



US Army Corps
of Engineers



Smart

AQUATIC PLANT CONTROL
RESEARCH PROGRAM

TECHNICAL REPORT A-90-5

EFFECTS OF SALINITY AND IRRADIANCE
CONDITIONS ON THE GROWTH, MORPHOLOGY
AND CHEMICAL COMPOSITION OF SUBMERSED
AQUATIC MACROPHYTES

by

Robert R. Twilley

Department of Biology
University of Southwestern Louisiana
Lafayette, Louisiana 70504

and

John W. Barko

Environmental Laboratory

DEPARTMENT OF THE ARMY
Waterways Experiment Station, Corps of Engineers
3909 Halls Ferry Road, Vicksburg, Mississippi 39180-6199



July 1990

Final Report

Approved For Public Release: Distribution Unlimited

Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

Destroy this report when no longer needed. Do not return
it to the originator.

The findings in this report are not to be construed as an official
Department of the Army position unless so designated
by other authorized documents.

The contents of this report are not to be used for
advertising, publication, or promotional purposes.
Citation of trade names does not constitute an
official endorsement or approval of the use of
such commercial products.

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION Unclassified		1b. RESTRICTIVE MARKINGS			
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION / AVAILABILITY OF REPORT			
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE		Approved for public release; distribution unlimited.			
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Technical Report A-90-5		5. MONITORING ORGANIZATION REPORT NUMBER(S)			
6a. NAME OF PERFORMING ORGANIZATION See reverse.	6b. OFFICE SYMBOL (if applicable)	7a. NAME OF MONITORING ORGANIZATION			
6c. ADDRESS (City, State, and ZIP Code) See reverse.		7b. ADDRESS (City, State, and ZIP Code)			
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Corps of Engineers	8b. OFFICE SYMBOL (if applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER			
8c. ADDRESS (City, State, and ZIP Code) Washington, DC 20314-1000		10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) Effects of Salinity and Irradiance Conditions on the Growth, Morphology, and Chemical Composition of Submersed Aquatic Macrophytes					
12. PERSONAL AUTHOR(S) Twilley, Robert R.; Barko, John W.					
13a. TYPE OF REPORT Final report	13b. TIME COVERED FROM _____ TO _____	14. DATE OF REPORT (Year, Month, Day) July 1990	15. PAGE COUNT 29		
16. SUPPLEMENTARY NOTATION Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.					
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)			
FIELD	GROUP	SUB-GROUP			
		See reverse.			
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p>The growth, morphology, and chemical composition of <i>Hydrilla verticillata</i>, <i>Myriophyllum spicatum</i>, <i>Potamogeton perfoliatus</i>, and <i>Vallisneria americana</i> were compared among different salinity and light conditions. Plants were grown in microcosms (1.2 m³) under ambient photoperiod adjusted to 50 and 8 percent of solar radiation. The culture solution in five pairs of tanks was gradually adjusted to salinities of 0, 2, 4, 6, and 12 ppt. With the exception of <i>H. verticillata</i>, the aquatic macrophytes examined may be considered euryhaline species that are able to adapt to salinities one third the strength of seawater.</p> <p>With increasing salinity, the inflorescence production decreased in <i>M. spicatum</i> and <i>P. perfoliatus</i>, yet asexual reproduction in the latter species by underground buds remained constant. Stem elongation increased in response to shading in <i>M. spicatum</i>, while</p> <p style="text-align: right;">(Continued)</p>					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION Unclassified			
22a. NAME OF RESPONSIBLE INDIVIDUAL		22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL		

6. NAME AND ADDRESS OF PERFORMING ORGANIZATION (Continued).

University of Southwestern Louisiana,
 Department of Biology, Lafayette, LA 70504;
 USAEWES, Environmental Laboratory,
 3909 Halls Ferry Road, Vicksburg, MS 39180-6199

18. SUBJECT TERMS (Continued).

Epiphytes	<i>Myriophyllum spicatum</i>
Hydrilla	Osmoregulation
Ion exclusion	Reproduction
Microcosm	Tissue nutrients
Morphology	<i>Vallisneria americana</i>

19. ABSTRACT (Continued).

shaded *P. perfoliatus* had higher concentrations of chlorophyll *a*. In association with high epiphytic mass, chlorophyll *a* concentrations in all species were greatest at 12 ppt. The concentration of sodium increased in all four species of aquatic macrophytes examined here, indicating that these macrophytes did not possess mechanisms to exclude this ion. The nitrogen content (Y) of the aquatic macrophytes tested increased significantly with higher sodium concentration (X), suggesting that nitrogen may be used in osmoregulation ($Y = X * 0.288 + 6.10, r^2 = 0.71$).

The tolerance of *V. americana* and *P. perfoliatus* to salinity was greater in this study than in other investigations. This may be associated with experimental methodology, whereby macrophytes were subjected to more gradual rather than abrupt changes in salinity. *Myriophyllum spicatum* and *V. americana*, the two macrophytes that adapted best to the estuarine conditions in this study, as shown by growth under salinity up to 12 ppt, also exhibited a greater degree of response in morphology, tissue chemistry (including chlorophyll content and total nitrogen), and reproductive output in response to varying salinity and light conditions.

Preface

The study reported herein was sponsored by the Headquarters, US Army Corps of Engineers (HQUSACE), through the Aquatic Plant Control Research Program (APCRP). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed by the US Army Engineer Waterways Experiment Station (WES), under the Environmental Resources Research and Assistance Programs, Mr. J. Lewis Decell, Manager. Technical Monitor for the study was Mr. James W. Wolcott, HQUSACE.

Principal investigators for this study were Dr. Robert R. Twilley of the University of Southwestern Louisiana and Dr. John W. Barko of the Environmental Laboratory (EL), WES. Experimental design, data analysis, interpretation, and report preparation were accomplished by Drs. Barko and Twilley. Reviews of the report were provided by Drs. Douglas Gunnison and Thomas L. Hart of the EL. Additional reviews were provided anonymously by members of the editorial board of the journal Estuaries.

The study was conducted at the University of Maryland, Horn Point Laboratory. The following Laboratory personnel provided assistance: Mr. Jim Lynch, Ms. Janaea Little, Ms. Julie Metz, Ms. Lori Staver, Ms. Jane Caffrey, and Mr. Greg Steyer. Dr. Joe F. Nix of Ouachita Baptist University performed nutrient analyses at the WES. The report was edited by Ms. Jessica S. Ruff of the WES Information Technology Laboratory.

This investigation was performed under the general supervision of Dr. John Harrison, Chief, EL, and Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division, and under the direct supervision of Dr. Thomas L. Hart, Chief, Aquatic Processes and Effects Group.

Commander and Director of WES was COL Larry B. Fulton, EN. Technical Director was Dr. Robert W. Whalin.

This report should be cited as follows:

Twilley, Robert R., and Barko, John W. 1990. "Effects of Salinity and Irradiance Conditions on the Growth, Morphology, and Chemical Composition of Submersed Aquatic Macrophytes," Technical Report A-90-5, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Contents

	<u>Page</u>
Preface.....	1
Introduction.....	3
Materials and Methods.....	4
Results.....	7
Discussion.....	17
Conclusions and Recommendations.....	21
References.....	22

EFFECTS OF SALINITY AND IRRADIANCE CONDITIONS ON THE
GROWTH, MORPHOLOGY, AND CHEMICAL COMPOSITION
OF SUBMERSED AQUATIC MACROPHYTES

Introduction

1. In the past 30 years there have been dramatic changes in abundance and dominance of submersed aquatic vegetation in Chesapeake Bay and its tributaries (Bayley et al. 1978, Kemp et al. 1983, Orth and Moore 1983). In the tidal Potomac River the areal distribution of submersed macrophytes in 1981 was less than 25 percent of that in 1960 (Haramis and Carter 1983). This loss included 10 to 15 native species and the exotic *Myriophyllum spicatum* L., which had previously dominated the littoral zone of the Chesapeake Bay region. During 1983 and 1984 there was a resurgence of macrophytes in the tidal fresh-water zone of the Potomac River estuary, including *Ceratophyllum demersum*, *Vallisneria americana*, *Zannichella palustris*, *Potamogeton pectinatus*, *Heteranthera dubia*, and *Myriophyllum spicatum* (Rybicki et al. 1985).

2. Changes in distribution and dominance of submersed macrophytes in the Potomac River estuary and other tributaries of Chesapeake Bay have been associated with various causes, most notably changes in water quality (Kemp et al. 1983, Twilley et al. 1985). Although many of these changes in macrophyte communities have occurred in estuarine regions of tributaries, the significance of salinity as a water quality factor is unknown since its effect is confounded with the presence of other environmental conditions (Haramis and Carter 1983).

