tissues. For example, aquatic plants exposed to identical concentrations of contaminant in the water column may bioaccumulate different concentrations of pollutant in their tissues, depending upon sediment composition and type, or plant genotype and sex, or ambient light conditions (Chapter 2 and 3; Lovett Doust *et al.*, 1994b; Biernacki *et al.*, 1995a,b). Analytic techniques alone can elucidate the chemical characteristics of an environmental sample, but give little information regarding ecological effects on biota.

In earlier laboratory trichloroethylene exposure studies, it was observed that increased concentrations of contaminant in sediment pore-water and water column caused increased bioaccumulation of contaminant in plant tissues (Chapter 2). Increased burdens of contaminant in plant tissues were significantly correlated with decreased survivorship, decreased rate of clonal growth, decreased flowering, decreased biomass of ramets and shorted lifespans of leaves and roots, and increased leaf-to-root surface area ratio (Chapter 2 and 3; Biernacki *et al.*, 1995a,b). In the present study, a significant

correlations between plant tissue contamination and measures of plant growth, development and reproduction were observed (Table 4.10). Unlike in the laboratory study with exposure to a single contaminant, in the field, plants were exposed to mixture of various contaminants. Due to substantial cost associated with GS-MS analytical techniques, organochlorine compound analysed in the present study form only a small fraction of 100,000 currently used industrial chemicals and new being registered at about 1000 a year (Pugsley *et al.*, 1985; Korte and Coulston, 1994). In the field, various contaminants differed in their effects on measures of plant performance. Highly significant correlations between plant contaminant burden and plant performance were found for organochlorines with relatively low hydrophobicity (QCB, HCB, OCS, pp'DDE, PCB #28). For contaminants with greater hydrophobicity (PCB #118, PCB #138), correlations were often marginally significant or not observed. It is possible, that highly hydrophobic contaminants were not readily bioavailable to plants, they transfer through plant tissue restricted and slow over relatively short growing period of *Vallisneria* ramets.

The significant correlations found between plant contaminant burdens and quantitatively measured plant performance form the logistic base for using *Vallisneria* as a biomonitor of aquatic ecosystems (Table 4.10). Plant performance expressed by measures of growth and reproduction may be used to predict relative contaminant burdens in plant tissues (Chapter 2; Lovett Doust *et al.*, 1994b; Biernacki *et al.*, 1995a,b). Submersed plants continuously probe surrounding water column and sediments over the growing season and thus are capable to deliver information about quality of both media simultaneously. In earlier studies, contamination of plant tissues was found to be significantly correlated to levels of bioavailable contaminants in the environment (Chapter 2; Lovett Doust *et al.*, 1994b; Biernacki *et al.*, 1995a, 1996). Aquatic macrophytes like *Vallisneria* may be consumed as a fresh mass by herbivores or utilized as a detritus by detritivores, bacteria and fungi, that may transfer contaminants present initially in plant tissues to following consumers in the food web (Cyr and Pace, 1993; Wetzel, 1995). If the food chain is considered a the major route of contaminant transfer to

top consumers (Rowan and Rasmussen, 1992; Madenjian *et al.*, 1994), then it is logical to monitor entry of contaminants into food web right at the base of the food chain - in plants. Thus, plants may be used as early warning biomonitor of pollution (Lovett Doust *et al.*, 1994b). If the goal of environmental management is ecosystem stability and sustainability, then maintenance of plant health should be of critical importance to the general health, diversity and productivity of aquatic ecosystem (Petr, 1993; Harris, *et al.*, 1994; Minns, *et al.*, 1994; Wetzel, 1995; Leslie and Timmins, 1995; Klinge *et al.*, 1995).

Plants in the transplant experiment had only been exposed to experimental conditions for one year, and there may be some "home site" carry-over effects. Most of the plants that were originally set up remained in place for subsequent harvest in the experiment. Future analyses should show the difference between this relatively short-term study and the effects of longer (chronic) exposure to a particular sediment and water column.

Overall, the contrasts are clear; growth at the Ecarte site is less than growth at Turkey Island. In general, growth of plants at either site but in sediment from the Ecarte site, was less than that of plants growing in sediment from Turkey Island. However, plants from Turkey Island grew more vigorously than plants from Ecarte at both sites.

According to the destructive harvest, clonal growth was reduced at Ecarte, and the presence of Ecarte sediment was detrimental to both Ecarte and Turkey Island plants. At Turkey Island, the negative effect of Ecarte sediment was less severe; plants that originated from Turkey Island still produced almost 50% more ramets than the alien Ecarte plants (which were, nevertheless, producing more ramets than they did at their native site).

In comparisons between ecotypes at "clean" environments, it have been found almost invariably that locals grew better than alien plants (see also Lovett Doust, 1981). Plants from the Turkey Island site were better adapted to the Turkey Island site than were the Ecarte plants. At the Ecarte site, the generally superior growth of plants from the Turkey site shows up in per capita measures such as leaves per plant, biomass per ramet, etc. However, it is important to note that, at the Ecarte site, the survival of plants from

Turkey Is. is significantly lower than it is for plants from Ecarte, despite the fact that clonal growth is typically higher for plants from the Turkey Island population (ramets per m² are greater for Ecarte natives, see Table 4.5). There are therefore more intrinsically resistant individuals at the Ecarte site, and it is possible that this greater resistance has a genetic basis.

Since plants can reflect the relative pollution of different areas, tracking their relative growth can provide an inexpensive indicator of exposure to contaminants. The examination in terms of "relative impairment" (Table 4.6) or selection coefficients (Table 4.7), can describe the degree of impairment using a single number, which makes it relatively simple to compare between sites. This could be of considerable assistance in prioritizing remedial actions in order to clean up polluted areas and to demonstrate successful remediation of polluted sites. Therefore, calculations of these simple indices of plant performance as a measure of local (and comparative) site conditions are highly recommended. Sufficient tub replicates remain in experiment that the present biomonitor study will be conducted for a further three years in order to evaluate chronic effects of exposure (to the experimental treatments) on plant growth, reproduction and survival.

Some of the possible selection pressures that may explain differential growth and reproduction at the two sites are:

1. shorter growing season at Ecarte (plants there are approximately two to three weeks behind those at Turkey Is. in terms of phenology);
2. different sediment properties at the two sites;
3. colder water at Ecarte; and
4. higher organochlorine contamination at Ecarte.

Several factors may be responsible for the greater mortality of plants from the Turkey Island site when they were grown at Ecarte. On the basis of the factorial transplant experiment, the most important factors are contamination of water followed by the effects of sediment, because detrimental effects on growth are qualitatively and quantitatively correlated with contaminant concentration in the plant tissues. This macrophyte is an abundant member of the plant community, and provides important food

resources for diving ducks and turtles as well as forming beds of vegetation that are used by fish as nursery areas. Although the leaves are not the most contaminated of the *Vallisneria* tissues, they may, as Manny and Kenaga (1991) and Manny *et al.* (1991) have pointed out, enter the drift and may transport contaminants downstream. In addition, uprooted turions and their attached roots may also convey contaminants downstream, concentrating contaminants in sediments when they establish and eventually decay, or transferring contaminants to other compartments of the food web when they are consumed by herbivores and detritivores.

It may be debated whether uptake by plants is best referred to as "bioaccumulation" or "bioconcentration". The use the term "bioaccumulation" is proper because bioconcentration simply refers to the uptake of pollutant from water, whereas bioaccumulation indicates there is uptake from food as well as water. Macrophytes are in contact with two media; sediment and the water column. They absorb nutrients by active transport through roots (from sediment, but via pore water), and to some extent across the surface of leaves and other tissues. They also may absorb complex organic substances from their surroundings. Therefore, it has been concluded that simple bioconcentration is an inadequate description of contaminant dynamics between plants, sediment and water.

In conclusion, the use of the macrophyte, *Vallisneria americana* is recommended as a biomonitor in the Great Lakes, specifically, using relative growth to provide impairment indices and selection coefficients as metrics of contamination at different places, and observing the effects of remedial actions undertaken through remedial action plans by tracking conditions at one site, over time.

Table 4.1. Organochlorine contamination in *Vallisneria* collected from natural populations at Ecarte (E) and Turkey Island (T) ($\mu\text{g}/\text{kg}$; in parentheses, $\mu\text{g}/\text{kg}$ of lipid).
The symbol (-) indicates results which were below detection limits.

Time	Tissue	Site	% Lipid			Trans			pp'DDE
			QCB	HCB	OCS	-non			
20 May	turion	T	0.037	-	0.02	-	-	0.08	
				(-)	(54)	(-)	(-)	(216)	
20 May	root	T	0.020	0.03	0.07	0.02	-	0.15	
				(150)	(350)	(100)	(-)	(750)	
22 May	turion	E	0.060	-	0.01	-	-	-	
				(0.1)	(17)	(-)	(-)	(-)	
22 May	root	E	0.050	0.05	0.37	1.11	0.02	-	
				(100)	(740)	(2220)	(40)	(5.0)	
5 July	stolon	E	0.110	-	-	-	-	-	
				(-)	(-)	(-)	(-)	(-)	
5 July	leaf	E	0.088	-	-	-	-	-	
				(-)	(0.2)	(0.2)	(-)	(-)	
5 July	root	E	0.121	0.05	0.37	1.11	0.04	0.25	
				(41)	(306)	(917)	(33)	(207)	
5 July	turion	E	0.070	-	-	-	-	-	
				(-)	(-)	(-)	(-)	(-)	
25 June	root	T	0.020	0.02	0.06	0.02	0.02	0.14	
				(100)	(300)	(100)	(100)	(700)	
25 June	leaf	T	0.050	-	0.02	0.01	-	0.09	
				(-)	(40)	(20)	(-)	(180)	
25 June	stolon	T	0.030	-	0.01	-	-	0.07	
				(-)	(33)	(-)	(-)	(233)	
25 June	turion	T	0.050	-	0.02	-	-	0.08	
				(-)	(40)	(-)	(-)	(160)	
2 Oct	turion	T	0.180	0.04	0.01	-	-	0.03	
				(22)	(6)	(-)	(-)	(17)	

Table 4.1. Continued

Time	Tissue	Site	%		Trans			
			Lipid	QCB	HCB	OCS	-non	pp'DDE
2 Oct	turion	T	0.160	0.05	0.01	-	-	0.03
				(31)	(6)	(-)	(-)	(19)
2 Oct	root	T	0.090	0.13	0.26	0.03	0.04	0.23
				(144)	(289)	(33)	(44)	(256)
2 Oct	leaf	T	0.020	0.06	0.05	0.11	0.01	0.09
				(300)	(250)	(550)	(50)	(450)
2 Oct	stolon	T	0.020	0.06	0.02	-	-	0.03
		& caudex		(300)	(100)	(-)	(-)	(150)
2 Oct	fruit	T	0.030	0.06	0.02	-	-	0.03
				(200)	(67)	(-)	(-)	(100)
4 Oct	turion	E	0.250	0.08	0.02	-	-	0.20
				(32)	(8)	(-)	(-)	(80)
4 Oct	root	E	0.190	0.26	1.02	1.75	0.05	0.42
				(137)	(537)	(921)	(26)	(221)
4 Oct	leaf	E	0.240	0.10	0.15	0.19	0.01	0.10
				(42)	(62)	(79)	(4)	(42)
4 Oct	stolon	E	0.140	0.17	0.04	0.04	-	0.14
		& caudex		(121)	(29)	(29)	(-)	(100)
4 Oct	fruit	E	0.310	0.06	0.02	-	-	0.10
				(19)	(6)	(-)	(-)	(32)
4 Oct	fruit	E	0.230	0.07	0.02	-	-	0.06
				(30)	(9)	(-)	(-)	(26)

Table 4.2A. PCB contamination of plants collected from the field population at Ecarte (E) and Turkey Island (T) ($\mu\text{g}/\text{kg}$; in parenthesis $\mu\text{g}/\text{kg}$ lipid). The symbol (-) indicates results which were below detection limits.

Time	Tissue	Site	%	Arochlor	PCB	PCB	PCB	PCB	PCB	PCB
			Lipid	1254:1260	#28	#52	#66/95	#101	#99	#87
20 May	turion	T	0.037	0.75 (2027)	0.04 (108)	0.23 (621)	- (-)	- (-)	- (-)	- (-)
20 May	root	T	0.020	1.08 (5400)	- (-)	0.05 (250)	- (-)	- (-)	- (-)	0.05 (250)
22 May	turion	E	0.060	0.10 (167)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
22 May	root	E	0.050	2.01 (4020)	0.06 (120)	0.12 (240)	0.17 (340)	0.15 (300)	0.07 (140)	- (-)
5 July	stolon	E	0.110	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	0.84 (764)
5 July	leaf	E	0.088	- (1.5)	- (0.1)	- (0.1)	- (0.1)	- (-)	- (-)	- (-)
5 July	root	E	0.121	2.49 (2058)	0.05 (41)	0.10 (83)	0.24 (198)	0.18 (149)	0.10 (83)	0.03 (25)
5 July	turion	T	0.070	0.10 (143)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
25 June	roots	T	0.020	1.08 (5400)	- (-)	0.05 (250)	0.07 (350)	0.05 (250)	0.03 (150)	- (-)
25 June	leaf	T	0.050	0.49 (980)	- (-)	0.03 (60)	0.04 (80)	0.02 (40)	- (-)	- (-)
25 June	stolon	T	0.030	0.50 (1677)	0.02 (67)	0.06 (200)	0.03 (100)	- (-)	- (-)	- (-)
25 June	turion	T	0.050	0.75 (1500)	0.04 (80)	0.06 (120)	0.05 (100)	0.03 (60)	- (-)	- (-)
2 Oct	turion	T	0.180	- (-)	0.04 (22)	0.04 (22)	- (-)	- (-)	- (-)	0.07 (39)

Table 4.2A. Continued

Time	Tissue	Site	%	Arochlor	PCB	PCB	PCB	PCB	PCB	PCB
			Lipid	1254:1260	#28	#52	#66/95	#101	#99	#87
2 Oct	turion	T	0.160	-	0.02	0.04	0.02	-	-	-
				(-)	(12)	(25)	(12)	(-)	(-)	(-)
2 Oct	root	T	0.090	1.75	0.05	0.09	0.22	0.13	0.05	-
				(1944)	(56)	(100)	(244)	(144)	(56)	(-)
2 Oct	leaf	T	0.020	0.46	0.02	0.11	0.06	0.07	0.03	-
				(2300)	(100)	(550)	(300)	(350)	(150)	(-)
2 Oct	stolon	T	0.020	-	0.02	0.04	0.02	-	-	-
				(-)	(100)	(200)	(100)	(-)	(-)	(-)
2 Oct	fruit	T	0.030	-	0.04	0.06	-	-	-	0.62
				(-)	(133)	(200)	(-)	(-)	(-)	(2067)
4 Oct	turion	E	0.250	-	0.04	0.13	0.09	0.05	-	-
				(-)	(16)	(52)	(36)	(20)	(-)	(-)
4 Oct	root	E	0.190	2.99	0.09	0.52	0.35	0.35	0.20	0.13
				(1574)	(47)	(274)	(184)	(184)	(105)	(68)
4 Oct	leaf	E	0.240	0.41	-	0.14	0.09	0.04	0.03	0.02
				(171)	(-)	(58)	(38)	(17)	(12)	(8)
4 Oct	stolon	E	0.140	-	0.06	0.11	0.06	0.05	-	-
				(-)	(43)	(79)	(43)	(36)	(-)	(-)
4 Oct	fruit	E	0.310	0.41	0.06	0.26	0.17	0.19	-	-
				(132)	(19)	(84)	(55)	(61)	(-)	(-)
4 Oct	fruit	E	0.230	-	0.04	0.44	0.32	-	-	-
				(-)	(17)	(191)	(139)	(-)	(-)	(-)

Table 4.2B. PCB contamination of plants collected from the field population at Ecarte (E) and Turkey Island (T) ($\mu\text{g}/\text{kg}$; in parenthesis $\mu\text{g}/\text{kg}$ lipid). The symbol (-) indicates results which were below detection limits.

Time	Tissue	Site	%	PCB	PCB	PCB	PCB	PCB	PCB	PCB
			Lipid	#110	#118	#153	#138	#182/187	#180	#170/190
20 May	turion	T	0.037	0.05	-	-	-	-	-	-
				(135)	(-)	(-)	(-)	(-)	(-)	(-)
20 May	root	T	0.020	0.07	-	-	-	-	-	-
				(350)	(-)	(-)	(-)	(-)	(-)	(-)
22 May	turion	E	0.060	-	-	-	-	-	-	-
				(-)	(-)	(-)	(-)	(-)	(-)	(-)
22 May	root	E	0.050	-	-	-	-	-	-	-
				(0.1)	(0.1)	(-)	(-)	(-)	(-)	(-)
5 July	stolon	E	0.110	0.20	0.01	0.07	0.18	0.11	0.04	-
				(182)	(9)	(64)	(164)	(100)	(36)	(-)
5 July	leaf	E	0.088	-	-	-	-	-	-	-
				(-)	(-)	(-)	(-)	(-)	(-)	(-)
5 July	root	E	0.121	0.07	0.03	0.08	0.08	0.02	0.04	-
				(58)	(25)	(66)	(66)	(17)	(33)	(-)
5 July	turion	T	0.070	0.04	-	0.01	0.04	-	-	-
				(57)	(-)	(14)	(57)	(-)	(-)	(-)
25 June	root	T	0.020	0.04	0.06	0.03	0.04	-	-	-
				(200)	(300)	(150)	(200)	(-)	(-)	(-)
25 June	leaf	T	0.050	0.04	-	0.02	0.06	-	-	-
				(80)	(-)	(40)	(120)	(-)	(-)	(-)
25 June	stolon	T	0.030	0.02	-	0.01	-	-	-	-
				(67)	(-)	(33)	(-)	(-)	(-)	(-)
25 June	turion	T	0.050	0.03	-	0.01	-	-	-	-
				(60)	(-)	(20)	(-)	(-)	(-)	(-)
2 Oct	turion	T	0.180	0.10	0.08	0.11	0.13	0.04	0.06	-
				(55)	(44)	(61)	(72)	(22)	(33)	(-)

Table 4.2B. Continued

Time	Tissue	Site	%	PCB	PCB	PCB	PCB	PCB	PCB	PCB
			Lipid	#110	#118	#153	#138	#182/187	#180	#170/190
2 Oct	turion	T	0.160	0.04 (25)	0.96 (600)	0.02 (12)	0.03 (19)	- (-)	- (-)	- (-)
2 Oct	root	T	0.090	0.02 (22)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
2 Oct	leaf	T	0.020	0.03 (150)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
2 Oct	stolon	T	0.020	- & caudex (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
2 Oct	fruit	T	0.030	0.11 (367)	0.01 (33)	0.05 (167)	0.12 (400)	0.10 (333)	0.03 (100)	- (-)
4 Oct	turion	E	0.250	0.09 (36)	- (-)	0.03 (12)	- (-)	- (-)	- (-)	- (-)
4 Oct	root	E	0.190	0.25 (132)	0.17 (90)	0.21 (111)	0.32 (168)	0.09 (47)	0.05 (26)	- (-)
4 Oct	leaf	E	0.240	0.06 (25)	0.04 (17)	0.02 (8)	0.03 (12)	- (-)	- (-)	- (-)
4 Oct	stolon	E	0.140	0.05 & caudex (36)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
4 Oct	fruit	E	0.310	0.06 (19)	0.03 (10)	0.03 (10)	0.03 (10)	- (-)	- (-)	- (-)
4 Oct	fruit	E	0.230	0.03 (13)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)

Table 4.3. Summary of analyses of variance (ANOVA) of plant growth in the *Vallisneria* reciprocal transplant-replant (biomonitor) experiment. Significance is indicated as follow: *= $p \leq 0.001$; **= $p \leq 0.01$; *= $p \leq 0.05$; NS=not significant. Only factors or interactions that were significant for at least one parameter are tabulated.**

TRAIT	FACTORS												
	Time (T)	Site (S)	Sedi- ment (E)	Plant Origin (P)	TxS	TxP	TxE	SxP	SxE	PxE	TxPxE	SxPxE	TxSxPxE
ramets/m ²	***	***	***	**	***	NS	***	***	NS	NS	NS	NS	NS
ramets/genet	***	***	***	***	***	***	***	***	***	*	NS	NS	NS
leaves/genet	***	***	***	***	NS	***	***	***	***	NS	*	NS	*
leaves/ramet	***	***	***	***	**	NS	**	*	NS	*	*	*	NS
leaves/m ²	***	***	***	***	***	NS	***	***	NS	**	NS	NS	NS
mean length of leaf/ramet	NS	*	NS	**	NS	NS	NS						
cumulative length of leaves/ramet	NS	**	NS	***	*	NS	NS	NS	NS	NS	NS	NS	NS
cumulative length of leaves/genet	***	***	***	***	NS	**	**	***	NS	***	NS	*	NS
flowering ramets/m ²	***	**	***	***	*	**	*	**	NS	**	NS	NS	NS

Table 4.4. Performance of plants in the reciprocal transplant-replant (biomonitor) experiment. July 1991.
For each factor, E = Ecarterie; T = Turkey Island.

Site	Ecarterie				Turkey Island			
	Ecarterie		Turkey Is.		Ecarterie		Turkey Is.	
Sediment	E	T	E	T	E	T	E	T
Plants								
Total length of leaves (per genet)	79.2	81.1	167.5	132.6	263.3	775.6	194.0	1090.2
Ramets per genet	2.2	2.4	4.3	2.2	4.8	8.8	3.5	9.7
Total length of leaves (per ramet)	35.2	33.4	39.0	58.9	54.9	88.1	55.4	112.4
Ramets/m ²	71.0	60.0	97.2	94.5	96.6	194.5	137.9	297.2

Table 4.5. Performance of plants in the reciprocal transplant-replant (biomonitor) experiment. September 1991.
For each factor, E = Ecarte; T = Turkey Island.

Site Sediment Plants	Ecarte		Turkey ls.		Ecarte		Turkey Island		Turkey ls.	
	E	T	E	T	E	T	E	T	E	T
Total length of leaves (per genet)	143.0	206.0	249.9	752.8	319.0	976.2	311.0	1930.0		
Ramets per genet	4.4	4.0	9.2	12.0	12.2	19.8	12.3	23.0		
Total length of leaves (per ramet)	32.7	51.5	27.2	62.7	26.1	49.3	25.3	83.9		
Ramets/m ²	343.5	236.6	573.1	515.9	579.3	673.8	771.0	1006.2		
Flowering ramets/m ²	0.0	0.0	1.38	9.66	0.0	2.28	0.62	8.97		

**Table 4.6. Relative impairment of plants in the reciprocal transplant experiment
(Impairment indices).**

Trait	Impairment due to Ecarte Water Column	Impairment due to Ecarte Sediment
Plants Originating from Turkey Island		
ramets per genet	62.6	32.0
ramet per m ²	55.2	40.2
leaves per ramet	-18.0	12.7
leaves per m ²	47.0	48.1
mean length of leaf per ramet	42.6	38.4
flowering ramets per m ²	14.3	87.7
Plants Originating from Turkey Island		
ramets per genet	44.6	22.7
ramet per m ²	32.1	31.4
leaves per ramet	-13.7	4.4
leaves per m ²	22.3	34.0
mean length of leaf per ramet	-2.1	-1.7
flowering ramets per m ²	-120.5	-100.0

Table 4.7. Coefficient of selection at Ecarte and Turkey Island sites.

Trait	Ecarte site	Turkey Is. site
ramet per genet	-0.179	0.427
ramets per m ²	0.179	0.196
leaves per ramet	-0.103	0.060
leaves per m ²	0.089	0.252
mean length of leaf	-0.451	0.612
flowering ramets per m ²	-5.997	0.944

Table 4.8. Organochlorine contamination in experimental *Vallisneria americana* (Reciprocal-Transplant-Replant experiment). The identity of site of exposure, site of plant origin, or sediment source is indicated as E (Ecarte site) or T (Turkey Island site) (µg/kg: in parentheses, µg/kg of lipid).

Sample Number	Time	Identity		Trans				
		(Site/Plant/ Sediment)	% Lipid	QCB	HCB	OCS	-non	pp'DDE
		T/T/T	0.131	-	0.03	0.04	0.02	0.14
(21)	July	T/T/T	0.131	-	0.03	0.04	0.02	0.14
				(-)	(23)	(31)	(15)	(107)
(22)	July	T/T/T	0.069	-	0.02	-	-	0.09
				(-)	(29)	(-)	(-)	(130)
(23)	July	T/T/T	0.050	0.03	0.08	-	0.05	0.30
				(60)	(160)	(-)	(100)	(600)
(24)	July	T/E/T	0.256	0.02	0.09	0.07	0.06	0.36
				(8)	(35)	(27)	(23)	(141)
(25)	July	T/E/T	0.314	-	-	-	-	0.72
				(-)	(-)	(-)	(-)	(229)
(26)	July	T/E/T	0.329	-	0.13	-	0.15	0.72
				(-)	(40)	(-)	(46)	(219)
(27)	July	T/T/E	0.131	0.04	0.09	0.06	0.03	0.15
				(31)	(69)	(46)	(23)	(115)
(28)	July	T/T/E	0.093	0.03	0.04	0.11	0.02	0.19
				(32)	(43)	(118)	(22)	(204)
(29)	July	T/T/E	0.031	0.03	0.11	0.02	0.02	0.14
				(97)	(355)	(65)	(65)	(452)
(30)	July	T/E/E	0.188	0.04	0.11	0.13	0.04	0.22
				(21)	(58)	(69)	(21)	(117)
(31)	July	T/E/E	0.136	-	0.15	0.45	-	0.44
				(-)	(110)	(331)	(-)	(324)
(32)	July	T/E/E	0.031	0.08	0.21	1.65	0.11	0.46
				(258)	(677)	(5323)	(355)	(1484)

Table 4.8. Continued

Sample Number	Time	Identity		Trans					
		(Site/Plant/ Sediment)	Lipid	% QCB		HCB	OCS	-non	pp'DDE
(38)	July	E/E/T	0.240	0.06	0.25	0.74	0.03	0.14	
				(25)	(104)	(308)	(12)	(58)	
(39)	July	E/E/T	0.190	-	0.29	0.19	-	0.35	
				(-)	(153)	(100)	(-)	(184)	
(40)	July	E/E/T	0.180	-	0.16	0.25	-	0.23	
				(-)	(89)	(139)	(-)	(128)	
(41)	July	E/E/E	0.300	0.09	0.47	0.55	-	0.38	
				(30)	(157)	(183)	(-)	(127)	
(42)	July	E/E/E	0.530	-	0.85	1.06	-	0.91	
				(-)	(160)	(200)	(-)	(172)	
(43)	July	E/E/E	0.360	0.56	1.78	3.06	-	-	
				(156)	(494)	(850)	(-)	(-)	
(44)	July	E/T/T	0.252	0.05	0.32	0.27	0.04	0.14	
				(20)	(127)	(107)	(16)	(56)	
(45)	July	E/T/T	0.234	-	0.14	0.10	-	0.09	
				(-)	(60)	(43)	(-)	(38)	
(46)	July	E/T/T	0.383	-	0.26	0.21	-	0.28	
				(-)	(68)	(55)	(-)	(73)	
(47)	July	E/T/E	0.453	0.72	0.36	0.41	-	0.15	
				(159)	(80)	(90)	(-)	(33)	
(48)	July	E/T/E	0.410	-	0.71	1.11	-	-	
				(-)	(173)	(271)	(-)	(-)	
(49)	July	E/T/E	0.510	-	0.44	2.15	-	-	
				(-)	(86)	(422)	(-)	(-)	
(50)	Sept	E/T/T	0.190	0.04	0.27	0.16	0.04	0.13	
				(21)	(142)	(84)	(21)	(68)	
(51)	Sept	E/T/T	0.110	-	0.10	0.02	-	0.06	
				(-)	(91)	(18)	(-)	(54)	

Table 4.8. Continued

Sample Number	Time	(Site/Plant/ Sediment)	Identity		Trans			
			(Site/Plant/ % Lipid		QCB	HCB	OCS	-non pp'DDE
(52)	Sept	E/T/T	0.100	0.04	124.84	0.12	0.03	0.17
					(40)	(124840)	(120)	(30) (170)
(53)	Sept	E/E/T	0.103	0.03	0.15	0.15	0.04	0.29
					(29)	(146)	(146)	(39) (282)
(54)	Sept	E/E/T	0.100	-	0.03	0.12	-	0.23
					(-)	(30)	(120)	(-) (230)
(55)	Sept	E/E/T	0.040	0.09	0.62	0.28	0.14	0.31
					(225)	(1550)	(700)	(350) (775)
(56)	Sept	E/T/E	0.171	0.15	0.59	0.89	0.05	0.13
					(88)	(345)	(520)	(29) (76)
(57)	Sept	E/T/E	0.146	0.12	0.20	0.33	-	0.24
					(82)	(137)	(226)	(-) (164)
(58)	Sept	E/T/E	0.188	0.13	0.54	2.46	-	0.42
					(69)	(287)	(1308)	(-) (223)
(59)	Sept	E/E/E	0.159	-	0.35	0.93	-	0.25
					(-)	(220)	(585)	(-) (157)
(60)	Sept	E/E/E	0.172	0.16	0.24	0.26	-	0.49
					(93)	(140)	(151)	(-) (285)
(61)	Sept	E/E/E	0.570	3.38	70.00	12.35	0.54	2.04
					(593)	(12281)	(2167)	(95) (358)
(62)	Sept	T/E/T	0.040	-	0.05	0.03	0.02	0.14
					(-)	(125)	(75)	(50) (350)
(63)	Sept	T/E/T	0.060	-	0.04	-	-	0.15
					(-)	(67)	(-)	(-) (250)
(64)	Sept	T/E/T	0.060	-	0.16	-	-	0.25
					(-)	(267)	(-)	(-) (417)
(65)	Sept	T/E/T	0.120	0.04	0.11	0.39	0.02	0.24
					(34)	(92)	(325)	(17) (200)

Table 4.8. Continued

Sample Number	Time	Identity		Trans					
		(Site/Plant/ Sediment)		Lipid	QCB	HCB	OCS	-non	pp'DDE
		%							
(66)	Sept	T/E/E	0.040	0.04	0.09	0.20	0.02	0.25	
				(100)	(225)	(500)	(50)	(625)	
(67)	Sept	T/E/E	0.040	0.08	0.36	6.83	-	0.19	
				(200)	(900)	(17075)	(-)	(475)	
(68)	Sept	T/T/T	0.100	-	0.04	0.03	-	0.10	
				(-)	(40)	(30)	(-)	(100)	
(69)	Sept	T/T/T	0.100	-	0.03	-	-	0.06	
				(-)	(30)	(-)	(-)	(60)	
(70)	Sept	T/T/T	0.500	0.03	0.11	-	0.02	0.16	
				(6)	(22)	(-)	(4)	(32)	
(71)	Sept	T/T/E	0.180	-	0.05	0.16	-	0.10	
				(-)	(28)	(89)	(-)	(56)	
(72)	Sept	T/T/E	0.170	0.07	0.30	1.68	0.04	0.11	
				(41)	(176)	(988)	(24)	(65)	
(73)	Sept	T/T/E	0.090	0.03	0.11	0.17	-	0.20	
				(33)	(122)	(189)	(-)	(222)	

Table 4.9A. PCB contaminants in experimental *Vallisneria americana* (Reciprocal-Transplant-Replant experiment). The identity of site of exposure, site of plant origin, or sediment source is indicated as E (Ecarte site) or T (Turkey Island site)($\mu\text{g}/\text{kg}$: in parentheses, $\mu\text{g}/\text{kg}$ of lipid).

		Identity								
Sample Number	Time	(Site/Plant/ Sediment)	% Lipid	Arochlor 1254:1260	PCB #28	PCB #52	PCB #66/95	PCB #101	PCB #99	
(21)	July	T/T/T	0.131	1.37 (1046)	0.02 (15)	0.92 (702)	0.35 (267)	0.14 (107)	0.08 (61)	
(22)	July	T/T/T	0.069	1.48 (2145)	0.03 (44)	0.12 (174)	0.10 (145)	0.08 (116)	0.04 (58)	
(23)	July	T/T/T	0.050	1.76 (3520)	- (-)	0.16 (320)	0.33 (660)	0.12 (240)	0.09 (180)	
(24)	July	T/E/T	0.256	1.88 (734)	0.07 (27)	0.21 (82)	0.53 (207)	0.30 (117)	0.23 (90)	
(25)	July	T/E/T	0.314	4.70 (1516)	- (-)	0.60 (194)	0.43 (139)	0.34 (110)	- (-)	
(26)	July	T/E/T	0.329	11.91 (3620)	0.52 (158)	1.79 (544)	1.48 (450)	0.47 (143)	0.24 (73)	
(27)	July	T/T/E	0.131	1.48 (1130)	0.05 (38)	0.11 (84)	0.15 (114)	0.17 (130)	0.07 (53)	
(28)	July	T/T/E	0.093	0.97 (1043)	0.07 (75)	0.22 (237)	0.19 (204)	0.14 (150)	0.10 (108)	
(29)	July	T/T/E	0.031	1.30 (4193)	0.04 (129)	0.11 (355)	0.10 (323)	0.07 (226)	0.04 (129)	
(30)	July	T/E/E	0.188	3.33 (1771)	0.08 (43)	0.20 (106)	0.22 (117)	0.23 (122)	0.10 (53)	
(31)	July	T/E/E	0.136	3.17 (2331)	0.22 (162)	0.39 (287)	0.31 (228)	0.30 (221)	- (-)	
(32)	July	T/E/E	0.031	4.65 (15000)	0.20 (645)	0.59 (1903)	0.44 (1419)	0.30 (968)	0.23 (742)	

Table 4.9A. Continued

		Identity							
Sample Number	(Site/Plant/ Sediment)	% Lipid	Arochlor 1254:1260	PCB #28	PCB #52	PCB #66/95	PCB #101	PCB #99	
(38)	July	E/E/T	0.240	2.18 (908)	0.12 (50)	0.14 (58)	1.22 (508)	0.21 (88)	0.72 (300)
(39)	July	E/E/T	0.190	4.90 (2579)	0.25 (132)	0.50 (263)	0.34 (179)	0.19 (100)	- (-)
(40)	July	E/E/T	0.180	4.43 (2461)	0.20 (111)	0.68 (378)	0.17 (94)	- (-)	- (-)
(41)	July	E/E/E	0.300	3.98 (1327)	0.21 (70)	0.22 (73)	0.26 (87)	0.11 (37)	- (-)
(42)	July	S/S/S	0.530	22.81 (4304)	0.42 (79)	0.45 (85)	0.47 (89)	- (-)	- (-)
(43)	July	E/E/E	0.360	33.36 (9267)	0.62 (172)	1.18 (328)	1.82 (506)	- (-)	- (-)
(44)	July	E/T/T	0.252	1.66 (659)	0.11 (44)	0.15 (60)	0.18 (71)	0.11 (44)	0.06 (24)
(45)	July	E/T/T	0.234	2.91 (1244)	0.13 (56)	0.17 (73)	0.16 (68)	- (-)	- (-)
(46)	July	E/T/T	0.383	8.76 (2287)	0.28 (73)	0.44 (115)	0.30 (78)	- (-)	- (-)
(47)	July	E/T/E	0.453	2.92 (645)	0.10 (22)	0.21 (46)	0.25 (55)	0.16 (35)	0.11 (24)
(48)	July	E/T/E	0.410	6.60 (1610)	0.14 (34)	0.40 (98)	0.52 (127)	- (-)	- (-)
(49)	July	E/T/E	0.510	7.77 (1524)	0.22 (43)	0.85 (167)	0.40 (78)	- (-)	- (-)
(50)	Sept	E/T/E	0.190	1.65 (868)	- (-)	0.10 (53)	0.53 (279)	0.11 (58)	0.07 (37)
(51)	Sept	E/T/T	0.110	2.05 (1864)	0.05 (45)	0.09 (82)	0.09 (82)	0.04 (36)	- (-)

Table 4.9A. Continued

		Identity								
Sample Number	(Site/Plant/Time)	% Lipid	Arochlor 1254:1260	PCB #28	PCB #52	PCB #66/95	PCB #101	PCB #99		
(52)	Sept	E/T/T	0.010	2.06 (20600)	0.07 (700)	0.88 (8800)	0.79 (7900)	0.07 (700)	0.04 (400)	
(53)	Sept	E/T/T	0.103	1.82 (1767)	0.09 (87)	0.26 (252)	0.33 (320)	0.11 (107)	0.06 (58)	
(54)	Sept	E/E/T	0.100	2.75 (2750)	0.12 (120)	0.30 (300)	0.29 (290)	0.12 (120)	- (-)	
(55)	Sept	E/E/T	0.040	1.71 (4275)	0.08 (200)	0.10 (250)	0.40 (1000)	0.26 (650)	0.29 (725)	
(56)	Sept	E/T/E	0.171	2.41 (1409)	0.20 (117)	0.20 (117)	0.20 (117)	0.18 (105)	0.12 (70)	
(57)	Sept	E/T/E	0.146	5.99 (4103)	0.26 (178)	0.28 (192)	0.13 (89)	- (-)	- (-)	
(58)	Sept	E/T/E	0.188	11.63 (6186)	0.40 (213)	0.73 (388)	0.31 (165)	0.20 (106)	- (-)	
(59)	Sept	E/E/E	0.159	3.45 (2170)	0.21 (132)	0.13 (82)	0.26 (164)	0.09 (57)	- (-)	
(60)	Sept	E/E/E	0.172	11.78 (6849)	0.56 (326)	0.26 (151)	0.19 (110)	- (-)	- (-)	
(61)	Sept	E/E/E	0.570	31.11 (5458)	1.17 (205)	2.01 (353)	3.49 (612)	1.31 (230)	0.94 (165)	
(62)	Sept	T/E/T	0.040	0.89 (2225)	0.14 (350)	0.09 (225)	0.47 (1175)	0.08 (200)	0.05 (125)	
(63)	Sept	T/E/T	0.060	0.44 (733)	0.05 (83)	0.13 (217)	0.06 (100)	0.08 (133)	0.04 (67)	
(64)	Sept	T/E/T	0.060	1.05 (1750)	0.25 (417)	0.27 (450)	0.17 (283)	0.08 (133)	0.08 (133)	
(65)	Sept	T/E/T	0.120	1.32 (1100)	0.03 (25)	0.13 (108)	0.95 (792)	0.09 (75)	0.08 (67)	

Table 4.9A. Continued

		Identity							
Sample Number	Time	(Site/Plant/ Sediment)	% Lipid	Arochlor 1254:1260	PCB #28	PCB #52	PCB #66/95	PCB #101	PCB #99
(66)	Sept	T/E/E	0.040	0.51 (1275)	0.03 (75)	0.18 (450)	0.12 (300)	0.06 (150)	0.06 (150)
(67)	Sept	T/E/E	0.040	2.52 (6300)	0.14 (350)	0.41 (1025)	0.26 (650)	0.20 (500)	0.12 (300)
(68)	Sept	T/T/T	0.100	0.43 (430)	0.04 (40)	0.06 (60)	0.05 (50)	0.05 (50)	- (-)
(69)	Sept	T/T/T	0.100	- (-)	0.03 (30)	0.09 (90)	0.05 (50)	0.03 (30)	- (-)
(70)	Sept	T/T/T	0.050	0.99 (1980)	0.05 (100)	0.13 (260)	0.10 (200)	0.07 (140)	0.04 (80)
(71)	Sept	T/T/E	0.180	- (-)	0.05 (28)	0.08 (44)	0.05 (28)	0.02 (11)	- (-)
(72)	Sept	T/T/E	0.170	2.00 (1176)	0.07 (41)	0.34 (200)	0.24 (141)	0.16 (94)	0.08 (47)
(73)	Sept	T/T/E	0.090	3.58 (3978)	0.03 (33)	0.11 (122)	0.09 (100)	0.09 (100)	0.05 (56)

Table 4.9B. PCB contaminants in experimental *Vallisneria americana* (Reciprocal-Transplant-Replant experiment) ($\mu\text{g}/\text{kg}$: in parentheses, $\mu\text{g}/\text{kg}$ of lipid).