3. Along with the resurgence of submersed macrophytes in the tidal freshwater region of the Potomac River estuary was the introduction of a new species, monoecious *Hydrilla verticillata* (Steward et al. 1984, Rybicki et al. 1985). Because of the highly competitive nature of the dioecious biotype of this species, common in the southeastern United States, monoecious *H. verticillata* may outcompete native aquatic plants in the Potomac River and other regions of Chesapeake Bay.

4. Physiological and morphological adaptations of dioecious *H. verticillata* enable it to exist in low-light environments; furthermore, its formation of a dense canopy can inhibit the growth of other submersed macrophytes

(Bowes et al. 1977; Van et al. 1977; Barko and Smart; Barko, Hardin, and Matthews 1982). Yet, many of these adaptations have not been examined for monoecious *H. verticillata*, and one of the key questions concerning the distribution of this species in the Potomac River is its salinity tolerance. Light is another important consideration in relation to the distribution of submersed macrophytes in turbid estuaries.

5. The objective of this study was to compare the growth of *Hydrilla verticillata*, *Myriophyllum spicatum*, *Vallisneria americana*, and *Potamogeton perfoliatus* under various salinity and light conditions. Relative success among the species tested in this study was evaluated by comparing biomass (root and shoot), morphology, and reproduction. This approach was designed to examine plant response under controlled yet nearly ambient conditions to better predict their importance in the distribution of freshwater macrophytes in estuarine ecosystems.

Materials and Methods

6. An outdoor microcosm approach was used to study the influence of salinity and light on the growth of selected submersed aquatic macrophytes at Horn Point Environmental Laboratory, University of Maryland (38°54.4' N 76°0.7' W). Aquatic macrophytes were grown in ten 1.2-m³ fiberglass tanks (0.9 m W × 1.5 m L × 0.9 m D) maintained outdoors under ambient sunlight and temperature. Water in a pair of tanks was recirculated to a reservoir located in a laboratory, which reduced water temperature differences among each pair of tanks, and reservoirs were maintained at one of five salinities (0, 2, 4, 6, and 12 ppt). Neutral-density fiberglass screens were used to modify light to 50 and 8 percent of ambient for one of the tanks, respectively, in each pair. Water temperature was allowed to fluctuate to an upper temperature limit of 30° C controlled by circulating water through a heat exchanger. Air was continuously pumped into each reservoir and distributed through air stones.

7. *Vallisneria americana* Michx., *Myriophyllum spicatum* L., and the monoecious biotype of *Hydrilla verticillata* (L.f.) Caspary were collected on 18 July 1985 from the Potomac River adjacent to the Mount Vernon Parkway, and *Potamogeton perfoliatus* L. var. *bupleuroides* (Fern.) Farw. was collected from an impoundment adjacent to the Chesapeake Bay bridge. Each species was

planted in groups of five 10-cm-long plants in 80-cm² plastic containers. Each container held 450 ml of sediment collected from Kingston Landing on the Choptank River estuary (freshwater to 1-ppt region of the estuary). Sediment was sieved to pass a 1-mm mesh and mixed with sand, resulting in the following characteristics: bulk density, 0.79 g/cm³; sand composition, 36.6 percent; carbon and nitrogen concentrations, 4.89 and 0.40 percent dry mass, respectively.

8. Individuals of *M. spicatum* and *H. verticillata* used for most plantings were rooted branches, although some rootless material was used; *V. americana* shoots with attached roots were used in all plantings. Following planting, clean sand was added to the surface of each container to a depth of 2 cm to retard nutrient loss from the sediments and to minimize suspension of sediments during the placement of containers in the tanks (Smart and Barko 1985). Four containers of each species were randomly placed in each tank on 22 July and allowed to equilibrate in a culture solution (Smart and Barko 1985) for 1 week.

9. On 29 July, salinity adjustments were initiated using Instant Ocean (Aquarium Systems, Inc., Eastlake, OH) mixed with deionized water. Salinity adjustments were made at approximately 1 ppt per day up to 6 ppt, and then at 2 ppt per day up to the final salinity of 12 ppt (Figure 1).

10. Plants were harvested on 29 August, approximately 5 weeks following the initiation of the study. Aboveground portions of the plants were clipped at the sediment surface and rinsed; stem density and length were determined for each individual. Observations were made for reproductive structures, including inflorescences, turions, and belowground tubers. The apical 10 cm of single plants from three of the four containers was arbitrarily chosen, cleaned of epiphytes, and frozen for chlorophyll analysis. Roots and rhizomes were rinsed with tap water and collected in a 1-mm sieve. All plant samples were dried for several days at 60° C and weighed to 0.001 g.

11. Prior to harvesting, the epiphytic mass of each species was sampled in triplicate by gently placing individual intact plants into 1-l plastic containers. The containers were capped and shaken; any epiphytic mass remaining on the plants was removed by hand. The solution with epiphytes was stirred, while duplicate 50-ml aliquots were subsampled and filtered through preweighed and ashed glass fiber filters (1.1- μ particle size) for determination of total suspended solid (TSS) concentration. The filters were rinsed with distilled

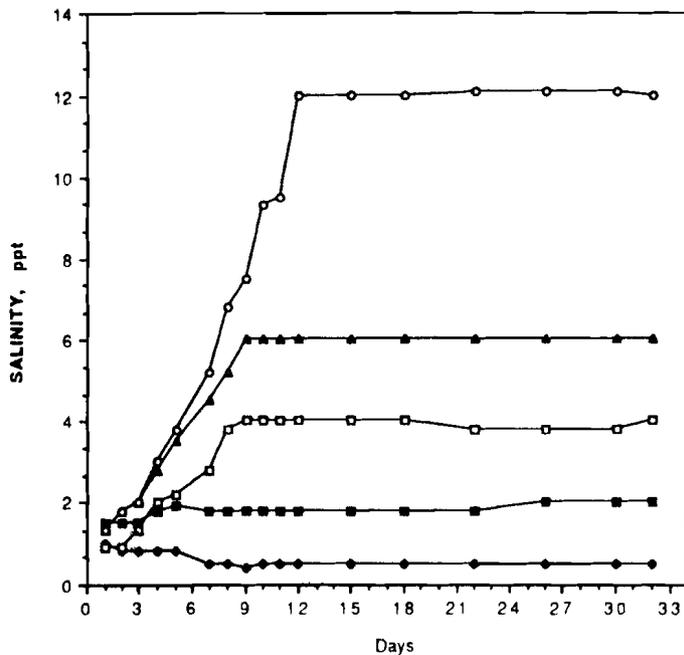


Figure 1. Measurements of salinity (in parts per thousand) in the reservoirs of five pairs of tanks

water to remove salt and were dried for 48 hr at 60° C. Levels of epiphytic material were corrected for background concentrations of TSS in tank water and calculated on the basis of ash-free dry mass (afdm) of host plants.

12. Plant samples were ground through a 40-mesh screen with a Wiley mill, and 1-g subsamples were ashed at 550° C overnight and weighed to 0.001 g. Elemental analyses of aboveground plant material were performed on wet digested samples using modified procedures from Allen et al.

(1974). Ammonium and phosphate were assayed on a Technicon Auto-Analyzer II system using standard techniques (US Environmental Protection Agency 1979). Potassium (K) and sodium (Na) were assayed by flame photometry on an atomic absorption spectrophotometer. The method and precision of the chemical analyses were as described in Barko and Smart (1983, p 164). Chlorophyll *a* and *b* were extracted from plant tissue ground in 90-percent acetone and assayed using the trichromatic method (Strickland and Parsons 1972) on a Beckman 510 spectrophotometer.

13. Attenuation of photosynthetically active radiation was measured with a LiCor 195S underwater sensor and meter. Salinity and temperature were monitored in reservoirs and tanks with a Beckman Osmometer, and pH with an Orion Model 407A meter and glass electrodes. Dissolved oxygen and temperature were measured with the Orbisphere Model 2603 polarographic electrode and meter system.

14. Two- and three-way analyses of variance (ANOVA) with interactions were computed using SAS (Statistical Analysis System 1982). Rank means from significant ANOVA were determined with Duncan's Multiple Range Test.

Results

15. Except for salinity, very few differences in water quality were noted among the tanks during the study (Figure 1, Table 1). Concentrations of ammonium, nitrite plus nitrate, and phosphate on 7 August, following an adjustment in salinity, were slightly higher in the 6- and 12-ppt treatments, but by less than 1 $\mu\text{g-at}/\ell$ (Table 1). During the remainder of the study, there was no trend for dissolved inorganic nitrogen among salinity treatments. Values of pH ranged from 8.70 to 9.30 and decreased with higher salinities.

16. The concentration of ash in the dry mass of all species increased significantly ($P < 0.05$) at higher salinity. In fresh water, nearly 80 percent of the dry mass of all the plants was ash free, but at 12 ppt the ash-free portion declined to less than 40 percent of the dry mass. Thus, the correction of biomass to ash-free dry mass was important to correct interpretation of our results. All values of biomass reported hereafter represent ash-free estimates and have also been corrected for the initial material planted in the pots.

17. Total biomass of *H. verticillata* and *P. perfoliatus* decreased with increasing salinity under both light conditions (Figure 2). Total ash-free dry mass of *H. verticillata* decreased by 80 percent between 0 and 12 ppt, while the decrease in *P. perfoliatus* was about 50 percent. Peak biomass of *M. spicatum* occurred at 12 ppt in the high-light treatment and was significantly greater than the aboveground mass at 0, 4, and 6 ppt. However, there were no differences in total biomass of this species among salinity treatments in the low-light condition. *Vallisneria americana* exhibited no significant differences in total biomass among the five salinity treatments in either light treatment.

18. Although salinity influenced the total biomass produced in only two of the species, it had a significant influence on root:shoot ratio in all four species (Figure 2). *Hydrilla verticillata* in both light levels and *P. perfoliatus* at the high-light level had significantly greater root:shoot ratios at 12 ppt. In contrast, *M. spicatum* and *V. americana* at both light levels exhibited significantly lesser root:shoot ratios than at 12 ppt, due primarily to a decrease in belowground biomass with increasing salinity.

19. Light was a significant factor in affecting both the total and belowground biomass of all species except *V. americana*; however, it had no

Table 1
Concentrations ($\mu\text{g-at}/\ell$) of Nutrients and pH of Water
in Each Salinity Treatment During the Study

<u>Date</u>	<u>Salinity, ppt</u>				
	<u>0</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>12</u>
	<u>Nitrite Plus Nitrate</u>				
7 Aug	0.07	0.06	0.17	0.13	0.98
14 Aug	0.08	0.04	0.17	0.14	0.34
	<u>Phosphate</u>				
7 Aug	0.00	0.00	0.05	0.00	0.12
14 Aug	0.10	0.00	0.19	0.00	0.24
20 Aug	0.56	0.12	0.08	0.18	0.27
	<u>Ammonium</u>				
7 Aug	0.09	0.33	0.05	0.73	0.29
14 Aug	0.27	0.29	0.10	0.49	0.21
20 Aug	0.62	1.03	0.39	0.40	0.40
27 Aug	1.63	0.21	0.61	0.61	0.48
	<u>pH</u>				
7 Aug	9.10	8.82	8.83	8.66	8.68
14 Aug	9.36	9.07	8.97	8.90	8.66
20 Aug	9.43	9.36	9.06	9.07	8.73
27 Aug	9.29	9.30	9.14	9.19	8.88

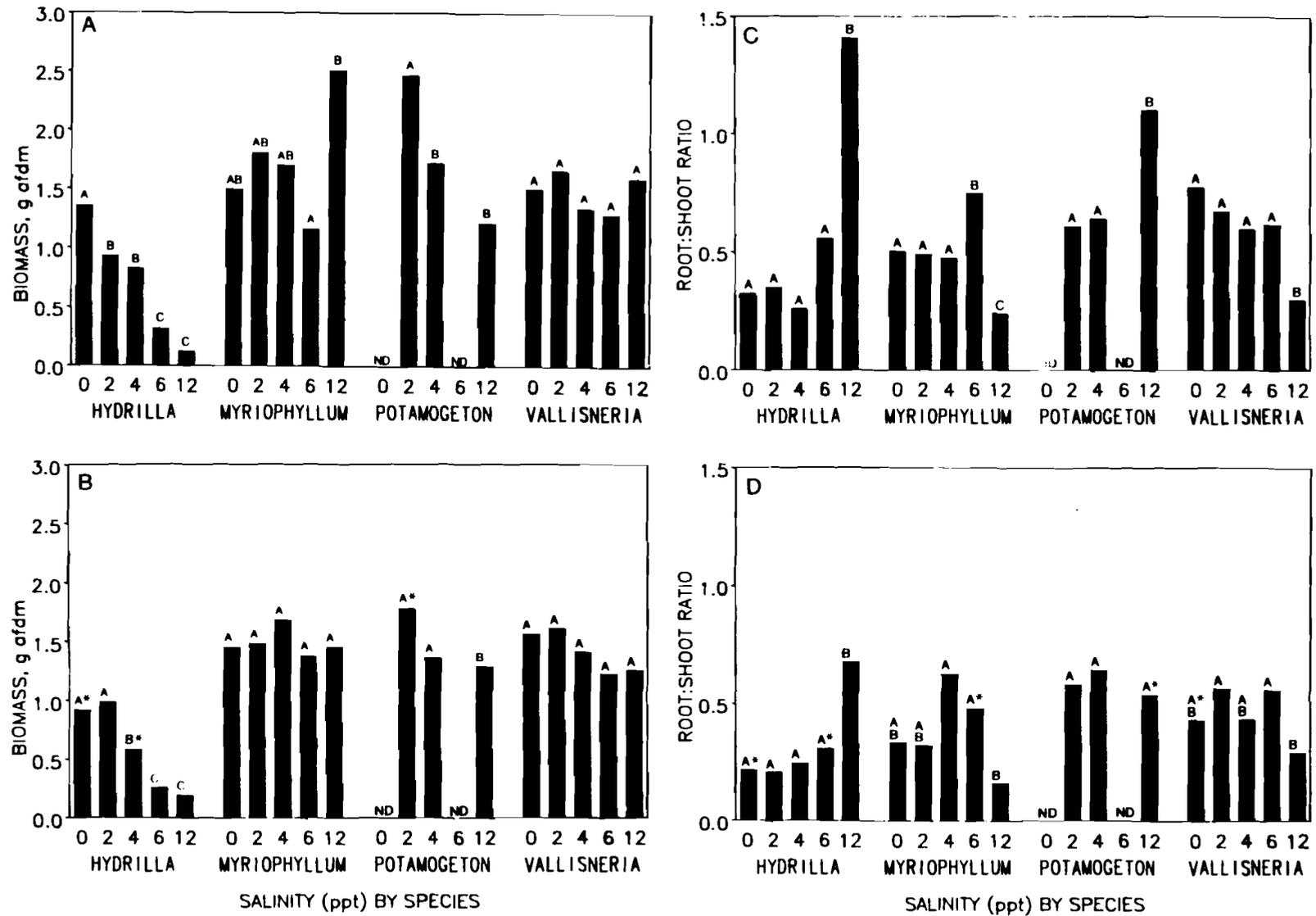


Figure 2. Total biomass (A and B, grams afdm/pot) and root:shoot ratio (C and D) of plant species at five salinities under high (A and C) and low (B and D) light levels. (ND, no data collected. Capital letters above bars denote significant differences among salinity treatments at $P < 0.05$; asterisk denotes significant difference ($P < 0.05$) between light treatments for each species and salinity effect)

influence on aboveground biomass of any species (Figure 2). In all species (across salinity levels), mean belowground biomass in the low-light treatments was less than that in the high-light treatments. Likewise, the root:shoot ratios in all species (across salinities) in the low-light treatment were less than in the pooled high-light treatment. When results of all salinities were pooled, there was a significant effect of light on the root:shoot ratio for all species, with lower ratios occurring in the low-light treatment.

20. Epiphytic mass increased to peak concentrations at 12 ppt for all species under both high- and low-light treatments (Figure 3). These increases were greatest for *H. verticillata* and *P. perfoliatus*, the two species most affected by salinity. Lowest levels of epiphytic mass were measured on *M. spicatum* and *V. americana*. In all four species at both light levels, significant differences in epiphytic mass occurred only at salinities of 6 or 12 ppt. Epiphytic mass was significantly lower in the low-light treatment than in the high for all species. Chlorophyll *a* concentrations in all species at the high-light treatment were greatest at 12 ppt (Figure 3). However, these differences were significant for *P. perfoliatus* and *M. spicatum*. No clear trends in chlorophyll *a* were observed with respect to salinity at the low-light level since data for 12 ppt existed only for *P. perfoliatus*.

21. The effects of light, salinity, and species on stem length were significant, but no effect was noted for stem density (Figure 4). Greatest stem lengths occurred in *M. spicatum* and *V. americana*, with values up to 40 cm/plant in the former occurring in the low-light treatments. The lowest stem lengths occurred in *H. verticillata*, with all values less than 20 cm/plant; in some treatments, stem lengths were less than 5 cm/plant. Stem lengths were generally greater in the low-light treatments for all four species, particularly for *M. spicatum* at the lower salinity treatments. However, at 12 ppt, the effect of light level on stem length was minimum. In general, stem density was much greater in *H. verticillata* and *V. americana* than in *P. perfoliatus* and *M. spicatum*; the latter species had the lowest stem densities, at less than 10 plants/pot.