Sample Number	PCB #87	PCB #110	PCB #118	PCB #153	PCB #138	PCB #182/187	PCB #180	PCB #170/190	PCB #194
(21)	0.06	0.11	0.12	0.26	0.10	-	0.02	-	0.02
	(46)	(84)	(92)	(198)	(76)	(-)	(15)	(-)	(15)
(22)	0.04	0.09	0.04	0.05	0.11	-	-	-	-
	(58)	(130)	(58)	(72)	(159)	(-)	(-)	(-)	(-)
(23)	0.42	0.13	0.07	0.11	0.13	0.05	0.05	-	-
	(840)	(260)	(140)	(220)	(260)	(100)	(100)	(-)	(-)
(24)	0.09	0.16	0.27	0.13	0.14	-	0.04	-	-
	(35)	(63)	(105)	(51)	(55)	(-)	(16)	(-)	(-)
(25)	-	0.30	-	0.30	0.35	-	-	-	-
	(-)	(97)	(-)	(97)	(113)	(-)	(-)	(-)	(-)
(26)	0.33	0.43	0.25	0.50	0.87	-	0.19	-	-
	(100)	(131)	(76)	(152)	(264)	(-)	(58)	(-)	(-)
(27)	0.05	0.11	0.07	0.10	0.11	-	0.03	0.03	-
	(38)	(84)	(53)	(76)	(84)	(-)	(23)	(23)	(-)
(28)	0.06	0.11	0.04	0.05	0.07	0.04	-	-	-
	(65)	(118)	(43)	(54)	(75)	(43)	(-)	(-)	(-)
(29)	0.07	0.11	0.05	0.09	0.10	0.02	0.02	-	-
	(226)	(355)	(161)	(290)	(323)	(65)	(65)	(-)	(-)
(30)	0.08	0.16	0.09	0.10	0.17	-	0.05	0.04	-
	(43)	(85)	(48)	(53)	(90)	(-)	(27)	(21)	(-)
(31)	-	0.26	0.06	0.20	0.23	-	-	-	-
	(-)	(191)	(44)	(147)	(169)	(-)	(-)	(-)	(-)
(32)	0.20	0.33	0.22	0.29	0.34	0.14	0.06	-	-
	(645)	(1065)	(710)	(935)	(1097)	(452)	(194)	(-)	(-)
(38)	0.06	0.14	0.07	0.06	0.16	-	0.03	-	-
	(25)	(58)	(29)	(25)	(67)	(-)	(12)	(-)	(-)
(39)	-	0.15	-	0.14	0.36	-	-	-	-
	(-)	(79)	(-)	(74)	(190)	(-)	(-)	(-)	(-)

Table 4.9B. Continued

Sample Number	PCB #87	PCB #110	PCB #118	PCB #153	PCB #138	PCB #182/187	PCB #180	PCB #170/190	PCB #194
(40)	-	0.14	-	-	0.33	-	-	-	-
	(-)	(78)	(-)	(-)	(183)	(-)	(-)	(-)	(-)
(41)	-	0.27	-	0.10	0.29	-	-	-	(-)
	(-)	(90.6)	(-)	(34.3)	(97.2)	(-)	(-)	(-)	(-)
(42)	-	0.41	-	-	1.67	-	-	-	-
	(-)	(77.9)	(-)	(-)	(315.2)	(-)	(-)	(-)	(-)
(43)	-	3.13	-	-	2.44	-	-	-	-
	(-)	(869)	(-)	(-)	(678)	(-)	(-)	(-)	(-)
(44)	0.05	0.15	0.07	0.04	0.12	-	-	-	-
	(20)	(60)	(28)	(16)	(48)	(-)	(-)	(-)	(-)
(45)	-	-	-	-	0.21	-	-	-	-
	(-)	(-)	(-)	(-)	(90)	(-)	(-)	(-)	(-)
(46)	-	0.24	-	0.23	0.64	-	-	-	-
	(-)	(63)	(-)	(60)	(167)	(-)	(-)	(-)	(-)
(47)	-	0.23	-	0.13	0.22	-	-	-	-
	(-)	(51)	(-)	(29)	(49)	(-)	(-)	(-)	(-)
(48)	-	0.31	-	-	0.48	-	-	-	-
	(-)	(76)	(-)	(-)	(117)	(-)	(-)	(-)	(-)
(49)	-	0.31	-	0.35	0.57	-	-	-	-
	(0)	(61)	(-)	(69)	(112)	(-)	(-)	(-)	(-)
(50)	0.06	0.29	0.12	0.05	0.12	-	-	-	-
	(32)	(153)	(63)	(26)	(63)	(-)	(-)	(-)	(-)
(51)	-	0.12	0.03	-	0.15	-	-	-	-
	(-)	(109)	(27)	(-)	(136)	(-)	(-)	(-)	(-)
(52)	0.05	0.07	0.04	0.09	0.15	0.06	-	-	-
	(500)	(700)	(400)	(900)	(1500)	(600)	(-)	(-)	(-)
(53)	0.05	0.18	0.08	0.05	0.13	-	0.02	-	-
	(49)	(175)	(78)	(49)	(126)	(-)	(19)	(-)	(-)
(54)	0.12	0.18	0.12	0.10	0.20	-	-	-	-
	(120)	(180)	(120)	(100)	(200)	(-)	(-)	(-)	(-)

Table 4.9B. Continued

Sample Number	PCB #87	PCB #110	PCB #118	PCB #153	PCB #138	PCB #182/187	PCB #180	PCB #170/190	PCB #194
(55)	0.10	0.32	0.10	0.15	0.13	-	0.06	-	-
	(250)	(800)	(250)	(375)	(325)	(-)	(150)	(-)	(-)
(56)	0.07	0.15	0.11	0.10	0.18	-	0.13	-	-
	(41)	(88)	(64)	(58)	(105)	(-)	(76)	(-)	(-)
(57)	-	0.14	0.09	0.11	0.85	-	-	-	-
	(-)	(96)	(62)	(75)	(587)	(-)	(-)	(-)	(-)
(58)	-	0.25	0.30	0.11	0.25	-	-	-	-
	(-)	(133)	(160)	(59)	(133)	(-)	(-)	(-)	(-)
(59)	-	0.16	-	-	0.86	-	-	-	-
	(-)	(101)	(-)	(-)	(541)	(-)	(-)	(-)	(-)
(60)	-	0.22	-	-	0.86	-	-	-	-
	(-)	(128)	(-)	(-)	(500)	(-)	(-)	(-)	(-)
(61)	1.07	1.78	1.11	0.54	2.28	0.30	-	-	-
	(188)	(312)	(195)	(95)	(400)	(53)	(-)	(-)	(-)
(62)	-	0.05	0.06	0.05	0.07	-	-	-	0.08
	(-)	(125)	(150)	(125)	(175)	(-)	(-)	(-)	(200)
(63)	0.04	0.06	0.02	0.03	0.03	-	-	-	-
	(67)	(100)	(33)	(50)	(50)	(-)	(-)	(-)	(-)
(64)	-	0.08	-	0.04	0.08	-	-	-	-
	(-)	(133)	(-)	(67)	(133)	(-)	(-)	(-)	(-)
(65)	0.06	0.07	0.17	0.08	0.10	-	0.03	-	-
	(50)	(58)	(142)	(67)	(83)	(-)	(25)	(-)	(-)
(66)	0.07	0.07	0.11	0.05	0.04	-	0.03	-	-
	(175)	(175)	(275)	(125)	(100)	(-)	(75)	(-)	(-)
(67)	0.10	0.19	0.43	0.09	0.19	0.12	-	-	0.80
	(250)	(475)	(1075)	(225)	(475)	(300)	(-)	(-)	(2000)
(68)	-	0.04	-	0.03	0.03	-	-	-	-
	(-)	(40)	(-)	(30)	(30)	(-)	(-)	(-)	(-)
(69)	-	0.05	0.03	-	-	-	-	-	-
	(-)	(50)	(30)	(-)	(-)	(-)	(-)	(-)	(-)

Table 4.9B. Continued

Sample Number	PCB #87	PCB #110	PCB #118	PCB #153	PCB #138	PCB #182/187	PCB #180	PCB #170/190	PCB #194
(70)	0.04	0.07	0.05	0.05	0.07	0.04	0.06	-	0.02
	(80)	(140)	(100)	(100)	(140)	(80)	(120)	(-)	(40)
(71)	-	0.04	0.04	-	-	-	-	-	-
	(-)	(22)	(22)	(-)	(-)	(-)	(-)	(-)	(-)
(72)	0.09	0.19	0.13	0.10	0.15	0.06	0.06	-	-
	(53)	(112)	(76)	(58)	(88)	(35)	(35)	(-)	(-)
(73)	0.10	0.09	0.16	0.39	0.26	0.86	1.68	0.59	0.64
	(111)	(100)	(178)	(433)	(289)	(956)	(1867)	(656)	(711)

Table 4.10. Results of Spearman rank correlation analysis of relative ranks of selected measures of plant performance in *Vallisneria americana* and ranks of organochlorine contaminant values in plant tissues collected from reciprocal transplant-replant experiment.
Only significant correlations are given (- p<0.001; ** - p<0.01; * - p<0.05; NS - not significant).**

TRAIT	CONTAMINANT													
	QCB	HCB	OCS	Trans-non pp'DDE	Arochlor 1254:1260	PCB #28	PCB #52	PCB #66/95	PCB #101	PCB #99	PCB #87	PCB #110	PCB #118	PCB #153
Non-destructive monitoring:														
Number of ramets per m ²	***	***	***	***	**	***	***	***	***	***	**	*	**	**
Number of leaves per m ²	***	***	***	***	**	***	***	***	***	***	**	*	NS	**
Number of flowers per m ²	***	***	***	***	**	***	***	***	***	***	**	*	**	*
Number of flowers per ramet	***	***	***	***	**	***	***	***	***	***	**	*	**	*
Rate of clonal growth	***	***	***	***	**	***	***	***	***	***	**	*	**	*
Destructive monitoring:														
Number of turions per m ²	***	***	***	***	**	***	***	***	***	***	*	*	*	*
Biomass of ramets per m ²	***	***	***	***	**	***	***	***	***	***	**	*	*	*
Number of roots per ramet	***	***	***	***	**	***	***	***	***	***	**	*	*	*
Biomass per ramet	***	***	***	***	**	***	***	***	***	***	**	*	*	*
Number of turions per ramet	***	***	***	***	**	***	***	***	***	***	**	*	*	*
Biomass of turions per ramet	***	***	***	***	**	***	***	***	***	***	**	*	*	*
Turions-to-ramet biomass ratio	***	***	***	***	**	***	***	***	***	***	**	*	*	*
Length of a leaf	**	**	*	*	*	**	**	**	**	**	*	*	*	NS
Biomass of a leaf	**	**	*	*	*	**	**	**	**	**	*	*	*	*
Biomass of a turion	**	**	*	*	*	**	**	**	**	**	*	*	*	*
Leaf-to-root biomass ratio	***	***	*	*	*	**	**	**	**	**	*	*	*	NS
Fraction of germinated turions	***	***	*	*	*	**	**	**	**	**	*	*	*	NS
Leaf-to-root surface area ratio	***	***	*	*	*	**	**	**	**	**	*	*	*	**

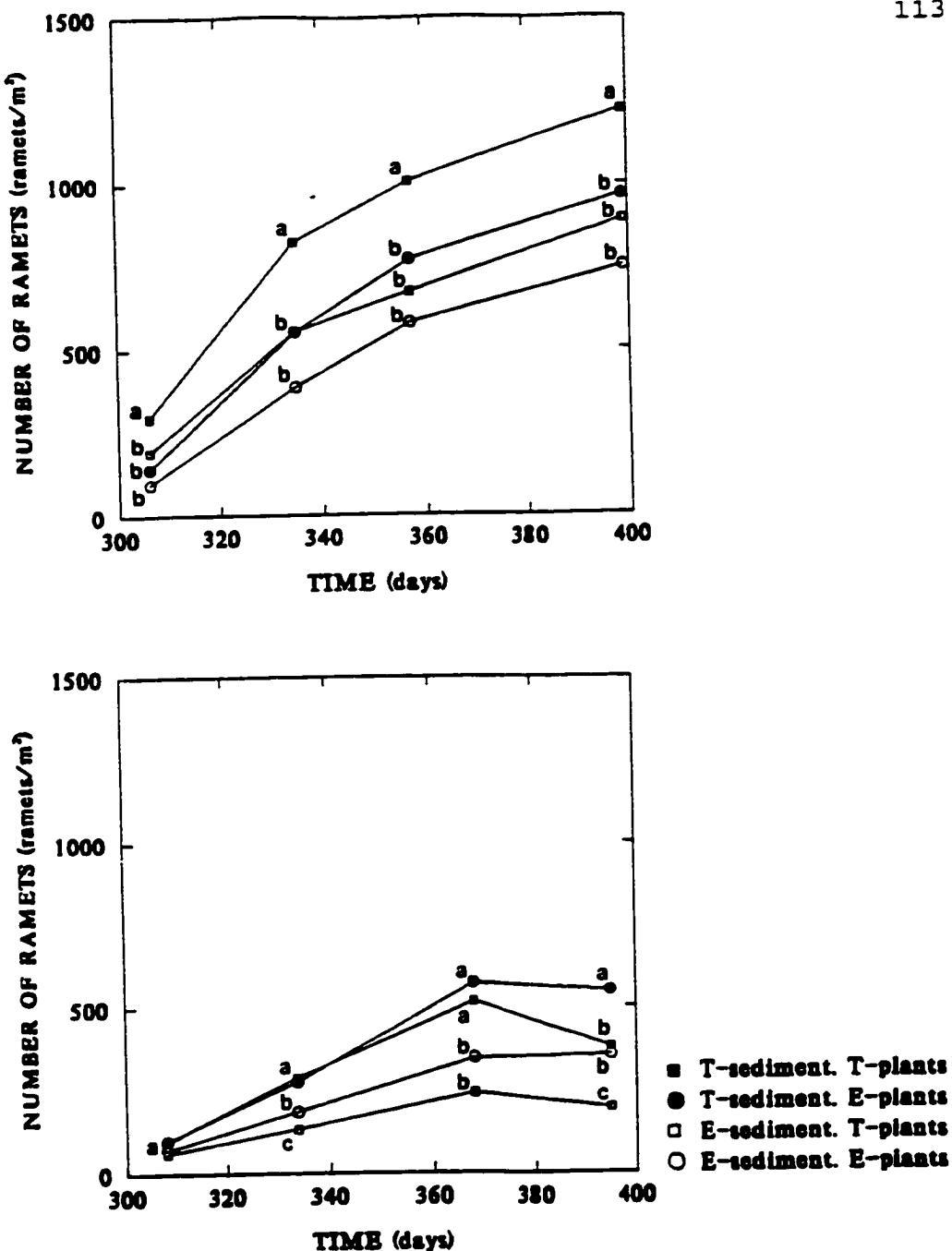


Figure 4.1. Number of ramets per m^2 at the Turkey Island site (upper), and the Ecarte site (lower), over time (in days post-initiation of the experiment). Different letters at single points in time indicate statistically significant differences according to Duncan's multiple range test ($p < 0.05$) between mean values. Note that statistical comparisons are not made between points in time.

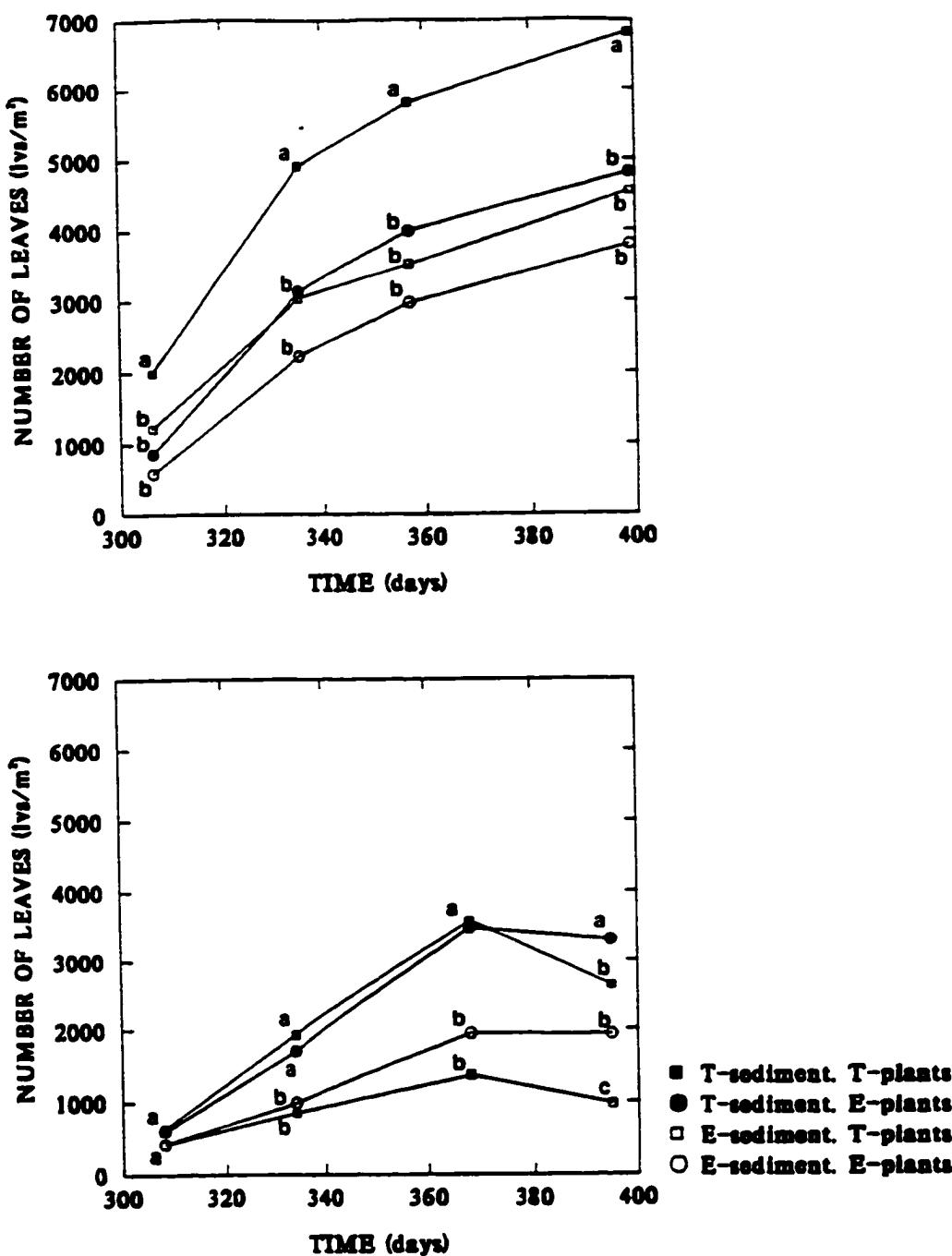


Figure 4.2. Number of leaves per m² at the Turkey Island site (upper), and the Ecarte site (lower), over time (in days post-initiation of the experiment). Different letters at single points in time indicate statistically significant differences according to Duncan's multiple range test ($p<0.05$) between mean values.

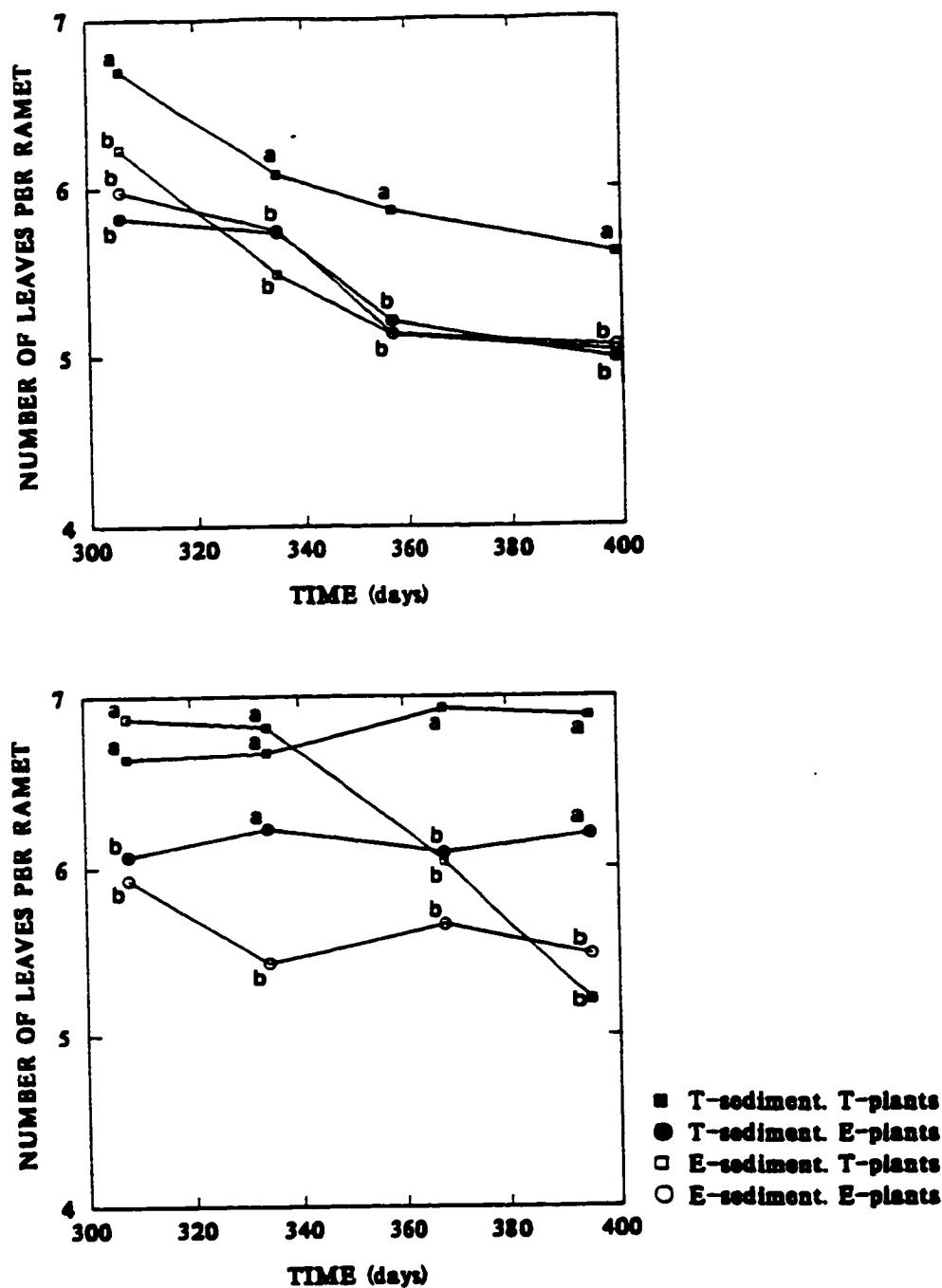


Figure 4.3. Number of leaves per ramet at the Turkey Island site (upper), and the Ecarter site (lower), over time (in days post-initiation of the experiment). Different letters at single points in time indicate statistically significant differences according to Duncan's multiple range test ($p<0.05$) between mean values.

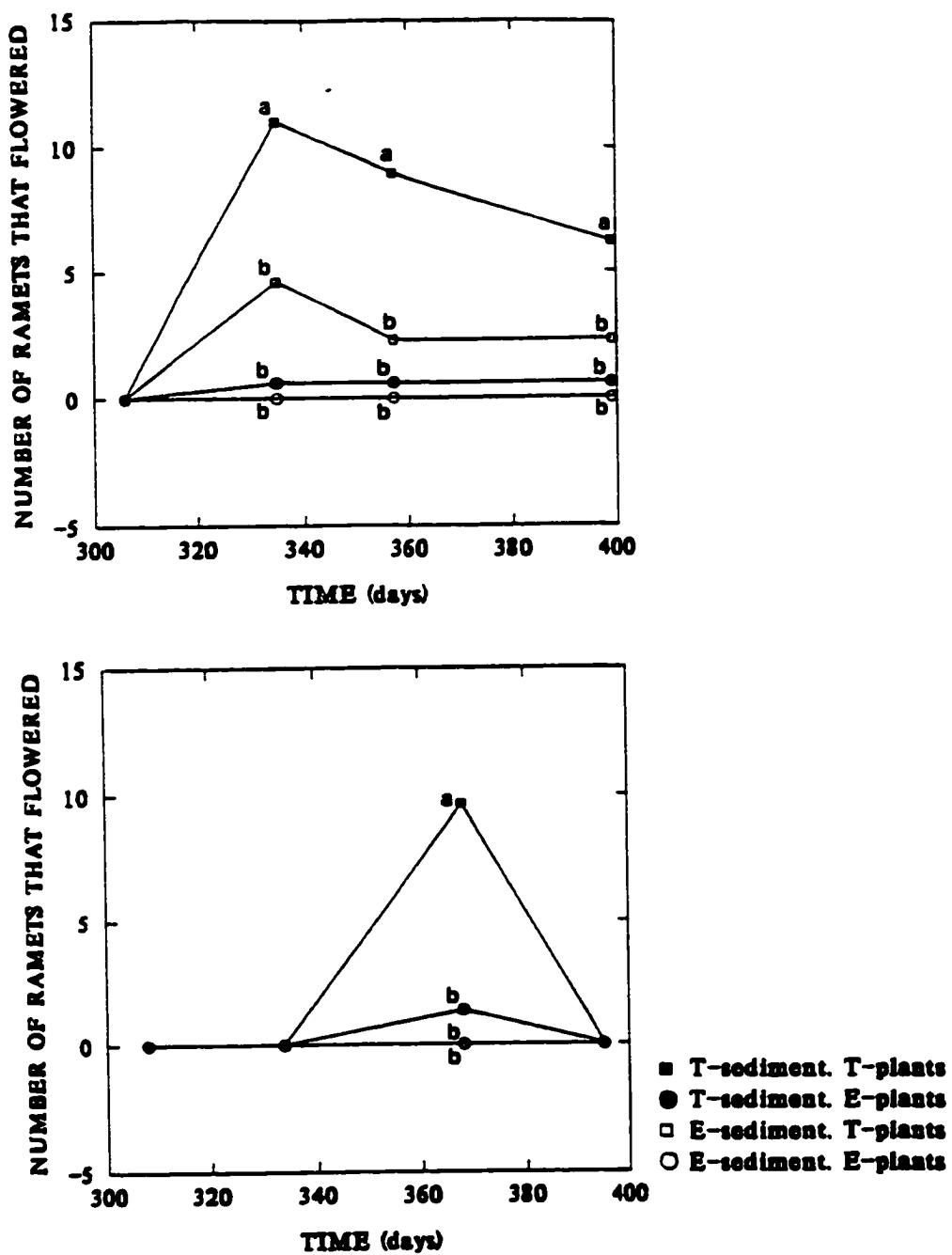


Figure 4.4. Proportion of ramets that flowered at the Turkey Island site (upper), and the Ecarte site (lower), over time (in days post-initiation of the experiment). Different letters at single points in time indicate statistically significant differences according to Duncan's multiple range test ($p<0.05$) between mean values.

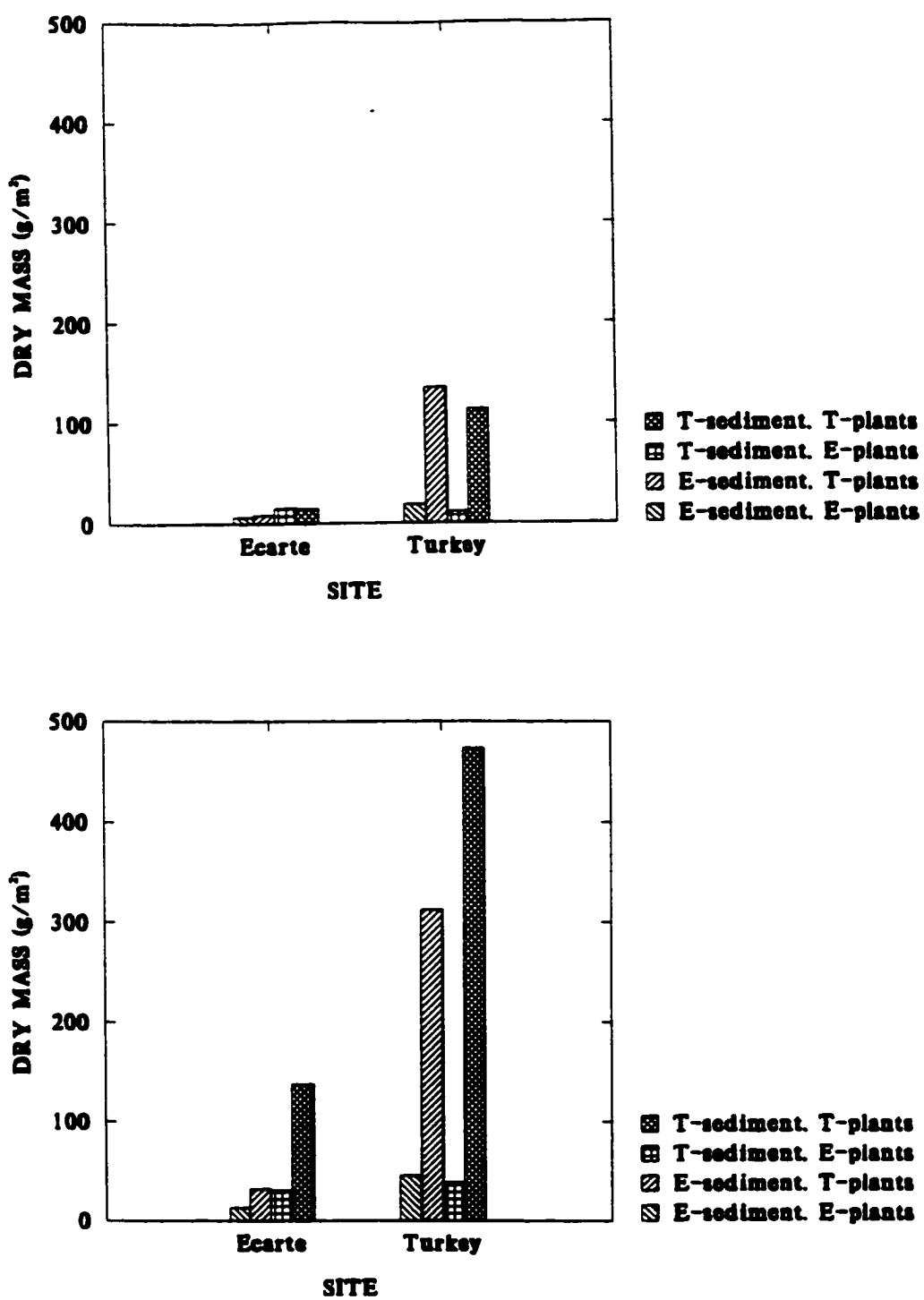


Figure 4.5. Dry mass of plants per m² at the Turkey Island site, and the Ecarte site, in July 1991 (upper), and in September 1991 (lower).

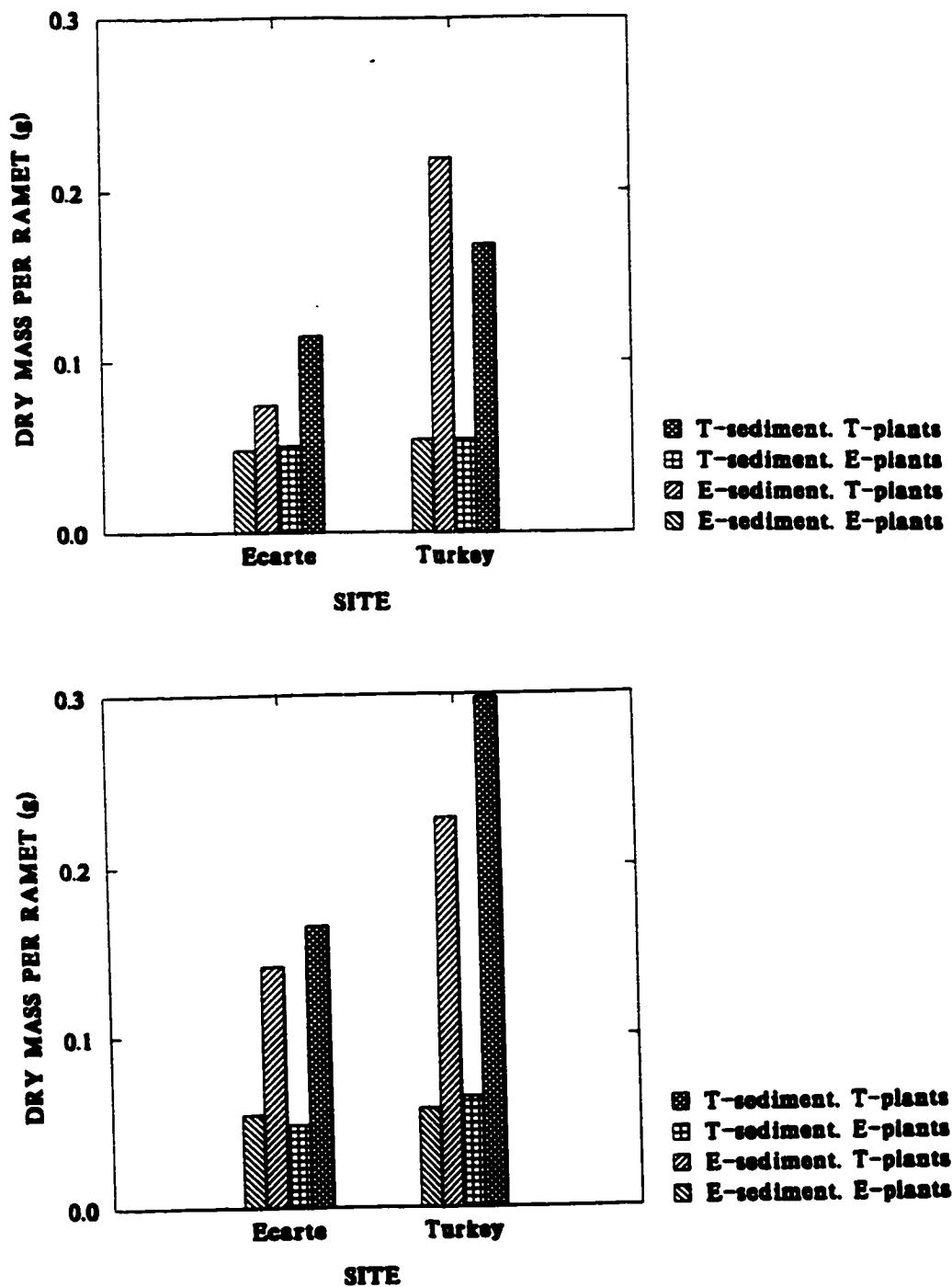


Figure 4.6. Dry mass per ramet at the Turkey Island site, and the Ecarter site, in July 1991 (upper), and in September 1991 (lower).

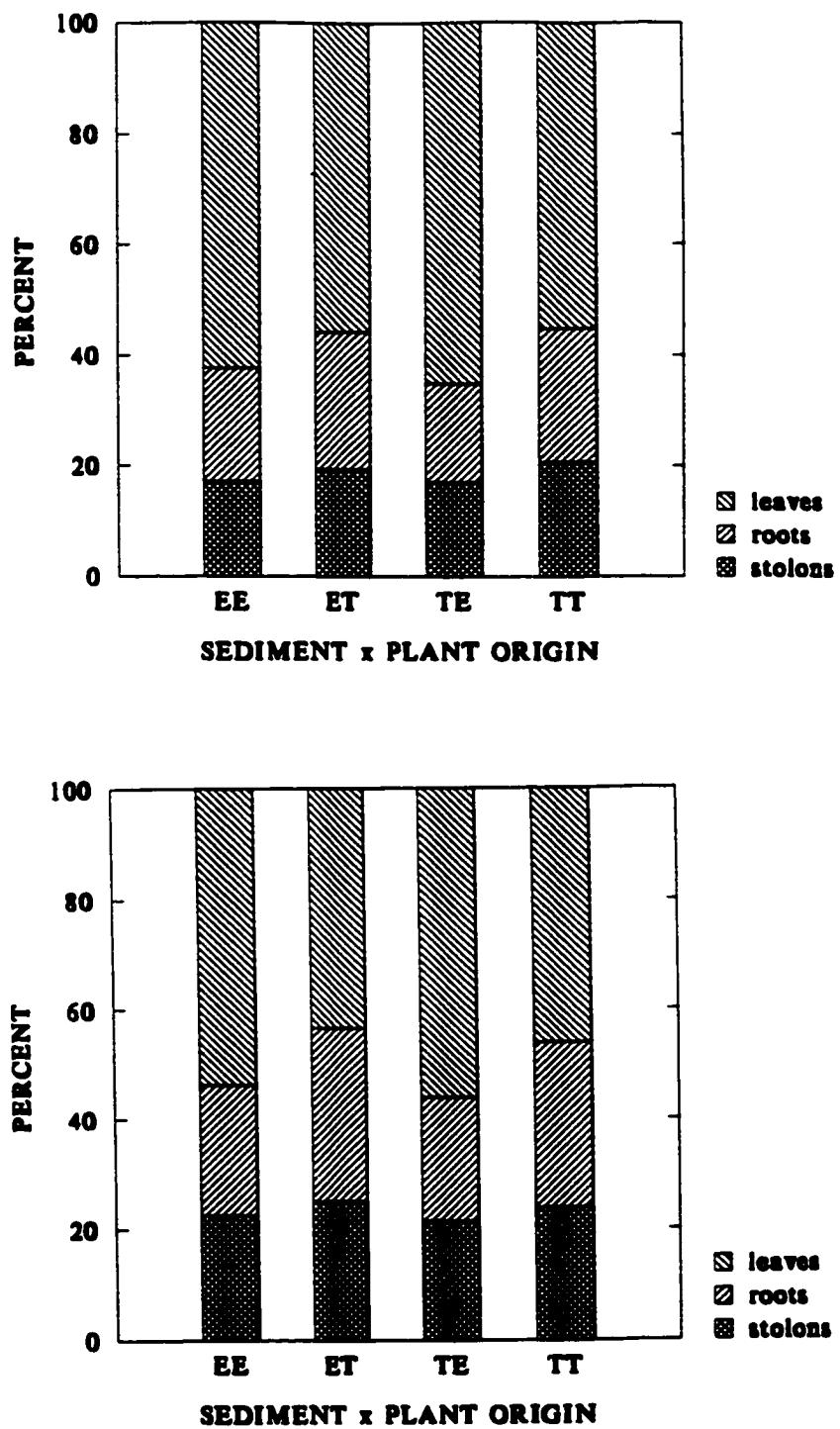


Figure 4.7. Proportionate contribution of leaves, roots and stolons to dry mass at the Turkey Island site (upper), and the Ecarte site (lower) in July 1991. Each bar represents a particular combination of plant source and sediment type as indicated.

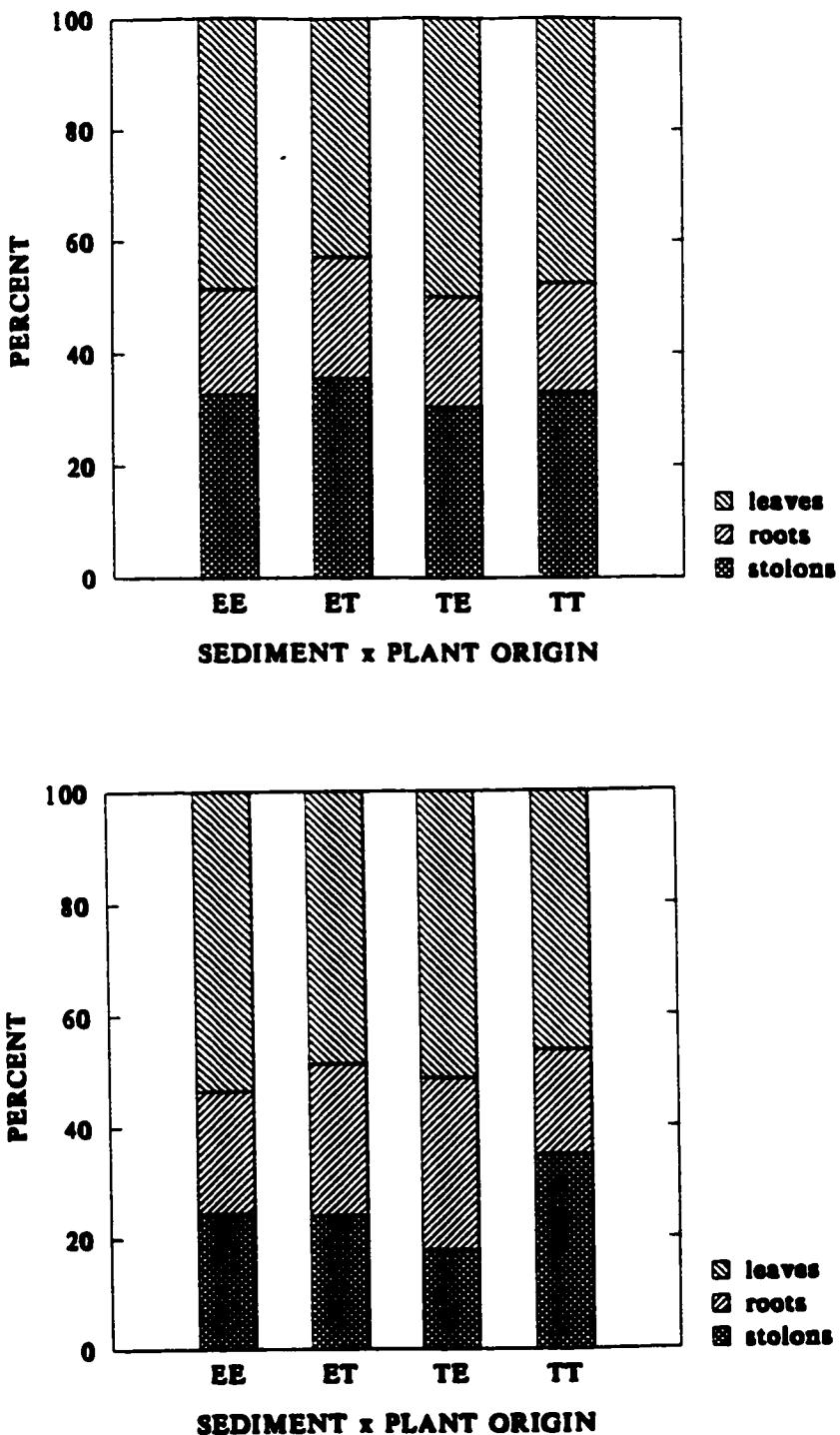


Figure 4.8. Proportionate contribution of leaves, roots and stolons to dry mass at the Turkey Island site (upper), and the Ecarte site (lower) in September 1991. Each bar represents a particular combination of plant source and sediment type as indicated.

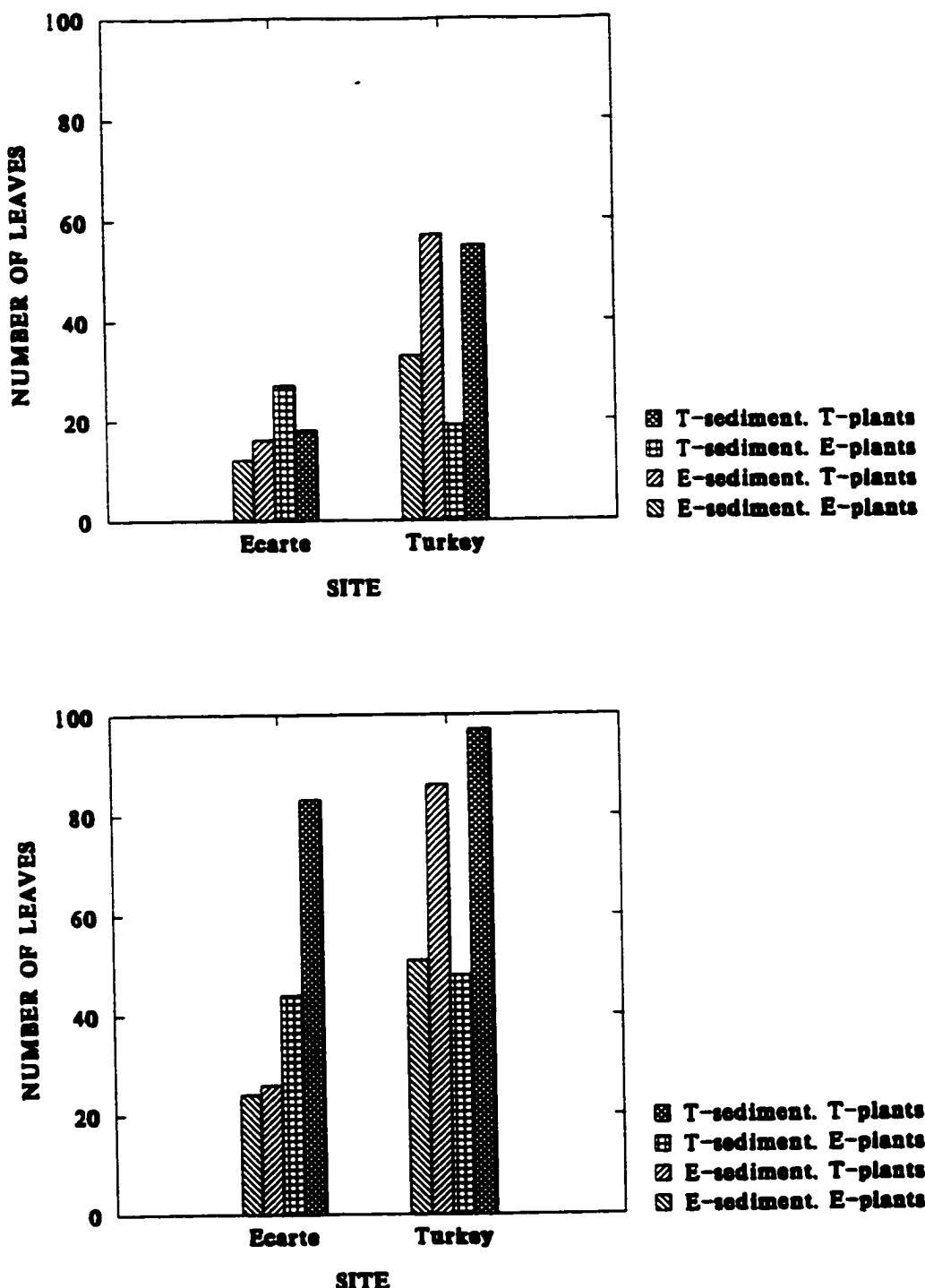


Figure 4.9. Number of leaves per genet in plants in each treatment, at each site in July 1991 (upper), and September 1991 (lower).

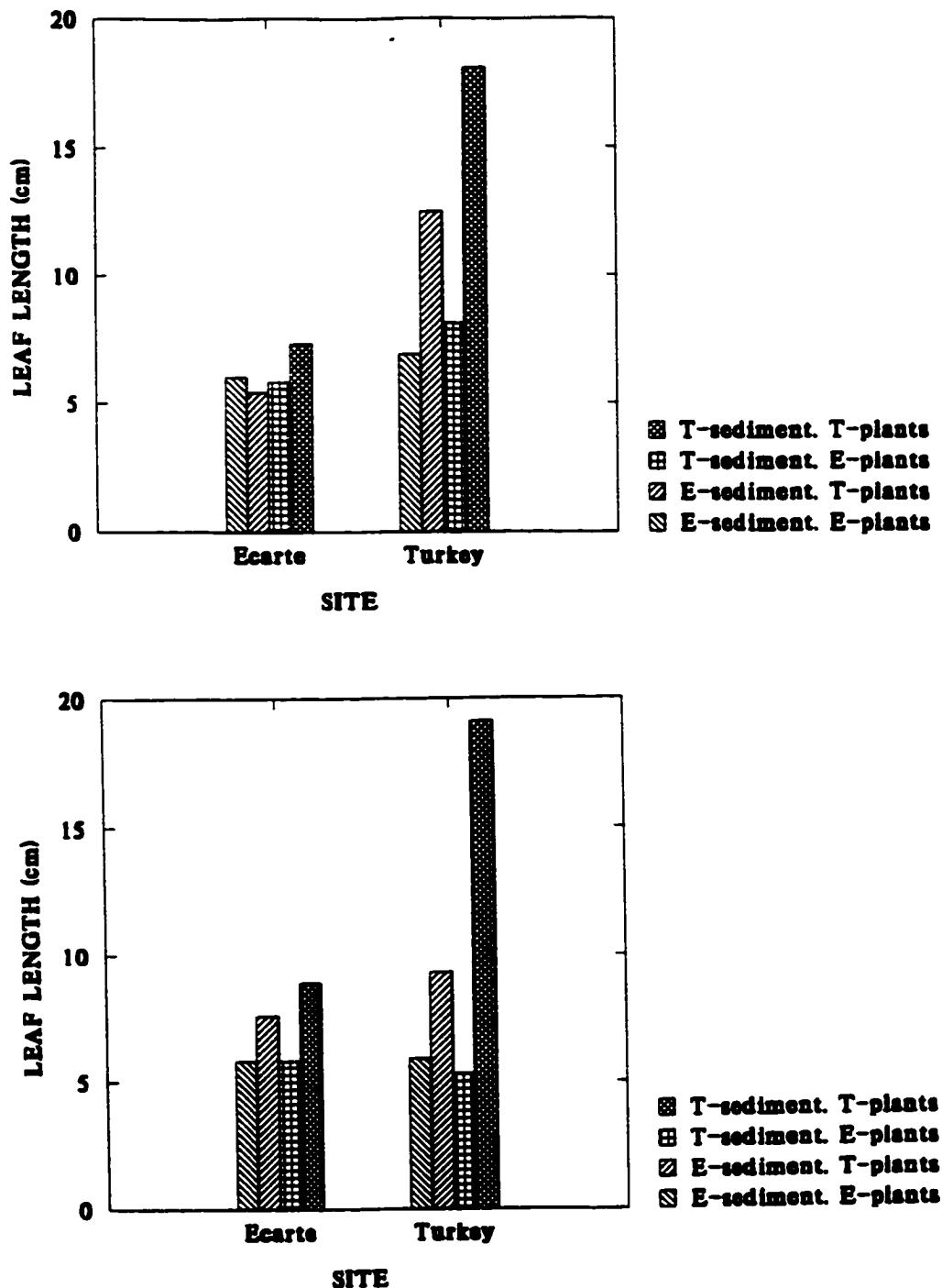


Figure 4.10. Mean length of a leaf in plants in each treatment, at each site in July 1991 (upper), and September 1991 (lower).