22. No significant differences in the production of inflorescences were noted between light levels. *Myriophyllum spicatum* had the greatest number of inflorescences at all salinity levels, followed by *P. perfoliatus* (Figure 5). Few inflorescences were observed in *V. americana*, and none in *H. verticillata*. The highest number of inflorescences in *M. spicatum* occurred at 4 ppt. At

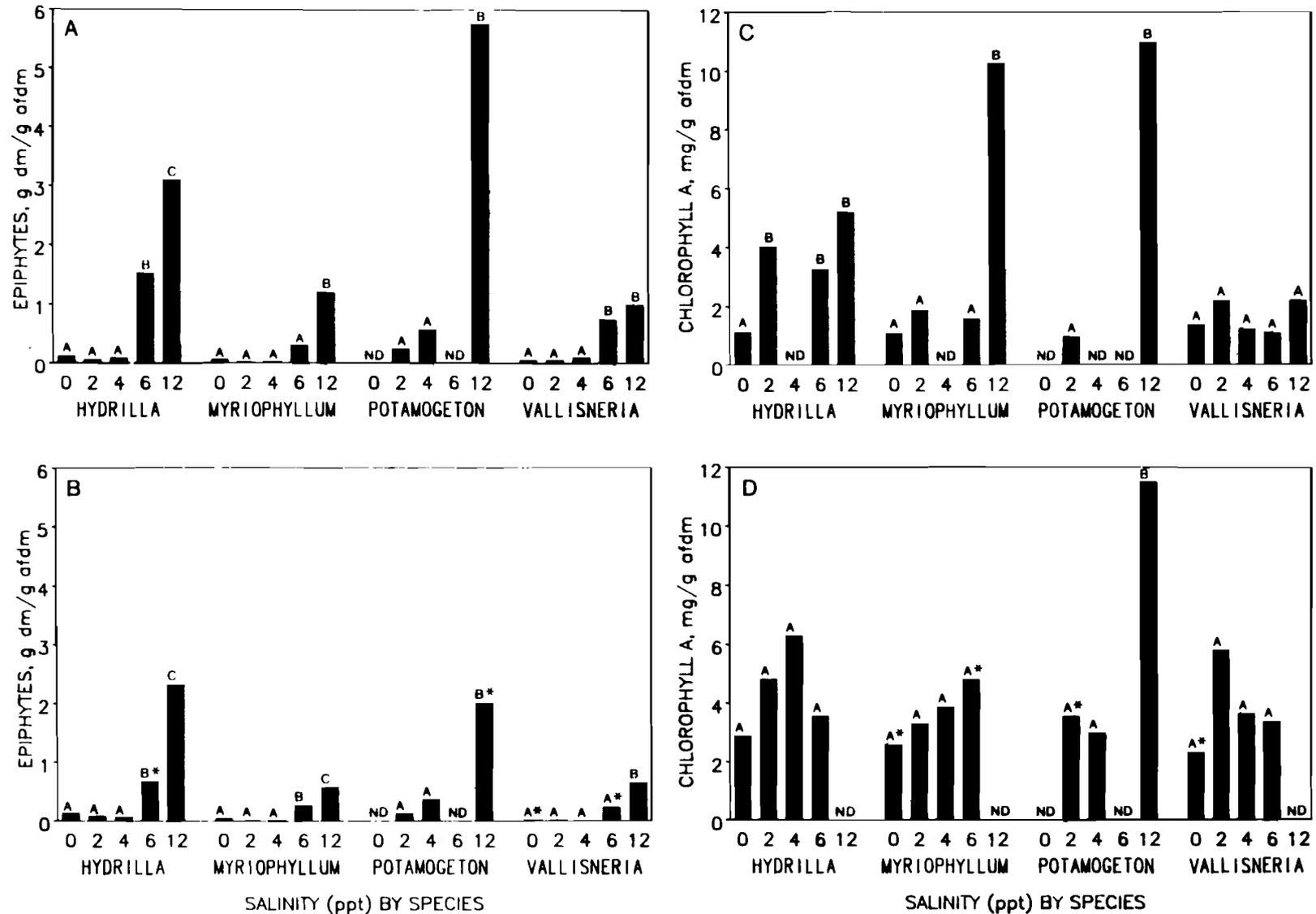


Figure 3. Epiphytes (A and B, g dry mass/g afdm of macrophyte) and chlorophyll *a* concentrations (C and D, mg dry mass/g afdm of macrophyte) of plant species at five salinities under high (A and C) and low (B and D) light levels. (ND, no data collected. Capital letters above bars denote significant differences among salinity treatments at $P < 0.05$; asterisk denotes significant difference ($P < 0.05$) between light treatments for each species and salinity effect)

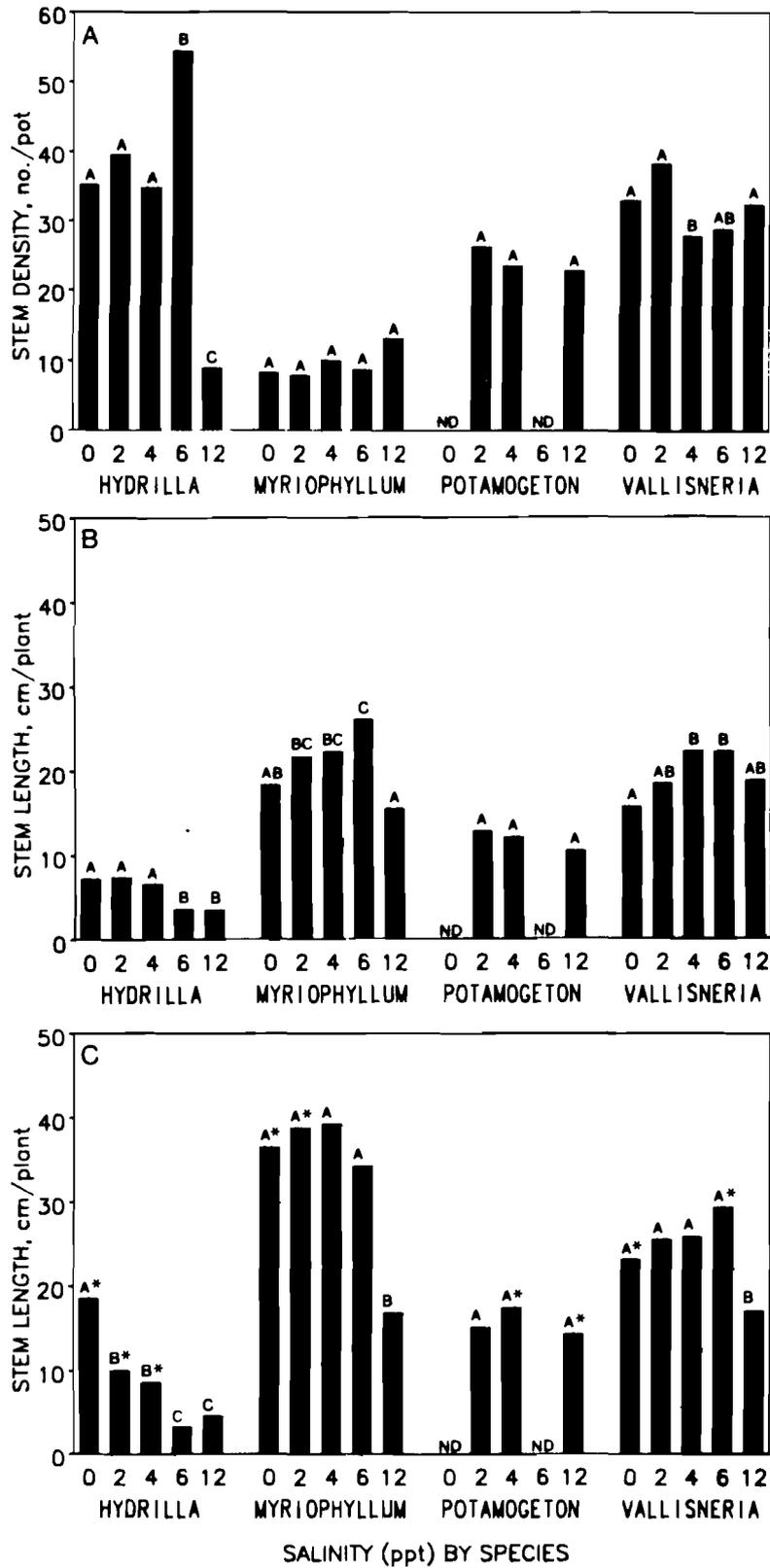


Figure 4. Stem density (A) and stem length (B and C) of plant species at five salinities under high (B) and low (C) light levels. Data were pooled for light treatments of stem density since no significant differences ($P < 0.05$) were observed

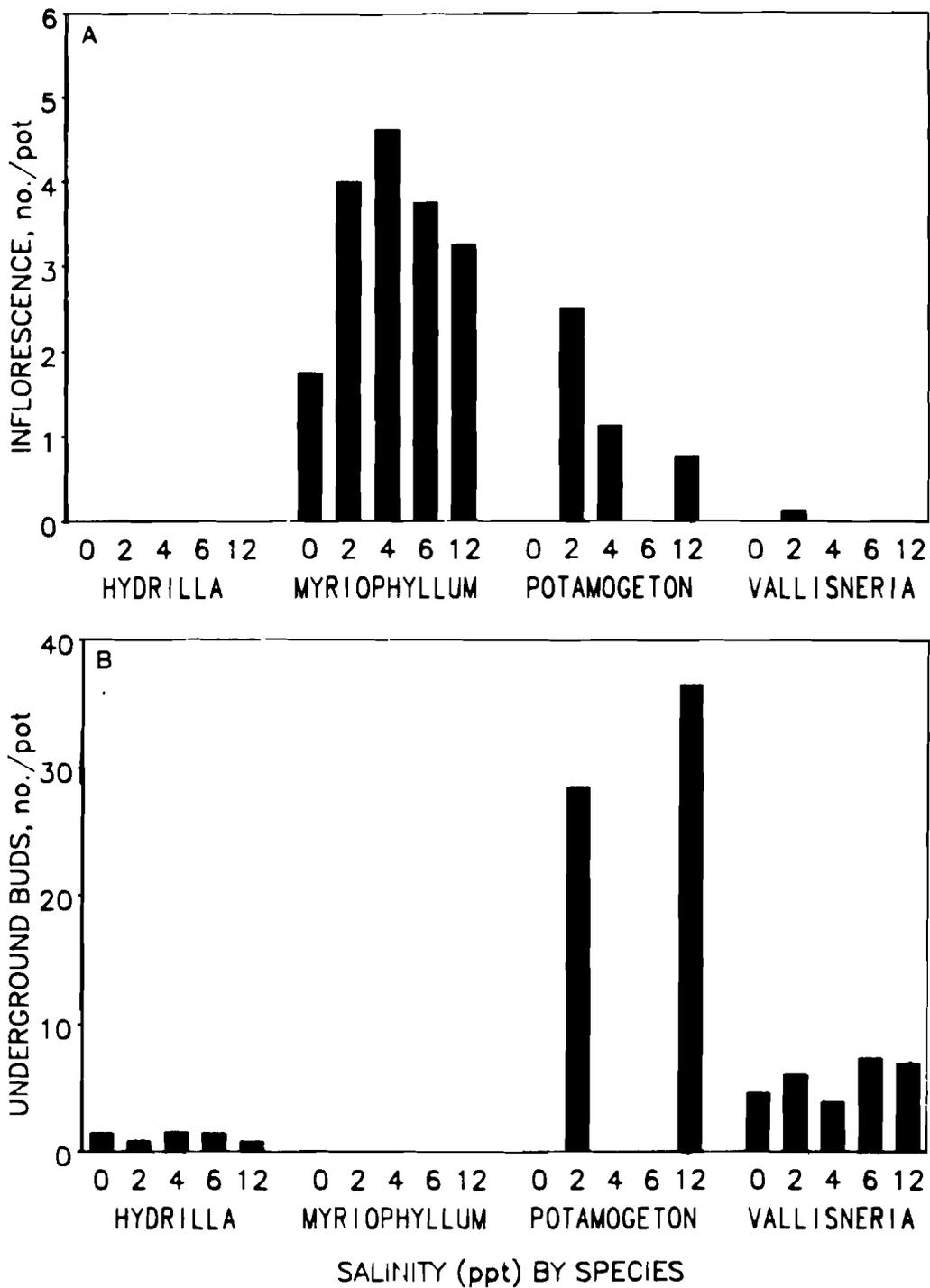


Figure 5. Number of inflorescences (A) and underground buds (B) of plant species at five salinities. (ND, no data collected. Data were pooled for light treatments since no significant differences ($P < 0.05$) were observed)

higher salinities, the number of inflorescences decreased only slightly in *M. spicatum*; however, for *P. perfoliatus*, the number of inflorescences at 4 and 12 ppt was less than half the frequency occurring at 2 ppt. Underground buds were observed in all species except *M. spicatum*, and the largest numbers occurred in *P. perfoliatus*. Salinity demonstrated very little effect on bud formation, in contrast to the effect of salinity on the number of inflorescences.

23. No effects of light were noted with regard to concentrations of any of the nutrients examined in the aboveground tissues of the four aquatic plants. Nitrogen concentrations in all four species increased significantly at the higher concentrations of salinity (Figure 6a). Peak concentrations of nitrogen at about 25 mg/g afdm were observed in *H. verticillata* at 4 ppt, in *M. spicatum* at 12 ppt, and in *P. perfoliatus* at 12 ppt. The peak concentration of nitrogen in *V. americana* was lower, at about 16 mg/g afdm.

24. In contrast, concentrations of phosphorus in the aboveground tissues did not increase with salinity for any species except *P. perfoliatus* (Figure 6b). Phosphorus concentrations were generally higher in *H. verticillata* over the range in salinity of 0 to 4 ppt, with values above 5 mg/g afdm. Because of the increase in nitrogen with increasing salinity, but relatively constant phosphorus concentration, there was a general increase in the N:P ratio with increasing salinity for all species except *P. perfoliatus* (Figure 6c). Peak ratios of nearly 17 occurred in *M. spicatum* and *V. americana* at 12 ppt, while ratios in *H. verticillata* and *P. perfoliatus* were less than 10 at all salinities.

25. Concentrations of sodium increased in the aboveground tissue of all species with increasing salinity up to at least 6 ppt (Figure 7a). Concentrations of sodium were significantly higher at 12 ppt in all species except for *V. americana*.

26. Potassium concentrations in the aboveground tissue of *H. verticillata*, *M. spicatum*, and *P. perfoliatus* ranged from 15 to 25 mg/g afdm and exhibited little difference among the salinity treatments. In contrast, tissue concentrations of potassium in *V. americana* decreased from 50 mg/g afdm, the peak concentration in this study at 0 ppt, to 27 mg/g afdm at 12 ppt (Figure 7b). The atomic ratios of sodium and potassium (Na:K) increased in all species with increasing salinity (Figure 7c). In freshwater solutions, ratios

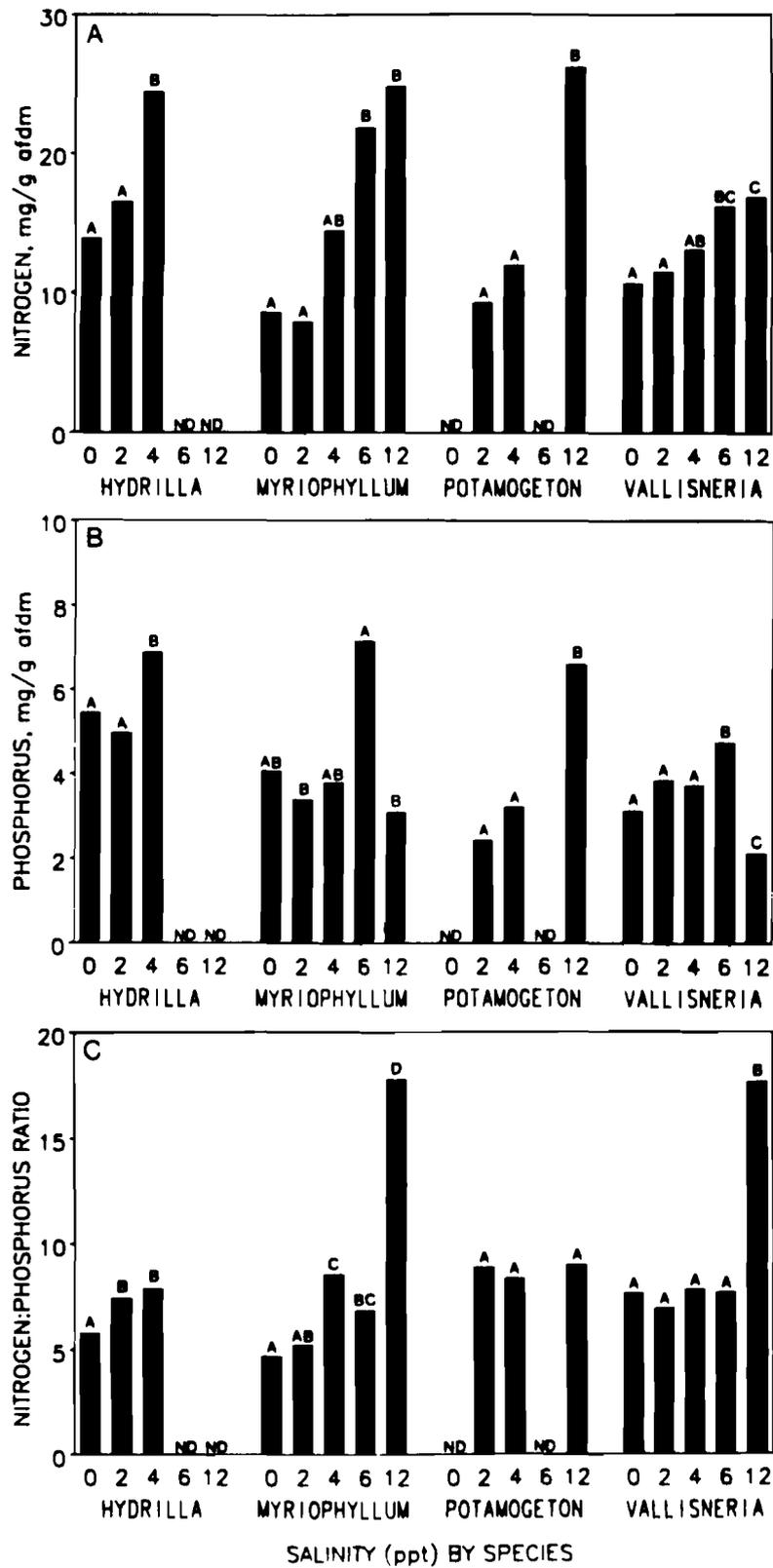


Figure 6. Concentration (mg/g afdm) of nitrogen (A) and phosphorus (B) and the N:P atomic ratio (C) in aboveground tissue of plant species at five salinities. Data were pooled for light treatments since no significant differences ($P < 0.05$) were observed