Chapter 5

EFFECT OF SEDIMENT, WATER COLUMN AND SITE OF PLANT ORIGIN ON GROWTH AND REPRODUCTION OF *Vallisneria americana*: LONG-TERM RECIPROCAL TRANSPLANT-REPLANT EXPERIMENT¹

ABSTRACT

This chapter gives the results of a 3-year extension of the 1-year experiment reported in Chapter 4. As described above, the experiment was carried out at two sites in the Huron-Erie Corridor, and was designed to assess the separate effects of sediment type, local water column, source population of plants, and duration of exposure upon growth and development in *Vallisneria americana*. The objective was to determine how long such studies would need to run to be of value in assessing site impairment, and whether year-to-year variations significantly affected the consistency of results in reciprocal-transplant-replant studies of active biomonitoring.

The relative rankings of the experimental treatments did not change over the four years. Measures of plant performance in the first year were significantly correlated with contamination of plant tissues. Detrimental effects on plant performance were primarily associated with exposure to a water column, and secondarily to the sediment. Plasticity in the length of leaves became evident only in year 2; any conclusions about ecotypic differences in leaf length based on a single year of study would therefore have been misleading.

Leaf-to-root surface area ratios provided a simple, reliable measure of environmental quality, and were shown to be consistent from year-to-year, as measures of

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environmental quality for the active biomonitor of this study. This supports conclusions based on the use of passive biomonitor of *Vallisneria* collected from the field (reported in Chapter 6).

INTRODUCTION

Macrophytes provide food, substrate, refuge, and breeding and nursery areas for numerous aquatic organisms. They form the base of the aquatic food web (Wetzel 1995); thus detrimental effects of pollutants may be found earlier in plants than in organisms higher in the food chain, although *Vallisneria* contains lower absolute concentrations of contaminants than organisms higher in the food chain (Mazak 1995).

In Chapter 4, the results of a one-year active biomonitoring experiment have been reported, where plants collected from two sites were deployed in a factorial design, either in native or alien sediment, and at their home site, or an alien site (Lovett-Doust *et al.*, 1994a). The objective was to determine whether, for *Vallisneria*, detrimental effects of polluted conditions were mediated primarily through sediment, or through the water column, and whether non-destructive measures of plant performance could indicate sub-lethal detrimental effects.

The present study is an extension of the one-year reciprocal transplant-replant study described in Chapter 4, also in Lovett-Doust *et al.* (1994a), and was designed to assess the utility of short- and long-term biomonitoring studies in providing assessment of environmental quality (see also Lovett-Doust *et al.*, 1994a,b). An objective of this study was to determine how long such studies would need to run to be useful in comparisons of site impairment, and whether year-to-year variations due to extrinsic factors would significantly affect the consistency of results. Although the two sites studied are in Areas of Concern, and (binational) Remedial Action Plans were in preparation, no direct remedial actions were undertaken over the four year period at these experimental sites.

MATERIALS AND METHODS

This long-term field experiment was intended to assess sublethal effects of local

water column (i.e., plants living at one site or another); sediment type; the site of origin of a plant; and duration of exposure (1 - 4 years), on growth, survivorship and reproduction in *Vallisneria americana* (see Catling *et al.*, 1994 and Lovett-Doust and LaPorte, 1991 for details of the biology of this species). The plants described in Chapter 4 (see also Lovett-Doust *et al.*, 1994a) were tracked for a total of four years, in order to determine whether the effects of sites and sediments, in terms of adverse effects on plant growth, were maintained.

The experiment was carried out at two sites in the Huron-Erie corridor of the Great Lakes. The first was Chenal Ecarte (42°37'N, 82°28'W) in the delta of the St Clair River, east of Walpole Island between Port Lambton and Wallaceburg, 37 km downstream of Sarnia. The second site was approximately 86 km downstream, in the lower Detroit River, on the south-eastern shore of Turkey Island (42°11'N, 83°28'W), just downstream of Fighting Island, 12 km downstream of Windsor, and upstream of Lake Erie (see Lovett-Doust 1994a). Plants collected from the two sites were placed in plastic tubs, either in their native sediment, or sediment taken from the other site. Labelled tubs were then placed, in a factorial array, at either the Turkey Island, or Chenal Ecarte site. There were initially 10 plants per tub, and 10 replicate tubs of each treatment combination. These replicate tubs were harvested over the four year period.

Non-destructive measurements

For four years, records of plant survival, clonal growth, leaf number per ramet and leaf number per unit area, were made at one-month intervals throughout the growing season (May- October). During flowering, the number of male inflorescences and female flowers per ramet, and per tub were recorded. On several occasions water temperature at the surface of the sediment was also recorded.

Destructive harvests

Once a year, in mid-September, a tub representing each combination of sediment and plants was harvested at each location. In the first year an additional destructive

harvest was made in July. In the fourth year of study, (September 1994), three tubs were collected for each treatment combination at each site, following the procedure in Chapter 4 (see also Lovett-Doust *et al.*, 1994a). Ramets from each tub were harvested separately. The number and length of leaves and roots, leaf width, root diameters and number of flowers were determined for each ramet. Biomass of leaves, roots, stolons, and turions (overwintering organs) was determined to observe the pattern of biomass distribution for each experimental treatment at each location.

Relative impairment of plants

The index of relative impairment of plants was described earlier in Chapter 4 (see also Lovett-Doust *et al.*, 1994a) and expresses relative plant performance, with the Turkey Island site or Turkey Island sediment as reference site or reference sediment. For plants originating from each of the two sites, final values of the index were estimated based on mean four-year plant performance at each exposure site or in each sediment. The significance of differences in relative plant performance according to water column and sediment were estimated using the nonparametric Kruskal-Wallis test on rankings of absolute values of relative impairment.

Comparison of short-term with long-term results

Short-term (single season) and long-term (four-year mean) results were compared using the Spearman Rank correlation test, based on relative rankings of plant performances in different treatments. Variance in the absolute measures of plant performances between growing seasons was also analysed. Data were analysed with SYSTAT for Windows version 5.03 (1992), using ANOVA and, where appropriate, differences between means were tested for significance by Tukey HSD pairwise comparison tests. In all ANOVA models, time was considered as a nesting factor.

RESULTS

Non-destructive monitoring

The period of exposure, site of plant growth, site of plant origin, and sediment type, as well as certain interactions had highly significant effects on plant performance (Table 5.1). Estimates of clonal growth and flower production were strongly affected by the main factors and their interactions, and therefore provide particularly sensitive measures of relative plant performance. Differences in water temperature did not have significant effects on overall differences in plant performance ($p>0.05$); however, the growing season typically begins two to three weeks earlier at the Turkey Island site than at Chenal Ecarte.

In general, site had the greatest effect on plant performance, with year, season, sediment type and site of plant origin also showing statistically significant effects (Table 5.1). For all treatments, and in most years, the number of ramets produced per m^2 was significantly greater at Turkey Island than at Chenal Ecarte ($p<0.001$; Figure 5.1). Each year, plants grown in Turkey Island sediment (T-sediment) produced significantly greater densities of ramets than those grown in Chenal Ecarte sediment (E-sediment) ($p<0.001$). There were significant differences in the rate of clonal growth (number of new ramets produced by parent rosettes over a growing season) between the two sites; plants grown at the Chenal Ecarte site had a significantly lower rate ($p<0.001$) than plants grown at the Turkey Island site. Transplanted to the Turkey Island site, plants from Chenal Ecarte had a significantly greater rate of clonal growth than plants originating from Turkey Island (Table 5.1). Thus alien plants did better in terms of clonal growth than native Turkey Island plants.

A similar pattern was seen for the number of leaves per m^2 at each site (Figure 5.2). Plants growing in Turkey Island sediment had significantly more leaves per m^2 than plants grown in Chenal Ecarte sediment ($p<0.001$). There were also significant differences between plants originating from different sites (Table 5.2). In three out of four growing seasons plants, growing at Turkey Island produced more than 5000 leaves per

m^2 . In contrast, plants that grew at the Chenal Ecarte site typically had fewer leaves per m^2 . The number of leaves per ramet (ranging from 5-7) was comparable at the two sites.

Different years and plant phenology over the growing season both had significant effects upon flower production in *Vallisneria* (Table 5.1, Figure 5.3). At the Ecarte site, in all plants grown in Ecarte sediment, no flowering occurred over the four year period; a small number of ramets growing in Turkey Island sediment at the Ecarte site flowered. In marked contrast, at the Turkey Island site, plants flowered every year, and ramets originating from Chenal Ecarte or Turkey Island were significantly more likely to flower if they were planted in Turkey Island sediment than in Chenal Ecarte sediment ($p<0.001$).

Destructive harvests

Over the four year period, the greatest mean biomass per m^2 was found in Turkey Island plants growing at Turkey Island, whether they were grown in Ecarte sediment or in sediment from Turkey Island. At the Chenal Ecarte site the greatest biomass per m^2 was recorded for Turkey Island plants growing in Turkey Island sediment. Biomass was also significantly affected by differences between years and by plant phenology (Table 5.1). Individual ramets from both sources were of significantly greater mean biomass when grown at Turkey Island compared to the Chenal Ecarte site ($p<0.001$). Individual ramets grown at either site in Turkey Island sediment had significantly greater biomass than those grown in Chenal Ecarte sediment ($p<0.001$). Both, year-to-year and seasonal differences had significant effects on individual ramet biomass (Table 5.1).

Cumulative leaf length of ramets per m^2 and per ramet were significantly greater at Turkey Island than at the Chenal Ecarte site ($p<0.001$, Figure 5.4). Over each growing season, plants grown at the Chenal Ecarte location in Turkey Island sediment developed significantly greater cumulative leaf length per m^2 than those grown in Chenal Ecarte sediment ($p<0.001$). The mean length of a leaf also remained significantly greater in ramets planted at the Turkey Island site than at Chenal Ecarte ($p<0.001$). In the first year of exposure of Chenal Ecarte plants at the Turkey Island location, mean leaf lengths were similar to those seen in undisturbed plants growing at Ecarte ($p>0.05$). However, in the

seasons that followed (1992-1994), the mean length of leaves on ramets collected from the Chenal Ecarte, but planted at Turkey Island, increased by up to 200%, becoming similar to the length of native ramets at that site.

There were significant differences in patterns of biomass allocation among experimental treatments. Every year, ramets grown at Ecarte allocated relatively more biomass to leaf tissue than plants at Turkey Island ($p<0.001$, Figure 5.5). At the Chenal Ecarte site, leaf-to-root biomass reached ratios of 10:1, whereas at Turkey Island the ratio did not exceed 4:1. Over the four-year period, ramets planted in Chenal Ecarte sediment had significantly more biomass in leaf tissue than ramets planted in Turkey Island sediment ($p<0.001$). The leaf-to-root mass ratio for ramets grown at Chenal Ecarte increased significantly from year 1 to subsequent years ($p<0.001$).

The number of turions per m^2 (evidence of successful clonal growth) was significantly higher at Turkey Island than at the Chenal Ecarte site ($p<0.001$, Figure 5.6). At Chenal Ecarte, the density of turions never rose above 900 in any treatment over any season, while at the Turkey Island site the density was greater than 1000 in three growing seasons, and over 1500 turions per m^2 in two seasons. Turion density was significantly affected by differences among growing seasons (Table 5.1). Plants grown in Turkey Island sediment produced significantly greater densities of turions ($p<0.001$). Ramets grown at Turkey Island also produced significantly more turions per ramet than plants grown at Chenal Ecarte ($p<0.001$). Turkey Island plants had significantly more turions per ramet at both locations compared to Chenal Ecarte plants ($p<0.001$). Plants grown in Turkey Island sediment at the Chenal Ecarte location produced significantly more turions per ramet than ramets planted in Ecarte sediment at that location over all four seasons ($p<0.01$). There was significant, progressive, increase in turion production per ramet in Chenal Ecarte ramets grown at Turkey Island site over the four years ($p<0.001$).

The biomass of turions produced per ramet was significantly greater at the Turkey Island location than at Chenal Ecarte, for plants of either site of origin and grown in either sediment type. Turion biomass per ramet produced by Turkey Island plants was significantly greater than that of Chenal Ecarte plants, at both locations and in both

sediment types. A similar pattern was observed for mean biomass per turion. Plants grown at Turkey Island produced turions of significantly greater individual mass compared to turions produced by plants grown at Chenal Ecarte. A significant difference was also found between sediment types, with mean mass per turion of plants grown in Turkey Island sediment greater at both locations. Plants from Chenal Ecarte had significantly lower mean mass per turion at both sites and in both sediment types compared to Turkey Island plants.

At Chenal Ecarte, a significantly greater proportion of biomass was allocated to turions by plants grown in Turkey Island sediment than in Chenal Ecarte sediment ($p<0.001$, Figure 5.7). At the Ecarte site there were no significant differences in biomass allocation to turions between plants from either source population. At the Turkey Island site, allocation of biomass to turions was significantly ($p<0.001$) different between plants originating from Turkey Island and Chenal Ecarte, but there was no significant effect due to the sediment in which plants were grown.

Over the study period, the fraction of turions produced at the end of each season that germinated the following spring was significantly higher at the Chenal Ecarte site, (regardless of plant origin and sediment types) compared to the Turkey Island location ($p<0.001$, Figure 5.8). At Chenal Ecarte, a significantly higher fraction of turions originating from Turkey Island plants germinated over the entire study period ($p<0.001$). Despite many differences at both locations in the fraction of turions which germinated, at the beginning of each growing season, on average, there were comparable initial densities of plants in the range of 100 to 300 per m^2 at the end of June.

The leaf-to-root surface area ratio was significantly different between sites ($p<0.001$, Figure 5.9) with greater values of this ratio in plants at the Chenal Ecarte site. Furthermore, plants grown in Chenal Ecarte sediment at either site had significantly greater leaf-to-root surface area ratios than plants grown in Turkey Island sediment ($p<0.001$). There was remarkable consistency in terms of this result from year-to year, for all treatment combinations. Leaf-to-root surface area ratio was the only plant trait of all those observed that was not significantly affected by differences between years, or by

differences in site of plant origin (Table 5.1, Figure 5.9).

Relative impairment of plants

Over the four years of observation, *Vallisneria* grew best at the Turkey Island site, thus performance at Turkey Island was used as the baseline for the calculation of relative impairment. A positive value of the index means that plant performance (for a particular trait) was greater at Turkey Island compared to Chenal Ecarte. A negative value for the index indicates that parameter had a larger value at Chenal Ecarte than at Turkey Island (this was true, for example, for leaf-to-root surface area ratio). Estimates of relative impairment of selected plant characteristics (based on four-year mean performance) are shown in Table 5.2. Exposure to the Chenal Ecarte water column was a significantly more damaging factor for plants from both source populations, than was exposure to Chenal Ecarte sediment ($p<0.001$; Kruskal-Wallis test). Over the four-year period, flowering, leaf-to-root biomass ratio, turion germination and leaf-to-root surface area ratio were, in terms of absolute magnitude, the most responsive to the effects of water column. The greatest sediment effects were on flowering, turion production, biomass of ramets per m^2 and cumulative leaf length per m^2 . Also, leaf-to-root surface area ratio was highly sensitive to changes in sediment characteristics.

Comparison between short-term and long-term results

A summary of the results of Spearman rank correlation analyses for ranks of *Vallisneria* performance in different growing seasons (years 1991, 1992, 1993, 1994) with ranks based on the mean performance over the whole four-year period is shown in Table 5.3. Rankings based on 1-year data sets with respect to leaf-to-root surface area ratio, biomass of a leaf, biomass of a turion, biomass allocation to turions, and rate of clonal growth were similar and highly correlated with ranks calculated from the long-term, four-year means ($p_s<0.001$). This was true for all experimental treatments, and each individual year of study. However, ranks based on ramet density, leaf density, leaf-to-root biomass ratio, and the fraction of turions that germinated varied between years, and for

some years (particularly year 2) were significantly different from ranks based on the four-year means. Local weather data for the years covered by this study provide some possible explanation of these results; at the nearby Windsor Airport the number of cumulative degree days over 5 °C was 14% lower (2228) in 1992 than average (2583) in the period 1990-1995. The drop in number of cumulative degree days over 18 °C was even more marked in 1992; the level was 221, representing a 46% drop relative to the 6-year mean of 412.

An ANOVA performed on absolute values of plant performance indicated that the only parameter in *Vallisneria* that did not differ significantly between years was leaf-to-root surface area ratio. Several other measures of plant performance did vary significantly between months of the growing season, and between years (Table 5.1).

DISCUSSION

Public and scientific attention often tends to focus upon rapid and dramatic changes in species diversity or population sizes, rather than on relatively slow and gradual change. Yet long-term, low-level, chronic exposure to contaminants is clearly an important agent of change, and chronic exposure may ultimately have more severe effects on organisms than short-term, high-level exposure to toxic contaminants (e.g., Meharg, 1994; Depledge, 1989, 1993). In the highly populated Great Lakes region, frequent toxic discharges by industry, agriculture or municipalities, from point and nonpoint sources, have been so common in the past that it is difficult to isolate and track single effects or the effects of single events, on local ecosystems (Edsall *et al.*, 1988; Manny and Kenaga, 1991). In polluted areas living organisms are affected continuously over long periods of time by a wide range of contaminants, often present in low concentrations, that have been continuously adsorbed and desorbed by sediments (Pugsley *et al.*, 1985; Oliver and Pugsley, 1986). It is reasonable to expect that the relatively higher levels of organic contamination found in the first year of this study at the Chenal Ecarte site, and relatively

lower contaminant levels in *Vallisneria* at the Turkey Island site (see Chapter 4 and also Lovett-Doust *et al.*, 1994a) persisted in the subsequent 3 years of the study. Repeat contaminant assessments were not made, because of the substantial cost.

Main factors affecting plant performance

Both non-destructive and destructive measures of plant performance showed numerous strong and significant main effects of year, month, site, sediment and plant origin. The greatest effect was that of site (water column); in all respects plants growing at Chenal Ecarte were impaired, regardless of where they came from, and in whatever sediment their roots were growing. Plants at Ecarte showed reduced clonal growth and leaf production, and those in Ecarte sediment did not even flower, let alone set seed.

Year 2 (1992) appears to have been an anomalous one for macrophyte growth at Turkey Island. That year, there was a lack of rank correlation with overall (4-year) rankings for ramet and leaf number, or for the fraction of turions that germinated (Table 5.3). In several respects growth at Turkey Island was reduced, although the fraction of turions that germinated was at its highest. This may represent a density dependent response to the poor growing conditions that held during the 1992 season (see meteorological data, presented above).

The water column had its most severe effect on the leaf-to-root surface area ratio (see Figure 5.9), the number of flowers per m², the leaf-to-root biomass ratio and the fraction of turions that germinated; sediment type primarily affected the number of flowers per m², the biomass of ramets per m², the number of turions produced per m², and the leaf-to-root surface area ratio of a ramet (Figure 5.9). The number of flowers per m² and the leaf-to-root surface area ratio of a ramet seem to be the most sensitive measures of environmental quality, reflecting changes in both local water column and sediment type (Table 5.2). However, flowering in *Vallisneria* was highly variable between growing seasons and may not be a useful comparative indicator of more polluted sites if significant contamination represses flowering altogether (as it appears to have done for the experimental plants at Ecarte).

The significant interaction effect between contaminants and absolute measures of plant performance noted in year 1 (Chapter 3; Lovett-Doust *et al.*, 1994a) suggests that plants at different stages of growth and development, exposed to the same contaminants, may respond in different ways depending on their phenologic stage (this observation is not true for the leaf-to-root surface area ratio). This suggests that, if plants at different sites are used for biomonitoring, and general measures of growth and performance are used (excepting the leaf-to-root surface area ratio), plants should be collected at a similar stage of development. Roots of *Vallisneria* were highly responsive to increased contamination in that, at the Ecarte site, there was reduced allocation of biomass to roots, fewer roots per ramet, less total length of roots, reduced root diameter and root surface area per gram of root tissue. A significant increase in leaf-to-root surface area ratio for ramets grown at Chenal Ecarte with increased levels of contamination was observed (Tables 5.1 and 5.2; Biernacki *et al.*, 1995a,b, 1996). A similar response in *Vallisneria* roots was caused under experimental conditions, by increased contaminant levels in controlled contaminant exposure studies (Biernacki *et al.*, 1995a,b).

Exposure of plants via the roots is particularly important because organic contaminants with high hydrophobicity tend to be adsorbed from the water column by sediments and concentrated there to high levels. In related laboratory studies, the Chenal Ecarte sediment, exposed to the same concentrations of the organic contaminant trichloroethylene (TCE) in the water column, accumulated greater concentrations of TCE than did Turkey Island sediment (Chapters 2 and 3; Biernacki *et al.*, 1995a,b). Organisms in direct contact with contaminated sediments can accumulate high contaminant burdens, which need not correlate with ambient concentrations in water (Ernst *et al.*, 1984). Since mobility of highly hydrophobic chemicals within plant tissues is very limited (Guilizzoni, 1991), they tend to remain, and accumulate in, the organs where they are absorbed (Chapter 4; Lovett-Doust *et al.*, 1994a).

Leaf-to-root surface area ratio

The ratio of leaf-to-root surface area was highly responsive to both water quality

and sediment type in the present study and provided a consistent and reliable ranking of experimental treatments (Tables 5.1-3, Figure 5.9). It is particularly useful that this ratio was not affected by differences among growing seasons or between source populations (see Tables 5.1-3). Wet biomass of individual ramets ranged over the study period from less than 0.5 g to 10 g, and density ranged from 50 to 1500 ramets per m², yet neither of these quantitative differences affected the leaf-to-root surface area ratio. Two main factors affected leaf-to-root surface area ratio: site of plant growth (water column effects) and sediment type (Tables 5.1 and 5.2). In a survey of leaf-to-root surface area ratios in *Vallisneria* at 243 sites in the Huron-Erie Corridor, a significant correlation was found between the rankings using this ratio and independent measures of site contamination (Biernacki *et al.*, 1996). Leaf-to-root surface area ratio was also a sensitive indicator of sediment contamination in laboratory exposure study (Chapter 3; Biernacki *et al.*, 1995b). This ratio therefore provides a simple measure that is independent of absolute plant size, year, and plant source, and that allows one to rank contaminated sites for a fraction of the cost of direct chemical monitoring of water, sediment and biota.

Effects of site of origin: genetic differentiation?

Lokker *et al.* (1995) have shown that there is significant genetic diversity among *Vallisneria* genets growing at Turkey Island, despite the prevalence of clonal growth as a means of vegetative reproduction. At the beginning of this study, there were some differences in the appearance of plants from the two sites; those from Ecarte were of lower stature, with shorter leaves. These differences were maintained for the first year, but from the second year onwards the leaves on plants from Ecarte transplanted to Turkey Island grew just as long as the native plants at that site. This indicates that leaf length was a plastic aspect of growth, rather than being a genetically determined “ecotypic” difference. The reciprocal statement does not hold, however; plants from Turkey Island growing in the Chenal Ecarte continued to produce longer leaves than the native plants. The generally poor performance of plants at the Ecarte site suggests that natural populations there may be largely sustained through clonal growth rather than local

sexual reproduction. However, naturally growing plants clearly survive and flower at the Ecarte site, indicating that there may be strong selection there for contaminant resistance.

The genetic makeup of plants (inferred from the effects of source of plant origin) also had significant effects on plant performance (Tables 5.2 and 5.3). These differences in the reactions of *Vallisneria* plants from the two populations (Tables 5.1 and 5.2) may have a genetic basis. Plants from these two sites may typically experience different growing seasons. Natural sediments also differed and the differences in sediment texture are likely to have influenced the rate at which each sediment would adsorb nutrients, as well as contaminants from the water column. There were also differences between sites in terms of the levels of contamination. The St. Clair River has a well-documented history of high contaminant loads, particularly downstream of the petrochemical industrial region that borders the St. Clair River (Pugsley *et al.*, 1985, Oliver and Pugsley, 1986; Edsall *et al.*, 1988; Manny *et al.*, 1988; Biernacki *et al.*, 1996). All of the above differences between sites could have served to produce genetic differentiation between the two populations of *Vallisneria*, although, as mentioned above, some of the apparent phenotypic differences disappear in the second and subsequent years.

Short-term vs. long-term biomonitoring

In the present study it was possible to assess how different the results would have been if a 1-year study had been carried out in any of four individual years (1991-1994), or in a long-term (four-year) study. In many respects additional data collected in years 2-4 simply served to underscore the results obtained in the first year of the study. It is well known from transplant studies that in the season immediately following experimental transplantation, plants may either suffer negative effects of "transplant shock" or may experience some "carry-over" effects attributable to conditions in the former (source) habitat (see, e.g., Lovett-Doust 1981). It was therefore important to test for consistency in plant performance in subsequent growing seasons.

In each year, most of the harvest-based data, and non-destructive measures of

plant performance (such as rate of clonal growth and flowering) produced the same rank order as that found in year 1, despite the fact that the magnitude of absolute measurements differed significantly between years (Tables 5.1 and 5.3). This finding is in agreement with the results of other long-term studies; for example Suns *et al.* (1993) studied fish contamination patterns in the Great Lakes (including the Detroit River) over 20 years; Bignert *et al.* (1993) studied fish contamination patterns over 20 years in the Baltic Sea; and Carignan *et al.* (1994) studied sediment cores spanning a 50-year period from the St. Lawrence River. All of these studies reported year-to-year fluctuations in absolute contaminant concentrations in the study areas, but the ranking of sites with respect to contamination levels, and measured effects on the biota, were consistent. In the present study, while data from different sites can usefully be ranked if they are collected in the same year (and season), data collected in different years cannot be pooled for the purposes of ranking. This is a consequence of the fact that, with one exception, the effect of year is significant ($p < 0.001$, Table 5.1).

There was one important exception to this, the pattern of leaf-to-root surface area ratio remained constant from year to year, and for most of the growing season. The leaf-to-root surface area ratio was also insensitive to site of plant origin. Leaf-to-root surface area ratios were highly responsive to sediment type and site of plant growth over the period of study. Changes in the ratio were also significantly correlated with contaminant concentrations found in plants tissues (Lovett-Doust *et al.*, 1994a). It has been shown elsewhere that the ratio of leaf-to-root surface area in *Vallisneria* is useful for comparative biomonitoring studies between sites, and at the same site between years (Biernacki *et al.*, 1996). Based on the present study the marginal seasonal effect on the ratio suggests that plants may be sampled at any time in the growing season (after June) without distorting the assessment of relative site quality, and that data collected in different years can validly be compared. Such a sampling procedure is therefore more robust in terms of sampling time than the other, more direct measures of growth and performance.

Vallisneria as an active biomonitor

The technical simplicity of biomonitoring protocol, focusing on the leaf-to-root surface area ratio, allows for multiple data collections from large samples of *Vallisneria* ramets in the field, at any point during the period of active growth. It has been shown that data from different years can be compared with respect to this measure, and that plants from different source populations show similar responses. The other metrics of growth and performance are less robust, and some show significant year-to-year variation. They are therefore not recommended for multi-year and multi-season comparisons of site quality.

To conclude, a one-year biomonitoring study based on leaf-to-root surface area ratio can reliably and repeatably assess relative site quality; also data collected in different years can be compared. Long-term exposure experiments are not necessary if this parameter is used. No remedial actions were undertaken in these experimental areas during the study, but, it is argued, long-term studies of leaf-to-root surface area ratios in *Vallisneria* biomonitor would be useful in areas where remedial actions will be carried out; thus changes in environmental conditions could thereby be inexpensively tracked by sampling sets of plants over several successive years.

Table 5.1. Summary of results of ANOVA for measures of growth and reproduction in *Vallisneria americana* in the reciprocal transplant-replant experiment. Only factors that were significant are included [Month(Y)=month nested in Year; Site(T)=site of growth nested in month and year]. (**=p≤0.001; **=p≤0.01; * = p≤0.05; NS= not significant).

TRAIT	FACTORS										
	Year (Y)	Month(Y) (T)	Site(T) (L)	Sediment (S)	Plant Origin (P)	T.S.	T.P.	L.S.	L.P.	S.P.	T.S.P.
Non-destructive monitoring:											*
Number of ramets per m ²	***	***	***	***	**	***	NS	NS	**	'*	***
Number of leaves per m ²	***	***	***	***	***	***	NS	NS	***	*	***
Number of flowers per m ²	***	***	***	***	***	***	**	NS	NS	NS	NS
Number of flowers per ramet	***	***	***	***	*	*	NS	**	NS	**	NS
Rate of clonal growth	***	***	***	***	**	**	NS	**	NS	**	*
Destructive monitoring:											NS
Number of turions per m ²	***	***	***	***	***	***	NS	NS	**	NS	NS
Biomass of ramets per m ²	***	***	***	***	*	**	NS	***	*	NS	NS
Number of roots per ramet	*	***	***	***	***	***	NS	**	NS	*	NS
Biomass per ramet	***	***	***	***	***	*	NS	**	NS	*	NS
Biomass per ramet	***	***	***	***	***	*	NS	NS	NS	NS	NS
Number of turions per ramet	***	***	***	***	***	*	NS	NS	**	NS	NS
Biomass of turions per ramet	***	***	***	***	***	*	NS	NS	**	NS	NS
Turions-to-ramet biomass ratio	***	***	***	***	***	*	NS	NS	**	**	**
Length of a leaf	***	***	***	***	***	*	NS	NS	**	NS	NS
Biomass of a leaf	***	***	***	***	***	*	NS	NS	NS	NS	NS
Biomass of a turion	*	***	***	***	***	***	NS	NS	**	NS	NS
Leaf length-to-leaf biomass ratio	***	***	***	***	***	*	NS	NS	**	NS	NS
Leaf-to-root biomass ratio	***	***	***	***	***	*	NS	**	**	*	NS
Fraction of germinated turions	***	***	***	***	***	*	NS	**	**	NS	NS
Leaf-to-root surface area ratio	NS	*	***	***	NS	NS	NS	NS	NS	NS	NS

Table 5.2. Relative impairment (%) (see definition in Chapter 4) of plants over four-year study in the reciprocal transplant-replant experiment. Only values of relative impairment significantly different from zero are shown.

Trait	Impairment due to Chenal Ecarte	
	Water Column	Sediment
Plants Originating from Chenal Ecarte site:		
No. of ramets per m ²	43.4	35.4
No. of leaves per m ²	42.3	36.9
Rate of clonal growth	32.3	-3.2
No. of flowers per m ²	99.0	58.2
No. of turions per m ²	65.3	41.4
Biomass of ramets per m ²	78.7	45.9
Cumulative leaf length per m ²	69.2	45.2
Cumulative leaf length per ramet	41.9	0.7
No. of leaves per ramet	0.7	0.7
Biomass of a ramet	60.5	7.5
No. of turions per ramet	32.0	2.7
Biomass of turions per ramet	57.1	19.4
Length of a leaf	47.1	-0.7
Biomass of a leaf	58.3	2.0
Biomass of a turion	36.7	19.5
Leaf-to-root biomass ratio	-99.3	-21.7
Fraction of turions that germinated	-73.4	11.5
Leaf-to-root surface area ratio	-144.0	-34.2
Plants Originating from Turkey Island site:		
No. of ramets per m ²	42.2	46.0
No. of leaves per m ²	23.6	55.8
Rate of clonal growth	29.0	13.0
No. of flowers per m ²	95.4	67.4
No. of turions per m ²	64.2	49.1
Biomass of ramets per m ²	60.1	56.0
Cumulative leaf length per m ²	32.7	60.3
Cumulative leaf length per ramet	1.2	25.9
No. of leaves per ramet	-5.6	6.0
Biomass of a ramet	33.2	21.0
No. of turions per ramet	29.5	16.6
Biomass of turions per ramet	49.5	30.7
Length of a leaf	25.9	21.6
Biomass of a leaf	32.9	15.0
Biomass of a turion	28.9	18.4
Leaf-to-root biomass ratio	-84.7	21.1
Fraction of turions that germinated	-146.6	-22.2
Leaf-to-root surface area ratio	-116.9	-60.0

Table 5.3. Results of Spearman rank correlation analysis of ranks of selected measures of *Vallisneria americana* performance in experimental treatments in different years of the experiment and ranks based on the four-year mean performance in the reciprocal transplant-replant experiment. Only significant correlations are given (***= $p \leq 0.001$; **= $p \leq 0.01$; *= $p \leq 0.05$; NS= $p > 0.05$). -

TRAIT	Year 1	Year 2	Year 3	Year 4
Non-destructive monitoring:				
Number of ramets per m ²	***	NS	**	***
Number of leaves per m ²	***	NS	**	***
Number of flowers per m ²	**	**	*	***
Number of flowers per ramet	**	*	**	***
Rate of clonal growth	***	***	***	***
Destructive monitoring:				
Number of turions per m ²	**	*	**	***
Biomass of ramets per m ²	**	**	**	***
Number of roots per ramet	**	**	***	***
Biomass per ramet	**	***	***	***
Number of turions per ramet	*	**	***	***
Biomass of turions per ramet	**	***	***	***
Turions-to-ramet biomass ratio	***	***	***	***
Length of a leaf	**	***	***	***
Biomass of a leaf	***	***	**	***
Biomass of a turion	***	***	***	***
Leaf length-to-leaf biomass ratio	***	**	***	***
Leaf-to-root biomass ratio	NS	*	NS	*
Fraction of turions that germinated	**	NS	**	
Leaf-to-root surface area ratio	***	***	***	***

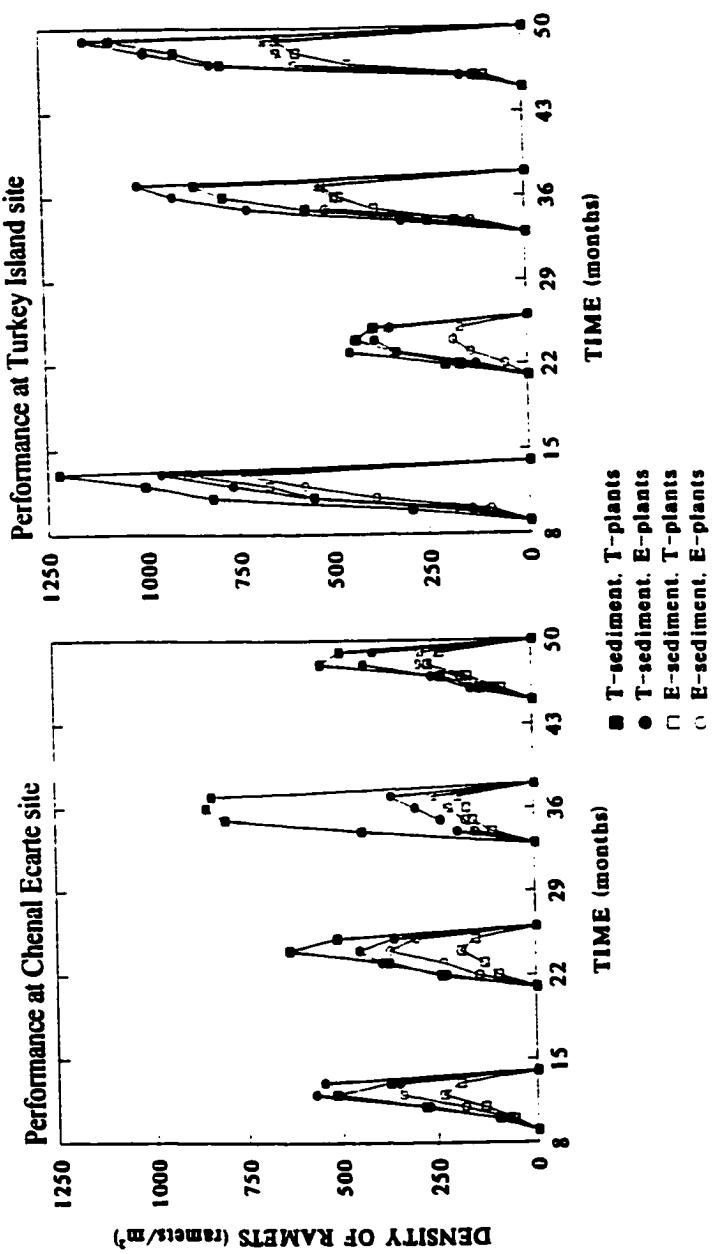


Figure 5.1. Density of *Vallisneria* ramets at the two sites.

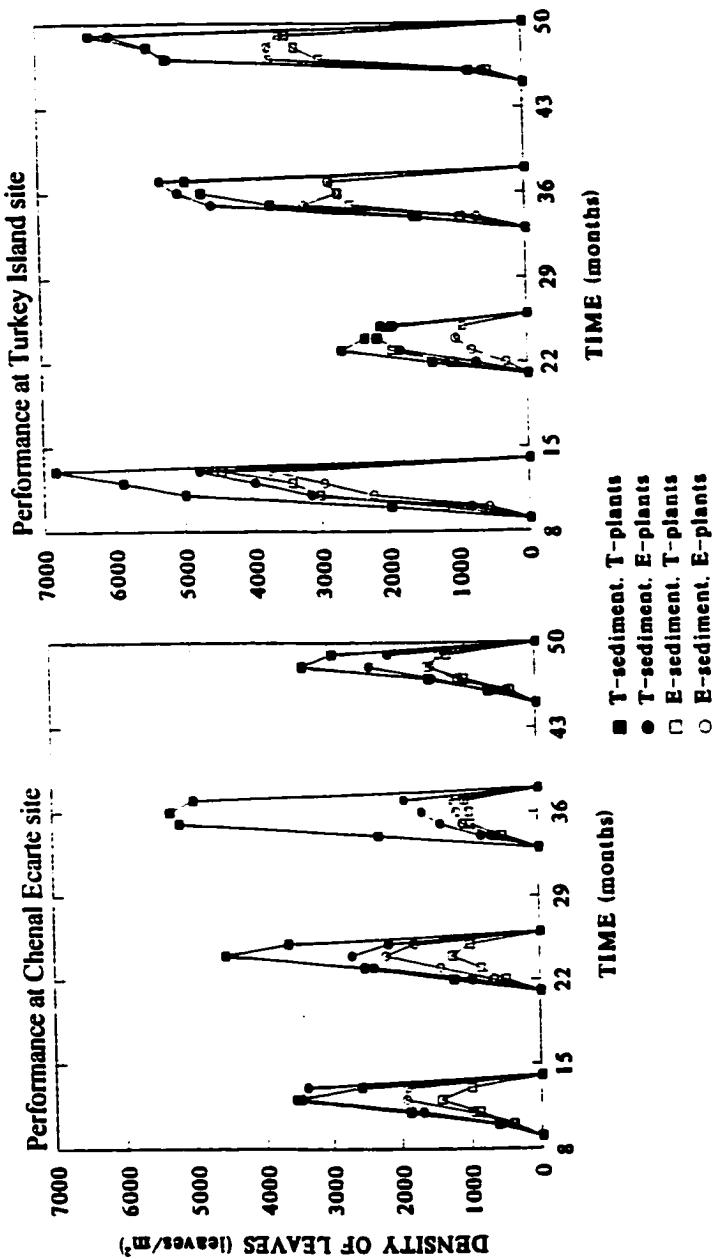


Figure 5.2. Density of leaves at the two sites.

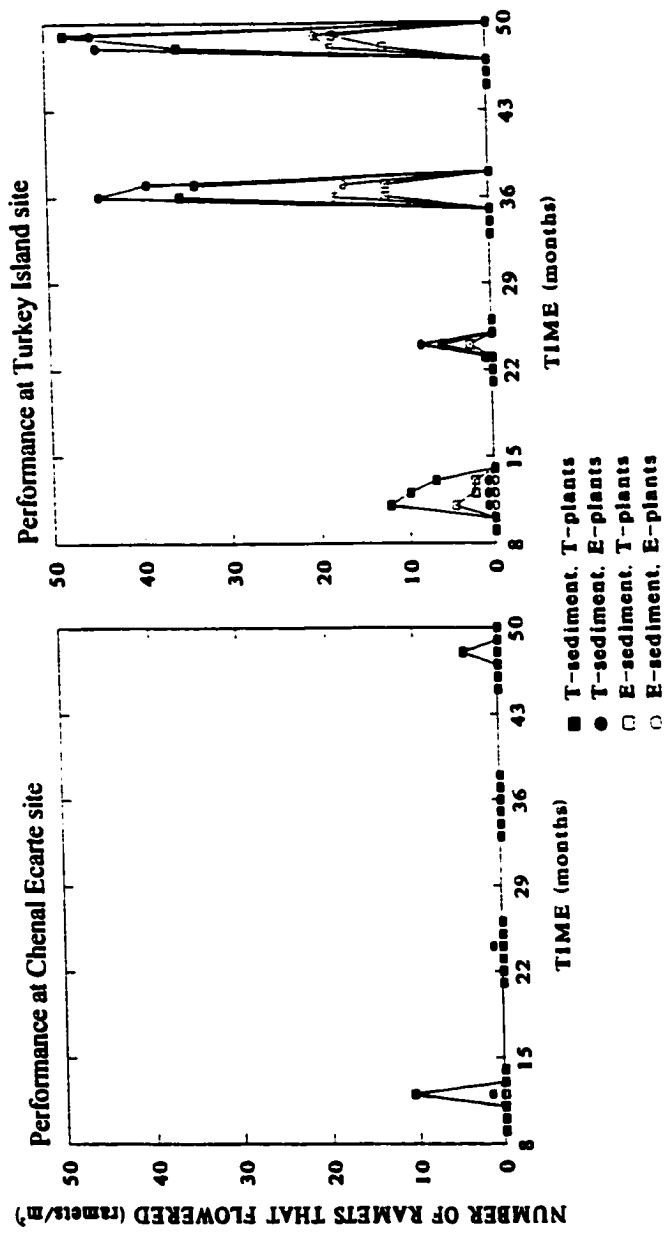


Figure 5.3. Frequency of flowering ramets at the two sites.

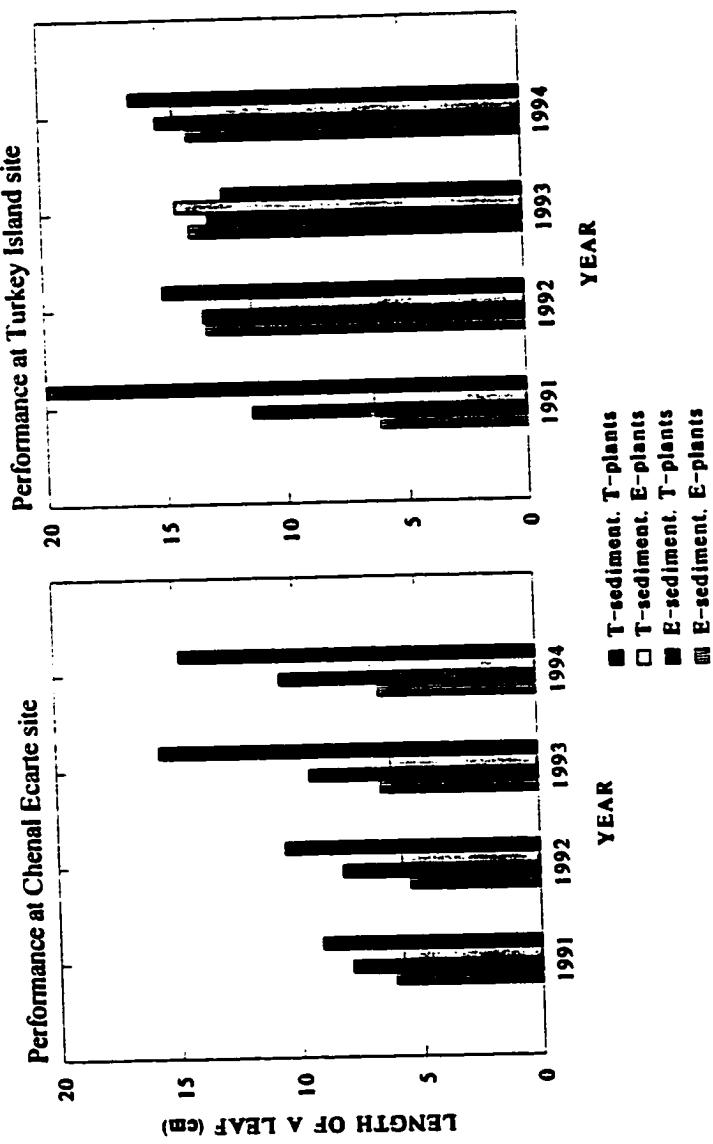


Figure 5.4. Mean length of a leaf on ramets grown at the two sites.