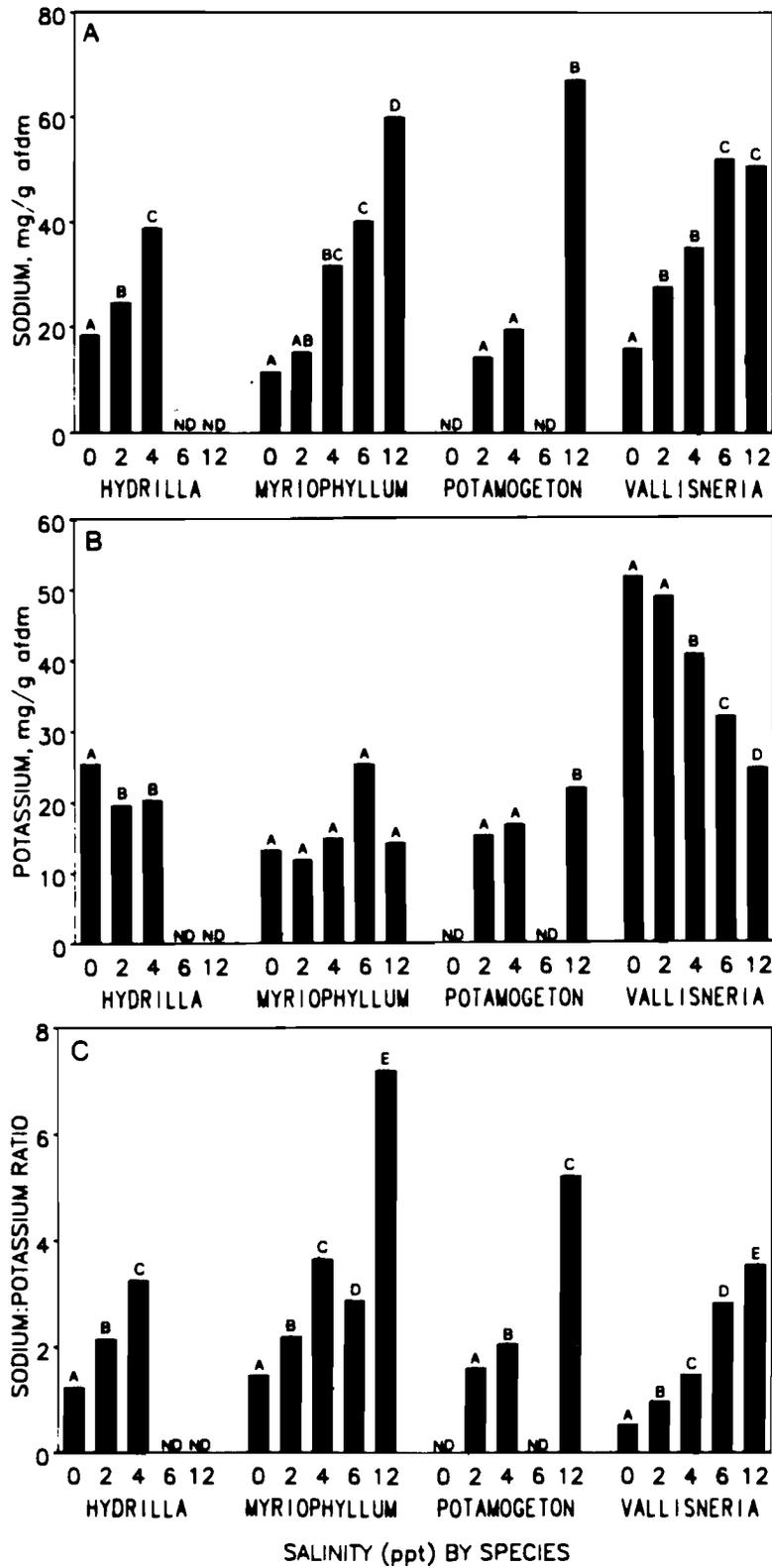


Figure 7. Concentration (mg/g afdm) of sodium (A) and potassium (B) and the Na:K atomic ratio (C) in above-ground tissue of plant species at five salinities. Data were pooled for light treatments since no significant differences ($P < 0.05$) were observed

ranged from 1 to 2, while at 12 ppt the ratio peaked at 7 in *M. spicatum*, and at 5.2 and 3.8 in *P. perfoliatus* and *V. americana*, respectively.

Discussion

27. Aquatic macrophytes can respond to lower levels of light by changing both their morphology and chlorophyll concentration. Increased stem length at lower light intensities was particularly evident for *M. spicatum* in this study, as has been observed by others (Titus and Adams 1979, Barko and Smart 1981). However, there was a lack of change in chlorophyll *a* concentrations in the shaded macrophytes. The increase in stem length of *P. perfoliatus* in lower light was less than observed for *M. spicatum*, but the effects of shading were overcome by an increase in chlorophyll *a* concentration. Thus, the relative response of morphology versus chlorophyll concentration as an adaptation to shading was species specific. Lack of stem elongation by *H. verticillata* at low light was unexpected, given its growth characteristics in the field (Haller and Sutton 1975). High light levels in our study may have reduced stem elongation, since the stem lengths in this species were nearly double those reported from studies conducted in the same facility the following year with an extra layer of screening.*

28. There was tremendous variation in epiphytic mass on each species among salinity treatments, even though nutrient concentrations were held constant. Epiphyte concentrations (dry mass) of 2 to 4 g/g afdm, observed at the higher salinities, can reduce incident light at the leaf surface by more than 50 percent of the original (Twilley et al. 1985), thus reducing the low-light treatment to less than 5 percent of the original light. These low light levels may explain the increase in chlorophyll *a* concentrations in the macrophytes in some cases as salinity increased. The increase in epiphytic mass may also be related to nutrient loss from macrophyte tissue at the higher salinities. Although the exact stimulus for epiphytic growth in this study is unknown, it is interesting that enhanced growth occurred with enrichment of less than 1 $\mu\text{g-at/l}$ of inorganic nitrogen and phosphorus.

* Personal Communication, 1989, J. C. Stevenson, Horn Point Laboratory, University of Maryland, Cambridge, MD.

29. *Hydrilla verticillata* and *V. americana* reproduced by underground buds, *M. spicatum* by inflorescences and turions, and *P. perfoliatus* by both underground buds and inflorescences. With increasing salinity, the production of inflorescences decreased in both *M. spicatum* and *P. perfoliatus*; however, even at the higher salinities, flowers were still present. Anderson (1964) observed that *M. spicatum* in Chesapeake Bay did not flower at a site with salinity ranging from 9 to 16.4 ppt. Among species in our study, the number of underground buds remained unchanged among the salinity treatments, or as in the case of *P. perfoliatus*, increased with salinity.

30. It appeared that sexual reproduction in *P. perfoliatus* was more susceptible to increases in salinity than asexual reproduction. Since *P. perfoliatus* is anemophilous, stems must reach the surface of the water to flower. However, the lack of flowering at the higher salinities was not due to effects on stem length. The loss of reproductive means by seeds as salinity increased most likely has minor effects on the distribution of these plants, since emergence of new plants from sediments is due mainly to asexual means (Sculthorpe 1967, Haag 1983).

31. With the exception of *H. verticillata*, the aquatic macrophytes examined may be considered eurysaline species that are restricted to waters from fresh to mixohaline composition (Den Hartog 1981). Den Hartog considers these macrophytes as true freshwater species that can intrude low-salinity waters, yet the growth and reproductive potential of three species indicates they can adapt to salinities up to one third the strength of seawater. Studies on the distribution (Moyle 1945; Luther 1951; Hynes 1960; Anderson 1964, 1972; Seddon 1972) and salinity tolerance (Haller et al. 1974) and *M. spicatum* corroborate our findings, indicating that this species can withstand a wide range of salinities up to 15 ppt. Haller et al. (1974) observed no growth of this species at 13.22 ppt, and McGahee and Davis (1971) found that salinities of 16 ppt significantly reduced its photosynthesis.

32. There is conflicting evidence for salinity tolerance in *P. perfoliatus*. Metcalf (1931) concluded from surveys of lakes in North Dakota that this species cannot withstand water with a salt content greater than 1.5 ppt. Bourn (1932) grew this macrophyte for 8 weeks in saline solutions and measured peak production at 4.2 ppt and an upper tolerance of 11.2 ppt. *Potamogeton perfoliatus* is not usually considered to be a species that can tolerate brackish waters (Hynes 1960), yet it is common in many estuaries in the United

States, including Chesapeake Bay (Anderson 1972, Stevenson and Confer 1978), Pamlico River estuary (Davis and Brinson 1976), and Currituck Sound (Davis and Carey 1981). *Potamogeton perfoliatus* was the dominant macrophyte in ponds flushed with water from the Choptank River estuary, with salinity ranging from 10 to 12 ppt (Twilley et al. 1985). In our study, *P. perfoliatus* did best over the lower salinity range, although it tolerated salinities up to 12 ppt.

33. The wide range in tolerance of *V. americana* to salinities from 0 to 12 ppt in our study contradicts several other reports on salinity tolerance of this macrophyte. Laboratory evidence from Haller et al. (1974) showed that growth occurred in this species over a range of 0.17 to 3.33 ppt, but no growth occurred at 6.66 ppt. Experimental studies by Bourn (1932, 1934) showed that growth of *V. americana* (identified as *V. spiralis*) peaked at 2.8 ppt, and no growth occurred above salinities of 8.4 ppt; yet, salt concentrations up to 15.8 ppt did not plasmolyze the cells of the leaf.

34. *Vallisneria americana* has been considered strictly a freshwater species (Metcalf 1931, Moyle 1945), although its distribution has been noted in the oligohaline regions of estuaries and saline lakes (Davis and Brinson 1976, Stevenson and Confer 1978, Stellar 1985). For example, along the north shore of the Pamlico River estuary, Davis and Brinson found *V. americana* in 78.1 percent of their quadrats in a region with a mean salinity of 5.3 ppt (range, 0 to 12.8 ppt), while no observations were made in a more saline region of 7.6 ppt (range, 2.2 to 13.9 ppt). Growth of *V. americana* in our study was unaffected by salinities up to 12 ppt. These results represent the first experimental evidence that this macrophyte may behave as a halophyte.

35. There is less evidence, based on either experimental data or plant distribution, to determine the tolerance of *H. verticillata* to salinity. Haller et al. (1974) observed that this species was stressed at low salinities, with no growth occurring at 6.66 ppt. We observed that a decrease in growth occurred with an increase in salinity, and little productivity occurred above 4 ppt. No communities of *H. verticillata* have been observed in the Potomac River estuary at salinities greater than 2 ppt (Rybicki et al. 1985), suggesting that this species is strictly a freshwater macrophyte. Yet, these results sharply contrast with the findings of Steward and Van (1987), which indicate that growth of *H. verticillata* can occur in salinities up to 13 ppt.

36. The concentration of sodium increased in all four species of aquatic macrophytes examined, suggesting that they do not possess mechanisms

for the exclusion of this ion. The three species that survived salinities of 12 ppt, *M. spicatum*, *P. perfoliatus*, and *V. americana*, all had similar sodium concentrations of about 60 mg/g afdm at the upper salinity treatment. Davis and Brinson (1976) observed changes in sodium concentration in *V. americana* along a salinity gradient in the Pamlico River estuary, with peak concentrations greater than about 33 mg/g afdm in higher salinity waters. Sodium concentrations of *V. americana* in fresh waters are normally less than about 6 mg/g afdm (Schuette and Alder 1927; Riemer and Toth 1968; Neal, Peterson, and Smith 1973). The relatively constant concentrations of potassium in all the species studied here, except for *V. americana*, suggest that the potassium concentration in *H. verticillata*, *M. spicatum*, and *P. perfoliatus* may be unaffected by salinity.

37. The uptake of sodium causes problems in the osmoregulation of plants, and halophytes have adaptations to control osmotic equilibrium in the cytoplasm. Low osmotic potentials in whole cells are maintained in many halophytes by the production of nitrogen compounds, particularly the amino acid proline (Stewart and Lee 1974, Storey and WynJones 1974, Treichel 1975, Cavalieri and Huang 1979, Husband and Hickman 1985). Brock (1979) showed an increase in proline in three species of *Ruppia* (*R. tuberosa*, *R. megacarpa*, and *R. polycarpa*) with increase in salinity among habitats, and *R. maritima* also demonstrated an increase in proline in response to increase in NaCl concentration (Stewart and Lee 1974). Both Treichel (1975) and Cavalieri and Huang (1979) have shown that osmoregulatory mechanisms, such as an increase in nitrogen concentrations, can respond to changes in Na and Cl concentrations within 24 hr.

38. The increase in total nitrogen concentration observed for the aquatic macrophytes in our study suggests that a nitrogen-based osmoregulatory mechanism occurred in response to an increase in internal sodium concentrations. We found a significantly ($P < 0.05$) positive relationship between internal sodium and nitrogen concentrations using results from all four species (Figure 8). This relationship suggests that a nitrogen:sodium atomic ratio of 0.72 was maintained within plants that existed in various saline environments. Field data on relative nitrogen and sodium concentrations of aquatic macrophytes in estuarine environments (e.g., Davis and Brinson 1976) may mask such a relationship due to different levels of eutrophy and the ability of plants for luxury storage of nitrogen (Gerloff and Krombholz 1966).

The low concentrations of nitrogen in the water and sediments in our study allowed us to discern the apparent chemical association of plant nitrogen with internal sodium concentrations.

39. Differences in reported salinity tolerances for submersed macrophytes may reflect differences in experimental methodology in relation to adaptations to increases in salinity. In our study, salinity was gradually changed at a rate of 1 ppt/day (2 ppt/day from 6 to 12 ppt), whereas in the experimental studies by Bourn (1932, 1934)

and Haller et al. (1974), plants were immediately exposed from fresh water to the desired salinity treatment. The protocol of gradually exposing aquatic macrophytes to changes in salinity may have enhanced the ability of *V. americana* to grow in a wide range of salinities by enabling some osmoregulatory mechanism to operate. The experimental results of our study are supported by the distribution of this species in the field. Fluctuation in salinity may be as important a factor as mean concentration in determining the distribution of these euryhaline macrophytes in estuarine waters.

Conclusions and Recommendations

40. As apparent in this investigation, irradiance conditions have an important influence on macrophyte growth form. Since it is quite often the growth form of submersed macrophytes that impute them as a nuisance, factors affecting form are as important as species composition in creating problems. Higher levels of underwater irradiance, while stimulating greater areal productivity of submersed aquatic vegetation, actually result in a more desirable (i.e., low-profile) growth form. Watershed disturbances that impart turbidity to the water column should be avoided in areas where canopy-forming species (e.g., *Hydrilla*) reside. Natural events such as droughts and heavy storms,

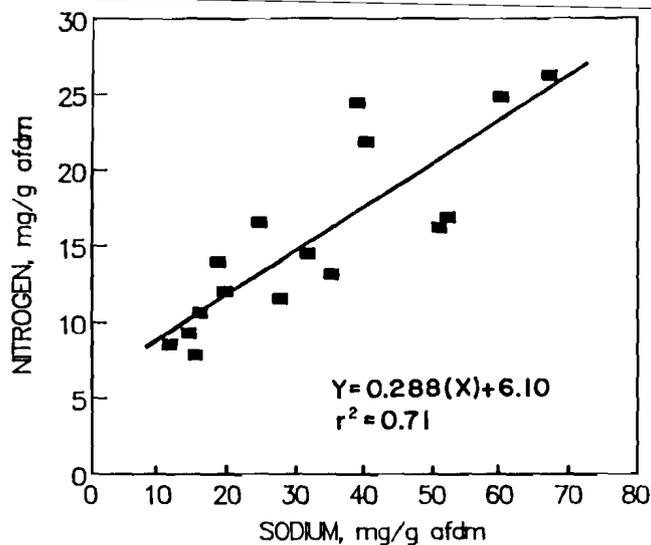


Figure 8. Relationship between the internal sodium and nitrogen concentrations of four aquatic macrophytes for which nutrient data existed (see Figures 6 and 7)

via effects on water clarity, can be expected to have important influences on the productivity and growth form of submersed macrophytes.

41. Nutrient loading to aquatic systems is known to stimulate production of epiphytes. However, in the present investigation, epiphytic development occurred in the absence of external nutrient inputs. Thus, the source of nutrients for these epiphytes appears to have been the macrophytes upon which they grew. Although there was no direct evidence of nutrient release from these plants, we conclude that the higher salinity levels resulted in nutrient loss from plant tissues, thereby stimulating epiphyte growth. Heavy epiphyte development, by decreasing the light available at leaf surfaces, can greatly diminish the growth of submersed macrophytes. Thus, reduced irradiance at leaf surfaces, in combination with osmotic stress, probably accounted for the diminished growth of intolerant macrophyte species with increasing salinity.