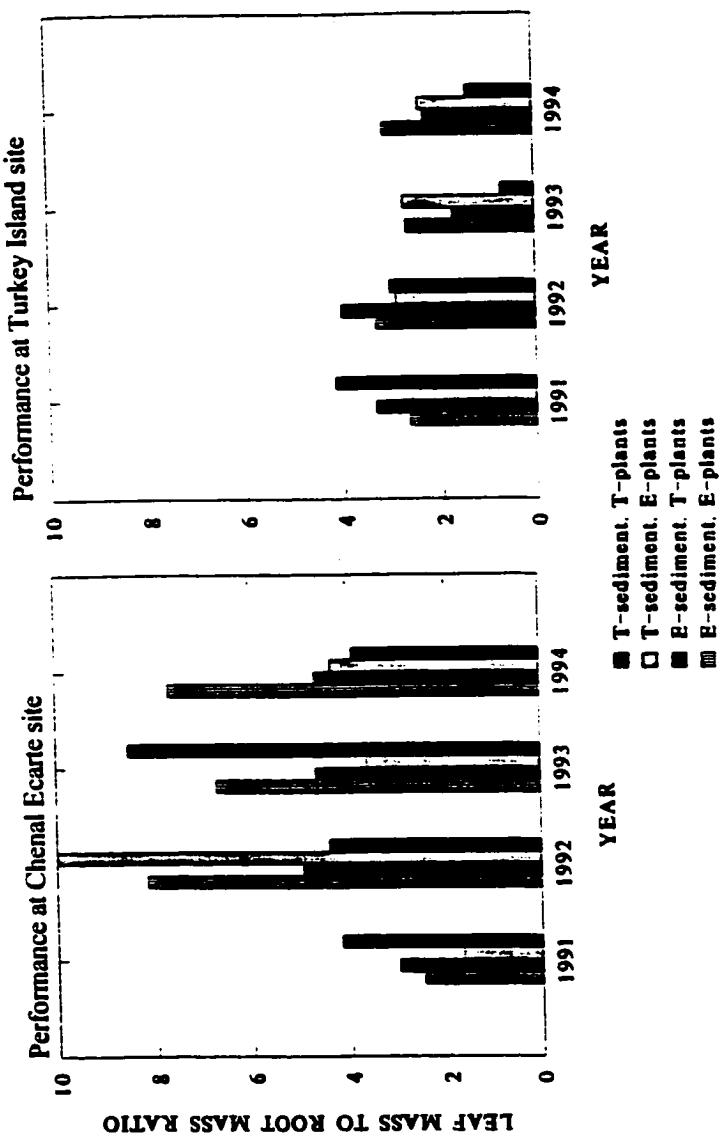


Figure 5.5. Leaf mass to root mass ratio of ramets grown at the two sites.

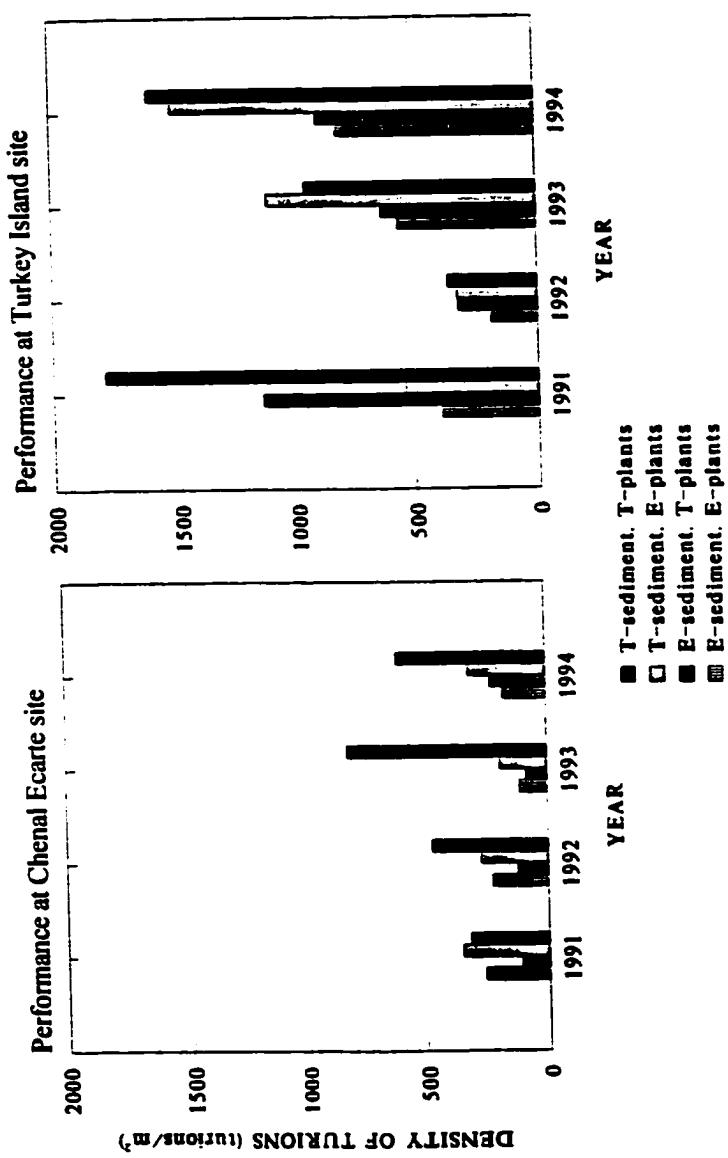


Figure 5.6. Density of turions for plants grown at the two sites.

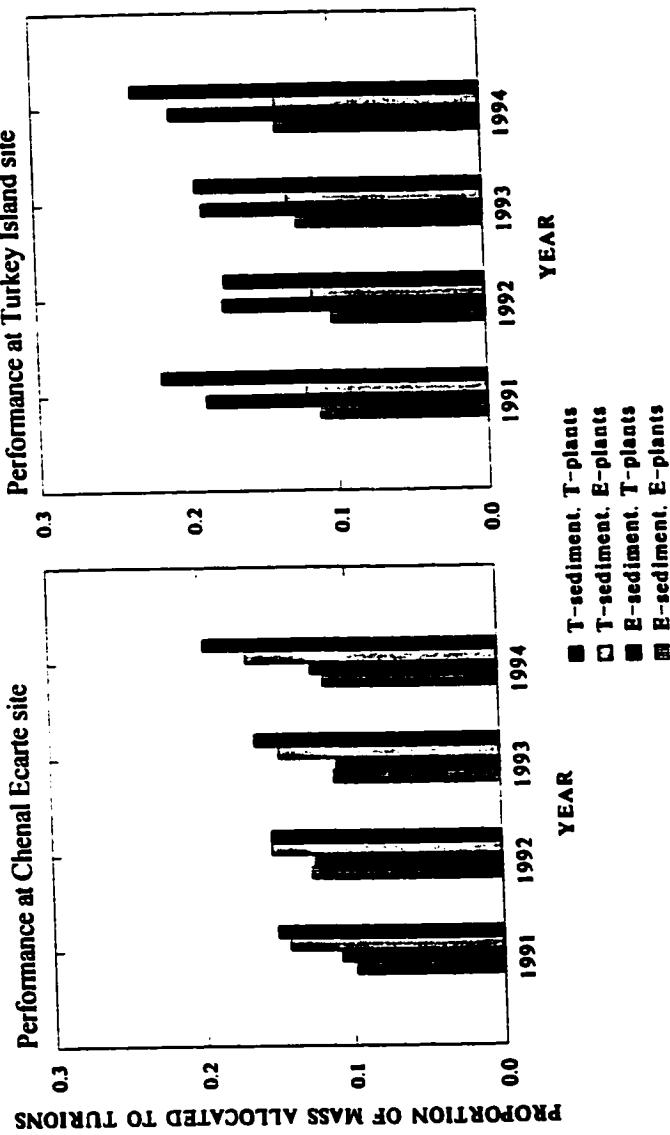


Figure 5.7. Proportion of mass allocated to turiions at the two sites.

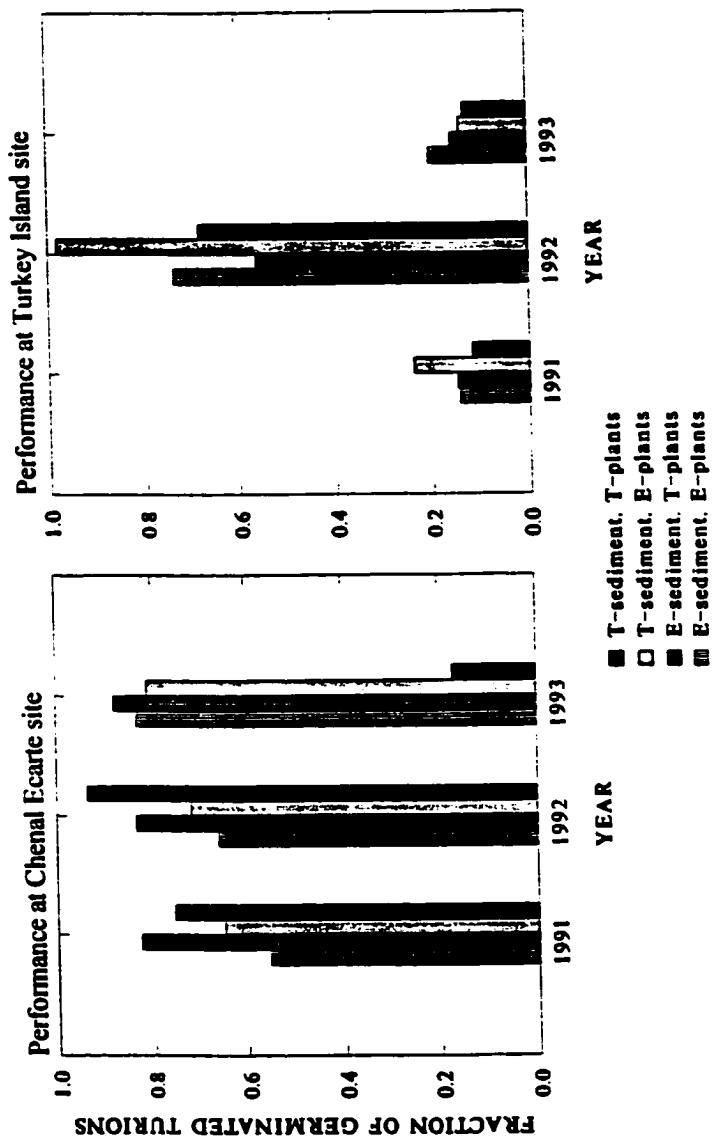


Figure 5.8. Turion germination at the two sites.

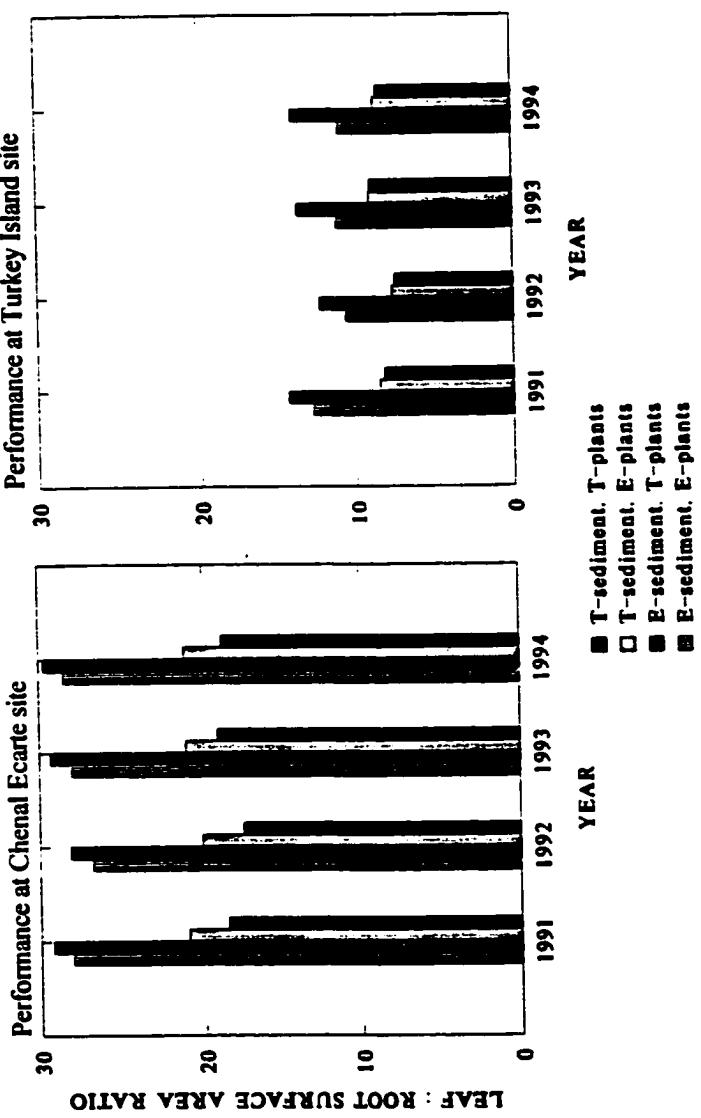


Figure 5.9. Leaf-to-root surface area ratio for plants grown at the two sites.

Chapter 6

CHANGES IN LEAF AND ROOT SURFACE AREA IN *Vallisneria americana* AS A TOOL FOR MONITORING ORGANIC CONTAMINANTS IN AQUATIC ECOSYSTEMS: A SURVEY OF NATURAL POPULATIONS IN THE HURON- ERIE CORRIDOR¹

ABSTRACT

Aquatic macrophytes are effective monitors of sub-lethal effects of organochlorine contaminants, and useful as early indicators of local environmental contamination. Ramets of *Vallisneria americana* were sampled from natural populations at 243 sites in the Huron-Erie corridor in August of 1993, to determine whether an index of leaf-to-root surface area ratios would be useful as a monitor of organic pollution in the field. The rankings of sites, based on the leaf-to-root surface area ratios in *Vallisneria* ramets were found to be significantly correlated with ranks of sites organochlorine contamination of biota or sediment, reported independently in the published literature. The ratio of leaf-to-root surface areas ranged from 2 to 92 with higher values associated with more polluted sites. The ratio changed little with water depth, from year to year, and seems to be independent of plant genotype. At highly polluted sites, there was a progressive increase in ratio over the growing season. Surveyed sites remained in the same relative ranking over the growing season. The results demonstrate that very simple estimation of ratios of leaf-to-root surface areas in *Vallisneria* may provide a rapid,

¹ The major results of this chapter have been published as follows:
Biernacki, M., Lovett Doust, J. and Lovett Doust, L. 1996. *Vallisneria americana* as a biomonitor of aquatic ecosystems: leaf-to-root surface area ratios and organic contamination in the Huron-Erie Corridor. *Journal of Great Lakes Research*, 22:289-303.

sensitive, convenient and inexpensive measure of site quality from the perspective of macrophytes, at the base of the food web. It may help to prioritize sites for remedial action, as well as to confirm environmental improvements due to remediation.

INTRODUCTION

Most studies of toxicity in the past have been based on the identification of a standardized mortality (LD_{50} or LC_{50}), the dose or concentration that caused 50% of individuals to die under laboratory conditions over a selected study period. Such tests may not have much field relevance. Acute levels of toxicants applied in laboratory tests are rare in field where chronic exposure to sublethal concentrations of pollutants are found more frequently (DiToro *et al.*, 1991). Measurements of morphology, growth and reproduction may be more sensitive in reflecting the subtle and gradual damage done by persistent low-level exposure of organism to contaminants than measures of mortality rates (Chapters 1, 2, and 3; Lovett Doust *et al.*, 1994a,b; Biernacki *et al.*, 1995a).

Submersed aquatic macrophytes grow at the interface of two distinct environments: the water column, and sediment. Their leaves are exposed to the water column, while roots are in the sediment. Both media, water column and sediment pore-water, are potential sources of uptake of contamination (Barko *et al.*, 1991; Guilizzoni, 1991). The use of plants as monitors of the environment provides an integrated picture of pollution within an ecosystem (Dennison *et al.*, 1993). Macrophytes can directly measure environmental quality at the base of the food web (Smith, 1991). Contaminants present in aquatic macrophytes may be transferred to higher levels of the food chain, when consumed as live plants by herbivores or as detritus by detritivores (Lodge, 1991; Wetzel, 1995). The use of biota for monitoring circumvents the need for assumptions to be made about the bioavailability of contaminants in sediment and water. Submersed aquatic plants have considerable potential for biomonitoring (Chapter 4; Lovett Doust *et al.*, 1994b). Several studies have used aquatic plants to monitor levels of heavy metals in water (Adams *et al.*, 1973; Guilizzoni, 1991). Macrophytes are also very useful for assessing organochlorine contamination in laboratory experiments (Chapters 2 and 3; Biernacki *et al.*, 1995a, b) and in the field (Chapter 4; Lovett Doust *et al.*, 1994a, b).

Vallisneria americana var. *americana* Michx. (Hydrocharitaceae) (Lowden, 1982; Catling *et al.*, 1994) is one of the most abundant submersed macrophytes in the Huron-

Erie Corridor (Schloesser *et al.*, 1985; Lovett Doust and LaPorte, 1991; Catling *et al.*, 1994), and it has already shown potential as a biomonitor of organic contamination in the field (Lovett Doust *et al.*, 1994a,b).

In field surveys, it has been shown that macrophytes accumulate significant amounts of organochlorines *in situ*, and that over the growing season contaminant concentrations and total "body burdens" increased (Chapter 4; Lovett Doust *et al.*, 1994a). The organochlorine bioaccumulation in submersed macrophytes may be very high (Painter, 1990; Lovett Doust *et al.*, 1994a). Contamination of *Vallisneria americana* was comparable to that of other aquatic plants in the Huron-Erie Corridor, including species of *Potamogeton*, *Najas*, *Myriophyllum* and *Elodea*. It has been confirmed that *Vallisneria americana* accumulates contaminants within its tissues and concentrations in roots were particularly high (Chapter 4; Lovett Doust *et al.*, 1994a). Exposure to sediment-borne organochlorine contaminants is particularly important for submerged rooted macrophytes (e.g. *Vallisneria americana*); due to hydrophobicity of organics, the sediments frequently contain higher concentrations of contaminants than the water (Baudo *et al.*, 1990; DiToro *et al.*, 1991; Burton, 1992). Consequently, plants grown in contaminated environments have much higher concentrations of pollutants in below-ground tissues than in foliage (Chapter 4; Lovett Doust *et al.*, 1994a). Different physico-chemical properties of sediments at different sites and history of their exposure determine concentrations of organic contaminants adsorbed by sediment (DiToro *et al.*, 1991; Burton, 1991). Macrophytes collected from different sites may contain different concentrations of contaminants within their tissues that reflect sediment and water contaminations at each location (Chapter 4; Lovett Doust *et al.*, 1994a).

In earlier greenhouse studies, ramets of *Vallisneria americana* were exposed to trichloroethylene (TCE) (Chapters 2 and 3; Biernacki *et al.*, 1995a,b), a common pollutant of the Great Lakes (Kaiser and Comba, 1986; Moore *et al.*, 1991). Exposure to TCE in the water column and sediment pore-water affected plant morphology, growth patterns and reproduction. A particularly responsive, and significantly associated with environment quality in the greenhouse study, was the ratio of leaf-to-root surface area in

Vallisneria ramets. The ratio increased with increasing TCE concentration in the water and sediment. The response of *Vallisneria* in terms of shift in the leaf-to-root surface area ratio was very rapid. Ramets achieved their final value of the ratio within a week of treatment (Chapter 3; Biernacki *et al.*, 1995b).

The objective of present study was to determine the spatial distribution pattern of leaf-to-root surface area ratio in *Vallisneria americana* collected in the Huron-Erie corridor, and whether the ratio, a useful indicator of contamination in greenhouse experiments, would also be a useful tool to monitor environmental quality in the field, where plants of different genotypes are exposed to mixtures of contaminants at various concentrations in the water and sediment and to various biotic and abiotic factors. The survey covered sites in Areas of Concern of the St. Clair and Detroit Rivers to include wide range of environmental qualities.

MATERIALS AND METHODS

The present survey of *Vallisneria americana* ramets from natural populations covered the area from the southern shores of Lake Huron, downstream to the western shores of Lake Erie (see insert Figure 6.1a). It included the St. Clair River (Figure 6.1a), Lake St. Clair (Figure 6.1b) and the Detroit River (and its tributary the Rouge River) (Figure 6.1c). A total of 243 sites were sampled once within a period of two weeks in the middle of August 1993. At each site 5 to 30 plants were collected. At each location, microsites that were at least 1 metre apart were sampled to increase the probability that different genotypes were being sampled (*Vallisneria* grows clonally) (Lokker *et al.*, 1994). All ramets were collected from depths of 0.8 to 1.2 metres so that they would have experienced similar light conditions. At each collecting site, a Secchi disc was used to assess water transparency and in all cases the disc was fully visible right down to the surface of the sediment. Plants were carefully removed from the substrate with a shovel; the sediment was carefully rinsed out of the roots, and only undamaged, fully grown

ramets were collected and stored. All plants were placed in labelled 1 L glass jars containing a 4% formalin solution. In pilot tests, it had been determined that storage of plants in the formalin solution did not ($p>0.05$) affect measurements of leaf and root dimensions or surface areas and shrinkage of leaves and roots was negligible. Each collecting site was marked on the map, and jars were labelled with date, site of collection, substrate type (gravel, sand, silt, clay, or organic), water depth and plant sex (if flowering occurred). Jars with ramets were stored in boxes at room temperature until they could be analysed in the laboratory.

At four of the sites (Chenal Ecarte, Peche Island, Rouge River, Turkey Island) (see Figures 6.1a and 6.1c), samples of *Vallisneria* were collected at monthly intervals over the growing season, from early June to late September 1993. These plants were growing at a depth of 1 m. In August 1993, further samples were collected at depths ranging from 0.5 m to 3.0 m. These ramets were collected to determine the influence of depth of growth, plant phenology, period of exposure, and flowering on the leaf-to-root surface area ratios. These samples were processed in the same manner as the survey samples.

To determine the effects of different growing seasons (year-to-year-variation), sediment types, and site of plant origin on changes in the leaf-to-root surface area ratio in *Vallisneria*, data from a separate long-term biomonitoring experiment were incorporated (Chapter 4; Lovett Doust *et al.*, 1994a). In this transplant-replant experiment ramets, originating from two sites (Turkey Island and Chenal Ecarte sites) were grown in each of the two sediment types (Turkey Island sediment or Chenal Ecarte sediment) and at each of the two sites in the Huron-Erie corridor (Turkey Island or Chenal Ecarte sites) over a five-year period.

All samples were analysed in the laboratory, with a minimum of five plants being analysed per site. Each plant was separated into leaves and roots. Before measurements, all plant parts were stored fully submersed in formalin solution to prevent shrinkage. Length and width of each leaf and length and diameter of each root were measured for each ramet using a digital micrometer (Mitutoyo Corporation, Tokyo, Model CD-6").

Root diameter was estimated as a mean of three measurements: at both ends and in the middle of each root. The fresh biomass (after preservation) of leaves and roots were also recorded for each plant. Approximately 2000 ramets of *Vallisneria* were measured. On the basis of these measurements, leaf and root projected surface areas for each plant were computed. The surface area of the ribbon-like leaves (Catling *et al.*, 1994) was calculated by multiplying leaf length by leaf width, and by 2 (to account for both sides of a leaf being exposed to the water column). The surface area of unbranched and fibrous roots (Catling *et al.*, 1994) was calculated by multiplying root length by π ($\text{pi} \approx 3.14$), and by root diameter. An index of leaf-to-root surface area was estimated for each site as arithmetic mean of the leaf-to-root surface area ratios for plants sampled at a particular site.

Statistical analyses

Data were analysed using statistical software SYSTAT for Windows ver. 5.03 (1992), through Spearman rank correlation coefficient analyses and ANOVA. Where appropriate, differences between means were tested for significance by Tukey HSD pairwise comparisons. Spatial pattern of distribution of index of leaf-to-root surface area ratio in *Vallisneria* ramets in present survey was compared to contaminant concentrations distribution patterns reported independently for sediments and biota in the Huron-Erie corridor to test for any correlation. Gobas *et al.* (1991) found a significant correlation between plant and biota contaminant concentrations on the lipid corrected basis, and described logistics of interpolation of contaminant levels between plant and other biota tissues (like fish or clams). A nonparametric Spearman correlation test was used to compare patterns of distribution of sediment or biota contamination rankings reported in independent studies and ranking of the ratio leaf-to-root surface area ratio found in the present study for matched sites. A nonparametric test was considered as the most appropriate because contaminant concentrations reported by different author were determine using different techniques, absolute contaminant concentrations could change since time of analysis, and direct interpolation of contaminant concentrations between

sediment, plants and other biota could also carried some errors. Detailed description of sites location and maps in published papers allowed us identify these sites in the field and if available, collect *Vallisneria* ramets. For the Spearman rank correlation coefficient analysis we correlated rankings of the leaf-to-root surface area ratio values found in present survey with the rankings of sites contamination determined independently on the basis of published data. Pugsley *et al.* (1985) reported sediment and clam (*Lampsilis radiata siliquoidea*) contamination by polychlorinated biphenyls (PCBs) and octachlorostyrene (OCS) for 102 sites in the Huron-Erie Corridor. Oliver and Pugsley (1986) reported sediment contamination with organochlorines; hexachloroethane (HCE), hexachlorobutadiene (HCBD), pentachlorobenzene (QCB), hexachlorobenzene (HCB), octachlorostyrene (OCS), perchloroethylene (PERC), trichloroethylene (TCE), carbon tetrachloride (CTC), dichlorobenzene (DSBs), trichlorobenzene (TCBs), tetrachlorobenzene (TeCBs), for 65 sites in the St. Clair River. Suns *et al.* (1993) collected over 20 years data on contamination with PCBs, DDT and mirex in Spottail Shiners (*Netropis hudsonius*) young-of-the-year for 6 sites in the Huron-Erie Corridor. Giesy *et al.* (1988) reported sediment toxicity data and result of three sediment toxicity assays (Microtox, *Daphnia magna*, and *Chironomus tentans*) for more then 100 sites in the Detroit River, including the Trenton Channel and Rouge River areas.

RESULTS

Spatial distribution of leaf-to-root surface area ratio

Populations of *Vallisneria* were less frequent and less extensive in highly polluted and disturbed areas. These areas were often adjacent to discharge pipes and industrial plants where access to the river banks was restricted. Figure 6.1a shows the values of leaf-to-root surface area ratio for *Vallisneria* ramets collected from different regions of the St. Clair River. Samples from Lake Huron and the vicinity of Sarnia Harbour had low ratios, ranging from 2 to 6. Ramets collected downstream of Sarnia had increased ratios,

up to 38. However, there was variation in leaf-to-root area ratio between sites. Sites south of Stag Island had ratios in the range of 20 to 38. The distribution pattern of the ratio values parallels pattern of contaminant levels in sediment and clams in the region reported by Pugsley *et al.* (1985) and Oliver and Pugsley (1986).

In Lake St. Clair (Figure 6.1b), the highest ratios were observed in samples from the delta of the St. Clair River. There was also an increase in the ratios near the mouths of larger creeks or rivers entering the lake (for example at the mouth of the Thames River, Ruscom River, Puce River, Pike Creek). Most of the populations in Lake St. Clair had low ratios, ranging from 4 to 7. All the populations surveyed were located around the perimeter of the lake because the survey was limited to sites comparable in depth (from 0.8 m to 1.2 m).

Figure 6.1c shows the values of leaf-to-root surface area ratios for *Vallisneria* further downstream in the Detroit River (including Rouge River). Samples collected from the Ontario shores never had ratios above 14, with the lowest values around Peche Island ranging from 4 to 7. Ratios for plants collected from the Michigan shores were generally much higher. The highest levels were recorded in the Rouge River and Zug Island area, and ranged from 25 to 92. The ratios were also very high in the area of the Trenton Channel, to the west of Grosse Ile, ranging from 22 to 74. The Rouge River and Trenton Channel areas had the highest ratio values observed in the entire Huron-Erie Corridor. The geographic distribution pattern of elevated leaf-to-root surface area ratios was similar to the pattern of increased sediment toxicity reported by Giesy *et al.* (1988), who used three separate biotic assays to determine the range and pattern of sediment toxicity in the lower Detroit River.

Seasonal patterns in leaf-to-root surface area ratio

At four sites (Rouge River, Peche Island, Turkey Island and Chenal Ecarte; see Figures 6.1a and 6.1c), the leaf-to-root surface area ratio was measured on five occasions, over the growing season. Figure 6.2 shows that the ratio increased significantly over the growing season, as plants matured, at three sites; Rouge River ($p < 0.001$), Chenal Ecarte

($p < 0.001$), and Turkey Island ($p < 0.01$). The rate of increase in ratio was different among sites, but the ranking of sites remained the same over the growing season. The greatest increase in the ratio over the growing season was observed at the Rouge River site, one of the most contaminated areas in the Detroit River (Giesy *et al.*, 1988).

Year-to-year patterns in leaf-to-root surface area ratio

The leaf-to-root surface area ratios of ramets collected at Chenal Ecarte and Turkey Island in the middle of September over four years from 1991 to 1994 did not change significantly ($p > 0.05$) over the study period (Figure 6.3). Higher ratio values in ramets from Chenal Ecarte were correlated with greater contaminant concentrations found in plants growing there than in ramets growing at Turkey Island site (see Chapters 4 and 5; Lovett Doust *et al.*, 1994a). Changes in the ratio and other measures in ramets collected in 1991 were significantly correlated with range of organochlorine contaminants measured in plant tissues (Table 6.3).

Sediment effects on changes in leaf-to-root surface area ratio

In the long-term experiment, sediment quality was one of the most important factors affecting plant growth and development. Significant differences ($p < 0.001$) in leaf-to-root surface area ratio in *Vallisneria* ramets were observed for plants grown in different sediments (from Turkey Island or Chenal Ecarte sites), but the same sites (exposed to the same water column). At both locations, the Turkey Island and the Chenal Ecarte sites plants planted in Chenal Ecarte sediment had significantly greater ratios compared to plants planted in Turkey Island sediment despite the fact that the plants were growing in the same water column (Figure 6.4). Chemical analyses showed that plants grown in Chenal Ecarte sediment had greater contaminant concentrations compare to ramets grown in Turkey Island sediment (Chapters 4 and 5; Lovett Doust *et al.*, 1994a). Significant differences between the leaf-to-root surface area ratio of ramets in each sediment type were observed over the four-year-long study period.

Ramets collected in present survey in the St. Clair and Detroit Rivers grown in silt

and clay substrates had significantly ($p<0.001$) greater ratio than those grown in gravel or sand. The ratio in plants collected in the Lake St. Clair (excluding delta of the St. Clair River) did not differ among substrate types ($p>0.05$).

Effects of plant origin on changes in leaf-to-root surface area ratio

Site of plant origin had significant effects on plant performance in the long-term experiment. Ramets collected from distinct populations of *Vallisneria* (Turkey Island and Chenal Ecarte), but planted in the same sediment type and at the same site, had similar leaf-to-root surface area ratios (Figure 6.5). Differences between the ratio values for plants from these two source populations were not significant ($p>0.05$) over the four year study period. However, all ramets had significantly ($p<0.001$) higher ratio values when grown at the Chenal Ecarte in the Chenal Ecarte sediment, compared to the ratios noted when plants were grown at the Turkey Island location and in the Turkey Island sediment.

Effects of depth of water column on changes in leaf-to-root surface area ratio

The ratio of leaf-to-root surface area increased significantly with increasing depth (from 0.5 to 1.0 m) for plants collected at the Rouge River site ($p<0.001$) and at the Chenal Ecarte ($p<0.01$) (Figure 6.6). However, there were no significant changes in ratios for plants collected at Peche Island and Turkey Island (as water depth increased from 0.5 to 3.0 m) and at the Chenal Ecarte as the depth increased from 1.0 m to 2.5 m. At the Rouge River site, *Vallisneria* ramets could not be found in depths greater than 1.0 m.

Comparison of male and female *Vallisneria* ramets

Flowering and nonflowering ramets sampled from the same site did not differ in leaf-to-root surface area ratio ($p>0.05$); neither did female and male flowering plants collected at the same site ($p>0.05$). However, flowering male ramets were very rare at sites where the leaf-to-root surface area ratio was higher than 35 (the Rouge River and Trenton Channel areas). Flowering female and vegetative (nonflowering) ramets together constituted nearly 100% of plants sampled at sites with leaf-to-root surface area ratio

higher than 40 (Figure 6.7). This difference was statistically significant ($p<0.001$).

Correlation of leaf-to-root surface area ratio with published data on site contamination

The results of Spearman rank correlation coefficient analysis of data for sites in the St. Clair River, Lake St. Clair and Detroit River are shown in Table 6.1. In all three regions of the Huron-Erie Corridor, the Spearman rank correlation coefficient between leaf-to-root surface area ratios in present survey and previous measures of contamination were highly significant for matched sites (Table 6.1). There is a significant correlation between the distribution of elevated leaf-to-root surface area ratios found in this survey and patterns of contamination reported by Pugsley *et al.* (1985, Table 6.1A), Oliver and Pugsley (1986, Table 6.1B), Suns *et al.* (1993, Table 6.1C), and Giesy *et al.* (1988, Table 6.1D). Thus, on the basis of Spearman rank correlation coefficient analysis, leaf-to-root surface area ratios of *Vallisneria* ramets agree with previous studies. The ratio can provide an accurate and independent measure of environmental quality. Correlation with other phenotypic traits varied. The biomass of sampled ramets ranged from less than 0.25 g to more than 35 g (wet mass) but there was no significant correlation between reported contamination level at a site and ramet biomass (Table 6.1). However, there was statistically significant rank correlation ($p<0.05$) between sediment or biota contamination level and ramet leaf-to-root mass ratios (fresh weight). There was a significant increase ($p < 0.01$) in leaf-to-root mass ratios in areas with higher leaf-to-root surface area ratios. Statistically significant increase ($p<0.05$) in leaf surface area per gram of leaf tissue and a significant decrease ($p<0.05$) in root surface area per gram of roots were observed in plants with elevated leaf-to-root surface area ratios.

The leaf-to-root surface area ratio of a ramet was significantly correlated with changes in such parameters as leaf-to-root mass ratio ($p<0.001$), number of roots per ramet ($p<0.001$), root diameter ($p<0.001$), number of leaves per ramet ($p<0.001$), surface area of a leaf ($p<0.001$), surface area of leaves per gram of leaf tissue ($p<0.001$), and

several other measures of plant growth, but there was no significant correlation with ramet biomass (Table 6.2).

DISCUSSION

Correlation of leaf-to-root surface area ratio with published contamination data

The present survey of *Vallisneria* ramets did not include all of the sites sampled by Pugsley *et al.* (1985), Oliver and Pugsley (1986), and Giesy *et al.* (1988) because some of their sites were too deep or there were no *Vallisneria* present. Access to some of the most highly polluted sites or river banks was restricted by rip-rap or other shoreline protection. Some of the waterways along the St. Clair River and Detroit River were dredged. The dredging resulted in diminished shallow, littoral areas suitable for aquatic macrophytes. As a result, it is possible that the most severely contaminated areas have not been sampled.

In the present study, changes in the leaf-to-root surface area ratios in *Vallisneria* were significantly correlated with independent measures of environmental contamination in the Huron-Erie Corridor. It is possible that contaminant levels changed over time, but a number of long term studies indicate that relative severity of site contamination have changed very little (Bignert *et al.*, 1993). Over a period of more than 20 years, Suns and others (1993) studied contamination patterns in the Great Lakes, including the Huron-Erie Corridor. They observed year-to-year fluctuations in contaminant concentrations in selected areas. However, with few exceptions, the ranking of sites with respect to contamination was constant over the study period. Suns *et al.* (1993) studied six of the sites that were sampled in this study, but did so over a 20-year period. Single survey of the leaf-to-root surface area ratios in *Vallisneria* ranked relative contamination of each site as well as independent measurements of contaminant concentrations reported over 20 year. Measured changes in the leaf-to-root surface area ratio of *Vallisneria* ramets reflect environmental quality in the field, just as it did in controlled, greenhouse study (Chapter

3; Biernacki *et al.*, 1995b). Specifically, roots of field grown *Vallisneria* were highly responsive to increased pollution in terms of reduced biomass, production of fewer roots per ramet, reduced root diameter, and reduced root surface area per gram of root tissue (Tables 6.1 and 6.2). Roots are the plant organs most strongly affected because hydrophobic organic contaminants are primarily absorbed from contaminated sediment and, since their mobility within the plant tissues is very limited (Guilizzoni, 1991), they tend to accumulate in roots (Chapter 4; Lovett Doust *et al.*, 1994a). Increased site contamination was also associated with an increased number of leaves per ramet, increased biomass allocation to leaf tissues, increased surface area per leaf, and an increase in the surface area of leaves per gram of leaf tissue (Table 6.2). In an earlier study, it was concluded that *Vallisneria* ramets allocated more biomass to leaf tissues exposed to the relatively less contaminated water than to roots surrounded by contaminated sediments (Chapters 2 and 3; Biernacki *et al.*, 1995a,b).

Geographic distribution of leaf-to-root surface area ratio values

The pattern of distribution of leaf-to-root surface area ratio in the St. Clair River (Figure 6.1a) was similar to contaminant distribution patterns reported by Pugsley *et al.* (1985) and Oliver and Pugsley (1986). The relatively less polluted shores of Lake Huron and the Sarnia Harbour area had plants with the lowest ratios. Sites on the St. Clair River, south of Sarnia, showed increased ratios. Contamination in this area by effluent and spills has been well documented (Edsall *et al.*, 1988; Oliver and Pugsley, 1986; Pugsley *et al.*, 1985). For some chemicals (e.g. TCE, PCE), sediments sampled in these area were the most contaminated of all collected in the Great Lakes and Canada (Moore *et al.*, 1991). Pugsley and others (1985) observed some variations in contaminant concentration at adjacent sites, which was attributed to differences in sediment composition and water movements. Sediments consisting of gravel and/or sand, had very low or non-detectable concentrations of contaminants (Pugsley *et al.*, 1985; Oliver and Pugsley, 1986). Therefore, biota inhabiting such substrates would be primarily exposed to water-column-borne contaminants. Measures of leaf-to-root surface area ratios reflected these

differences. *Vallisneria* ramets collected in present survey from sandy and/or gravel sediments had significantly lower leaf-to-root surface area ratios than plants that grew nearby but in different substrate (silt or clay).

In Lake St. Clair, leaf-to-root surface area ratios were greatest in the delta of the St. Clair River (Figure 6.1b). This area is known to be contaminated by organic compounds transported in the waters and sediments of the St. Clair River (Kaiser and Comba, 1986; Edsall *et al.*, 1988). The Canadian shores of the lake had plants with low ratios, suggesting diversion of the contaminant stream away from the eastern shores of Lake St. Clair (Kaiser and Comba, 1986; Edsall *et al.*, 1988), contaminant dilution, or contaminant removal from sediments by vegetation in the shallow littoral areas densely covered by aquatic macrophytes (Muir *et al.*, 1981).

In the Detroit River, the highest leaf-to-root surface area ratios were found along the Michigan shores (Figure 6.1c). The Rouge River, and the western shore of the Trenton Channel had the highest values. Earlier studies had found in these areas high concentrations of organic contaminants and heavy metals in the sediments (Giesy *et al.*, 1988; Baudo *et al.*, 1990). *Vallisneria* ramets collected from the field proved to be as useful for biomonitoring of sediment toxicity as other laboratory assays (Microtox, *Daphnia magna*, and *Chironomus tentans*) reported for this area (Giesy *et al.*, 1988). Survey of *Vallisneria* had advantages over other reported tests in that it did not require sediment sampling, processing, and additional laboratory testing.

In addition to contamination, a number of other factors may contribute to the shift in leaf and root surface areas that have been observed in *Vallisneria*. Maberly (1993) found that the surface area of leaves of *Potamogeton obtusifolius* increased with water depth because of changes in the quantity and quality of light available to the plant. Root surface area is responsive to sediment nutrient availability (Chambers and Kalff, 1987; Nicholson and Best, 1974) and sediment texture (Barko *et al.*, 1991). Both leaf and root surface areas are also affected by the concentration of inorganic carbon (Maberly, 1985), temperature (Moeller, 1980), pH of water and sediment (Titus, 1992), hydrostatic pressure of the water column (Titus and Stephens, 1983; Titus, 1983), plant density and

morphogenesis (Nicholson and Best, 1974), and current (Barko *et al.*, 1991). Variation in all of these factors is part of the natural heterogeneity of the environment, that could modify the leaf and root surface areas of *Vallisneria* ramets.

Effects of depth of water column on changes in leaf-to-root surface area ratio

Vallisneria occurs from 0.3 m to 7.0 m below water surface (Catling *et al.*, 1994). To reduce the effects of variation in water depth, pressure of the water column and differences in light flux, ramets were sampled from sites at similar depths (0.8 m to 1.2 m). The influence of depth on changes in leaf and root surface areas was assessed at four sites by sampling ramets from 0.5 m to 3.0 m deep water (Figure 6.6). Significant differences in the ratio between 0.5 m and 1 m depths at the Rouge River and Chenal Ecarte sites could be attributed to heterogeneity of substrate. At both locations gravel and recycled concrete were used for shoreline protection. Thus, plants growing in 0.5 m of water were rooted in a different substrate than at 1 m depth. The increase in leaf-to-root surface area ratio was negligible for plants grown at depths greater than 1 m at all sites.

Water temperature and incident light are generally lower at greater water depths. As a result, the half life of contaminants associated with sediments may be extended because of reduced bacterial decay, photolytic breakdown, and oxidation of contaminants (Guilizzoni, 1991; Moore *et al.*, 1991). Elevated levels of sediment-borne contaminants in greater depths may increase leaf-to-root surface area ratio in *Vallisneria* at deeper water.

Seasonal patterns in leaf-to-root surface area ratio

The statistically significant increase in the ratio at the more contaminated Chenal Ecarte and Rouge River sites (Figure 6.2) supports the finding that macrophytes continue to accumulate contaminants over the growing season, particularly in roots (Chapters 4, 2, and 3; Lovett Doust *et al.*, 1994a; Biernacki *et al.*, 1995a,b). In the long-term experiment, plant tissue contaminant concentrations were significantly correlated with different measures of root and leaf growth (Table 6.3). Contaminant effects on plant decreased as

compound octanol/water partition coefficient increased. Significant correlation between plant tissue contamination and the leaf-to-root surface area ratio was observed (see Chapters 4 and 5). The greater increase in the leaf-to-root surface area ratios at contaminated sites was probably associated with the steeper concentration gradient between plant and sediment for contaminants in more polluted sites. It is likely that in different years slope of increase in the ratio could be modified by weather conditions affecting rate of plant growth.

Year-to-year patterns in leaf-to-root surface area ratio

There were no significant changes in the ratio over a five year study period (1990-1994, Figure 6.3). This indicates that from the perspective of submersed macrophyte there was no significant change in environmental quality. Thus, routine monitoring using leaf-to-root surface area ratio need not to be carried out every year. The results do not allow to assume that *Vallisneria* responds to organic contaminants only. Present survey found a significant correlation between levels of organochlorine contaminants and changes in the ratio. It is very likely that other compounds like some metals and nutrients may also affect the ratio; however, these assumptions were not tested in the present study. The ratios have potential to measure site quality from the perspective of submersed macrophytes exposed at different sites to "cocktails" of contaminants present at various concentrations and also contaminant interactions with present nutrients, metals, or weather conditions. Chemical analysis alone or other derived methods (e.g QSAR) are not able to make such estimation including all possible interactions and from chemical concentrations predict effects on plants.

Sediment effects on changes in leaf-to-root surface area ratio

Several environmental factors such as water and sediment pH, water current, sediment texture, organic carbon and mineral composition were not measured and probably differed among sites. These factors are known to influence contaminant adsorption by sediments (Barko *et al.*, 1991; Moore *et al.*, 1991; Burton, 1991, 1992) and

Vallisneria growth (Barko *et al.*, 1991; Catling *et al.*, 1994), so they may contribute to overall site quality. In a greenhouse experiment (Chapter 3; Biernacki *et al.*, 1995b) it was determined that sediment composition may affect contaminant uptake by sediment, and subsequently bioavailability to plants. In laboratory study silica sand addition to sediment decreased TCE concentration in substrate and subsequently decreased leaf-to-root surface area ratio. In the field, it also was observed that tissue contamination was lower in plants grown in sediment with greater fraction of sand compared to sediments with increased fractions of silt and clay (Chapter 4; Lovett Doust *et al.*, 1994a). Organic carbon content in sediments was not determined; however, it is possible that a greater sand fraction in sediment diluted organic carbon content and fine particles content. Organic carbon and fine particles are known to be major factors that regulate adsorption of organic contaminants by sediments (Di Toro *et al.*, 1991; Burton, 1991, 1992).

Effects of site of plant origin on changes in leaf-to-root surface area ratio

Despite a capacity for extensive vegetative growth, populations of *Vallisneria* show significant genetic variability (Lokker *et al.*, 1994). It is possible that plants from different populations may respond to the same concentration and contaminant differently in terms of survival, growth and reproduction (Chapters 2, 4 and 5; Lovett Doust *et al.*, 1994a; Biernacki *et al.*, 1995a). However, isoenzymes study showed overlap between plants from natural populations at Chenal Ecarte and Turkey Island, transplanted ramets still reassembled plants at site of origin than plants at site they were transplanted to after five seasons. After five years of growth at the same site, plants from Chenal Ecarte and Turkey Island differed significantly in mean mass of ramets, leaf length, biomass allocation patterns, rate of flowering, rate of clonal growth, so despite overlap in isoenzymes they are likely to be genetically different. Transplanted and replanted ramets of *Vallisneria* originated from Turkey Island and the Chenal Ecarte, had similar ratios if grown at the same location and planted in the same sediment (Figure 6.5). This finding suggests that the leaf-to-root surface area ratio of a ramet is a general and reliable measure of environment quality, independent of plant genotype. Ramets of different

origin and of different phenotype ranked site quality in a similar way. It is possible that observed changes in leaf-to-root surface area ratios in *Vallisneria* in response to environmental contamination may represent general response of plants to environmental quality. There are reports of decrease in root mass and lengths of agricultural plants (Smith, 1991; Przymusinski and Gwozdz, 1994) and trees (Kodric, 1994) grown in contaminated soils.

Comparison of male and female *Vallisneria* ramets

The observation that, as the leaf-to-root surface area ratio increased, the fraction of flowering male ramets decreased (Figure 6.7) had not been anticipated. This suggests that female and male plants of *Vallisneria* may have different abilities to tolerate high levels of pollution, as measured by leaf-to-root surface area ratio, with females being tolerant, males susceptible. It cannot be distinguished whether male plants are unable to survive in highly contaminated areas, or if they are simply not able to flower, and persist, instead, as non-flowering (vegetative) plants.

Potential of *Vallisneria* as a biomonitor

Several previous studies have used changes in root length to assess plant growth as a measure of environmental quality and to detect toxic chemicals in aquatic and terrestrial systems (Byl and Klaine, 1991; Etzion and Neumann, 1993; Fiskesjo, 1993; Ryan *et al.*, 1993; Byl *et al.*, 1994; Przymusinski and Gwozdz, 1994). Short-term, seven day contaminant exposure studies found significant decrease in shoot and root lengths in aquatic macrophyte at lower contaminant concentrations before increase in measured enzyme activity occurred (Byl and Klaine, 1991; Byl *et al.*, 1994). For some contaminants, the greatest rate of change in root and shoot lengths was noticed at lower range of concentration, so changes in root and shoot lengths may have potential to monitor low contaminant concentrations. It was observed that increased contaminant concentration decreased not only root and leaf lengths but also leaf width and root diameter, leaf and root numbers and their masses in *Vallisneria* ramets (Table 6.3, see

also Biernacki *et al.*, 1995a,b). Measurement of leaf and root surface areas and estimation of leaf-to-root surface area ratio may be more sensitive and useful for monitoring changes in contaminant concentration than measurements of leaf or shoot and root lengths alone. In the long-term transplant-replant experiment, it was found that relative change in the leaf-to-root surface area ratio was 3 to 4 times greater than that of other measures of plant performance like rate of clonal growth, plant biomass, leaf number, or flowering in plants monitoring environmental quality at two different locations (Chapter 5). In laboratory studies, Devare and Bahadir (1994) concluded that the free-floating aquatic *Lemna minor* was more sensitive than terrestrial plants and cost efficient biomonitor. Number of studies suggest that submersed rooted macrophyte like *Vallisneria americana* are likely to be more sensitive than free-floating macrophytes or algae to lower concentrations of contaminants (Swanson *et al.*, 1991).

In the present survey, plants were sampled from natural populations (passive biomonitoring). However, active biomonitoring, with the use of genetically identical ramets, planted in reference and local test sediments could reduce variance in some factors that were not controlled for in the present study (Lovett Doust *et al.*, 1994b). The use of identical genotype at different locations and also over different years at the same location may increase precision and accuracy of environmental monitoring (Baird, 1992). Contaminant-tolerant but still responsive clones of *Vallisneria* could be particularly useful for biomonitoring in the most contaminated sites, where susceptible ramets would not survive. The use of standardized sediments in the field experiments would make possible to determine, separately, the effects of exposure to water-borne contaminants and sediment-borne contaminants (see chapters 4 and 5; Lovett Doust *et al.*, 1994a,b). Also, samples of sediment and water collected from the field could be tested using clones of *Vallisneria* in standardized, controlled laboratory environment, which would provide greater repeatability and precision of evaluation (Biernacki *et al.*, 1995a,b). Such a protocol would also allow testing of sediments and/or water collected from sites where *Vallisneria* does not grow naturally (for example; sediments collected from greater depths, beyond the limits of distribution of *Vallisneria*; groundwater samples, landfill

leachates, terrestrial soils). However, field tests are environmentally more relevant, because each site is unique and actual effect of pollutants on biota may vary with a particular combination of site characteristics. Field validated information is most useful for resource managers (Lovett Doust *et al.*, 1994a,b; Rowan and Rasmussen, 1992).

In conclusion, *Vallisneria americana* has great potential as a biomonitor of organic contamination. It is responsive to low contaminant concentrations found in the aquatic environment (Lovett Doust *et al.*, 1994a). A very simple estimation of leaf-to-root surface area ratios provides a convenient, localized and inexpensive measure of site quality, that incorporates the effects of both water and sediment quality. Higher ratios indicate higher levels of contamination, and these ratios can be compared between sites when managers are deciding how sites should be prioritized in terms of expenditures on remediation. The ratio can also be used to track and demonstrate incremental improvement in biotic aspects of site quality.

Table 6.1. Results of Spearman rank correlation coefficient analyses of selected morphological traits of *Vallisneria* at each site, and data on site-specific organochlorine contamination of biota or sediment, published for the Huron-Erie Corridor. Significance is indicated as: NS= no significant; *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$.

A. Correlation with levels of contamination in clams (*Lampsilis radiata siliquoidea*) and sediment, as reported by Pugsley *et al.*, 1985. N=48.

TRAIT	AREA		
	St. Clair River	Lake St. Clair	Detroit River
(No. of matched sites)	(19)	(20)	(9)
leaf-to-root surface area ratio	***	**	***
leaf-to-root mass ratio	**	*	**
mass of a ramet	NS	NS	NS
leaf surface area-to-leaf mass	*	NS	NS
root surface area-to-root mass	*	*	*
surface area of a leaf	NS	NS	NS

B. Correlation with levels of sediment contamination as reported by Oliver and Pugsley, 1986. N=24.

TRAIT	AREA
St. Clair River	
(No. of matched sites)	(24)
leaf-to-root surface area ratio	***
leaf-to-root mass ratio	*
mass of a ramet	NS
leaf surface area-to-leaf mass	*
root surface area-to-root mass	*
surface area of a leaf	NS

C. Correlation with levels of contamination in spottail shiners (*Netropis hudsonius*) as reported by Suns *et al.*, 1993. N=6.

TRAIT	AREA
Huron-Erie Corridor	
(No. of matched sites)	(6)
leaf-to-root surface area ratio	**
leaf-to-root mass ratio	*
mass of a ramet	NS
leaf surface area-to-leaf mass	NS
root surface area-to-root mass	NS
surface area of a leaf	NS

D. Correlation with levels of sediment toxicity as reported by Giesy *et al.*, 1988. N=28.

TRAIT	AREA
	Detroit River
(No. of matched sites)	(28)
leaf-to-root surface area ratio	***
leaf-to-root mass ratio	*
mass of a ramet	NS
leaf surface area-to-leaf mass	NS
root surface area-to-root mass	*
surface area of a leaf	NS

Table 6.2. Regression analyses of changes in leaf-to-root surface area ratio and correlation with other morphological parameters of *Vallisneria americana*. Analyses are based on mean values for ramets collected from 243 sites in the Huron-Erie Corridor (N=243). Significance of regression relationship is indicated as: *=p≤0.001; **=p≤0.01; *=p≤0.05; NS=no significant.**

VARIABLE	INTERCEPT	r^2	p
	OF REGRESSION		
Root diameter	-999.88	0.05	*
Leaf mass	1.23	0.03	*
Root mass	-21.60	0.06	**
Ramet biomass	1.05	0.03	NS
Surface area of a leaf	0.45	0.15	***
Surface area of a root	-27.53	0.08	**
Leaf-to-root mass ratio	0.19	0.27	***
Number of leaves	0.06	0.15	***
Number of roots	-0.51	0.09	***
Leaf-to-root number ratio	-0.03	0.05	*
Surface area of leaves per ramet	0.03	0.08	**
Surface area of roots per ramet	-0.19	0.04	*
Leaf surface area per leaf mass	0.06	0.11	***
Root surface area per root mass	0.04	0.04	*

Table 6.3. Results of Spearman rank correlation analysis of ranks of root and leaf measures in *Vallisneria americana* and ranks of organochlorine contamination in plant tissues collected from reciprocal transplant-replant experiment in September 1991. Only significant correlations are given as: *=p<0.001, **=p<0.01, *=p<0.05, NS=no significant.**

TRAIT	CONTAMINANT													
	QCB	HCB	OCS	Trans-non pp'DDE	Arochlor 1254:1260	PCB #28	PCB #52	PCB #66/95	PCB #101	PCB #99	PCB #87	PCB #110	PCB #118	PCB #153
Number of roots per ramet	***	***	***	***	***	***	***	***	***	***	***	***	***	**
Root diameter	**	***	***	***	***	***	***	***	***	***	***	NS	NS	*
Length of a root	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Surface area of a root	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Root mass	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Root surface area per ramet	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Number of leaves per ramet	*	*	*	*	*	*	*	*	*	*	*	*	*	NS
Length of a leaf	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Width of a leaf	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Surface area of a leaf	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Leaf surface area per ramet	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Biomass of a leaf	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Leaf surface area per leaf mass	***	***	***	***	***	***	***	***	***	***	***	***	***	NS
Root surface area per root mass	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Leaf-to-root biomass ratio	***	***	***	***	***	***	***	***	***	***	***	***	***	*
Leaf-to-root surface area ratio	***	***	***	***	***	***	***	***	***	***	***	***	***	*

St. Clair River

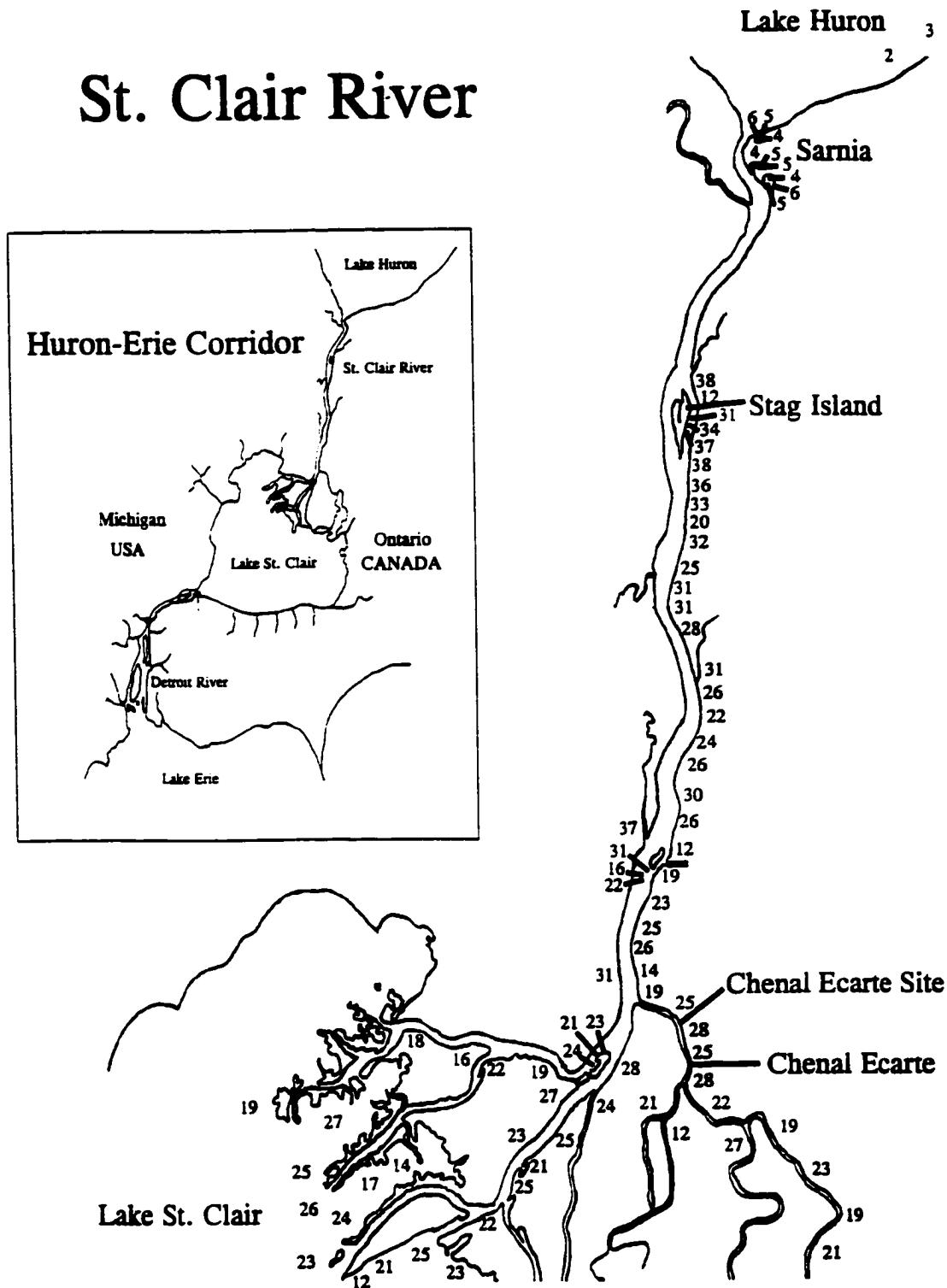


Figure 6.1a. Geographic distribution of leaf-to-root surface area ratio values in the St. Clair River.

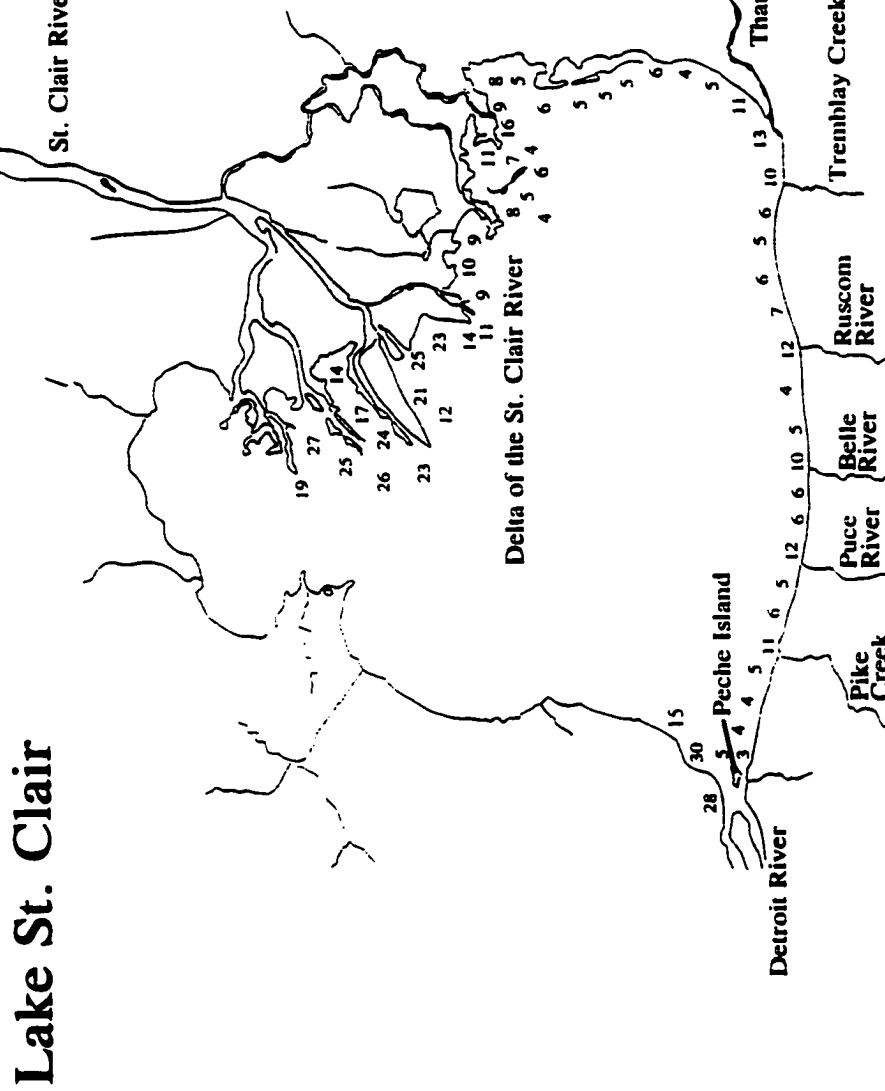


Figure 6.1b. Geographic distribution of leaf-to-root surface area ratio values in Lake St. Clair.

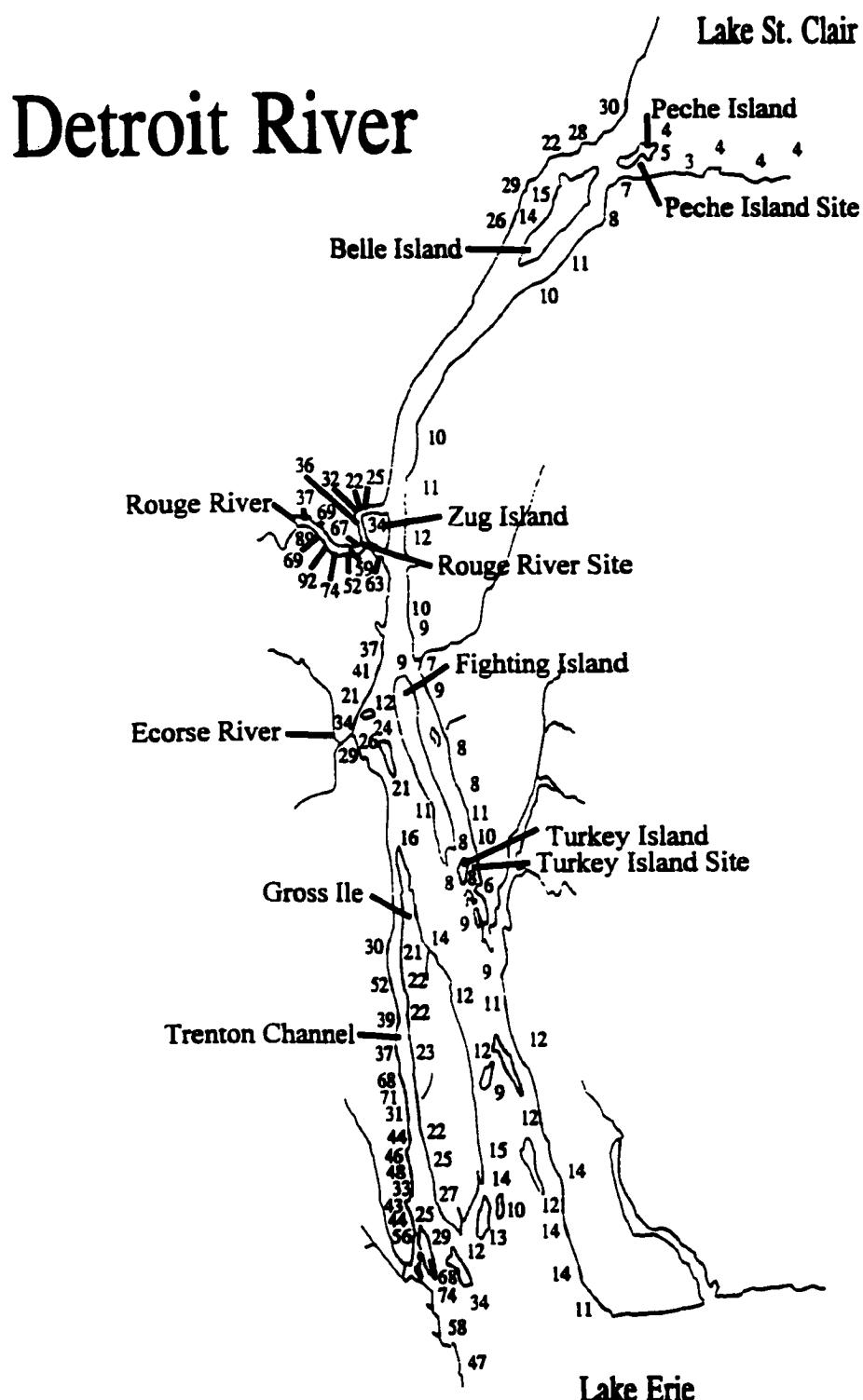


Figure 6.1c. Geographic distribution of leaf-to-root surface area ratio values in the Detroit River.

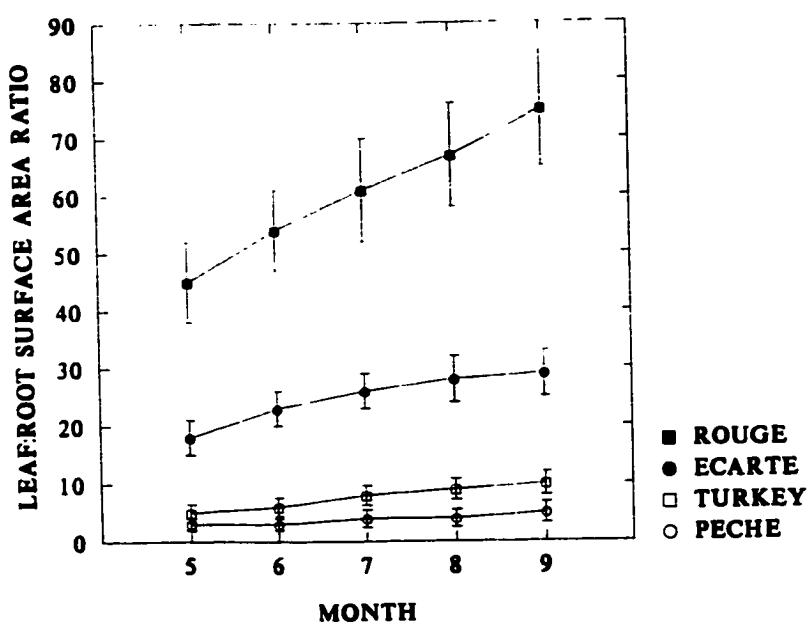


Figure 6.2. Changes in leaf-to-root surface area ratio over the 1993 growing season, at four sites.

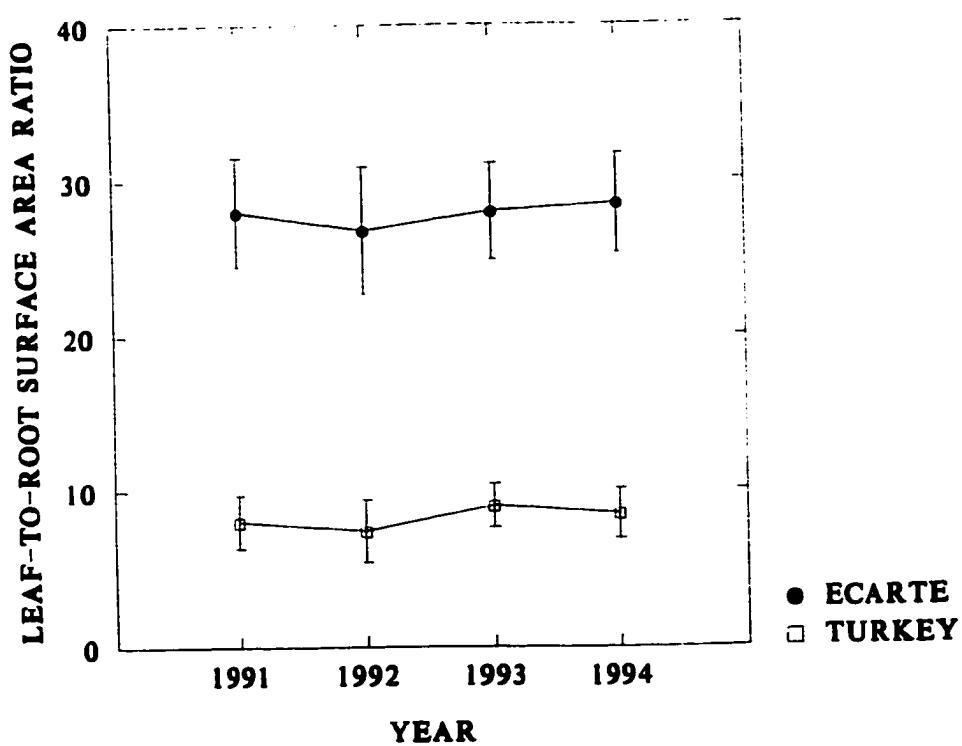


Figure 6.3. Changes in leaf-to-root surface area ratio over four growing seasons 1991-1994, at two sites (measurements taken in September).

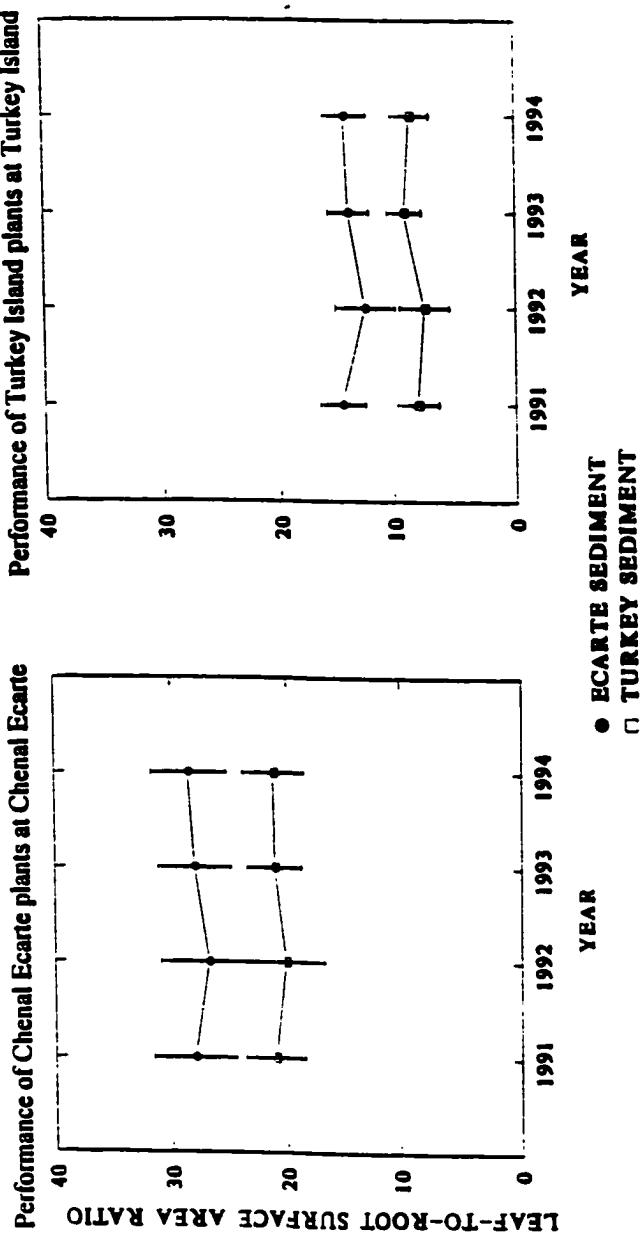


Figure 6.4. Changes in leaf-to-root surface area ratio in ramets from the Chenal Ecarte population, grown at the Chenal Ecarte site and in ramets from the Turkey Island population, grown at the Turkey Island site in sediments from Turkey Island or Chenal Ecarte over four growing seasons (measurements taken in September).

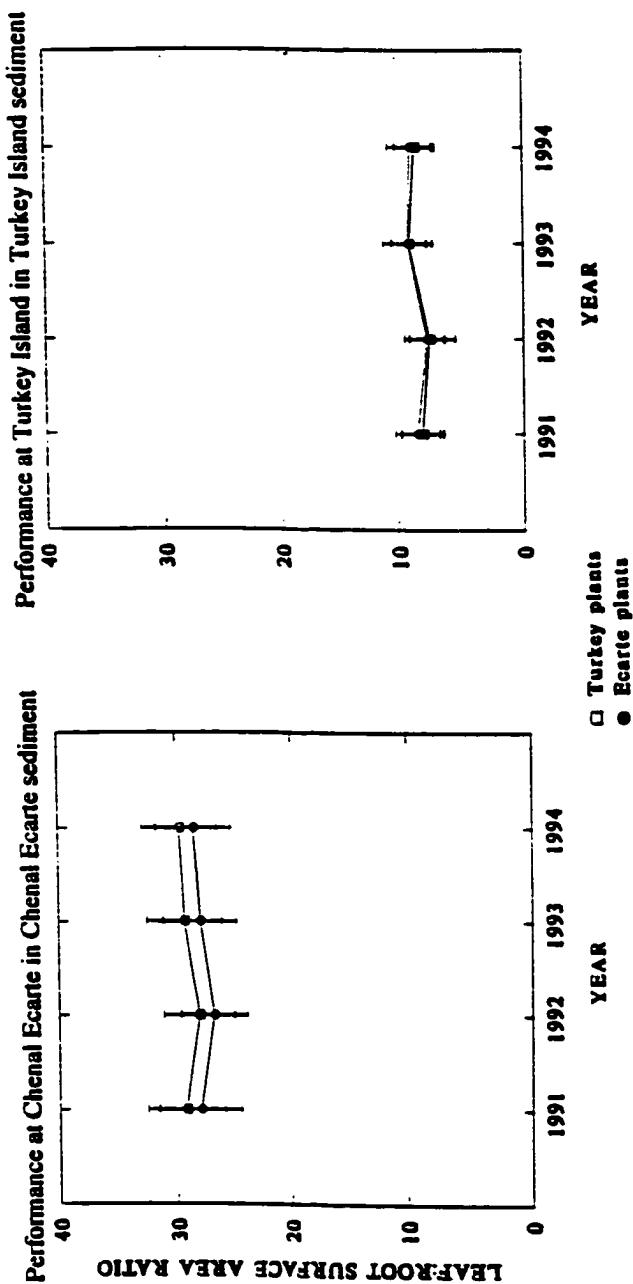


Figure 6.5. Leaf-to-root surface area ratio of ramets from the Chenal Ecarte and Turkey Island populations grown at the Chenal Ecarte site in Chenal Ecarte and Turkey Island site in Turkey Island sediment over four growing seasons (measurements taken in September).

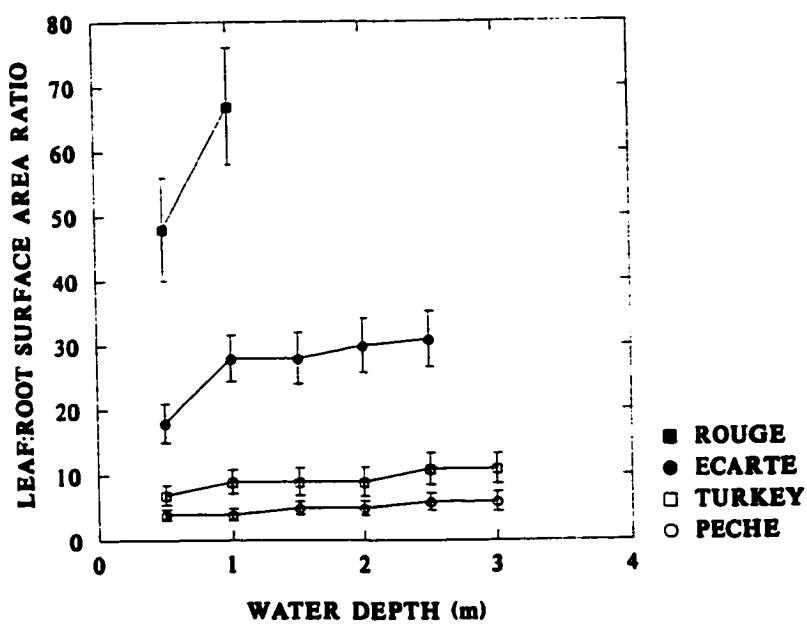


Figure 6.6. Changes in leaf-to-root surface area ratio with increased water depth, at four sites.

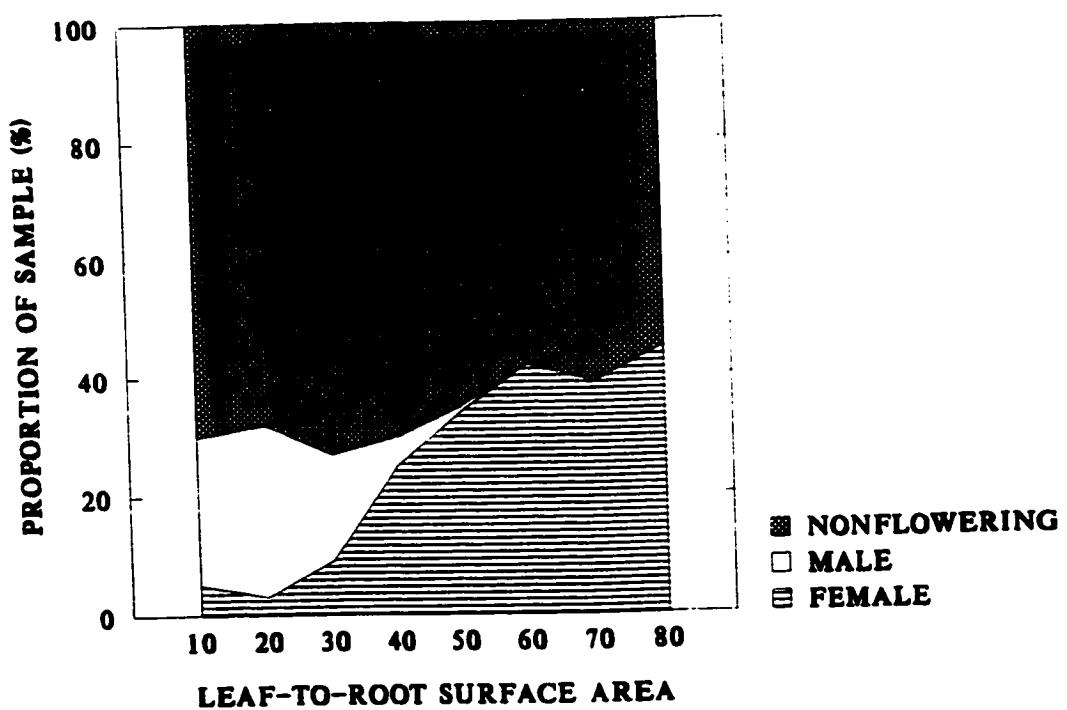


Figure 6.7. Proportion of flowering male and female ramets, and nonflowering ramets at sites having different leaf-to-root surface area ratio values.

Chapter 7

***Vallisneria americana* AS A BIOMONITOR OF AQUATIC ECOSYSTEMS: COMPARISON OF CLONED GENOTYPES¹**

ABSTRACT

The effects of local environmental quality on growth and development in six clones (genotypes) of *Vallisneria americana* replicated at five sites in the Huron-Erie Corridor were assessed. Ranks of environmental quality of sites were based on ranks of relative site contamination. Detrimental effects of local environment on plant performance (rate of clonal growth, leaf and root production, surface area of leaf and roots, plant biomass, rate of flowering and turion production) were correlated with local environmental quality and with plant genotype. At the Rouge River and Trenton Channel sites, detrimental effects of local environment were the greatest and at Peche Island and Turkey Island they were the least. All genets used in the study ranked environmental quality of five sites in the same order. Two genotypes which were tolerant to contaminants survived over the two-years of exposure at all sites, while others, not previously known to be contaminant tolerant died at the most contaminated sites within the first year of study. The use of cloned plants in biomonitoring studies can decrease variance, and increase precision and accuracy of assessment. Clones of *V. americana* that have evolved in local populations tolerant to contaminants have particular potential as inexpensive biomonitor.

¹ The major results of this chapter have been submitted for publication as follow:
Biernacki, M. and Lovett Doust, J. 1996. *Vallisneria americana* as a biomonitor of aquatic ecosystems: comparison of cloned genotypes.

INTRODUCTION

Submersed aquatic vascular plants are a natural and essential element of aquatic ecosystems, affecting biotic, physical, and chemical interactions. They provide oxygen, mineral nutrients, shade, substrate, shelter, breeding and nursing areas, and food for an array of organisms (see, e.g., Catling *et al.*, 1994). Macrophytes may also affect 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 which may alter sediment redox potentials, and local water and sediment pH. Plants are involved in nutrient cycling, and exchange between water and sediment, thus significantly affecting water and sediment quality (Barko *et al.*, 1991; Nichols 1991; Petticrew and Kalff 1992, Catling *et al.*, 1994).

One of the most common submersed macrophytes in the Huron-Erie Corridor of the Great Lakes is *Vallisneria americana* (Schloesser and Manny, 1990; Lovett Doust and LaPorte, 1991; Catling *et al.*, 1994). The species suffered a significant decline in abundance in the Detroit River and other areas (Schloesser and Manny, 1990; Manny and Kenaga, 1991); however, remaining 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 within a growing season (Catling *et al.*, 1994)

Submersed aquatic plants are capable of sequestering metals and organic chemicals and breakdown byproducts of, e.g., organochlorine pesticides, herbicides, PCBs, solvents, surfactants, and PAHs, from both the sediment and the water (Chapter 4; Crowder and Painter, 1991; Guilizzoni, 1991; Lovett Doust *et al.*, 1994a,b; Lewis, 1995). Changes in contaminant concentrations in plant tissues have been found to be significantly correlated with parameters of plant growth and development, and measures of plant performance have been used as a tool for inexpensive environmental monitoring (Chapters 2, 3, 4, 5, and 6; Lovett Doust *et al.*, 1994a,b; Biernacki *et al.*, 1995a,b, 1996).

The use of cloned, genetically identical individuals for biomonitoring may have many advantages. Baird (1992) and Lovett Doust *et al.*, (1994b) 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 a genetically mixed population. Forbes and Depledge (1992) and Guilizzoni (1991) expressed concern that selected clones may under-represent the range of responses of organisms within a population, particularly if the clones of experimental organisms used are more tolerant to contaminants than most organisms. In earlier field and greenhouse studies (Chapters 3 and 6; Biernacki *et al.*, 1995b, 1996) it has been determined that some individuals of *Vallisneria americana* could indeed tolerate significantly increased concentrations of contaminants. I studied clones of *Vallisneria americana* capable of surviving in increased concentrations of trichloroethylene (TCE), while most of the other individuals of *Vallisneria* did not survive over two growing seasons. Through selection at highly polluted areas, it is very likely that surviving local populations may evolve tolerance to contaminants. Furthermore, it is likely that use of such organisms could produce false sense of security in an observer, and significantly underestimate environmental degradation.

The present study was designed to estimate the utility of selected genotypes (i.e., those tolerant and those not tolerant to contaminants) in *V. americana* for biomonitoring and assessing environmental quality. Primary objective was to compare performance of selected genotypes at five experimental sites over two year 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 is well documented (Giesy *et al.*, 1988; Pugsley *et al.*, 1985; Manny and Kenaga,

1991). High levels of organic contaminants and 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 (IJC; 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 (Figure 7.1). The other four sites were located in the Detroit River; one on the southern shore of Peche Island, in 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, approximately 1 km upstream of Lake Erie; and a fourth in the delta of Rouge River (see Figure 7.1). At all locations, natural populations of *V. americana* var. *americana* were present.

Since summer 1991, selected genotypes of dioecious *V. americana* var. *americana* (see Catling *et al.*, 1994 for complete species description and Lowden, 1992 for taxonomy details) were cultivated in an area surrounding Turkey Island in the Detroit River (Figure 7.1). The following genotypes were used in the study:

1. A female plant originating from the Rouge River area, and found to be tolerant to increased contamination (Chapter 6; Biernacki *et al.*, 1996);
2. A male plant originating from Chenal Ecarte, and found to be tolerant to increased TCE concentrations (Chapter 2; Biernacki *et al.*, 1995b);
3. A female plant originating from Chenal Ecarte, and found to be tolerant to increased TCE concentrations (Chapter 2; Biernacki *et al.*, 1995b);
4. A randomly sampled female plant originating from Chenal Ecarte;
5. A randomly sampled male plant originating from Chenal Ecarte;
6. A randomly sampled male plant originating from Turkey Island.

In addition to field cultivation, selected genotypes of *V. americana* were grown in the greenhouse at the University of Windsor over three-year period to compare their performance in common sediment and environmental conditions. Plants were grown in aquaria filled with water, with no additional nutrients in the water or sediment. There were minimum three replicate aquaria for each genotype. Non-destructive data was collected in monthly intervals over growing cycle and destructive data once at the end of

each growing cycle. Plants grown in the greenhouse were able to complete two growing cycles per year.

Over two years (1991-1993), selected plants were grown clonally in the field and ramets were confined separately in large plastic tubs. In early August 1993, a series of ramets were collected from these tubs. Ramets were carefully removed from sediment and only 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, ramets were planted in plastic containers (36 cm W X 46 cm L X 12 cm H) filled with local sediment at one of the five locations.

Sediment was collected at each site and processed prior to planting. It was collected from a depth of 0.5 to 0.8 m and manually cleaned of any plants or plant parts. The cleaned sediment was placed in plastic tubs and stored in shallow water (0.6 m deep). *Vallisneria* was planted in the tubs after a minimum of two days sediment storage.

In August 1993, a total of 1500 *Vallisneria* ramets, 250 ramets of each of six genotypes, were deployed at five sites as follows. Plants were planted in 150 plastic tubs, initially with 10 ramets per tub. Thirty tubs were placed at each location. Each genet was planted in a separate tub. There were six genotypes planted at each site and five replicate tubs of each genotype. Tub were set on the sediment surface at a depth of 0.8 - 1.2 m, depending upon site. Plant leaves extended into the local water column and roots were growing in local sediment in the tubs. All tubs were permanently marked.

Over a two year period information was collected once a month throughout the growing season from May to October on plant survival, clonal growth (production of new shoots), leaf number per rame and leaf number per unit area. Information was collected individually for each replicate tub at each location. During flowering stage, information about the number of male and female flowers per shoot and per tub was recorded. Once a month, as information about plant performance was collected, water temperature at the surface of the sediment was measured.

In mid-September, 1994 and 1995, two tubs representing each plant genotype were harvested at each location. Plants were carefully removed from tubs and sediment

was gently washed away to minimize damage to roots, leaves and other plant parts. Plants were placed in water in plastic containers and stored, for a maximum of one day, in the coldroom at 6 °C before processing. Shoots from each tub were processed separately. The number of leaves and roots, their individual lengths, leaf width, root diameters and number of flowers were determined for each ramet. Biomass of leaf, root, stolon, and turion (overwintering organs) was determined to observe the pattern of biomass distribution for each experimental treatment at each location.

Coefficients of variation of mean plant measurements, using single-genotypes of *Vallisneria* were compared with parallel treatments from another study carried out in 1994 at the Turkey Island and Chenal Ecarte sites (see Chapters 4 and 5; Lovett Doust *et al.*, 1994a), but using initially a mixture of 10 different genotypes of *Vallisneria* planted per tub. Plants in both studies were exposed to the same sediment, local water column and research protocol. Significance of difference between coefficients of variation in the two studies (using either single-genotype, or multi-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 analysed with SYSTAT for Windows version 5.03 (1992), using ANOVA, and, where appropriate, differences between means were tested for significance by Tukey HSD pairwise comparison tests. In all ANOVA models, time was considered as a nesting factor. Spearman rank correlation analysis was used to relate the relative rank of contaminant concentration in plant tissues with the relative rank of plant performance in experimental treatments.

RESULTS

Selected genotypes of *Vallisneria americana* grown at greenhouse in common sediment type and the same environmental conditions differed significantly from each other at least in one out of eight basic measurements of plant growth or reproduction

observed over six growing cycles (Table 7.1). There were no significant differences in plant growth and reproduction between replicate aquaria of the same genotype.

Weather pattern did not differ significantly between the 1994 and 1995 growing seasons (Environment Canada, 1994, 1995). Also, water temperature measured at the end of July, August and September was not significantly different among the sites. However, there was a significant difference in water temperature between Chenal Ecarte and other sites at the end of June, and plants at Chenal Ecarte were phenologically one to two weeks behind other sites at the beginning of the growing season. At all experimental sites, Secchi disk was visible at the bottom, from 0.8 m to 1.2 metres.

Analysis of variance revealed significant effects of the main factors and their interaction on plant performance (Table 7.2). Plant phenology (described as a monthly effect), site of plant growth, plant genotype, and interaction of site and plant genotype all significantly affected plant growth and development. Performance of the three 'tolerant' genotypes was significantly different from the three 'susceptible' clones ($p<0.001$). Female plants did significantly better than males ($p<0.01$), however power of this comparison is low due to fact that two out of three 'tolerant' clones were females, and only three genotypes of each sex were tested.

The number of ramets produced per m^2 was significantly greater ($p<0.001$) for all genets at Peche and Turkey Island sites, and least at Trenton Channel and Rouge River sites (Figure 7.2). Genotype #1 had significantly greater densities of ramets per m^2 at Trenton Channel and Rouge River than any other genotype at these sites. Genets #4 and #5 did not survive the first growing season at Trenton Channel; nor did genets #4, #5, and #6 at the Rouge River site. All replicates of genet #2 died at the Rouge River site in the second season.

There were significant differences in the rate of clonal growth (i.e., number of new ramets produced by parent rosettes over a growing season) between different sites; plants grown at Peche and Turkey Island sites had the greatest rates of clonal growth and at Trenton Channel and Rouge River, the lowest (Figure 7.3). The rate of clonal growth was greatest for genet #1 at all sites and least for genet #6. However, while there was

variation in the rate of clonal growth within growing seasons, there were no significant differences between the two years.

A similar pattern, as for ramet density, was observed for the number of leaves per m² at each site (Figure 7.4). Plants growing at Peche and Turkey Island sites had significantly ($p<0.001$) more leaves per m² than plants grown for two years at the Trenton Channel and Rouge River sites. Chenal Ecarte site was intermediate. Genet #1 had nearly three times as much leaves per m² as any other genet at the same location. The number of leaves per ramet differed among the sites; plants had fewest leaves per ramet at Peche and Turkey Island sites and most at Trenton Channel and Rouge River sites, over two years (Figure 7.5). Genet #1 had nearly twice the number of leaves per ramet than any other genotype.

Both, different growing seasons and plant phenology (i.e., “monthly” differences) had significant effects upon flower production in *Vallisneria* (Table 7.2). At Chenal Ecarte, Trenton Channel, and Rouge River flowering had decreased compared to the Peche and Turkey Island sites, where plants of both sexes flowered each year. At the Rouge River site, only female genet #1 produced flowers over two growing seasons.