42. Among the species included in the present investigation, *Hydrilla* was the least tolerant of high salinity. Based on the conclusion that this species in nature will not tolerate salinities much above about 6 ppt, it is recommended that this value be used as a criterion in assessing the distribution potential of *Hydrilla* in estuarine and other brackish water systems.

References

- Allen, S. E., Grinshaw, H. M., Parkinson, J. A., and Quarmby, C. 1974. Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford.
- Anderson, R. R. 1964. Ecology and mineral nutrition of *Myriophyllum spicatum* L. MS thesis, University of Maryland, College Park.
- _____. 1972. Submerged vascular plants of the Chesapeake Bay and tributaries. Chesapeake Sci. 13:S87-S89.
- Barko, J. W., and Smart, R. M. 1981. Comparative influences of light and temperature on the growth and metabolism of selected submersed freshwater macrophytes. Ecological Monographs 51:219-235.
- _____. 1983. Effects of organic matter additions to sediment on the growth of aquatic plants. Journal of Ecology 71:161-175.
- Barko, J. W., Hardin, D. G., and Matthews, M. S. 1982. Growth and morphology of submersed freshwater macrophytes in relation to light and temperature. Canadian Journal of Botany 60:877-887.
- Bayley, S., Stotts, V. D., Springer, P. F., and Steens, J. 1978. Changes in submerged aquatic macrophyte populations at the head of Chesapeake Bay, 1959-1975. Estuaries 1:73-84.

- Bourn, W. S. 1932. Ecological and physiological studies of certain aquatic angiosperms. *Contrib. Boyce Thompson Inst.* 4:425-496.
- _____. 1934. Sea-water tolerance of *Vallisneria spiralis* L. and *Potamogeton foliosus* Raf. *Contrib. Boyce Thompson Inst.* 6:303-308.
- Bowes, G., Van, T. K., Garrad, L. A., and Haller, W. T. 1977. Adaptation to low light levels by *H. verticillata*. *J. Aquat. Plant Manage.* 15:32-35.
- Brock, M. A. 1979. Accumulation of proline in a submerged aquatic halophyte, *Ruppia* L. *Oecologia* 51:217-219.
- Cavaliere, A. J., and Huang, A. H. C. 1979. Evaluation of proline accumulation in the adaptation of diverse species of marsh halophytes to the saline environment. *American Journal of Botany* 66:307-312.
- Davis, G. J., and Brinson, M. M. 1976. The submersed macrophytes of the Pamlico River estuary, North Carolina. Report No. 112, Water Resour. Res. Inst. Univ. North Carolina, Raleigh.
- Davis, G. J., and Carey, D. F., Jr. 1981. Trends in submersed macrophyte communities of the Currituck Sound: 1977-1979. *J. Aquat. Plant Manage.* 19:3-8.
- Den Hartog, C. 1981. Aquatic plant communities of poikilosaline waters. *Hydrobiologia* 81:15-22.
- Gerloff, G. C., and Krombholz, P. H. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. *Limnol. Oceanogr.* 11:529-537.
- Haag, R. W. 1983. The ecological significance of dormancy in some rooted aquatic plants. *Journal of Ecology* 67:727-738.
- Haller, W. T., and Sutton, D. L. 1975. Community structure and competition between *Hydrilla* and *Vallisneria*. *Hyacinth Control Journal* 13:48-50.
- Haller, W. T., Sutton, D. L., and Barlowe, W. C. 1974. Effects of salinity on growth of several aquatic macrophytes. *Ecology* 55:891-894.
- Haramis, G. M., and Carter, V. 1983. Distribution of submersed aquatic macrophytes in the tidal Potomac River. *Aquat. Bot.* 15:65-79.
- Husband, B. C., and Hickman, M. 1985. Growth and biomass allocation of *Ruppia occidentalis* in three lakes, differing in salinity. *Canadian Journal of Botany* 63:2004-2014.
- Hynes, H. B. N. 1960. The biology of polluted waters. University Press, Liverpool.
- Kemp, W. M., Boynton, W. R., Twilley, R. R., Stevenson, J. C., and Means, J. C. 1983. The decline of submerged vascular plants in Chesapeake Bay: A summary of results concerning possible causes. *Mar. Techn. Soc. J.* 17:78-89.
- Luther, H. 1951. Verbreitung und Okologie der höheren Wasserpflanzen im Brackwasser der Ekenas-Gegend in Sudfinnland. *Acta Bot. Fenn.* 49:1-23.
- McGahee, C. F., and Davis, G. J. 1971. Photosynthesis and respiration in *Myriophyllum spicatum* L. as related to salinity. *Limnology and Oceanography* 16:826-829.

- Metcalf, F. P. 1931. Wild-duck foods in North Dakota lakes. US Department of Agriculture Tech. Bull. 221.
- Moyle, J. B. 1945. Some chemical factors influencing the distribution of aquatic plants in Minnesota. *Amer. Midl. Nat.* 34:402-420.
- Neal, J. K., Peterson, S., and Smith, W. 1973. Weed harvest and lake nutrient dynamics. Office of Research and Development, US Environmental Protection Agency, Washington, DC.
- Orth, R. J., and Moore, K. A. 1983. Submerged aquatic vegetation of the Chesapeake Bay: Past, present and future. *Science* 222:51-53.
- Riemer, D. N., and Toth, S. J. 1968. A survey of the chemical composition of aquatic plants in New Jersey. Bulletin 320, New Jersey Agricultural Experiment Station, College of Agricultural and Environmental Sciences, Rutgers University, New Brunswick, NJ.
- Rybicki, N. B., Carter, V., Anderson, R. T., and Trombley, T. J. 1985. *Hydrilla verticillata* in the tidal Potomac River, Maryland, Virginia, and the District of Columbia, 1983 and 1984. US Geological Survey Open-File Report 85-77, Washington, DC.
- Schuetz, H. A., and Alder, H. 1927. Notes on the chemical composition of some of the larger aquatic plants of Lake Mendota; II. *Vallisneria* and *Potamogeton*. Transactions of the Wisconsin Academy of Science, Arts and Letters 24:135-139.
- Sculthorpe, C. D. 1967. The biology of aquatic vascular plants. Edward Arnold Ltd., London.
- Seddon, B. 1972. Aquatic macrophytes as limnological indicators. *Freshwater Biology* 2:107-130.
- Smart, R. M., and Barko, J. W. 1985. Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aquat. Bot.* 21:251-263.
- Statistical Analysis System. 1982. SAS user's guide: Statistics. SAS Institute Inc., Cary, NC.
- Stellar, D. L. 1985. Production and nitrogen dynamics of a *Vallisneria americana* grass bed in Lake Pontchartrain, Louisiana. Ph.D. thesis, Louisiana State University, Baton Rouge.
- Stevenson, J. C., and Confer, N. 1978. Summary of available information on Chesapeake Bay submerged vegetation. FWS/OBS 78/66, US Department of the Interior.
- Steward, K. K., and Van, T. K. 1987. Comparative studies of monoecious and dioecious *Hydrilla* (*Hydrilla verticillata*) biotypes. *Weed Science* 35:204-210.
- Steward, K. K., Van, T. K., Carter, V., and Pieterse, A. H. 1984. *Hydrilla* invades Washington, D.C., and the Potomac. *American Journal of Botany* 71:162-163.
- Stewart, G. R., and Lee, J. A. 1974. The role of proline accumulation in halophytes. *Planta* 120:279-289.
- Storey, R., and WynJones, R. G. 1975. Betaine and choline levels of plants and their relationship to NaCl stress. *Plant Sci. Lett.* 4:161-168.

Strickland, J. D. H., and Parsons, T. R. 1972. A practical handbook of seawater analysis, 2nd ed. Fish. Res. Bd. of Canada Bull. 167.

Titus, J. E., and Adams, M. S. 1979. Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria americana* Michx. Am. Midl. Nat. 102:263-272.

Treichel, S. 1975. The effect of NaCl on the concentration of proline in different halophytes. Z. Pflansenphysiol. Bd. 76:56-68.

Twilley, R. R., Kemp, W. M., Staver, K. W., Stevenson, J. C., and Boynton, W. R. 1985. Nutrient enrichment of estuarine submersed vascular plant communities; 1. Algal growth and effects on production of plants and associated communities. Mar. Ecol. Prog. Ser. 23:179-191.

US Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. EPA-600/4-79-020, US Environmental Protection Agency, Cincinnati, OH.

Van, T. K., Haller, W. T., Bowes, G., and Garrard, L. A. 1977. Effects of light quality on growth and chlorophyll composition in *Hydrilla*. J. Aquat. Plant Manage. 15:29-31.