Genet #1 growing at Peche and Turkey Island sites achieved the greatest mean biomass per m². Other clones also reached their maximum biomass at these two sites over two years of study (Figure 7.6). At the Trenton Channel and Rouge River sites biomass per m² of surviving genets was lowest. Despite this, genet #1 was still able to produce as much biomass per m² as other genotypes did at Peche Island site. There were significant differences in fresh mass of individual ramets at each location (Figure 7.7). At each site, the biomass of ramets of genet #1 was greatest. Two patterns in ramet biomass were evident. Ramets of genets #2 to #6 were of greatest biomass at Peche and Turkey Island sites and least at Trenton Channel and Rouge River; and in contrast ramets of genet #1 had greatest biomass at Trenton Channel and Rouge River sites and lowest at Peche and Turkey Island sites.

There were significant differences in patterns of biomass allocation in ramets among experimental sites (Figure 7.8). The pattern did not differ between the two years

of study and nor did proportional allocation to leaf and root differ significantly between genotypes at each site. Also, there were no significant differences between ramets grown at Peche and Turkey Island sites or between Trenton Channel and Rouge River grown plants. However, it was significantly different between ramets grown at Trenton Channel and Rouge River sites compare to ramets grown at other sites. Pattern of biomass allocation was marginally different between Chenal Ecarte site compared to Peche and Turkey Island sites. At the Rouge River site, plants had up to 80 times as much biomass in leaf compared to root (per ramet), whereas at the Peche and Turkey Island sites this ratio did not exceed four.

The number of turions per m² was greatest at Peche and Turkey Island sites least at Trenton Channel and Rouge River, and at Chenal Ecarte site intermediate ($p<0.001$, see Figure 7.9). At Trenton Channel and Rouge River sites, the density of turions never rose above 500 in any genet over either season, while at the Turkey Island site the density exceeded 1000 and at the Peche Island site there were 1500 turions per m² in both seasons. Turion production by genet #1 was significantly greater at Chenal Ecarte, Trenton Channel and Rouge River than that of other genets at these sites.

The leaf-to-root surface area ratio was significantly different among experimental sites ($p<0.001$), with lowest values found at Peche Island and greatest values at Rouge River (Figure 7.11). There was some variation in the ratios 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 found at each location did not differ significantly between both growing seasons. Leaf-to-root surface area ratio was the only plant trait of all those studied which was not significantly affected by differences among genotypes (Table 7.2).

There was a significant difference in the coefficient of variation for plant performance between single-genotype and multi-genotype test designs ($p<0.001$; Kruskal-Wallis test). Coefficient of variation ranged from five to ten times greater in multi-genotype experimental treatments than in the single-genotype tests.

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* (Table 7.1). Thus, it is assumed, that these differences indicate that plants may indeed be genetically different from each other. Lokker *et al.* (1994) used cellulose acetate gel electrophoresis technique to characterize the allozyme phenotypes in *Vallisneria*. She observed high genetic diversity in this clonal aquatic plant sampled in the Huron-Erie corridor.

Most biomonitoring protocols assume that experimental species form a homogenous and uniform category (Solomon *et al.*, 1996). This approach totally ignores the fact that individuals used in such tests may be genetically distinct and thus the reaction to a particular contaminant dose or concentration may vary significantly among individuals. Results of the present study indicate that the variation of measures of *Vallisneria* performance may be five to ten times greater in an experiment using mixtures of genotypes compared with study using standardized plant genotypes. Most natural populations have a unique history of exposure to toxic metals and organic contaminants as a result local selection pressures due to such contamination will strongly affect the genotypes of the surviving biota.

Some researchers have recognized that mixed cultures of experimental organisms kept in the laboratory tend toward monoculture over time (Baird *et al.*, 1991; Baird, 1992). Others have pointed out that only the use of mixtures of individual genotypes may truly represent genetic diversity extant in nature (Forbes and Depledge, 1992). However, there are some 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 strong bias in genotype composition, and of course may not represent the array of genotypes from others sites. In particular, due to physical limitations, laboratory studies use small numbers of individuals in an experimental design and this significantly limits the range of genetic variation studied. In practice, the number

of individuals decreases as the size of experimental organisms increases. Often it is assumed in ecotoxicological studies that less polluted or relatively pristine sites will be reflected in high genetic diversity, but this has rarely been confirmed experimentally (see Forbes and Depledge, 1992). Some studies have shown that individuals highly sensitive to one contaminant are not necessarily the most sensitive to other contaminants (Baird *et al.*, 1991). Based on this logic, it is worth pointing out the danger of using organisms assumed to be tolerant to contaminants, because tests using such tolerant individuals may underestimate the extent of environmental deterioration and detrimental effects to biota (Guilizzoni, 1991; Forbes and Depledge, 1992). However, in highly polluted areas locally evolved contaminant-tolerant biota may form the majority or even total of all of the genotypes present there (e.g. see Antonovics *et al.*, 1971), and "the most sensitive" genotypes are unlikely to be present. 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 suggests that degradation and contamination of experimental sites differed among sites (Giesy *et al.*, 1988; Pugsley *et al.*, 1985; Edsall *et al.*, 1988; Manny *et al.*, 1988; Manny and Kenaga, 1991; Biernacki *et al.*, 1996). On the basis of literature documentation, the relative ranking of sites by contamination level was the same as inferred from results of the present study based on the relative performance of *Vallisneria* genets. Peche Island site was the cleanest site relative to others, followed by Turkey Island and Chenal Ecarte sites. Trenton Channel and Rouge River sites were relatively most impaired and in others studies were found to be the most contaminated (Giesy *et al.*, 1988) (this order was used in all figures). The pattern of response differed somewhat between tolerant plants. However, on the basis of most measures of plant performance site ranking was the same. Genets #2 and #3 had decreased biomass of individual ramets and turions at the most polluted sites while genet #1 had greatest biomass of ramets and turions when growing at the most contaminated Rouge River site (Figures 7.7 and 7.10). Ramets found at natural populations in the Rouge River had greatest biomass (up to 35 g fresh mass) compared with any other highly contaminated area in Ontario, Canada, that

were surveyed.

“Tolerant” genets differed in the degree of their tolerance to contaminants. A genet “tolerant” to TCE (genet #2) did not survive in the field at the Rouge River site although genets #1 and #3 did. Tolerant genets proved useful for biomonitoring, and at some sites even more useful than more sensitive “susceptible” genotypes, because they were able to survive in the most polluted sites and measure relative impairment, where other, more susceptible genets, did not survive and ranking of impairment was not possible.

Results of study using selected genetic individuals allows one to precisely measure plant responses at each location as well as determining whether genotypes were able to survive at a location. In the present study, the density of ramets (Figure 7.2), the rate of clonal growth (Figure 7.3), density of leaves per unit area (Figure 7.4), biomass of ramets per unit area (Figure 7.6), and density of turions produced per unit area (Figure 7.9) were all excellent predictors of relative site quality.

One of the most useful parameters of plant performance was the leaf-to-root surface area ratio (Figure 7.11, Table 7.2). It was highly responsive to site quality and independent of plant genotype. The leaf-to-root surface area ratio did not differ between growing seasons, and nor did the ratio in the present study differ from that found in 1993 survey of *Vallisneria* ramets from natural populations including the same sites as the present study (Chapter 6; Biernacki *et al.*, 1996). Thus, ramets grown in tubs did not differ in their exposure to environmental factors compared with naturally grown ramets at the study sites.

In natural populations, individuals are exposed to continuously varying selection pressures and this determines a dynamic balance in population genetic structure. Hence, a focus upon individuals is more likely to reveal true patterns of response and environmental quality than using uncontrolled, randomly assembled mixtures of individuals. Plants (but also other organisms, such as *Daphnia*) can grow vegetatively by means of clonal growth, producing many replicate copies of the same genotype. Macrophytes are relatively easy to cultivate. Through clonal growth, *Vallisneria* can

produce a sufficient number of genetically identical ramets for large field or laboratory investigation. Tolerant genotypes of *Vallisneria* may survive in the most contaminated sites and allow for properly replicated experimental designs.

In summary, it is concluded that the use of selected genotypes of *Vallisneria americana* in biomonitoring of environmental quality may increase precision, accuracy and reliability of assessment of relative site quality. Tolerant genotypes were very useful in the most highly contaminated sites, where no susceptible genotypes could survive. In particular, the measurement of leaf-to-root surface area ratios in *Vallisneria* has great potential for tracking changes in environmental quality.

Table 7.1. Summary of measures of growth and reproduction in different genotypes of *Vallisneria americana* grown in common sediment in aquaria (31 cm W x 91.5 cm L x 61 cm H) over six growing cycles in years 1992-1995 at the greenhouse of the Department of Biological Sciences of the University of Windsor. Mean values (standard error) of selected measures of plant performance are shown.

TRAIT	GENOTYPE					
	#1	#2	#3	#4	#5	#6
Plant gender	Pistillate	Staminate	Pistillate	Pistillate	Staminate	Staminate
	Rouge River	Ecarte	Ecarte	Ecarte	Ecarte	Turkey Island
Site of plant origin	Yes	Yes	Yes	No	No	No
Tolerance to TCE	6.2 (1.6)	1.6 (0.3)	3.4 (0.6)	1.4 (0.5)	2.9 (0.6)	3.5 (0.8)
Rate of clonal growth	9.3 (3.1)	2.1 (0.4)	1.9 (0.5)	2.9 (0.7)	3.1 (0.6)	2.2 (0.5)
Number of leaves per ramet	18.4 (4.4)	12.4 (2.1)	11.3 (2.2)	13.6 (3.4)	15.1 (2.9)	16.6 (3.6)
Number of flowers per ramet	84 (26)	47 (14)	61 (12)	36 (12)	29 (14)	51 (19)
Number of roots per ramet	9.9 (1.2)	1.9 (0.5)	3.2 (0.6)	2.4 (0.7)	2.3 (0.6)	4.7 (1.3)
Biomass per ramet [g]	5.7 (2.1)	2.3 (0.3)	1.7 (0.2)	2.7 (0.5)	3.3 (0.6)	2.2 (0.4)
Number of turions per ramet	67 (36)	27 (14)	41 (19)	38 (17)	34 (19)	62 (28)
Length of a leaf [cm]	1.69 (0.31)	0.29 (0.05)	0.32 (0.07)	0.19 (0.03)	0.21 (0.04)	0.82 (0.12)
Biomass of a turion [g]						

Table 7.2. Summary of results of ANOVA for measures of growth and reproduction in different genotypes of *Vallisneria americana*. Only factors that were significant are included [Month(Y)=month nested in Year; Site(T)=site of growth nested in month and year]. Significance is indicated as follow: ***= $p \leq 0.001$; **= $p \leq 0.01$; * = $p \leq 0.05$; NS= not significant.

TRAIT	FACTORS				
	Year (Y)	Month(Y) (T)	Site(T) (S)	Plant Genotype (G)	SxG
Non-destructive monitoring:					
Number of ramets per m ²	NS	***	***	***	***
Number of leaves per m ²	NS	***	***	***	***
Number of leaves per ramet	NS	***	***	***	***
Number of flowers per m ²	**	***	***	***	***
Number of flowers per ramet	*	***	***	**	***
Rate of clonal growth	NS	***	***	***	***
Destructive monitoring:					
Number of turions per m ²	NS		***	***	***
Biomass of ramets per m ²	NS		***	***	***
Number of roots per ramet	NS		***	***	***
Biomass per ramet	NS		***	***	***
Number of turions per ramet	NS		***	***	***
Biomass of turions per ramet	NS		***	***	***
Turion-to-ramet biomass ratio	NS		***	***	***
Length of a leaf	NS		***	***	***
Biomass of a leaf	NS		***	***	***
Biomass of a turion	NS		***	***	***
Leaf length-to-leaf biomass ratio	NS		***	***	***
Leaf-to-root biomass ratio	NS		***	***	***
Fraction of germinated turions	NS		**	**	***
Leaf-to-root surface area ratio	NS		***	NS	NS

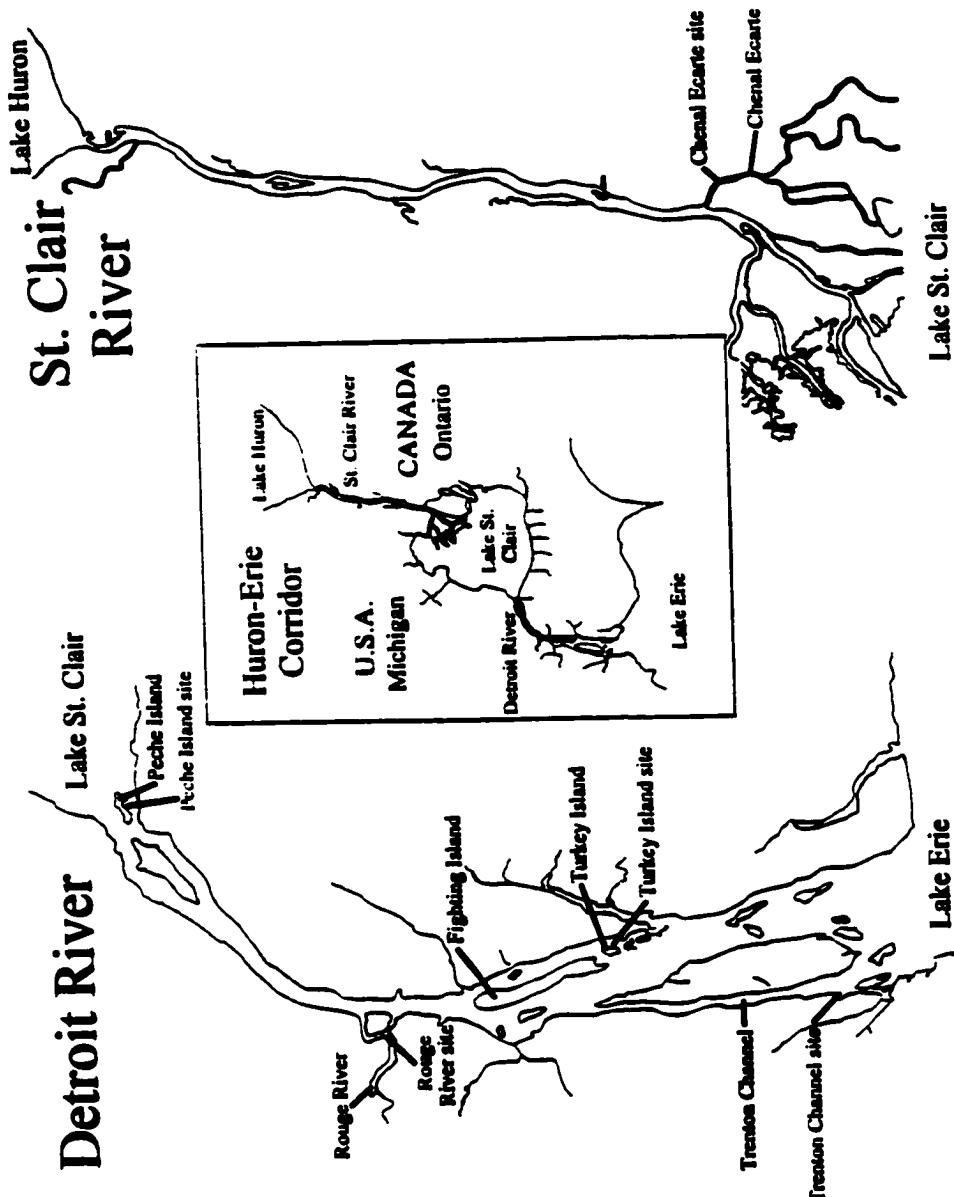


Figure 7.1. Map of Huron-Erie corridor with location of experimental site.

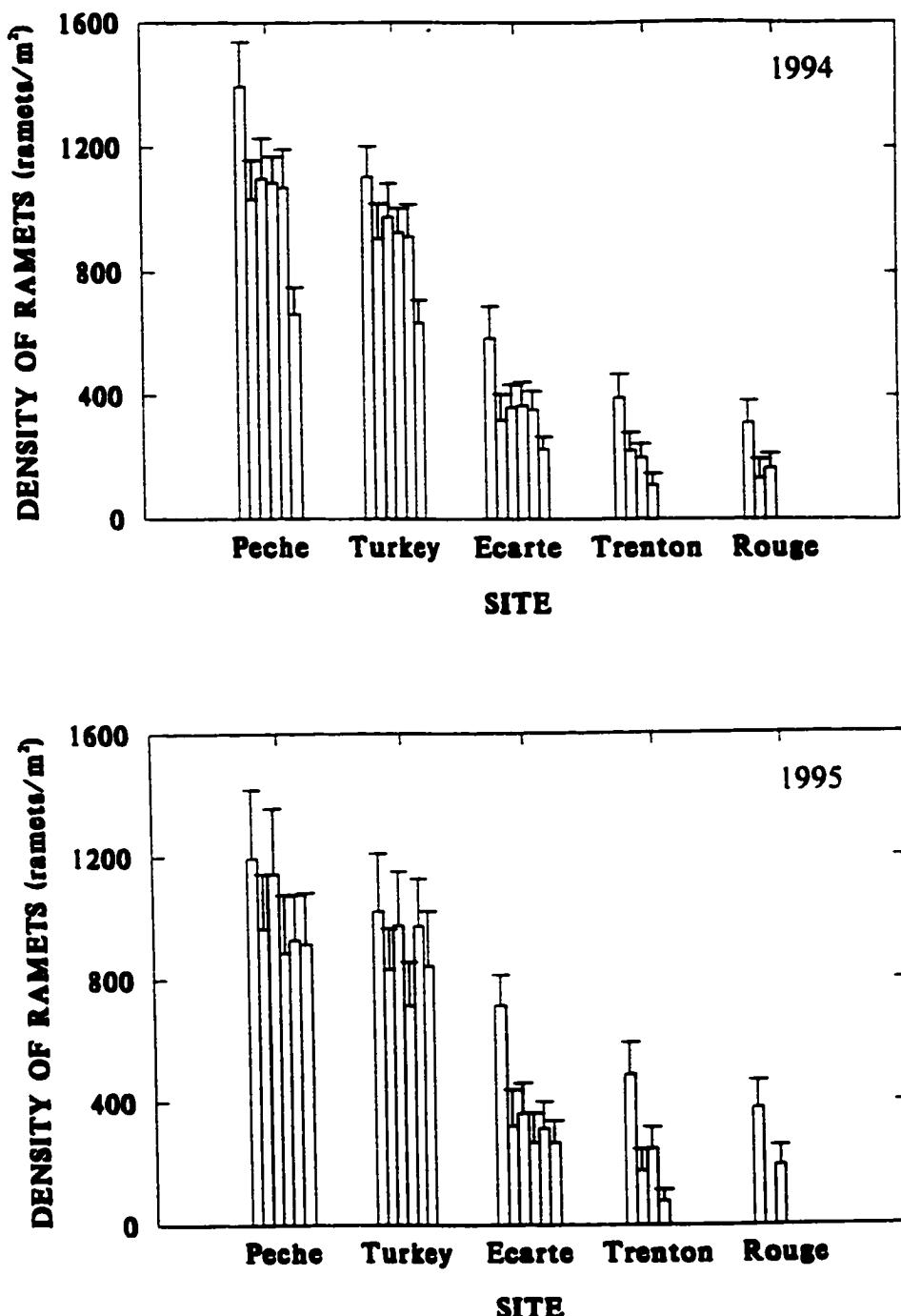


Figure 7.2. Mean density (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

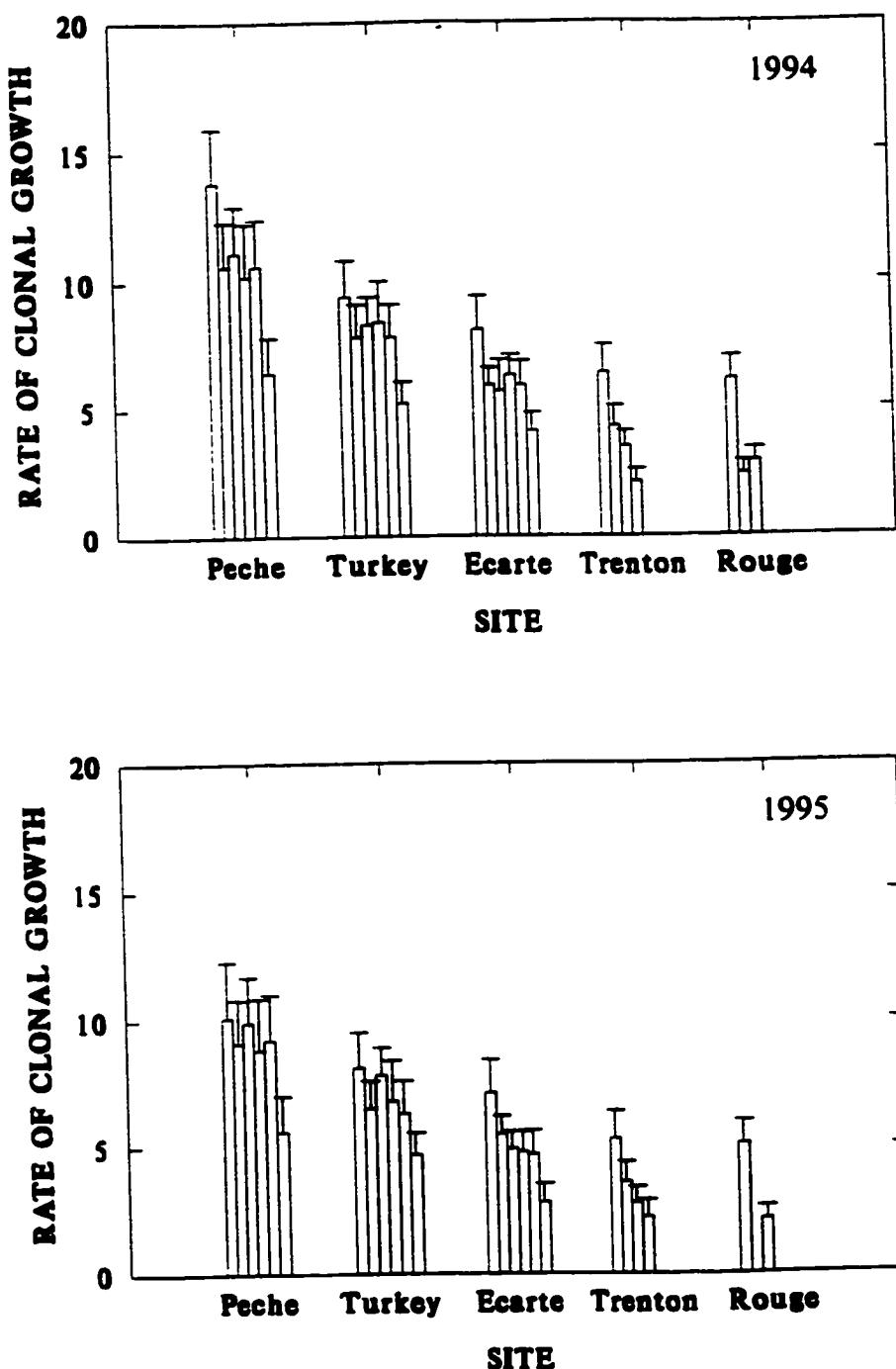


Figure 7.3. Mean rate of clonal growth (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

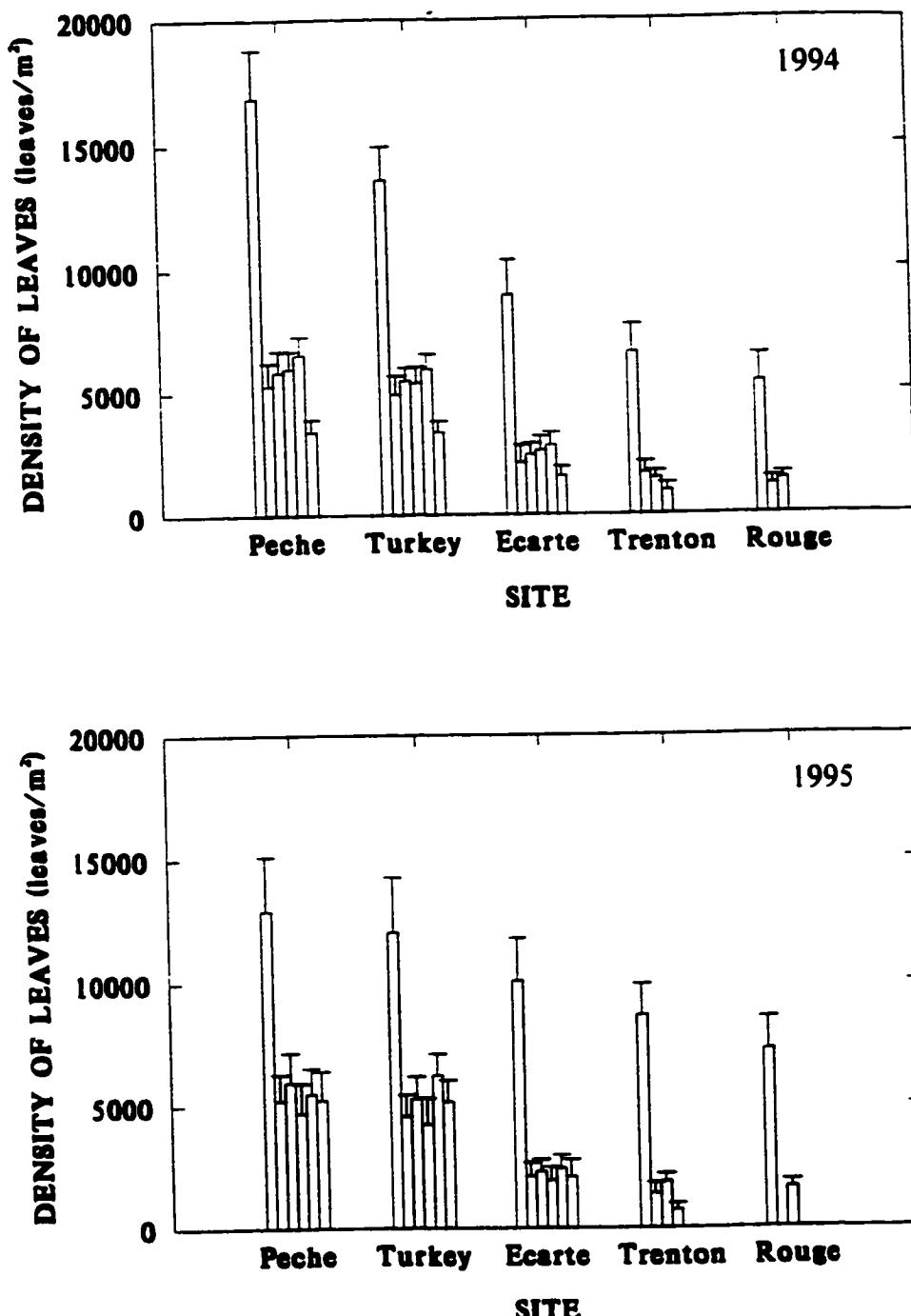


Figure 7.4. Mean density of leaves (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

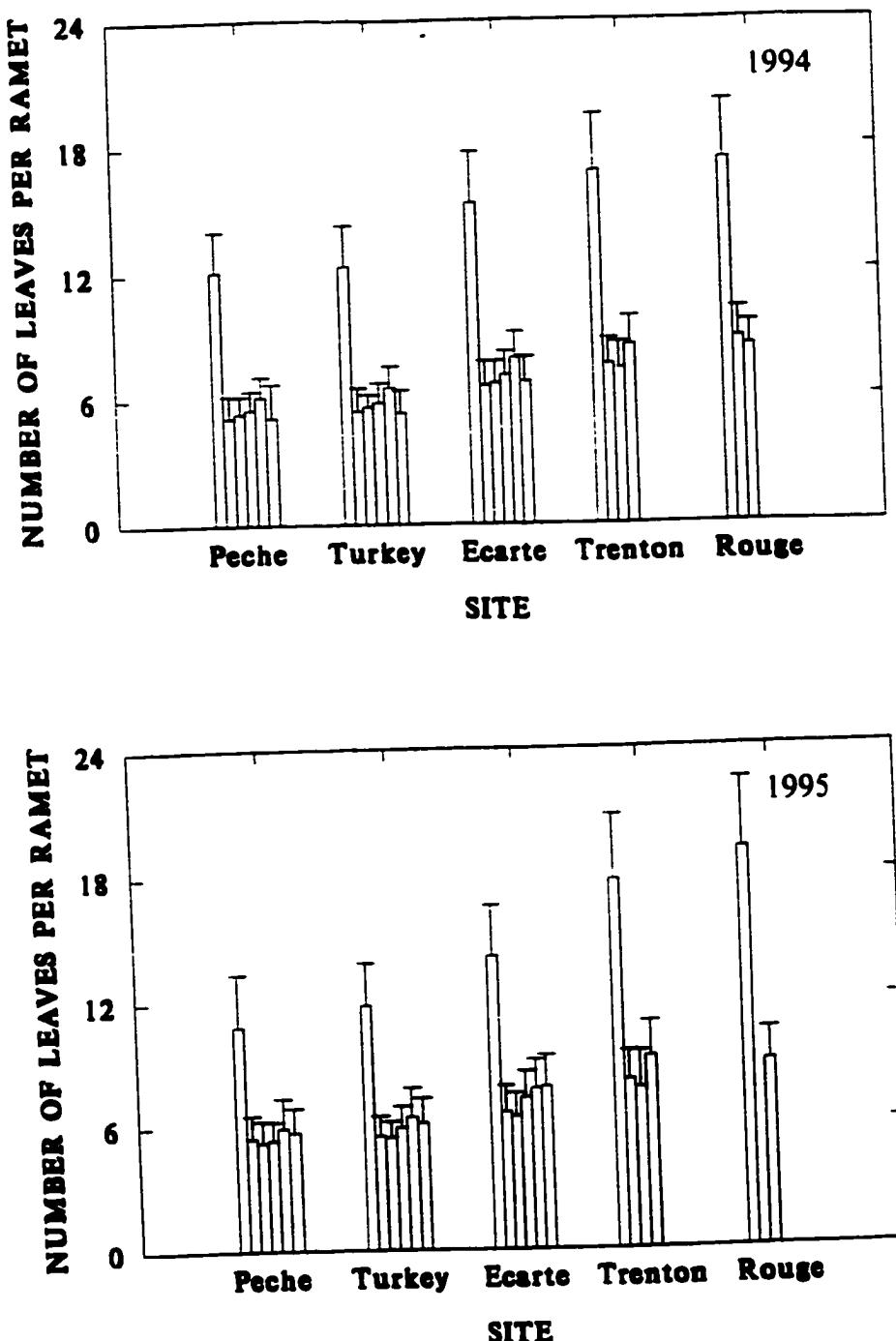


Figure 7.5. Mean number of leaves per ramet (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

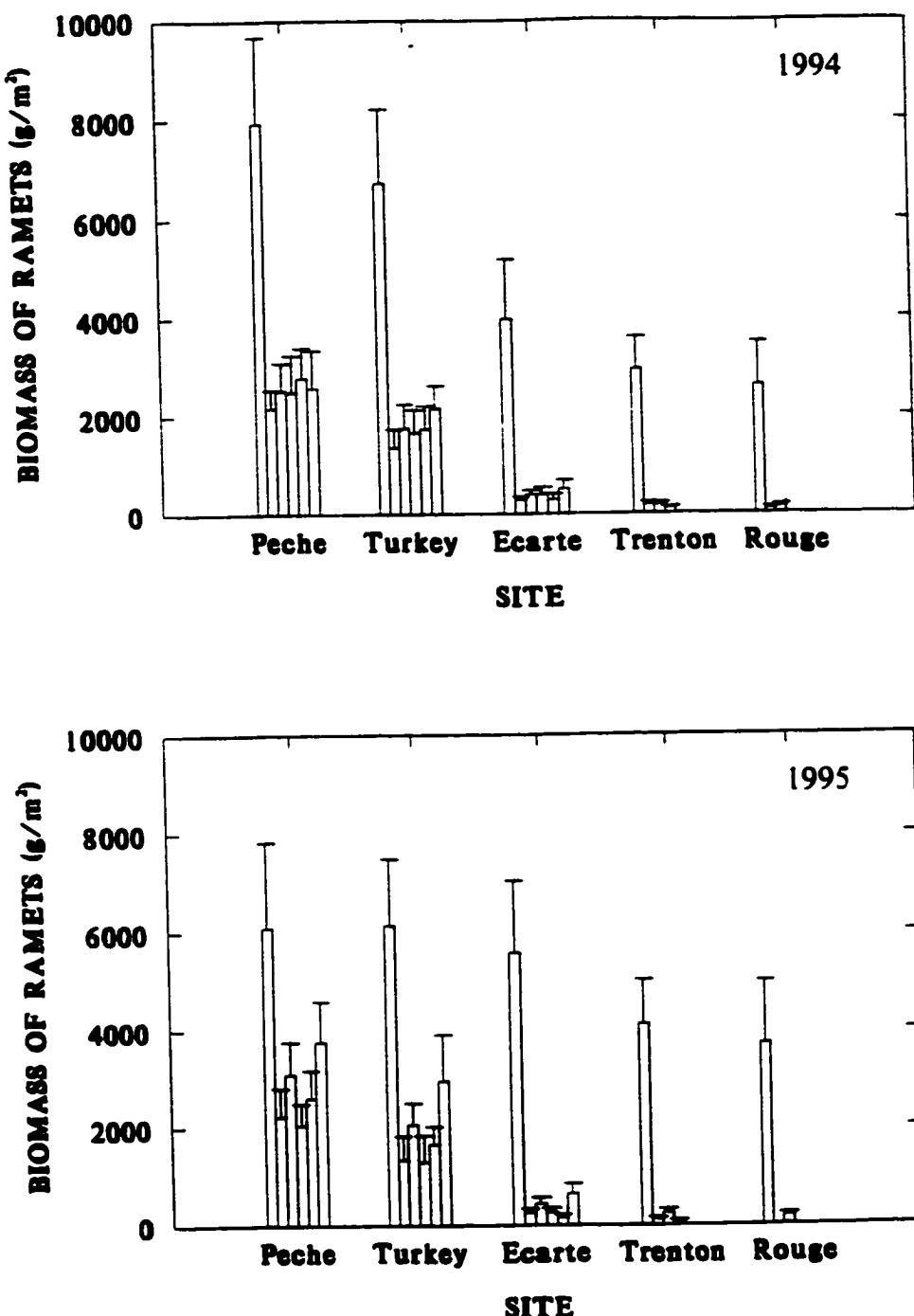


Figure 7.6. Mean biomass of ramets (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

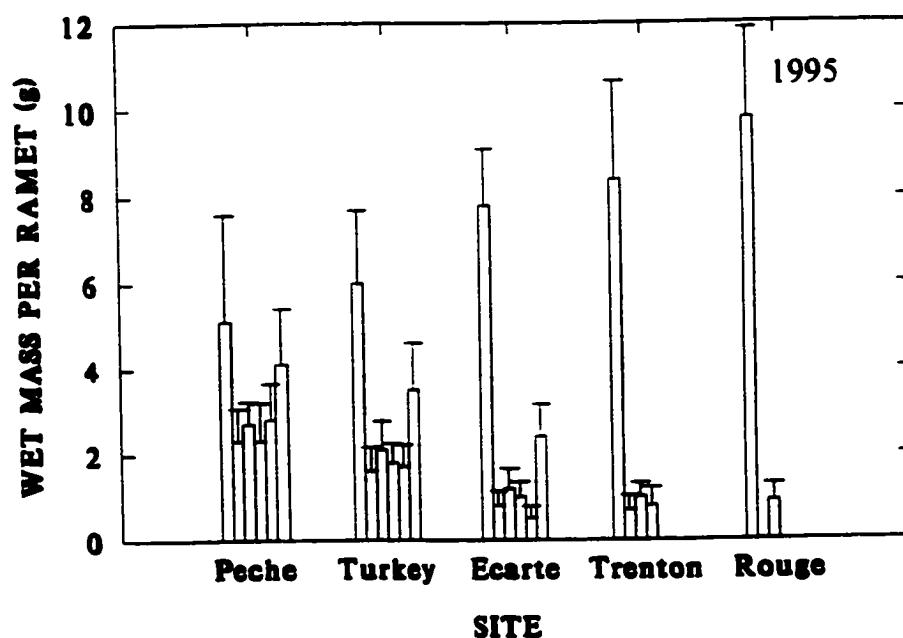
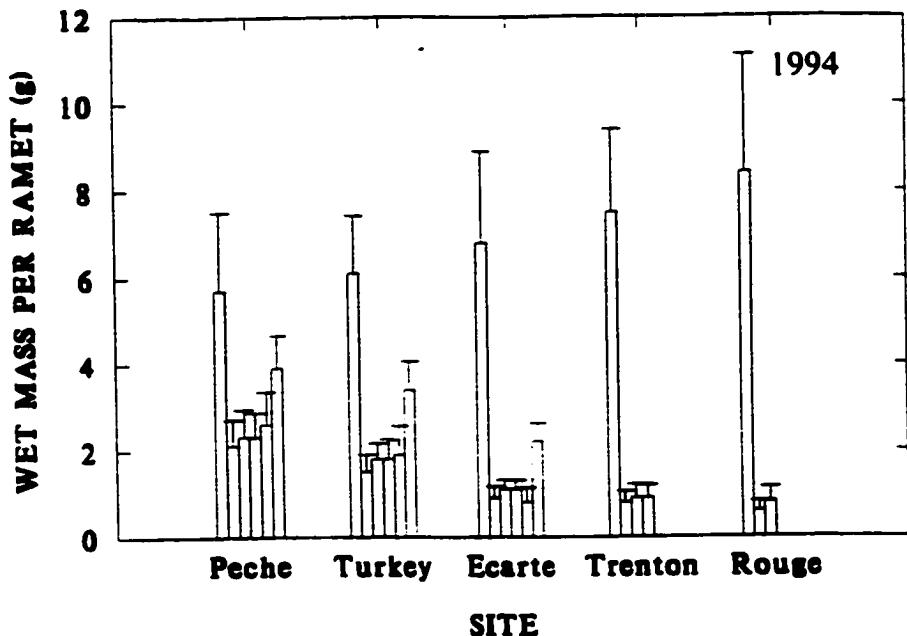


Figure 7.7. Mean biomass per ramet (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

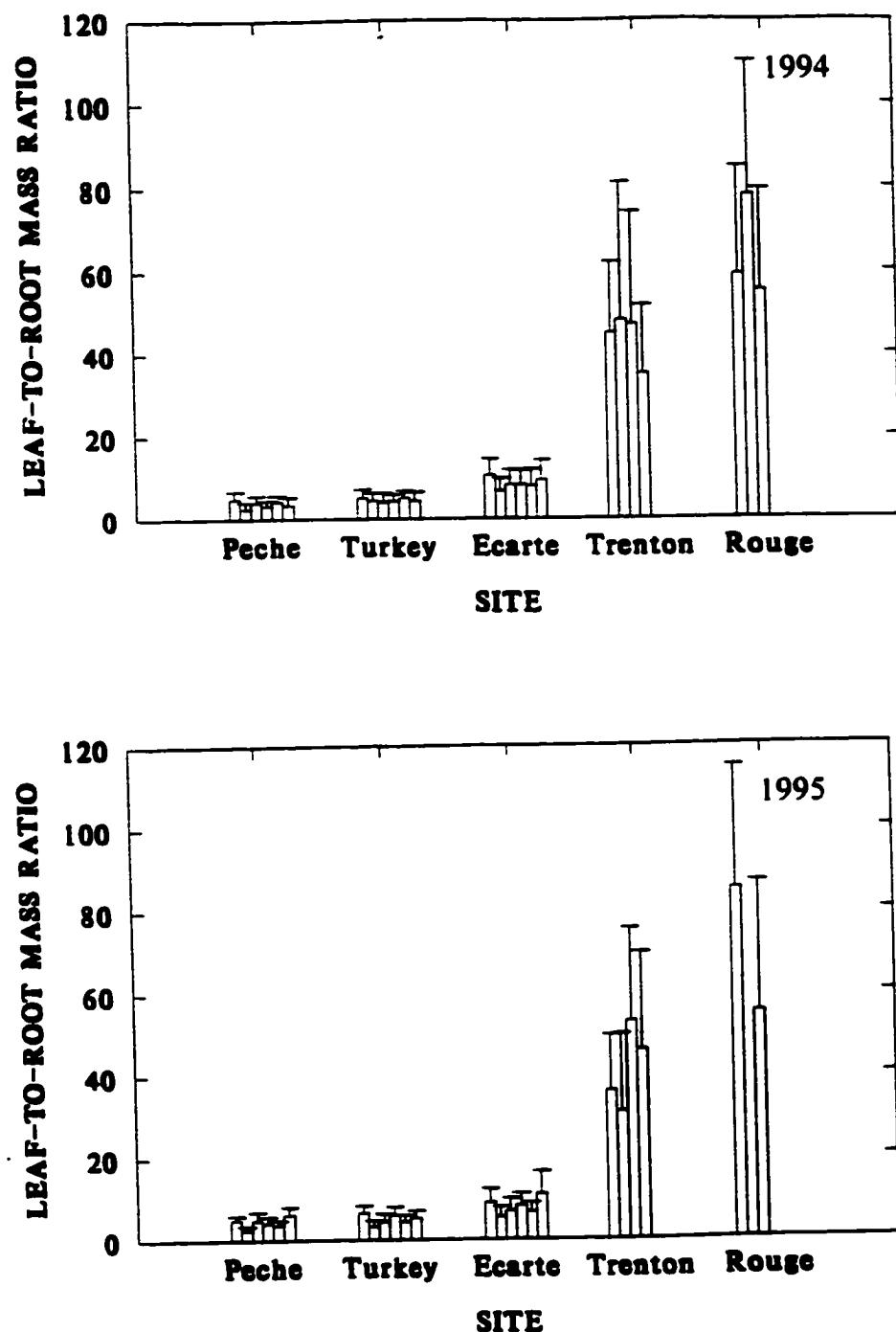


Figure 7.8. Mean leaf-to-root mass ratio (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

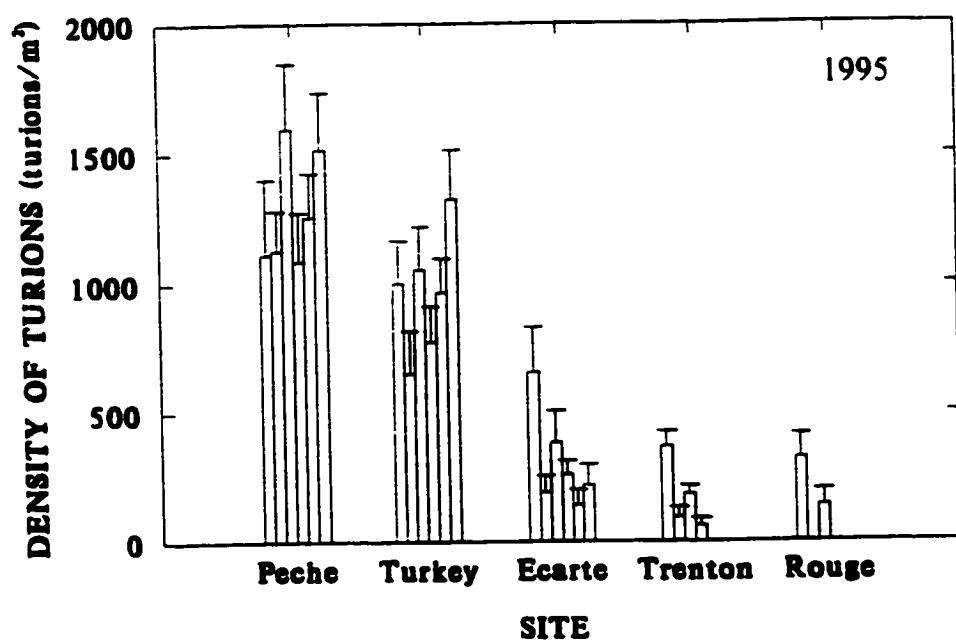
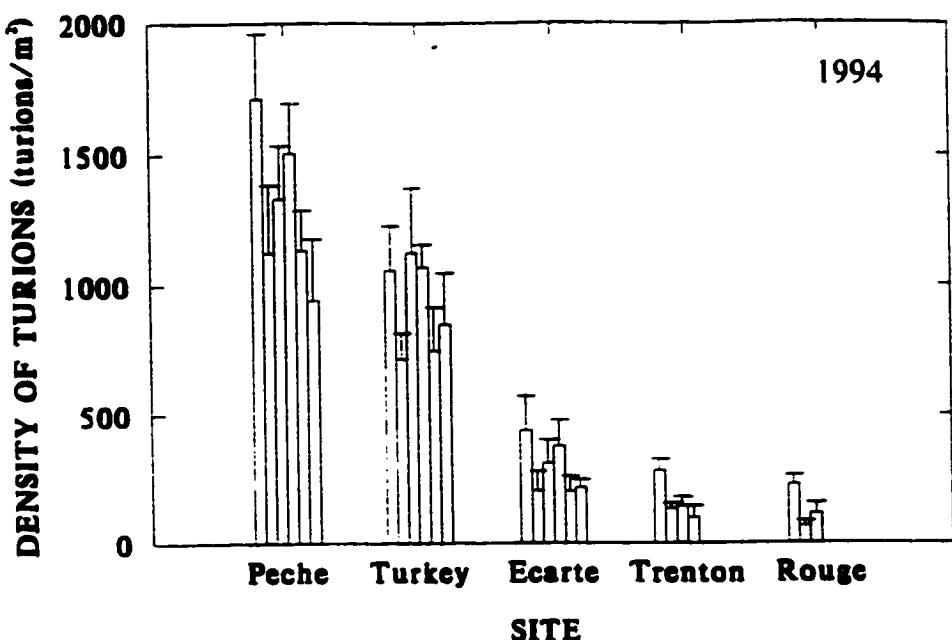


Figure 7.9. Mean density of turions (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

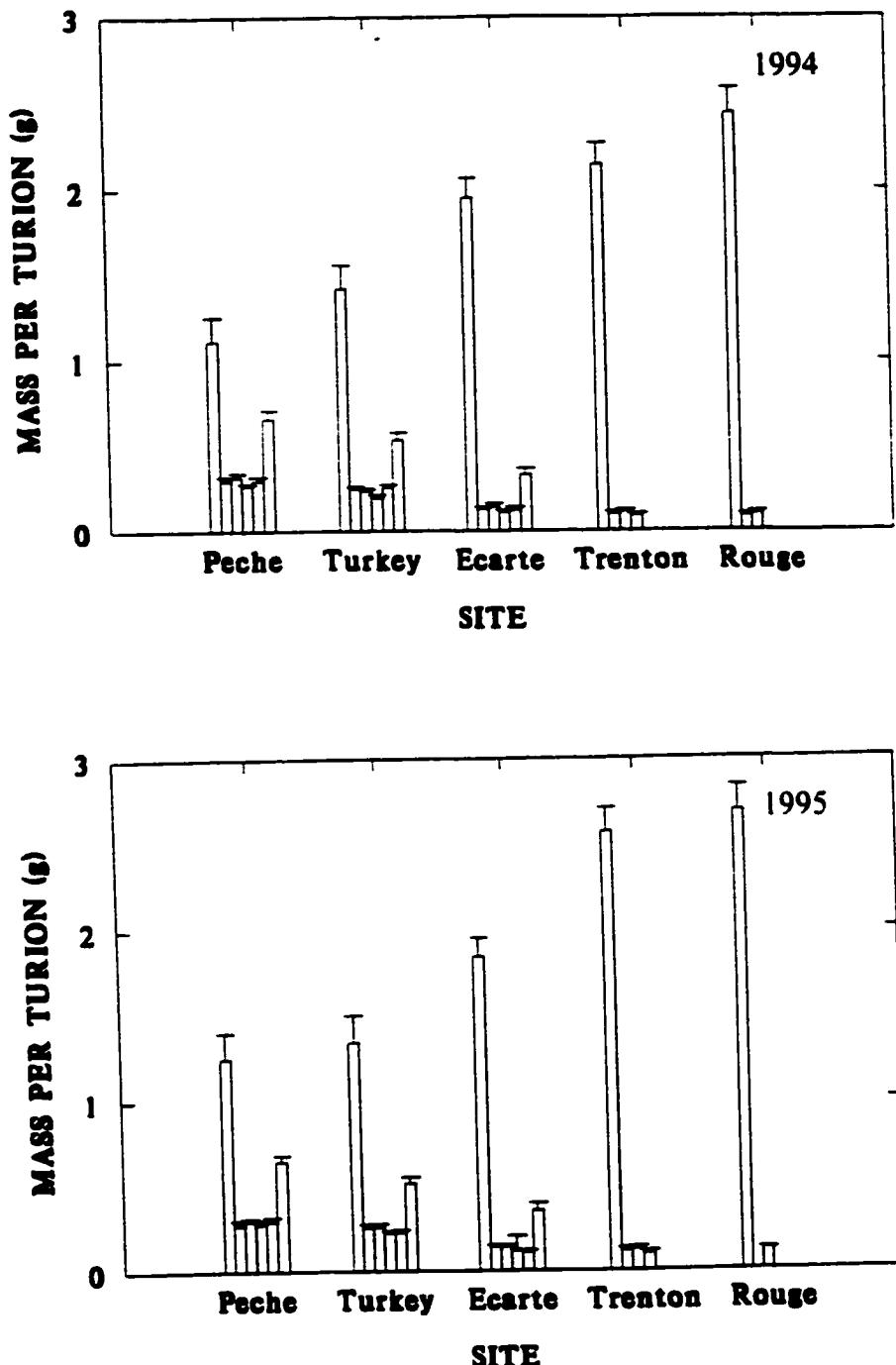


Figure 7.10. Mean biomass of turions (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

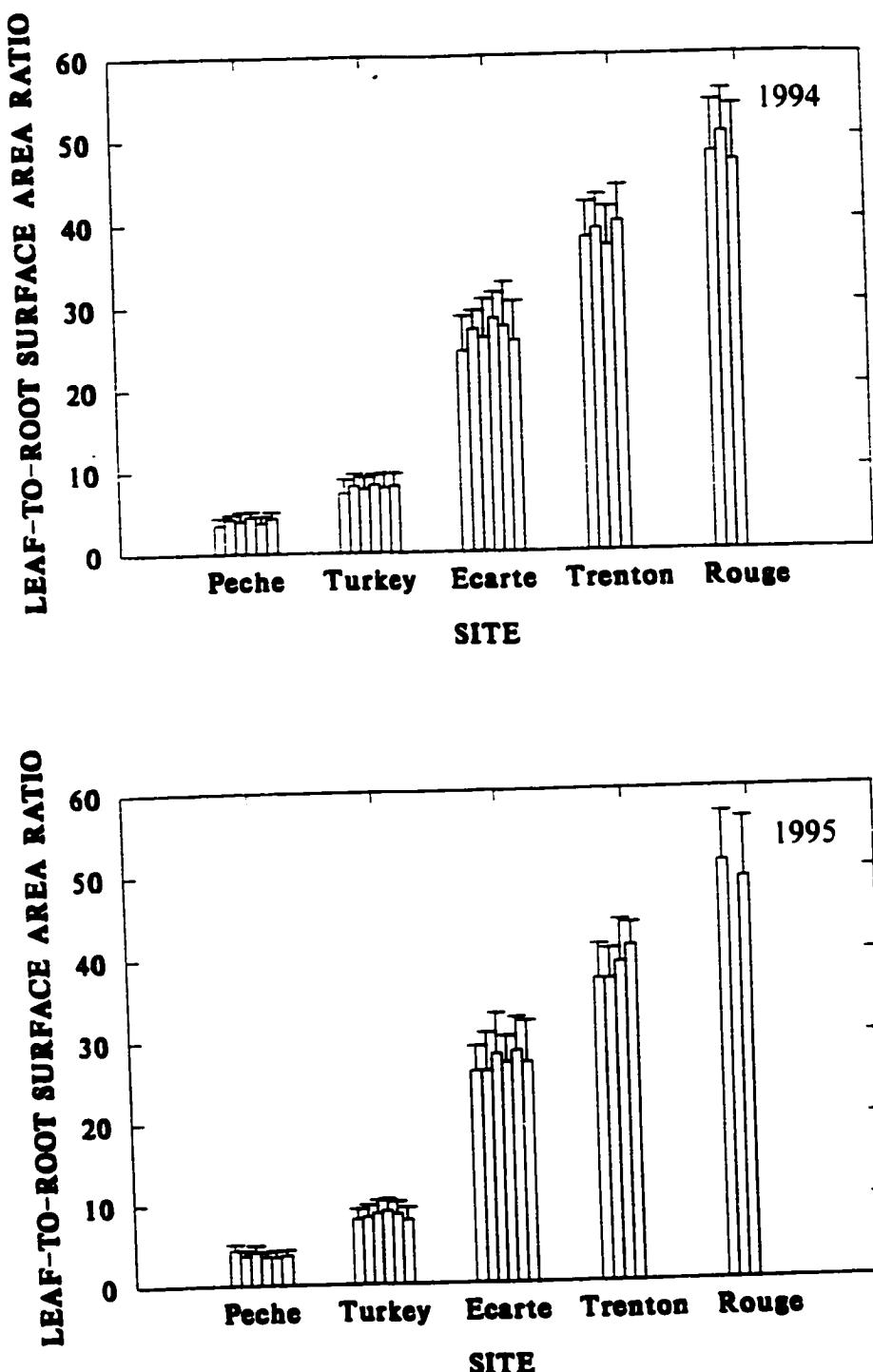


Figure 7.11. Mean leaf-to-root surface area ratio (\pm SE) of *Vallisneria* genotypes raised at different sites. Individual 6 bars represent genotypes 1-6 arranged in order, by site.

Chapter 8

SHORT-TERM SEDIMENT PHYTOTOXICITY TESTING USING *Vallisneria americana* IN THE LABORATORY¹

ABSTRACT

In contrast to their ecological importance, submersed rooted macrophytes have been largely overlooked in environmental science. Presently, the array of phytotoxic bioassays includes the use of only one free-floating vascular macrophyte (*Lemna*) and several algal species. A short-term and inexpensive assay was studied for feasibility in evaluating sediment quality. Cloned ramets of the macrophyte *Vallisneria americana* were used to test the phytotoxicity of sediments collected at different locations in the Detroit River. Ramets were planted in sediment samples and placed in greenhouse aquaria. After a week of exposure, ramets were destructively sampled, preserved, and leaf and root surfaces areas were calculated for each ramet; plant biomass was also recorded. An index of the leaf-to-root surface area ratio proved to be a reliable predictor of sediment phytotoxicity; the ratio of leaf-to-root mass was also useful, but was less consistent. Results of ANOVA indicate that the index of leaf-to-root surface area ratio responded to sediment quality but was not significantly affected by either plant genotype or by interaction between sediment quality and plant genotype. Ramets grown in sediments that were relatively less contaminated with organic compounds had lower values of the leaf-to-root surface area ratio, while plants grown in relatively more contaminated sediments had higher values. There was a significant correlation ($p<0.001$) between rank ordering of results of the present greenhouse study and similar data for

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plants that had been surveyed in the field.

INTRODUCTION

Submersed rooted macrophytes have recently been shown to be involved in the uptake, bioaccumulation and movement of toxic metals and organochlorine contaminants in aquatic ecosystems (Guilizzoni, 1991; Manny *et al.*, 1991; Lovett Doust *et al.*, 1994a,b; St-Cyr *et al.*, 1993, 1994). However, the ability of plants to bioaccumulate and, in some cases, biotransform toxic compounds into less toxic forms is largely unexplored. The possibility of "phytoremediation" of sediments and/or water in areas contaminated with nutrients, metals and organics (Walton and Anderson, 1992; Brix, 1994; Salt *et al.*, 1995; Brown *et al.*, 1995; Barber *et al.*, 1995; Cunningham *et al.*, 1996). Living at the boundary between the sediment layer and the water column, rooted plants have great potential for biomonitoring toxic metals (Guilizzoni, 1991; Manny *et al.*, 1991) and organochlorine contaminants (Lovett Doust *et al.*, 1994a,b; Biernacki *et al.*, 1995a,b; Biernacki *et al.*, 1996). They may be superior to algae or free-floating plants when contaminated sediments are the major source of impairment. Finally, some studies have shown that species of rooted macrophytes may be more sensitive to lower concentrations of herbicides and organochlorine contaminants than free floating plants (Swanson *et al.*, 1991; Lovett Doust *et al.*, 1994b).

Despite many functional roles that plants play in aquatic ecosystems and their general ecological importance, submersed rooted macrophytes tend to be overlooked in remediation studies, rarely used in ecosystem assays, and not required in standard screening procedures for the introduction and registration of new chemicals, including even herbicides that target vascular plants in particular (Swanson *et al.*, 1991; Freemark and Boutin, 1994; Lewis, 1995; Boutin *et al.*, 1995). There are many reasons for this neglect. The major obstacles to their use in laboratory assays are their large size, relatively slow growth, relatively long growth cycle, technical difficulties in studying roots, lack of standard analytical methods for tissue contaminant analyses, a lack of standardized plant material, and a lack of established test methods and assay endpoints. In several recently published studies using the submersed rooted macrophyte *Vallisneria*

americana in both greenhouse experiments (Biernacki *et al.*, 1995a,b) and field studies (Lovett Doust *et al.*, 1994a,b; Biernacki *et al.*, 1996), a number of the above problems were resolved. These studies showed that *Vallisneria americana* is noticeably responsive to changes in environmental quality and potentially very useful for both short- and long-term studies of environmental quality. Plant growth have been standardized and a number of assays of growth utilized, which correspond significantly with levels of contaminants in both the external environment and the plant tissues (see Chapters 2 and 3).

Several studies have shown a significant correlation between rates of root and/or shoot elongation in plants exposed to contaminants, and concentrations of contaminant (Byl and Klaine *et al.*, 1991; Smith, 1991; Etzion and Neumann, 1993; Fiskesjo, 1993; Ryan *et al.*, 1993; Byl *et al.*, 1994; Przymusinski and Gwozdz, 1994). Earlier studies showed that contaminants affect not only elongation of roots and leaves but also changes in the number of leaves and roots per ramet, leaf width, root diameter, and patterns of biomass allocation to leaves and roots (Chapter 2 and 3; Biernacki *et al.*, 1995a,b). Some of these changes in plant morphology may be reflected in estimates of changes in leaf and root surface areas per ramet. Shifts in leaf and root dimensions may occur very rapidly; significant differences can be observed within less than a week (Chapter 3; Byl and Klaine, 1991; Biernacki *et al.*, 1995a). Furthermore, even at very low concentrations of organic contaminants, a significant change in relative root and shoot growth can occur before measureable increases in enzyme activity (Byl and Klaine, 1991; Byl *et al.*, 1994).

The objectives of this study were to develop a simple method for controlled sediment evaluation, to estimate the potential of selected clones of *Vallisneria americana* for use in a short-term bioassay and, by comparison with field results to evaluate the reliability of the method.

MATERIALS AND METHODS

This experiment was carried out in June 1995 in the greenhouse of the University of Windsor. Ramets of *Vallisneria americana* were planted in sediments collected at different locations (see below). During the experimental period, water temperature ranged from 22°C to 24°C (measured at noon at the bottom of aquaria). Plants were exposed to the natural photoperiod, and light quanta ranged from 2000 $\mu\text{mol s}^{-1} \text{m}^{-2}$ on sunny days to 400 $\mu\text{mol s}^{-1} \text{m}^{-2}$ on cloudy days (measured at noon at floor level at the bottom of aquaria). Dechlorinated tap water ($\text{pH} = 7.1$) was used and no additional nutrients were added. To minimize evaporation, aquaria were covered with plexiglass.

Sediment

Sediments were collected from six sites in the Detroit River (Figure 8.1). Two samples were taken from the Trenton Channel (sites TC1 and TC2), two from the Rouge River (RR1 and RR2), one off Turkey Island (TI) and one off Peche Island (PI). Samples of sediment were collected at 1 m water depth from the top 12-15 cm of the sediment layer, placed in sealed polyethylene bags, and stored for transport in large, dark plastic containers. Within two - three hours following collection, sediments were stored in a coldroom at 6 °C. Within a week, sediments were placed in 500 mL glass jars (9.5 cm tall) and set up in the aquaria filled earlier with water. Each aquarium (capacity 175 L; 92 cm (L) x 31 cm (W) x 62 cm (H)) contained six jars filled with sediment originating from the same site. There were three replicate aquaria for each sediment treatment (each with six jars). A total of 18 aquaria were used (with a total of 108 ramets planted individually in jars).

Experimental plants

Plants of *V. americana* var. *americana* (native to North America) and of *V. americana* var. *biwaensis* (native to South America and Asia) (Hydrocharitaceae) (classified after Lowden, 1982) were used in this study. Ramets of selected genets had

been cultivated prior to the study. Individual genets are capable of producing many identical ramets each growing season (Catling *et al.*, 1994). Only well-developed undamaged ramets were used in the study. Genets of six *Vallisneria* lines were selected:

1. A male plant of *V. americana* var. *americana*, originating from the Chenal Ecarte site of the St. Clair River and found to be tolerant of high concentrations of trichloroethylene (TCE) (see Chapter 2; Biernacki *et al.*, 1995a) and subsequently cultivated in the greenhouse;
2. The same genetic individual as above, but cultivated in the field;
3. A female plant of *V. americana* var. *americana*, originating from Rouge River and cultivated in the greenhouse;
4. the same genetic individual as above, but cultivated in the field;
5. A female plant of *V. americana* var. *americana*, originating from Turkey Island and cultivated in the field;
6. A plant of *V. americana* var. *biwaensis*, of unknown sex (plant did not flower), and cultivated in the greenhouse.

In order to standardize initial plant conditions, all ramets of each genet were planted in the greenhouse in containers filled with a mix of 80% sand by volume, 18% silt, 2% clay (pH = 7.2), and grown two weeks. Ramets selected for the study initially had four or five leaves. One ramet was planted per jar. Each aquarium had plants of the six genetic lines.

Data collected

Prior to the experiment, samples of three ramets from each of the six lines were preserved in 4% formaldehyde to determine initial levels of growth. After a week of exposure to the experimental sediments, one ramet from each of the six lines was removed from aquarium and preserved. When ramets were removed, the sediment around roots was carefully washed in order to recover intact plants and undamaged roots. The preserved plants were subsequently analysed individually and the number of leaves, width and length of each leaf, the number of roots, diameter and length of each root, and the biomass of leaves and roots were determined. Leaf and root dimensions of harvested

ramets were measured using a digital micrometer (Mitutoyo Corporation, 1992). A total of 126 ramets was measured: 18 ramets that had been collected prior to the experiment (controls, to characterize plants before exposure to TCE, indicated on figures as "C") and 108 ramets exposed to sediments. Surface areas of leaves and roots were calculated and the means of three ramets per line were used for all subsequent analyses. Measures of plant performance were used to estimate adverse effects of each sediment on plant growth.

Comparison of laboratory-derived data with field observations

To draw conclusions from this short-term laboratory test, results were compared with data from an independent survey of *Vallisneria* ramets in the field, at the same sites of sediment collection, carried out in 1993 (Chapter 6; Biernacki *et al.*, 1996). Plants surveyed in 1993 in the field at sites of sediment collection were measured and leaf-to-root surface area ratio was calculated in the same way as were plants in this phytotoxicity test. To compare field and laboratory studies, Spearman rank correlation analysis was used; for both studies sediments were ranked from those that caused the greatest leaf-to-root surface area ratio to those that induced lowest ratio.

Comparison with Microtox assay

Giesy *et al.* (1988) reported results of a Microtox assay for 136 sediment samples from the Detroit River including Trenton Channel and Rouge River. Sediment collected from site RR1 in present study was the same as site #203 in Giesy *et al.* (1988) study; site RR2 was site #198 in Giesy *et al.* (1988); site TC1 was site #110 in Giesy *et al.* (1988); TC2 was site #42 in Giesy *et al.* (1988); site #83 in Giesy *et al.* (1988) study could be compared to site TI. However, sediments for both studies were collected in different years, hence results were compared using nonparametric Spearman rank correlation analyses performed on ranks of relative sediment toxicity reported in both studies. Sediments from Giesy *et al.* (1988), using the Microtox assay, were ranked in increasing order from the most toxic to the least toxic, and sediments in present study were ranked

also in increasing order from one that induced the greatest leaf-to-root surface area ratio in *Vallisneria* to that with the lowest.

Statistical analyses

Data were analyzed using SYSTAT for Windows version 5.03 (1992), through ANOVA, and where appropriate, differences between mean values were tested for significance by Tukey HSD pairwise comparison tests. Nonparametric Spearman rank correlation coefficient analysis was also used.

RESULTS

ANOVA revealed significant effects of the sediment and *Vallisneria* line, and though less significantly, their interaction, on nearly all variables (Table 8.1). Number of leaves per ramet did not change significantly in ramets. The plant measures most significantly affected were the indices of leaf-to-root surface area ratio, the leaf-to-root biomass ratio, surface area of leaf per gram of leaf tissue, and the surface area of root per gram of root tissue. However, the only measure that responded to sediment effects but was not affected by other factors (*Vallisneria* line and interaction) was the ratio of leaf-to-root surface area. Additional analyses showed no significant differences between female and male genets ($p>0.05$) and no significant differences between plants which had been cultured in the field and in the greenhouse ($p>0.05$) in leaf-to-root surface area ratio. However, plants cultured in the field had significantly ($p<0.001$) greater biomass than plants cultured in the greenhouse (see also Figure 8.2).

Regression analyses of leaf-to-root surface area ratio with other measures of plant performance revealed significant correlations (Table 8.2). The leaf-to-root biomass ratio and the surface area of leaf per gram of leaf tissue were significantly correlated with changes in leaf-to-root surface area ratio. Across all of the ramets, changes observed in leaf-to-root biomass ratio accounted for 31% of variance in leaf-to-root surface area ratio.

In order to observe if this correlation was affected by total biomass of a ramet, further analysis including only smaller ramets (below 4 g) indicated that changes in leaf-to-root biomass ratio explained nearly 65% of the variance in leaf-to-root surface area ratio.

Ramet mass changed measurably over the relatively brief study period. Final biomass of ramets tended to be lower following growth in sediment originating from the Rouge River and the Trenton Channel, than when grown in Turkey Island or Peche Island sediment (Figure 8.2). However, only for smaller ramets (those below 4 g, N = 44) were these differences significant ($p < 0.01$). Overall, there were no significant differences in final biomass of ramets, and changes in biomass of ramets were not used in ranking sediment quality.

Sediment type significantly affected changes in leaf-to-root surface area ratio (Table 8.1 and Figure 8.3). The ratio ranged from about 2 to 15. Results for all ramets from different *Vallisneria* lines resulted in sediments being ranked in the same order, and there were no significant differences between *Vallisneria* lines in leaf-to-root surface area ratio for the same sediment sample. The lowest value of leaf-to-root surface area ratio was with the Peche Island sediment sample, the highest was for Rouge River sediment collected at RR1 site. Values of the ratio were three-to-five times greater for Trenton Channel and Rouge River sediment samples than for Peche Island and Turkey Island sediments.

Comparison of laboratory-derived data with field observations

In a large field survey, leaf-to-root surface area ratio in *Vallisneria* ranged, at six sites of sediment sampling in the present study, from 4 at the Peche Island location to 89 at the RR1 site (Biernacki *et al.*, 1996). In the present laboratory test, values ranged from two to 15 for the six different sediment samples studied. There was no significant difference in the relative rankings of sediment quality in the field survey and the present laboratory test (Spearman rank correlation analysis, $p_s < 0.001$; with Bartlett Chi-square statistics $p < 0.001$).

Comparison of *Vallisneria* phytotoxicity test with Microtox assay

Spearman rank correlation analysis carried out on the rankings of relative toxicity of sediments in a Microtox assay, reported by Giesy *et al.* (1988) for sediments sampled at the same locations as in the present study, and the rankings of relative phytotoxicity of these sediments to *Vallisneria* in the present study, revealed a marginally significant correlation between the two sets of data ($p_s < 0.05$; with Bartlett Chi-square statistics $p < 0.05$). Ranking of sediments due to toxicity to *Photobacterium phosphoreum* in the Microtox assay, and ranking of sediments due to phytotoxicity to *Vallisneria* in present test thus were similar for sediments sampled at the same locations in the two studies.

DISCUSSION

In an earlier laboratory study (Chapter 2; Biernacki *et al.*, 1995a), it had been shown that addition of the organic contaminant, trichloroethylene, into the water column of aquaria was subsequently adsorbed to sediments and caused a rapid increase in the leaf-to-root surface area ratio in *V. americana* shoots (Chapter 3; Biernacki *et al.*, 1995b). Plants responded differently to different contaminant concentrations and each contaminant concentration was associated with characteristic leaf-to-root surface area ratios in exposed plants. Sediments adsorbed contaminant to greater concentrations than the water column, thus ramets were exposed to higher concentrations through root tissues than through leaves. Subsequently in contaminated treatments, turnover of roots and leaves increased due to decrease of their lifespans (Biernacki *et al.*, 1995a,b). Ramets exposed to contaminants allocated more biomass to new leaf tissues than to new root tissues, also their leaf surface area increased and root surface area decreased, depending on contaminant concentrations found in the water column and sediment-pore-water (Biernacki *et al.*, 1995b). Also, it was confirmed in the field survey that increased concentrations of organic contaminants at different sites were significantly correlated with increased leaf-to-root surface area ratios in ramets collected throughout the Huron-

Erie Corridor of the Great Lakes of North America (Biernacki *et al.*, 1996).

Recently, environmental managers and in particular wetland conservation authorities have been studying approaches to the assessment of freshwater environmental quality from the perspective of submersed macrophytes (Smith, 1991; Swanson *et al.*, 1991; Lewis, 1993, 1995; Boutin *et al.*, 1995). Measure of leaf-to root surface area ratio in *Vallisneria* ramets has potential as a biomonitoring tool for laboratory and field studies of pollution monitoring and remediation. There is need for a more standardized protocol for submersed macrophyte assay.

In the present study, results were compared with results of a survey in 1993 of *Vallisneria* sampled from many natural populations in the Detroit River (Biernacki *et al.*, 1996) and with results of sediment Microtox toxicity tests which had been carried out in the same areas (Giesy *et al.*, 1988). It is possible that over time, since the 1988 study was carried out, the absolute contaminant concentrations have changed, but long term studies have suggested that relative contamination of sites changes little over time (Suns *et al.*, 1993). A nonparametric test was used to evaluate correlations between the results of the present study and those of Giesy *et al.* (1988). The correlation was significant ($p<0.05$). All six of the sites for sediment sampling in this study were also sampled in earlier survey (Biernacki *et al.*, 1996). There was again a significant correlation between the two patterns, suggesting that the present laboratory test may be used to predict the relative levels of contamination of sediments in the field. Present laboratory assay may be used for testing polluted sediments or soil samples collected at sites where *Vallisneria* does not occur naturally (e.g. dredged sediments, retrieved sediments from greater depth, terrestrial soils, underground samples) or at times in the year when *Vallisneria* does not grow actively (late fall, winter, and early spring). Also, the present assay has potential for testing of new chemicals in laboratory-prepared sediments.

Lower values leaf-to-root surface area ratio in the greenhouse were observed than in the field. This is likely a result of the fact that plants in the field had spent entire life living in their sediment, not just one week, as in the bioassay test group. There are also other differences between greenhouse and field conditions. In the greenhouse,

temperature was higher. Temperature could promote increase mobility and bioavailability of contaminants present in the sediment. Furlong *et al.* (1988) reported a range of organic contaminants at high concentrations, including many PAHs, PCBs, PCNs, and PCTs, in sediment samples taken at the same locations as in present study.

Currently, for registration of new pesticide, routine tests using aquatic plants are not required, and even newly-introduced herbicides (targeting higher vascular plants) need not be tested with submersed plants (Swanson *et al.*, 1991; Freeman and Boutin, 1993; Boutin *et al.*, 1995). In a number of comparative toxicity studies submersed aquatic plants were found to be a few orders of magnitude more sensitive to lower concentrations of herbicides (e.g. atrazine) than benthos, zooplankton, or fish (Solomon *et al.*, 1996). In comparison to other seven species of aquatic macrophytes (*Potamogeton perfoliatus*, *P. pectinatus*, *Lemna gibba*, *L. minor*, *Myriophyllum spicatum*, *Elodea canadensis*, *Ceratophyllum demersum*), *V. americana* was reported to be the most sensitive to atrazine (Swanson *et al.*, 1991; Solomon *et al.*, 1996). Submersed rooted macrophytes may be particularly suitable for testing contaminated sediments (Lovett Doust *et al.*, 1994a,b; Lewis, 1993, 1995; Boutin *et al.*, 1995; Biernacki *et al.*, 1994a,b, 1996).

Toxicologists initially indicated that results from algae (*Selenastrum capricornutum* was the most frequently used) may be representative for higher plants; however, many tests using algae do not predict responses of aquatic macrophytes (Swanson *et al.*, 1991; Guilizzoni, 1991; Lewis, 1993, 1995). This may explain only-marginal significant correlation between ranks of sediment quality using the Microtox assay, and ranks of *Vallisneria* leaf-to-root surface area ratios in the present study. Also, the number of comparisons was low and this will greatly affect the level of significance. Many existing assays can readily discriminate between relatively clean and relatively contaminated sediments, but for sediment samples of common origin, results of toxicity tests differed among assays due to differences in sensitivity to and effects of particular contaminants on different organisms (Giesy *et al.*, 1988). Interpolation of results from one group of organisms to another is difficult. The necessity for use of submersed macrophytes as test species should not be overlooked due to their ecological importance.

Different lines of *V. americana* as well as interaction between line and sediment, had significant effects on all measures of plant performance (Table 8.1). Of all measures, only the index of leaf-to-root surface area was not affected by genetic line of the ramet or its interaction with sediment quality. In biomonitoring studies, the use of genetically identical ramets may increase precision and accuracy of toxicity testing (Chapter 7; Baird *et al.*, 1991; Baird, 1992; Lovett Doust *et al.*, 1993, 1994b). Standardization of plant genotypes would improve comparability of results from different places as well as facilitating the use of particular traits (large or small ramets, female or male, tolerant to particular chemicals or nontolerant). Genets of *V. americana* var. *biwaensis* continue to produce ramets throughout the entire year. By comparison, *V. americana* var. *americana* typically will produce turions (overwintering buds) in the fall and not initiate new growth until May or June of the following year. However, fully developed ramets may be stored for a long period in the greenhouse at light compensation point and thus become available over the entire year.

Significant cultivation effects were observed for the two *Vallisneria* line in which this was considered. Ramets cultured in the field had greater biomass compared with the same genetical individual but pre-cultured in the greenhouse. Smaller ramets (below 4 g) are easier to maintain. They are particularly useful for measurement of leaf-to-root mass ratio as a substitute for leaf-to-root surface area ratio since in these plants changes in leaf-to-root mass ratio accounts for nearly two thirds of variance in leaf-to-root surface area ratio. Mass of leaves and roots may be easier to determine than leaf and root surface areas. Furthermore, measurement of leaf and root surface areas may be simplified using photometric techniques (Watala and Watala, 1994) or image analysis technique (Tagliavini *et al.*, 1993; Gerber *et al.*, 1994).

Submersed aquatic macrophyte *Vallisneria americana* has great potential as a biomonitor and should be considered in an assay for screening toxicities of new chemicals or in environmental samples. It has been shown that a laboratory assay using *V. americana* is simple, rapid, cost-efficient, and can deliver field-relevant results.

Table 8.1. Summary of ANOVA of effects of sediment and *Vallisneria* line on morphological parameters in *Vallisneria americana*. Analysis is based on measurements of 108 ramets (N=108). Significance is indicated as: ***= $p \leq 0.001$; **= $p \leq 0.01$; *= $p \leq 0.05$; NS=non significant.

VARIABLE	SEDIMENT	<i>Vallisneria</i> LINE	S x G
	(S)	(G)	
Number of leaves	NS	*	NS
Number of roots	*	*	NS
Leaf-to-root number ratio	*	*	NS
Leaf mass	*	*	*
Root mass	*	*	*
Ramet biomass	*	*	*
Leaf-to-root mass ratio	**	*	NS
Root diameter	*	NS	*
Surface area of a leaf	*	*	*
Surface area of a root	*	*	*
Surface area of leaves per ramet	*	*	*
Surface area of roots per ramet	*	*	*
Leaf-to-root surface area ratio	***	NS	NS
Leaf surface area per leaf mass	**	*	*
Root surface area per root mass	**	*	*

Table 8.2. Regression analyses of changes in leaf-to-root surface area ratio and correlation with other morphological parameters in *Vallisneria americana*. Only significant correlations are shown. Analyses were based on measurements collected from 108 ramets (N=108). Significance of regression is indicated as: *=p≤0.001; **=p≤0.01; *=p≤0.05.**

VARIABLE	r ²	p
Number of roots	0.09	*
Leaf-to-root number ratio	0.11	*
Root mass	0.09	*
Leaf-to-root mass ratio	0.31	***
Root diameter	0.10	*
Surface area of a leaf	0.14	*
Surface area of a root	0.09	*
Surface area of leaves per ramet	0.19	**
Surface area of roots per ramet	0.12	*
Leaf surface area per leaf mass	0.18	***
Root surface area per root mass	0.21	**

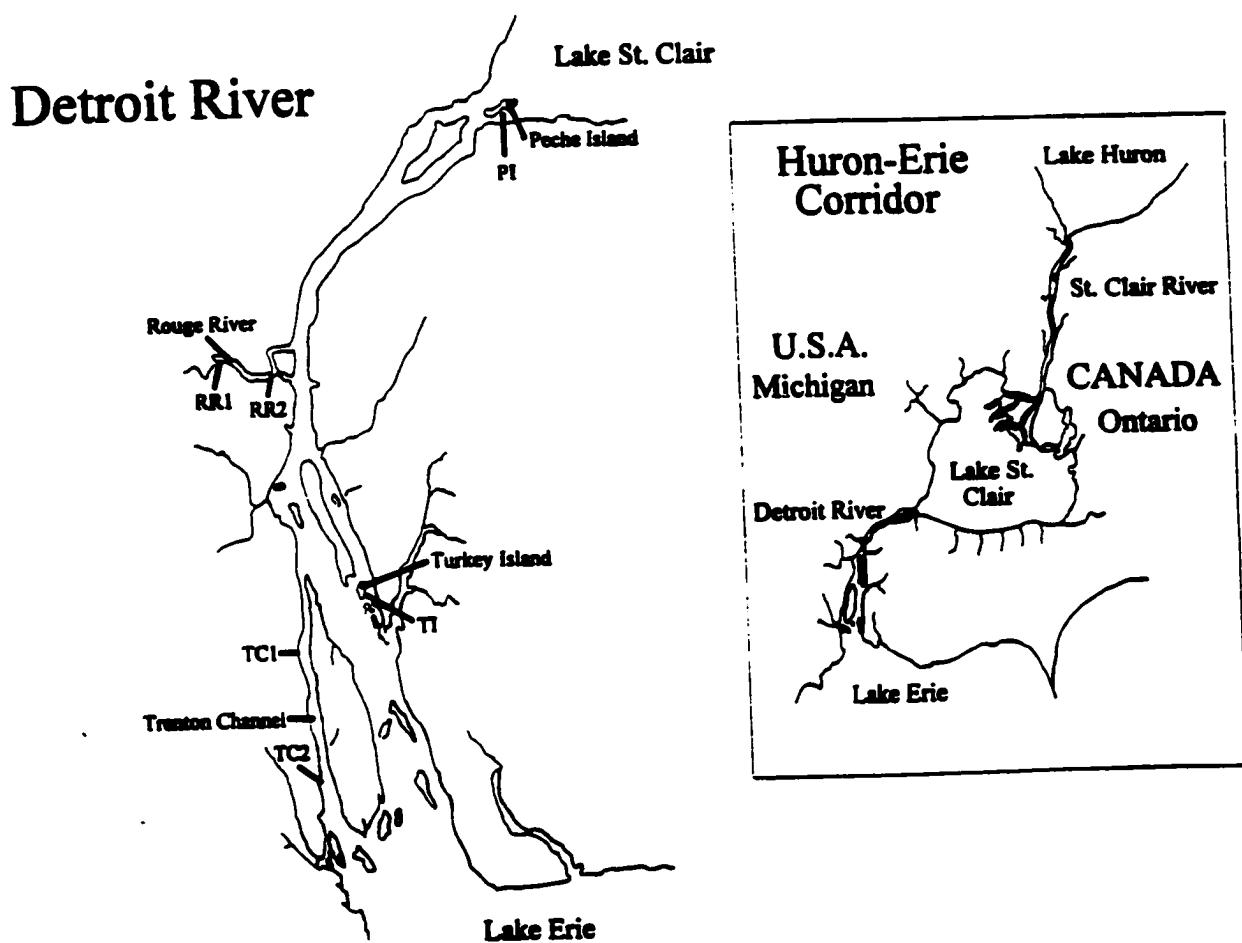


Figure 8.1. Map of the Detroit River indicating sites of sediment sampling. Sites are labelled in the Rouge River as RR1 and RR2, in the Trenton Channel as TC1 and TC2, at Turkey Island as TI, and at Peche Island as PI. Insert shows location of Detroit River in the Huron-Erie Corridor.

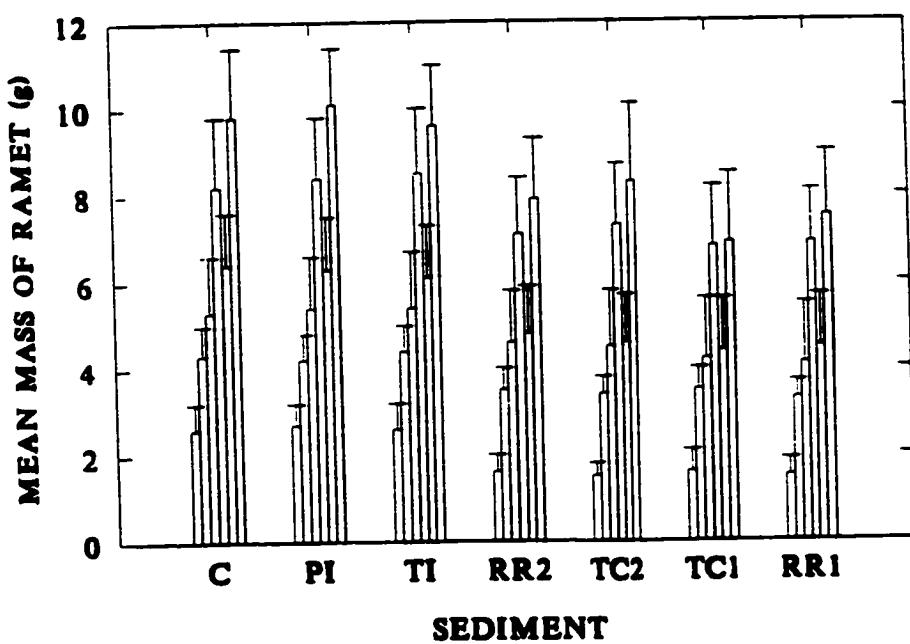


Figure 8.2. Mean biomass (\pm SE) of ramets planted in different sediments: C (control before initiation of experiment), PI (Peche Island sediment), TI (Turkey Island sediment), TC1 and TC2 (Trenton Channel sediments), RR1 and RR2 (Rouge River sediment). Six bars per sediment represent one of each of the six *Vallisneria* lines (from 1 on the left to 6 on the right, see Methods for description of lines) used in the study.

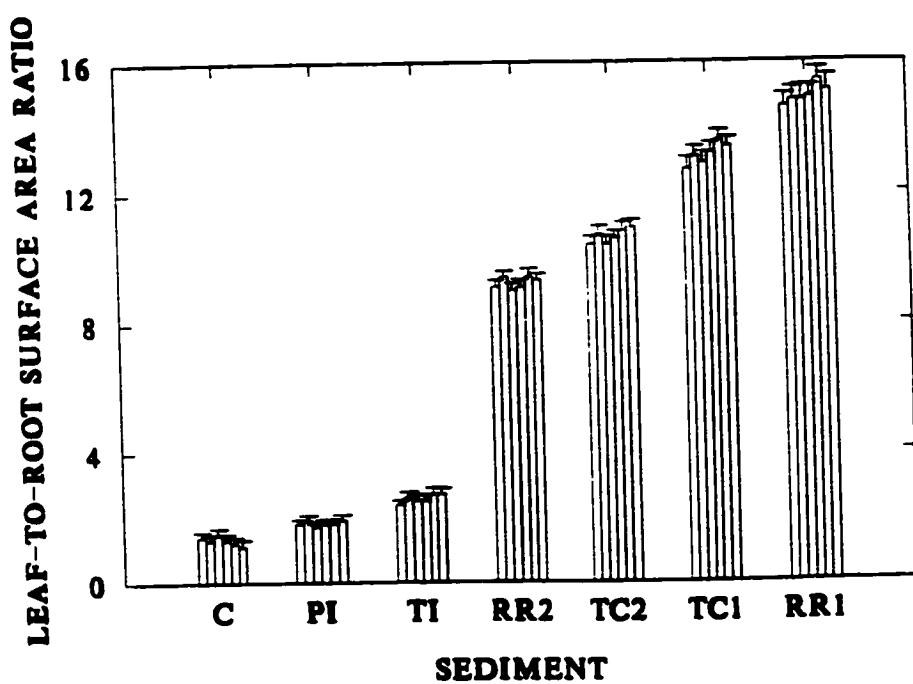


Figure 8.3. Mean leaf-to-root surface area ratios (\pm SE) of ramets planted in different sediments: C (control before initiation of experiment), PI (Peché Island sediment), TI (Turkey Island sediment), TC1 and TC2 (Trenton Channel sediments), RR1 and RR2 (Rouge River sediment). Six bars per sediment represent the *Vallisneria* lines (1 to 6) used in the study.

Chapter 9

GENERAL DISCUSSION

In all ecosystems, plant production dominates the total biomass. Phytomass in terrestrial and freshwater aquatic systems may be up to 2000 times greater than that of animal production (Sitte *et al.*, 1991). Thus, it is logical to study contaminant effects on plants and contaminant transfers through the plants at the base of the trophic hierarchy, to higher organisms in the food chain that either feed directly on plants as herbivores or eat plant-derived detritus, as detritivores (Cyr and Pace, 1993; Wetzel, 1995; Cebrian and Duarte, 1995). Since plant biomass dominates the total biomass of ecosystems, it seems reasonable that the mass of plant-associated contaminants may also dominate, compared to the mass of contaminants associated with other biota (Trapp and Mc Farlane, 1995). In the past, studies of contaminant effects on aquatic ecosystems were focused exclusively on animals and only rarely included attention to vascular plants, despite their ecological importance (Chapter 1; Lovett Doust *et al.*, 1994b; Trapp and Mc Farlane, 1995). Plants play a central role in carbon cycling and energy flow through the biosphere (Moriarty, 1990; Trapp and Mc Farlane, 1995; Wetzel, 1995).

Aquatic macrophytes are capable of affecting many of the physico-chemical characteristics of the aquatic environment. Plants provide habitat and food for numerous biota (Lodge, 1991; Korschgen and Green, 1988; Leslie and Timmins, 1995). Higher vascular plants are also necessary for energetic stability in aquatic ecosystems (Cyr and Pace, 1993; Wetzel, 1995; Cebrian and Duarte, 1995). It has been generally assumed that algae were the dominant source of energy in the majority of freshwater ecosystems (Hairston and Hairston, 1993; Boutin *et al.*, 1995). However, more recent studies which review the importance of macrophyte-derived dissolved organic carbon (DOC) in the energy flow in freshwater ecosystems indicate that macrophyte-derived DOC often dominates this energy flow and, on average, concentrations of DOC have been found to

be 6 to 10 times greater than of particulate organic carbon. The particulate organic carbon was considered in earlier studies as a major route of energy flow in freshwater systems (Hairston and Hairston, 1993; Wetzel, 1995).

Plants grow and develop from meristems and this determines their modular construction (Chapter 1; Silvertown, 1989). Studies of plant performance and the effects of contaminants on plants may therefore be focused on metapopulation demography of plant modules, including leaves, roots, shoots, and flowers. Growth and development of plant parts collectively determine the overall performance of an individual plant. Thus, while the effects of contaminants on plant modules have detrimental effects on the plant as a whole, the effects may be evaluated in terms of the effects on particular components. Leaves are the major photosynthetic organs, flowers the major reproductive units, and ramets are the major organs of clonal growth. Each of these modules shows a distinct pattern of birth and persistence, and a distinct pattern of growth due to such factors as sediment, population source of origin, and degree of organic contamination (Chapters 1, 2, and 3; Lovett Doust *et al.*, 1994b).

In numerous studies, submersed plants have been shown to improve aspects of the aquatic environment by water and sediment purification, cycling of essential nutrients, and increasing sedimentation of suspended particles within macrophyte beds.

Furthermore, plants provide shelter and nursery areas for numerous biota. All these services are even more important and evident in highly polluted areas (Korschgen and Green, 1988; Crowder and Painter, 1991; Leslie and Timmins, 1995). Other studies have concluded that remediation of impaired freshwater systems is not possible without including recovery of submersed macrophytes as a keystone in the process (Grimm and Backx, 1990; Klinge *et al.*, 1995), and that sustainable fisheries are also not possible without recovery of beds of aquatic macrophytes (Petr, 1993; Klinge *et al.*, 1995; Roos *et al.*, 1995; Ligtvoet *et al.*, 1995).

Historically, ecotoxicology has developed as a science focused on contaminant effects on animals, and this has affected the early definitions of basic terms used in ecotoxicology (see Moriarty, 1990). For example, consider the following fundamental

parameters of contamination in an aquatic ecosystem (see Moriarty, 1990 and also Suter *et al.*, 1993). Loading expresses the total amounts of contaminants that enter the system, getting diluted in the water column and/or adsorbed by sediments. Concentration describes the amount of contaminant present by unit mass or volume. Bioconcentration has been defined as the increase of contaminant concentration when passing directly from water into organisms. Bioaccumulation represents the intake of contaminant from food as well as from water by aquatic organisms (the use of word "food" assumes that this is an animal). Biomagnification indicates the increase in concentration of contaminant in animal tissues in successive levels of a food chain (theoretically it may be also applied to carnivorous plants).

In the present studies, plants of *Vallisneria* were exposed to contaminants dissolved in the water column and adsorbed by sediments. It is assumed that the term bioaccumulation best expresses the relevant pathways of plant exposure, taking into account two media of plant exposure: water and sediment. Plants may uptake contaminants from the water column by leaves and from the sediments by roots (see Chapters 2 and 4). It is difficult to define sediment as a "food" (the term used above in the standard definition of bioaccumulation), but plants may readily uptake sediment-associated contaminants by secreting enzymes, changing rhizosphere redox potential, or via rhizosphere-associated microorganisms (Barko *et al.*, 1986, 1991). Bioconcentration, by definition, assumes only a single route of exposure of an organism to contaminants present in the water, and it does not accurately reflect the routes of contaminant transfer to rooted plants.

At present, there is an increased need for information about environmental quality in aquatic systems. Biomonitoring, using biota to measure local environmental impairment and contaminant concentrations, seems the most ecologically relevant way of estimating environmental quality. Chemical analyses alone, despite significant costs, are not able to predict bioavailability of contaminants present in the environment or to predict the extent of their effect on development, growth and reproduction of aquatic organisms. Likewise, contaminant concentrations alone cannot be used to predict their effects on

ecological integrity of the aquatic ecosystem. In the present studies, the submersed rooted macrophyte *Vallisneria americana* has been used to measure impairment on plant performance due to organochlorine contaminants and associated contaminant concentrations in plant tissues (see Chapters 2 and 4). Biomonitoring may be further divided into passive biomonitoring and active biomonitoring (for more details see Lovett Doust *et al.*, 1994b). If a biomonitoring study is based on measures of performance using randomly sampled plants from natural populations, it is defined as passive biomonitoring (see Chapter 6). In contrast, studies in which factors are controlled experimentally, and involve selected individuals of plants being raised in environments other than their 'native' habitats of conditions, are termed active biomonitoring (see Chapters 4, 5, 7; also Lovett Doust *et al.*, 1994b).

Effects of anthropogenic disturbance on submersed macrophytes

Increased industrialization is associated with greater consumption of energy, services and products; this typically produces a great amount of wastes, often toxic, and pollutes surface and ground water resources. Recently, with programs eliminating the release of persistent toxic substances, most management strategies focus on pollution prevention rather than on the difficult and costly clean-up of contaminated aquatic systems (IJC, 1992). However, contaminants released in the past will continue impair aquatic ecosystems for a long time. In particular, organochlorine compounds (e.g., PCBs) decay at a very slow rate and may be cycled in aquatic systems for many years (Diamond *et al.*, 1996).

Submersed macrophytes are affected by abiotic factors characteristic of the site of their growth. Any contaminants present in the ecosystems have potential to affect plant growth. Increased contaminant concentration may cause increase in plant mortality, change in species composition, impair plant growth and reproduction and also decrease plant biomass (see Chapters 1, 2, 4, and 7; Lovett Doust *et al.*, 1994a,b; Barko *et al.*, 1986, 1991; Crowder and Painter, 1991). These changes will have effects on aquatic organisms that depend on plants as a substrate, habitat, or source of organic carbon and

energy (Wetzel, 1995).

Contaminant transfer through submersed macrophytes

Contaminants present in aquatic environments are associated primarily with sediments (Burton, 1991, 1992; Diamond *et al.*, 1996). Of all the biota, aquatic plants constitute the greatest biomass (see Edwards *et al.*, 1989; Trapp and Mc Farlane, 1995). As a result, although, contaminant concentrations in plant tissues may be lower than that found in animal tissues, the total mass of contaminants associated with plants will be greater than those associated with animals (Moriarty, 1990; Trapp and Mc Farlane, 1995). Surprisingly, plants are seldom included in mass balance models developed for aquatic systems, despite the fact that they may carry the greatest load of contaminants compared to other organisms and despite their ecological significance. Remediation plans, focused upon the cleanup of highly contaminated areas and based on mass balance models developed without knowledge of plant contaminant burdens, may lead to disappointing results, public distrust and frustration with scientific research.

Significance of aquatic macrophytes in highly disturbed areas of aquatic ecosystems

Recently, many restoration projects of aquatic ecosystems and remediation programs have been initiated worldwide (e.g., National Research Council, 1992). As anticipated, in most cases successful restoration of animal populations in impaired areas has been significantly dependent upon local plant populations, their qualities and quantities. In numerous remedial actions, plant community restoration has been shown to be a basic and absolutely necessary step for successful restoration of animal communities. Services provided by aquatic macrophytes to other biota (see Chapter 1) are of critical value for the restoration of herbivory and predation within aquatic food webs (National Research Council, 1992; Leslie and Timmins, 1995; Klinge *et al.*, 1995). Remedial and restoration programs focused on top predators only (e.g., the commercially significant top consumers in aquatic food chains) may temporarily repair the effects of ecosystem impairment, but in the long-term continuous management of such "restored" systems will

be required.

If the goal of environmental management is to restore and develop sustainable aquatic ecosystems, the fundamental importance of aquatic plants needs to be acknowledged (Petr, 1993; Klinge *et al.*, 1995; Korschgen and Green, 1988). The quality and quantity of aquatic macrophytes will directly determine the subsequent quality and quantity of animal populations, including most commercially significant species (National Research Council, 1992; Leslie and Timmins, 1995).

Aquatic macrophytes that evolve tolerance to contaminants present in aquatic ecosystems may be of particular value (Moriarty, 1990; Lovett Doust *et al.*, 1994b; Biernacki *et al.*, 1996). Resistant plants are able to grow in highly contaminated and impaired areas and may form the basis for natural recovery of aquatic ecosystems. Tolerant plants may have also potential for phytoremediation of contaminant-impaired sediments and water (Salt *et al.*, 1995; Anderson *et al.*, 1995; Cunningham *et al.*, 1996).

The ability of plants to tolerate toxic compounds (like metals) has been studied for a long time (Antonovics *et al.*, 1979). The basis of any tolerance by aquatic plants to anthropogenic organic contaminants remains largely unexplored (Moriarty, 1990; Lovett Doust *et al.*, 1994b). On the basis of a relatively small number of studies, it is believed that plants metabolize most xenobiotics in a similar way (Trapp and Mc Farlane, 1992). Plants passively uptake xenobiotics by leaves and roots, transport the contaminants through xylem and phloem to different tissues and cells, where enzymes (like cytochrome P₄₅₀ monogenases and glutathione S-transferases) oxidize, hydrolyse and reduce them. Subsequently, enzyme-transformed xenobiotics are conjugated, and in the final phase, transformed contaminants are compartmentalized in plant vacuoles or the cell wall (Trapp and Mc Farlane, 1992). This general metabolic pathway of contaminant transfer suggests that potentially plants may be tolerant of a broad range of anthropogenic contaminants, rather than just selected compounds (see Chapter 7). In contrast, studies of tolerance of plants to toxic metals show a very specialised tolerance to some metals and no tolerance to others ("narrow" tolerance, see Antonovics *et al.*, 1979). Studies of plant tolerance to xenobiotics are clearly a fertile ground for future research.

Potential of *Vallisneria americana* as a biomonitor

As was shown in a number of studies, *V. americana* has great potential for biomonitoring in aquatic ecosystems. Measures of plant growth, development and reproduction have proved to be useful in this situation. In particular, leaf-to-root surface area ratio in *Vallisneria* appears as a highly robust and reliable measure of environmental quality. The ratio was found to be unaffected by the year of biomonitoring and so may be used in making long term comparisons of environmental quality; also plant genotype did not affect this ratio. Using the leaf-to-root surface area ratio, it was possible to probe local environment within a short period of a week or two following exposure. It was found to be useful in passive biomonitoring of environmental quality in the field, as well as in active biomonitoring studies, in both field and laboratory experiments (Chapters 3, 4, and 6).

Advantages of using *Vallisneria* as a biomonitor

A submersed rooted plant, like *V. americana*, may have a number of advantages for biomonitoring. Submersed plants tend to accumulate greater concentrations of metals, pesticides and organochlorine contaminants than free-floating aquatic plants like *Lemna minor*, which has been used in toxicity tests (Swanson *et al.*, 1991; Lewis, 1993, 1995). Highly hydrophobic compounds, in particular, tend to accumulate in root tissue rather than in other tissues of submersed macrophytes (Guilizzoni, 1991; Harrass *et al.*, 1991; Lovett Doust *et al.*, 1994a). This may result from the fact that rooted macrophytes are exposed to sediment pore water and its associated pollutants, whereas non-rooted plants are only exposed to toxicants in the water column. Depending upon texture and composition, sediment may adsorb contaminants from the water column to much higher concentrations than that present in the water column (Giesy *et al.*, 1988; Moore *et al.*, 1991; Biernacki *et al.*, 1995a,b). Since sediment may have significantly higher concentrations of hydrophobic contaminants than the water column, exposure of free-floating plants to water-column-borne pollutants may significantly underestimate levels of contaminant exposure of rooted macrophytes, and, subsequently through other

compartments of the food web, to herbivorous or detritivorous food chains (Stewart *et al.*, 1992; Lovett Doust *et al.*, 1994a; Wetzel, 1995).

The technical simplicity of biomonitoring protocols allows for multiple data collections from large samples of *Vallisneria* ramets, non-destructively over the growing season, perhaps using genetically identical plants (clones). Extensive vegetative reproduction in *Vallisneria* allows one to produce particular genotypes (e.g., larger or smaller individuals, tolerant to particular contaminants or non-tolerant, male or female) depending upon needs. Uniform genotypes of plants used may increase reliability, accuracy and precision of biomonitoring (Chapters 7 and 8; Lovett Doust *et al.*, 1994b). A well-designed biomonitoring study using *V. americana* could yield important data in North American Areas of Concern of the Great Lakes to monitor progress in remediation in these areas. These areas, as the most contaminated "hotspots", support lower densities of animals; often there have been problems obtaining representative sample sizes and proper replication in studies using animal biomonitorors at these sites (e.g., Pugsley *et al.*, 1985; Suns *et al.*, 1993; Minns *et al.*, 1994). Using clonally reproduced organisms for biomonitoring studies, minimizes impact on the genetic diversity of the studied organism. If unique individuals of an organism are sampled destructively from impaired areas, they are sacrificed and removed from populations.

Freely-moving animal species may have different contaminant levels despite being sampled at the same areas (Rowan and Rasmussen, 1992; Madenjian *et al.*, 1994). Cageing animals that are normally freely-moving for use in biomonitoring studies, may significantly alter their contaminant burdens because stress, artificially changed behaviour, or limited access to natural food items and sites used for cover or refuge. By contrast, using aquatic plants of controlled genotypes planted in particular sediment, allows for monitoring precisely a particular spot.

Vallisneria is native to the North American continent, but also to other continents (see Lowden, 1982) and there are few restrictions to their distribution and establishment. It is even highly desirable for restoration and reestablishment of beds of aquatic vegetation important to wildlife (Korschgen and Green, 1988). Compared to exotics,

Vallisneria may be legally used in different countries, in the field as well as in laboratories.

Possible directions for future studies

Increasingly, the potential of aquatic macrophytes to neutralize and remove nutrients and toxic substances from the water column and from sediment have been studied and used in a number of biotechnologies (Dunbabin and Bowmer, 1992; de Casabianca-Chassany *et al.*, 1992; Brix, 1993; Anderson *et al.*, 1995). Phytoremediation, using submersed plants, may have potential for the remedial process, in natural systems as well as in artificial wetlands. There are some suggestions that abilities of aquatic vascular plants for bioremediation may be greatly increased in systems where plants are associated with microorganisms (Anderson *et al.*, 1995; Cunningham *et al.*, 1996). This area remains largely unexplored. Biomonitoring studies associated with these new remedial techniques may be used to monitor optimum plant harvest time, to signal the presence of intermediate products of contaminant decay, and also to monitor progress of the remediation process.

Increased knowledge about the effects of aquatic macrophytes on aquatic ecosystems is associated with an increased need for information about the effects of anthropogenic factors on their biology. Aquatic plants are increasingly being included on lists of nontarget plant tests required for registration of newly introduced chemicals. Despite an urgent need for aquatic macrophytes assays, progress in this area is slow (but see Chapter 8; also Boutin *et al.*, 1995).

Studies of macrophyte resistance to organic contaminants may have potential to increase our general understanding of modes of resistance to anthropogenic contaminants (e.g., narrow or broad resistance to contaminants), and may increase knowledge about the genetic basis of such resistance in plants, and may also have an impact on development of new biotechnologies involving resistant organisms (in bioremedial technologies). Studies of genetic diversity of resistant organisms should be of value for development of effective biodiversity conservation plans. Resistant plants could form the base of natural recovery

of stressed ecosystems and support local fauna in highly impaired systems.

Information about the effects of contaminants on aquatic macrophytes and contaminant bioaccumulation in plant tissues may also form the basis for development of contaminant mass models for aquatic ecosystems. In littoral areas, but also in many aquatic ecosystems, macrophytes may dominate total biomass. Thus, contaminants associated with plants may also dominate compared to the mass of contaminants associated with other biota found in local food webs. Ignoring contaminants associated with plants may significantly underestimate contaminant movement through ecosystems and decrease realism and predictive values of mass balance models. At present, mass balance models of contaminant fate, developed for remedial purposes, and using food chain models do not include vascular plants at all, despite the great biomass of vascular macrophytes (e.g., see model developed for Bay of Quinte in Lake Ontario, by Diamond *et al.*, 1996; this area is known to be densely covered by submersed macrophytes [see Crowder and Painter, 1991]).

In the area of environmental management, the words “species control” have been used to describe methods of removal or destruction of a particular species at particular locations. At present, newly developed restoration projects expand the meaning of “species control”; often it may express the need to re-establish locally extinct or threatened native species (National Research Council, 1992). Planned wetland restoration projects will require more detailed information about macrophyte biology and cultivation. There is an urgent need to develop efficient technologies and methods for the re-establishment of aquatic plants in the field, in such ways that they have a chance to withstand competition from exotics and stresses associated with anthropogenically altered environment. Unfortunately, the destruction of species and plant communities is more likely than their recovery.

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APPENDIX

Table A. Mean values and standard errors of selected measures of plant growth in TCE exposure experiment (Chapter 2).

TCE Treatment	Leaf Cohort	Leaf Lifespan	SE
CONTROL	1	70.681	9.895
CONTROL	2	92.229	12.912
CONTROL	3	103.569	14.500
CONTROL	4	93.103	13.034
CONTROL	5	89.94	12.592
CONTROL	6	98.844	13.838
CONTROL	7	90.465	12.665
CONTROL	8	107.465	15.045
CONTROL	9	105.576	14.781
CONTROL	10	92.959	13.014
CONTROL	11	82.000	11.48
CONTROL	12	78.485	10.988
CONTROL	13	80.333	11.247

CONTROL	14	84.000	11.760
CONTROL	15	76.000	10.64
CONTROL	16		
CONTROL	17		
CONTROL	18		
CONTROL	19		
CONTROL	20		
CONTROL	21		
CONTROL	22		
LOW	1	57.013	9.692
LOW	2	84.833	14.422
LOW	3	84.856	14.425
LOW	4	83.569	14.207
LOW	5	82.382	14.005
LOW	6	79.71	13.551
LOW	7	62.143	10.564
LOW	8	79.286	13.479
LOW	9	65.278	11.097
LOW	10	61.524	10.459
LOW	11	33.091	5.625
LOW	12	39.322	6.685
LOW	13	36.825	6.260

LOW	14	32.256	5.484
LOW	15	28.000	4.760
LOW	16	26.324	4.475
LOW	17	23.476	3.991
LOW	18	21.039	3.577
LOW	19	16.388	2.786
LOW	20	11.023	1.874
LOW	21	8.659	1.472
LOW	22		
MEDIUM	1	58.693	10.565
MEDIUM	2	72.422	13.036
MEDIUM	3	72.667	13.080
MEDIUM	4	73.173	13.171
MEDIUM	5	75.358	13.564
MEDIUM	6	64.714	11.649
MEDIUM	7	77.556	13.960
MEDIUM	8	90.373	16.267
MEDIUM	9	73.655	13.258
MEDIUM	10	70.078	12.614
MEDIUM	11	45.481	8.187
MEDIUM	12	50.159	9.029
MEDIUM	13	50.909	9.164

MEDIUM	14	48.000	8.640
MEDIUM	15	47.000	8.460
MEDIUM	16	38.132	6.864
MEDIUM	17	32.947	5.930
MEDIUM	18	28.672	5.161
MEDIUM	19	19.371	3.487
MEDIUM	20	14.695	2.645
MEDIUM	21		
MEDIUM	22		
HIGH	1	42.453	8.066
HIGH	2	38.509	7.317
HIGH	3	35.474	6.740
HIGH	4	31.798	6.042
HIGH	5	25.817	4.905
HIGH	6	28.772	5.467
HIGH	7	39.75	7.553
HIGH	8	36.364	6.909
HIGH	9	36.444	6.924
HIGH	10	32.352	6.147
HIGH	11	27.958	5.312
HIGH	12	26.556	5.046
HIGH	13	25.048	4.759

HIGH	14	25.778	4.898
HIGH	15	23.2	4.408
HIGH	16	17.355	3.297
HIGH	17	14.558	2.766
HIGH	18	12.476	2.370
HIGH	19	9.788	1.860
HIGH	20	8.232	1.564
HIGH	21		
HIGH	22		

Table A. Continued

Treatment	TCE	Week of Exposure	Number of ramets per genets produced by			
			Turkey	Ecarte	Plants	SE
CONTROL	1	0.875	0.16625	0.896	0.188	
CONTROL	2	1.000	0.19	0.937	0.196	
CONTROL	3	1.104	0.20976	1.083	0.227	
CONTROL	4	1.292	0.24548	1.229	0.258	
CONTROL	5	1.458	0.27702	1.313	0.275	
CONTROL	6	1.625	0.30875	1.354	0.284	
CONTROL	7	1.896	0.36024	1.438	0.301	
CONTROL	8	2.229	0.42351	1.688	0.354	
CONTROL	9	2.396	0.45524	1.75	0.367	
CONTROL	10	2.521	0.47899	1.917	0.402	
CONTROL	11	2.667	0.50673	2.083	0.437	
CONTROL	12	2.729	0.51851	2.063	0.433	
CONTROL	13	3.792	0.72048	2.021	0.424	
CONTROL	14	2.958	0.56202	2.021	0.424	
CONTROL	15	2.729	0.51851	2.001	0.420	

CONTROL	16	2.501	0.475	1.833	0.384
CONTROL	17	2.512	0.475	2.042	0.428
CONTROL	18	2.417	0.45923	1.729	0.363
CONTROL	19	2.396	0.45524	1.729	0.363
CONTROL	20	2.229	0.42351	1.479	0.310
CONTROL	21	2.104	0.39976	1.667	0.350
CONTROL	22	1.646	0.31274	1.146	0.240
CONTROL	23	1.396	0.26524	0.813	0.170
CONTROL	24	0.521	0.09899	0.25	0.052
CONTROL	25	0.188	0.03572	0.104	0.021
LOW	1	0.833	0.15827	0.875	0.183
LOW	2	0.917	0.17423	0.958	0.201
LOW	3	1.063	0.20197	1.104	0.231
LOW	4	1.104	0.20976	1.188	0.249
LOW	5	1.271	0.24149	1.271	0.266
LOW	6	1.313	0.24947	1.313	0.275
LOW	7	1.354	0.25726	1.271	0.266
LOW	8	1.313	0.24947	1.063	0.223
LOW	9	1.313	0.24947	1.083	0.227
LOW	10	1.396	0.26524	1.042	0.218
LOW	11	1.396	0.26524	1.042	0.218
LOW	12	1.417	0.26923	1.042	0.218

LOW	13	1.417	0.26923	1.042	0.218
LOW	14	1.375	0.26125	1.000	0.210
LOW	15	1.375	0.26125	1.000	0.210
LOW	16	1.438	0.27322	1.000	0.210
LOW	17	1.438	0.27322	1.000	0.210
LOW	18	1.438	0.27322	1.000	0.210
LOW	19	1.625	0.30875	1.000	0.210
LOW	20	1.417	0.26923	1.000	0.210
LOW	21	1.354	0.25726	1.000	0.210
LOW	22	1.188	0.22572	0.938	0.196
LOW	23	1.083	0.20577	0.813	0.170
LOW	24	0.542	0.10298	0.271	0.056
LOW	25	0.375	0.07125	0.229	0.048
MEDIUM	1	0.833	0.15827	1.000	0.210
MEDIUM	2	1.021	0.19399	1.167	0.245
MEDIUM	3	1.083	0.20577	1.292	0.271
MEDIUM	4	1.188	0.22572	1.479	0.310
MEDIUM	5	1.375	0.26125	1.583	0.332
MEDIUM	6	1.417	0.26923	1.583	0.332
MEDIUM	7	1.417	0.26923	1.292	0.271
MEDIUM	8	1.271	0.24149	1.208	0.253
MEDIUM	9	1.25	0.2375	1.271	0.266

MEDIUM	10	1.333	0.25327	1.271	0.266
MEDIUM	11	1.375	0.26125	1.313	0.275
MEDIUM	12	1.396	0.26524	1.375	0.288
MEDIUM	13	1.396	0.26524	1.417	0.297
MEDIUM	14	1.417	0.26923	1.396	0.293
MEDIUM	15	1.438	0.27322	1.354	0.284
MEDIUM	16	1.5	0.285	1.292	0.271
MEDIUM	17	1.5	0.285	1.271	0.266
MEDIUM	18	1.479	0.28101	1.25	0.262
MEDIUM	19	1.438	0.27322	1.25	0.262
MEDIUM	20	1.396	0.26524	1.188	0.249
MEDIUM	21	1.375	0.26125	1.104	0.231
MEDIUM	22	1.188	0.22572	0.771	0.161
MEDIUM	23	1.042	0.19798	0.583	0.122
MEDIUM	24	0.562	0.10678	0.208	0.043
MEDIUM	25	0.396	0.07524	0.208	0.043
HIGH	1	0.792	0.15048	0.854	0.179
HIGH	2	0.917	0.17423	1.021	0.214
HIGH	3	0.979	0.18601	1.25	0.262
HIGH	4	1.042	0.19798	1.292	0.271
HIGH	5	1.229	0.23351	1.313	0.275
HIGH	6	1.188	0.22572	1.188	0.249

HIGH	7	0.958	0.18202	0.938	0.196
HIGH	8	0.75	0.1425	0.688	0.144
HIGH	9	0.479	0.09101	0.646	0.135
HIGH	10	0.438	0.08322	0.5	0.105
HIGH	11	0.375	0.07125	0.375	0.078
HIGH	12	0.479	0.09101	0.313	0.065
HIGH	13	0.458	0.08702	0.313	0.065
HIGH	14	0.458	0.08702	0.313	0.065
HIGH	15	0.5	0.095	0.313	0.065
HIGH	16	0.5	0.095	0.271	0.056
HIGH	17	0.5	0.095	0.271	0.056
HIGH	18	0.5	0.095	0.271	0.056
HIGH	19	0.5	0.095	0.271	0.056
HIGH	20	0.479	0.09101	0.208	0.043
HIGH	21	0.437	0.08303	0.208	0.043
HIGH	22	0.375	0.07125	0.187	0.039
HIGH	23	0.333	0.06327	0.187	0.039
HIGH	24	0.188	0.03572	0.062	0.013
HIGH	25	0.062	0.01178	0.000	0.000

Table B. Mean values and standard errors of selected measures of plant growth in short-term TCE exposure study (Chapter 3).

TCE Treatment	Day of Exposure	Leaf-to-root Surface Area Ratio	SE
CONTROL	0	2.85	0.28
CONTROL	1	2.85	0.28
CONTROL	2	2.86	0.28
CONTROL	3	2.86	0.28
CONTROL	4	2.86	0.28
CONTROL	5	2.88	0.28
CONTROL	6	2.88	0.28
CONTROL	8	2.89	0.28
CONTROL	10	2.92	0.29
CONTROL	14	2.93	0.29
CONTROL	20	2.94	0.29
CONTROL	30	2.94	0.29
CONTROL	40	2.94	0.29
CONTROL	60	2.95	0.29
MEDIUM	0	2.86	0.28
MEDIUM	1	3.6	0.36

MEDIUM	2	4.8	0.48
MEDIUM	3	5.6	0.56
MEDIUM	4	5.9	0.59
MEDIUM	5	6.12	0.61
MEDIUM	6	6.18	0.61
MEDIUM	8	6.22	0.62
MEDIUM	10	6.3	0.63
MEDIUM	14	6.38	0.63
MEDIUM	20	6.4	0.64
MEDIUM	30	6.41	0.64
MEDIUM	40	6.43	0.64
MEDIUM	60	6.44	0.64
HIGH	0	2.83	0.28
HIGH	1	4.7	0.47
HIGH	2	5.86	0.58
HIGH	3	6.52	0.65
HIGH	4	7.41	0.74
HIGH	5	7.9	0.79
HIGH	6	8.12	0.81
HIGH	8	8.16	0.81
HIGH	10	8.2	0.82
HIGH	14	8.24	0.82

HIGH	20	8.27	0.82
HIGH	30	8.29	0.82
HIGH	40	8.3	0.83
HIGH	60	8.31	0.83

Table C. Mean values and standard errors of selected measures of plant growth in long-term reciprocal-transplant-replant experiment (Chapters 4 and 5).

Year	Site of Exposure		Source of Sediment	Origin of Plants	Plant		Leaf	
					Density	SE	Density	SE
1991	Ecarte	Ecarte	Ecarte	Ecarte	228.956	41.212	1252.525	300.606
1991	Ecarte	Ecarte	Turkey	Turkey	215.488	38.788	1407.407	337.778
1991	Ecarte	Turkey	Ecarte	Ecarte	612.795	110.303	2882.155	691.717
1991	Ecarte	Turkey	Turkey	Turkey	808.081	145.455	5569.024	1336.566
1992	Ecarte	Ecarte	Ecarte	Ecarte	377.104	67.879	1643.098	394.343
1992	Ecarte	Ecarte	Turkey	Turkey	107.744	19.394	511.785	122.828
1992	Ecarte	Turkey	Ecarte	Ecarte	505.051	90.909	2040.404	489.697
1992	Ecarte	Turkey	Turkey	Turkey	343.434	61.818	1690.236	405.657
1993	Ecarte	Ecarte	Ecarte	Ecarte	350.168	63.03	1373.737	329.697
1993	Ecarte	Ecarte	Turkey	Turkey	255.892	46.061	1185.185	284.444
1993	Ecarte	Turkey	Ecarte	Ecarte	289.562	52.121	1326.599	318.384
1993	Ecarte	Turkey	Turkey	Turkey	1326.599	238.788	6040.404	1449.697
1994	Ecarte	Ecarte	Ecarte	Ecarte	363.231	65.382	1373.737	329.697
1994	Ecarte	Ecarte	Turkey	Turkey	235.432	42.378	1185.185	284.444
1994	Ecarte	Turkey	Ecarte	Ecarte	578.561	104.141	1326.599	318.384

1994	Ecarte	Turkey	Turkey	356.599	64.188	6040.404	1449.697
1991	Turkey	Ecarte	Ecarte	760.943	136.97	3144.781	754.747
1991	Turkey	Ecarte	Turkey	1333.333	240	5744.108	1378.586
1991	Turkey	Turkey	Ecarte	579.125	104.242	2296.296	551.111
1991	Turkey	Turkey	Turkey	1548.821	278.788	6498.316	1559.596
1992	Turkey	Ecarte	Ecarte	282.828	50.909	1286.195	308.687
1992	Turkey	Ecarte	Turkey	350.168	63.03	1346.801	323.232
1992	Turkey	Turkey	Ecarte	397.306	71.515	1663.3	399.192
1992	Turkey	Turkey	Turkey	208.754	37.576	801.347	192.323
1993	Turkey	Ecarte	Ecarte	323.232	58.182	1164.983	279.596
1993	Turkey	Ecarte	Turkey	235.69	42.424	821.549	197.172
1993	Turkey	Turkey	Ecarte	404.04	72.727	1723.906	413.737
1993	Turkey	Turkey	Turkey	181.818	32.727	565.657	135.758
1994	Turkey	Ecarte	Ecarte	323.232	58.182	1164.983	279.596
1994	Turkey	Ecarte	Turkey	235.69	42.424	821.549	197.172
1994	Turkey	Turkey	Ecarte	404.04	72.727	1723.906	413.737
1994	Turkey	Turkey	Turkey	181.818	32.727	565.657	135.758

Table C. Continued.

Year	Site of Exposure	Source of Sediment	Origin of Plants	Cumulative Leaf		Mean	
				Plants	Length	SE	Ramets
1991	Ecarte	Ecarte	Ecarte	7703.703	1771.852	188.552	35.8249
1991	Ecarte	Ecarte	Turkey	11097.643	2552.458	343.434	65.2525
1991	Ecarte	Turkey	Ecarte	16828.282	3870.505	444.444	84.4444
1991	Ecarte	Turkey	Turkey	50693.602	1165.529	1515.152	287.879
1992	Ecarte	Ecarte	Ecarte	9010.101	2072.323	202.02	38.3838
1992	Ecarte	Ecarte	Turkey	4202.020	966.465	204.04	38.7677
1992	Ecarte	Turkey	Ecarte	11865.319	2729.024	362.29	68.835
1992	Ecarte	Turkey	Turkey	17892.255	4115.219	655.219	124.492
1993	Ecarte	Ecarte	Ecarte	9070.707	2086.263	159.663	30.336
1993	Ecarte	Ecarte	Turkey	11292.929	2597.374	316.633	60.1603
1993	Ecarte	Turkey	Ecarte	8202.020	1886.465	177.374	33.701
1993	Ecarte	Turkey	Turkey	94801.346	21804.31	2808.081	533.535
1994	Ecarte	Ecarte	Ecarte	9070.707	2086.263	159.663	30.336
1994	Ecarte	Ecarte	Turkey	11292.929	2597.374	316.633	60.1603
1994	Ecarte	Turkey	Ecarte	8202.020	1886.465	177.374	33.701
1994	Ecarte	Turkey	Turkey	94801.346	21804.31	2808.081	533.535

1991	Turkey	Ecarte	Ecarte	19333.333	4446.667	666.667	126.667
1991	Turkey	Ecarte	Turkey	65737.373	1511.596	3400.673	646.128
1991	Turkey	Turkey	Ecarte	14659.932	3371.785	565.657	107.475
1991	Turkey	Turkey	Turkey	129966.329	2989.256	5245.791	996.7
1992	Turkey	Ecarte	Ecarte	17016.835	3913.872	522.559	99.2862
1992	Turkey	Ecarte	Turkey	18026.936	4146.195	555.556	105.556
1992	Turkey	Turkey	Ecarte	18949.494	4358.384	634.343	120.525
1992	Turkey	Turkey	Turkey	12060.606	2773.939	510.438	96.9832
1993	Turkey	Ecarte	Ecarte	16121.212	3707.879	580.471	110.29
1993	Turkey	Ecarte	Turkey	10720.538	2465.724	637.71	121.165
1993	Turkey	Turkey	Ecarte	24875.420	5721.347	801.347	152.256
1993	Turkey	Turkey	Turkey	7037.037	1618.519	533.333	101.333
1994	Turkey	Ecarte	Ecarte	16121.212	3707.879	580.471	110.29
1994	Turkey	Ecarte	Turkey	10720.538	2465.724	637.71	121.165
1994	Turkey	Turkey	Ecarte	24875.420	5721.347	801.347	152.256
1994	Turkey	Turkey	Turkey	7037.037	1618.519	533.333	101.333

Table D. Mean values and standard errors of selected measures of plant growth in field survey of *Vallisneria* (Chapter 6).

Site	Depth	Water	Leaf-to-root Surface	SE
		Area Ratio		
ECARTE	0.5	18		1.6
ECARTE	1	28		2.4
ECARTE	1.5	28		2.5
ECARTE	2	30		2.7
ECARTE	2.5	31		2.7
ECARTE	3			
PECHE	0.5	4		0.4
PECHE	1	4		0.3
PECHE	1.5	5		0.4
PECHE	2	5		0.4
PECHE	2.5	6		0.5
PECHE	3	6		0.4
ROUGE	0.5	48		4.3
ROUGE	1	67		6.0
ROUGE	1.5			

ROUGE	2		
ROUGE	2.5		
ROUGE	3	.	
TURKEY	0.5	7	0.6
TURKEY	1	9	0.8
TURKEY	1.5	9	0.8
TURKEY	2	9	0.7
TURKEY	2.5	11	0.9
TURKEY	3	11	0.8

Table E. Mean values and standard errors of selected measures of plant growth in field study using selected genotypes of *Vallisneria* (Chapter 7).

Year	Site	Tolerance	Genet	Sex	Origin	Rate of Clonal	
						Growth	SE
1994	Peché	tolerant	1	female	Rouge	13.8	2.1
1994	Turkey	tolerant	1	female	Rouge	9.4	1.41
1994	Ecarte	tolerant	1	female	Rouge	8.1	1.315
1994	Trenton	tolerant	1	female	Rouge	6.4	1.1
1994	Rouge	tolerant	1	female	Rouge	6.1	0.915
1994	Peché	tolerant	2	male	Ecarte	10.6	1.7
1994	Turkey	tolerant	2	male	Ecarte	7.8	1.1
1994	Ecarte	tolerant	2	male	Ecarte	5.9	0.7
1994	Trenton	tolerant	2	male	Ecarte	4.3	0.8
1994	Rouge	tolerant	2	male	Ecarte	2.4	0.5
1994	Peché	tolerant	3	female	Ecarte	11.1	1.8
1994	Turkey	tolerant	3	female	Ecarte	8.3	1.1
1994	Ecarte	tolerant	3	female	Ecarte	5.7	0.7
1994	Trenton	tolerant	3	female	Ecarte	3.5	0.4
1994	Rouge	tolerant	3	female	Ecarte	2.9	0.4
1994	Peché	nontolerant	4	female	Ecarte	10.2	2.05

1994	Turkey	nontolerant	4	female	Ecarte	8.4	1.6
1994	Ecarte	nontolerant	4	female	Ecarte	6.3	0.8
1994	Trenton	nontolerant	4	female	Ecarte	2.1	0.5
1994	Rouge	nontolerant	4	female	Ecarte	0	0
1994	Peché	nontolerant	5	male	Ecarte	10.6	1.8
1994	Turkey	nontolerant	5	male	Ecarte	7.8	1.3
1994	Ecarte	nontolerant	5	male	Ecarte	5.9	0.95
1994	Trenton	nontolerant	5	male	Ecarte	0	0
1994	Rouge	nontolerant	5	male	Ecarte	0	0
1994	Peché	nontolerant	6	male	Turkey	6.4	1.4
1994	Turkey	nontolerant	6	male	Turkey	5.2	0.85
1994	Ecarte	nontolerant	6	male	Turkey	4.1	0.731
1994	Trenton	nontolerant	6	male	Turkey	0	0
1994	Rouge	nontolerant	6	male	Turkey	0	0
1995	Peché	tolerant	1	female	Rouge	10.1	2.2
1995	Turkey	tolerant	1	female	Rouge	8.1	1.41
1995	Ecarte	tolerant	1	female	Rouge	7.1	1.315
1995	Trenton	tolerant	1	female	Rouge	5.3	1.1
1995	Rouge	tolerant	1	female	Rouge	5.1	0.915
1995	Peché	tolerant	2	male	Ecarte	9.1	1.7
1995	Turkey	tolerant	2	male	Ecarte	6.5	1.1
1995	Ecarte	tolerant	2	male	Ecarte	5.5	0.7

1995	Trenton	tolerant	2	male	Ecarte	3.6	0.8
1995	Rouge	tolerant	2	male	Ecarte	0	0
1995	Peche	tolerant	3	female	Ecarte	9.9	1.8
1995	Turkey	tolerant	3	female	Ecarte	7.8	1.1
1995	Ecarte	tolerant	3	female	Ecarte	4.9	0.7
1995	Trenton	tolerant	3	female	Ecarte	2.8	0.4
1995	Rouge	tolerant	3	female	Ecarte	2.12	0.4
1995	Peche	nontolerant	4	female	Ecarte	8.8	2.05
1995	Turkey	nontolerant	4	female	Ecarte	6.8	1.6
1995	Ecarte	nontolerant	4	female	Ecarte	4.8	0.8
1995	Trenton	nontolerant	4	female	Ecarte	2.2	0.5
1995	Rouge	nontolerant	4	female	Ecarte	0	0
1995	Peche	nontolerant	5	male	Ecarte	9.2	1.8
1995	Turkey	nontolerant	5	male	Ecarte	6.3	1.3
1995	Ecarte	nontolerant	5	male	Ecarte	4.7	0.95
1995	Trenton	nontolerant	5	male	Ecarte	0	0
1995	Rouge	nontolerant	5	male	Ecarte	0	0
1995	Peche	nontolerant	6	male	Turkey	5.6	1.4
1995	Turkey	nontolerant	6	male	Turkey	4.7	0.85
1995	Ecarte	nontolerant	6	male	Turkey	2.8	0.731
1995	Trenton	nontolerant	6	male	Turkey	0	0
1995	Rouge	nontolerant	6	male	Turkey	0	0

Table E. Continued.

Year	Site	Tolerance	Genet	Sex	Origin	Ramets	Density of SE
1994	Peché	tolerant	1	female	Rouge	1392.6	222.816
1994	Turkey	tolerant	1	female	Rouge	1103.9	176.624
1994	Ecarte	tolerant	1	female	Rouge	586	93.76
1994	Trenton	tolerant	1	female	Rouge	393	62.88
1994	Rouge	tolerant	1	female	Rouge	311	54.56
1994	Peché	tolerant	2	male	Ecarte	1033	165.28
1994	Turkey	tolerant	2	male	Ecarte	908.2	145.312
1994	Ecarte	tolerant	2	male	Ecarte	321	51.36
1994	Trenton	tolerant	2	male	Ecarte	221	35.36
1994	Rouge	tolerant	2	male	Ecarte	132	21.12
1994	Peché	tolerant	3	female	Ecarte	1098.9	175.824
1994	Turkey	tolerant	3	female	Ecarte	976.6	156.256
1994	Ecarte	tolerant	3	female	Ecarte	362	57.92
1994	Trenton	tolerant	3	female	Ecarte	198	31.68
1994	Rouge	tolerant	3	female	Ecarte	165	26.4
1994	Peché	nontolerant	4	female	Ecarte	1085.7	173.712
1994	Turkey	nontolerant	4	female	Ecarte	927	148.32

1994	Ecarte	nontolerant	4	female	Ecarte	367	58.72
1994	Trenton	nontolerant	4	female	Ecarte	111	17.76
1994	Rouge	nontolerant	4	female	Ecarte	0	0
1994	Peche	nontolerant	5	male	Ecarte	1069.2	171.072
1994	Turkey	nontolerant	5	male	Ecarte	913.9	146.224
1994	Ecarte	nontolerant	5	male	Ecarte	354	56.64
1994	Trenton	nontolerant	5	male	Ecarte	0	0
1994	Rouge	nontolerant	5	male	Ecarte	0	0
1994	Peche	nontolerant	6	male	Turkey	661.65	105.864
1994	Turkey	nontolerant	6	male	Turkey	634.6	101.536
1994	Ecarte	nontolerant	6	male	Turkey	228	36.48
1994	Trenton	nontolerant	6	male	Turkey	0	0
1994	Rouge	nontolerant	6	male	Turkey	0	0
1995	Peche	tolerant	1	female	Rouge	1194	222
1995	Turkey	tolerant	1	female	Rouge	1022	187
1995	Ecarte	tolerant	1	female	Rouge	715	98
1995	Trenton	tolerant	1	female	Rouge	489	105
1995	Rouge	tolerant	1	female	Rouge	379	90
1995	Peche	tolerant	2	male	Ecarte	965	179
1995	Turkey	tolerant	2	male	Ecarte	833	133.28
1995	Ecarte	tolerant	2	male	Ecarte	321	79
1995	Trenton	tolerant	2	male	Ecarte	174	56

1995	Rouge	tolerant	2	male	Ecarte	0	0
1995	Peche	tolerant	3	female	Ecarte	1143	212
1995	Turkey	tolerant	3	female	Ecarte	976.6	175
1995	Ecarte	tolerant	3	female	Ecarte	362	87
1995	Trenton	tolerant	3	female	Ecarte	246	51
1995	Rouge	tolerant	3	female	Ecarte	193	49
1995	Peche	nontolerant	4	female	Ecarte	887	189
1995	Turkey	nontolerant	4	female	Ecarte	715	141
1995	Ecarte	nontolerant	4	female	Ecarte	267	62
1995	Trenton	nontolerant	4	female	Ecarte	78	36
1995	Rouge	nontolerant	4	female	Ecarte	0	0
1995	Peche	nontolerant	5	male	Ecarte	929	148.64
1995	Turkey	nontolerant	5	male	Ecarte	973	155.68
1995	Ecarte	nontolerant	5	male	Ecarte	314	57
1995	Trenton	nontolerant	5	male	Ecarte	0	0
1995	Rouge	nontolerant	5	male	Ecarte	0	0
1995	Peche	nontolerant	6	male	Turkey	916	166
1995	Turkey	nontolerant	6	male	Turkey	844	178
1995	Ecarte	nontolerant	6	male	Turkey	266	62
1995	Trenton	nontolerant	6	male	Turkey	0	0
1995	Rouge	nontolerant	6	male	Turkey	0	0

Table F. Mean values and standard errors of selected measures of plant growth in laboratory study using selected genotypes of *Vallisneria* (Chapter 8).

Sediment		Plant	
Source	Genet	Mass	SE
C	1	2.6	0.6
PI	1	2.7	0.5
TI	1	2.6	0.6
RR2	1	1.6	0.4
TC2	1	1.5	0.3
TC1	1	1.6	0.5
RR1	1	1.5	0.4
C	2	4.3	0.7
PI	2	4.2	0.6
TI	2	4.4	0.6
RR2	2	3.5	0.5
TC2	2	3.4	0.4
TC1	2	3.5	0.5
RR1	2	3.3	0.4
C	3	5.3	1.3

PI	3	5.4	1.2
TI	3	5.4	1.3
RR2	3	4.6	1.2
TC2	3	4.5	1.3
TC1	3	4.2	1.4
RR1	3	4.1	1.4
C	4	8.2	1.6
PI	4	8.4	1.4
TI	4	8.5	1.5
RR2	4	7.1	1.3
TC2	4	7.3	1.4
TC1	4	6.8	1.4
RR1	4	6.9	1.2
C	5	6.4	1.2
PI	5	6.3	1.2
TI	5	6.1	1.2
RR2	5	4.8	1.1
TC2	5	4.6	1.1
TC1	5	4.4	1.2
RR1	5	4.5	1.2
C	6	9.8	1.6
PI	6	10.1	1.3

TI	6	9.6	1.4
RR2	6	7.9	1.4
TC2	6	8.3	1.8
TC1	6	6.9	1.6
RR1	6	7.5	1.5

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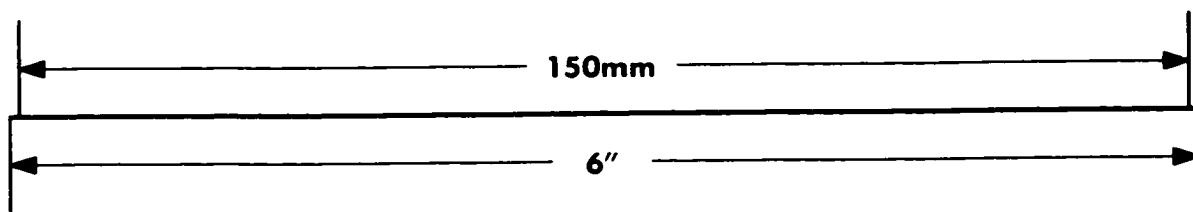
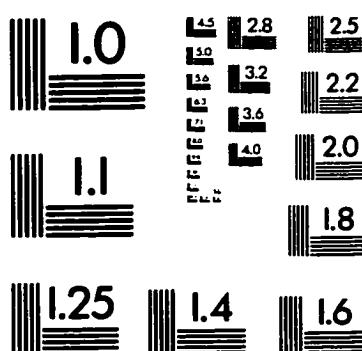
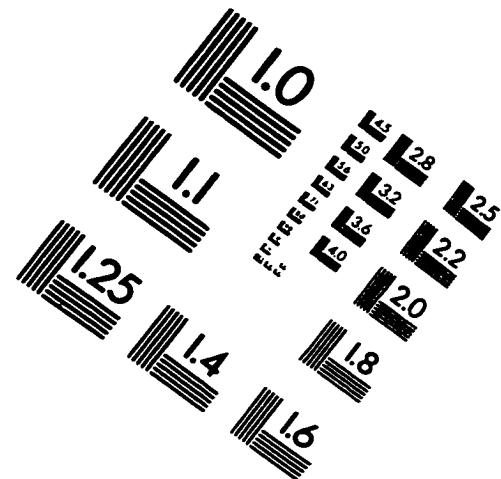
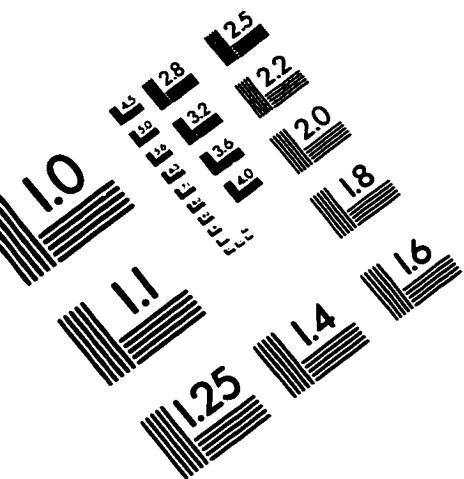
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IMAGE EVALUATION TEST TARGET (QA-3)



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