SEDIMENT AND LIGHT REQUIREMENTS OF FOUR SPECIES OF NATIVE SUBMERGED MACROPHYTES OCCURRING IN FLORIDA LAKES

By

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To my family – Carlos, Eddie, Maria, Mom and Daddy – for your undying support and willingness to help no matter what needed to be done. We did it!

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SEDIMENT AND LIGHT REQUIREMENTS OF FOUR SPECIES OF NATIVE SUBMERGED MACROPHYTES OCCURRING IN FLORIDA LAKES

By

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The purpose of this research was to investigate sediment and light growth requirements, two key factors in the establishment of native submersed macrophytes in shallow eutrophic Florida lakes. The study plants were *Najas guadelupensis*, *Potamogeton illinoensis*, *Vallisneria americana* and *Chara* sp., all Florida native submerged species commonly occurring state-wide. The selected species exhibited a variety of morphometries and life histories. The results will be of value to lake managers for use in the development of a more systematic approach to the establishment of diverse communities of desirable native species.

This study was divided into two main objectives. In Objective 1, the study species were cultured outdoors in growth tanks in south Florida for three separate 9-week culture periods on inorganic sediments collected three different times from four littoral stations in Lake Hollingsworth and on artificial control sediments. The results suggested that the

inorganic sediments collected from Lake Hollingsworth had sufficient nutrient levels to support the growth of the study species. The findings further indicated that late spring was the ideal time to introduce plant propagules into restored systems. Submerged macrophyte growth in this study appeared to be most significantly affected by a combination of factors including light, water temperature and sediment nutrients.

In Objective 2, shade cloth was used to establish four light treatment groups in an outdoor growth tank in south Florida. The light requirements of mature plants and vegetative propagules were investigated during three culture periods for each group. The photosynthetic photon flux density (PPFD) for no net growth of mature plants ranged from 14 to 416 μmol photons s^{-1} m^{-2} (2 to 50% incident irradiance). The PPFD for no net growth of propagules ranged from 25 to 183 μmol photons s^{-1} m^{-2} (3 to 22% incident irradiance). *V. americana* exhibited the lowest minimum light requirement for growth, 2 to 18% incident irradiance. Propagules of *P. illinoensis* and *V. americana* had higher light requirements as compared with mature plants. Both mature and propagule plants exhibited the greatest capability for growth at low light levels during the summer culture periods.

CHAPTER 1
INTRODUCTION

Background Information

Submersed macrophytes are important ecological components of aquatic systems. These primary producers provide excellent habitat for epiphytes (Cattaneo and Kalff 1980, Wetzel 1999b), invertebrates (Soszka 1975, Wetzel 2001), fish (Wiley et al. 1984) and a variety of other aquatic organisms (van der Velde 1987). Aquatic macrophytes also play a critical role in the nutrient dynamics of aquatic ecosystems (Carpenter and Lodge 1986).

Macrophyte growth is affected by a variety of abiotic and biotic factors including light availability, water depth, nutrient availability and sediment composition (Spence 1967, Canfield et al. 1983, Chambers and Kalff 1987). Biotic factors such as the degree of colonization by epiphytes (Sand-Jensen and Borum 1984, Sand-Jensen 1990), competition with phytoplankton (Fitzgerald 1969, Allen 1971) and inter-specific competition (McCreary 1991) also impact macrophyte production. Herbivory and grazing often play an integral role in macrophyte community dynamics in many systems (cf. Lodge 1991). There is currently considerable evidence that indicates that light (Canfield et al. 1985, Duarte and Kalff 1986, Kalff 2002) and sediment physical and nutrient composition (Barko and Smart 1986, Short 1987, Barko et al. 1991) are among the most significant abiotic factors controlling submersed macrophyte growth.

Realization of the pivotal role that submersed macrophytes play in determining the alternative stable state of shallow productive lakes and ponds (cf. Moss 1990, Scheffer et al. 1993, Donnabaum et al. 1999) has led to an increased interest in the nutritional ecology of submersed macrophytes. However, a review of the literature indicates that, to date, little research has been done on the sediment and light requirements for growth of submersed macrophytes especially species native to subtropical and tropical climes. Information on the growth requirements of native submersed macrophytes will be of particular value to lake managers for use in the development of a more systematic approach to the establishment of desirable aquatic vegetation in restored systems.

Natural sediments investigated in this study were collected from Lake Hollingsworth. Lake Hollingsworth is a 144 ha shallow (z_{mean}= 1.2 m) (City of Lakeland 1988-2000) urban lake located in central Florida (28°01'30", 81° 56'45") (Figure 3-1). The lake is a solution basin roughly circular in shape with a mean depth of 1.2 m (Lake Hollingsworth Diagnostic Feasibility Study 1994). Water inputs to the lake are in the form of rainfall, groundwater seepage, stormwater runoff and inputs from Lakes Morton and Horney. Groundwater recharge contributes up to 85% of the total water budget for the lake (Romie 1994). Surface drainage flows southeast into Lake Bentley. Paleolimnological study indicated that Lake Hollingsworth is naturally eutrophic (Brenner et al. 1995). This is probably due to the fact that the lake lies in the Bartow Embayment division of the Central Lakes District where the underlying bedrock consists of the phosphatic sands and clays of the Bone Valley region of Central Florida (Canfield and Hoyer 1992). During the course of the study, Lake

Hollingsworth was being restored using whole-lake dredging in order to remove a layer of flocculent organic measuring up to 15 feet in thickness in some areas of the lake. The lake is a highly valued resource, serving as the site for a variety of social, educational and recreational activities for the City of Lakeland and the surrounding community. The lake also supports sustenance, commercial and sport fisheries.

Statement of Purpose

The goal of this research was to investigate two key factors in the establishment of native submersed macrophytes in shallow eutrophic urban Florida lakes: sediment and light growth requirements. This study was divided into two main objectives. The first objective was to investigate the effect of sediment chemical composition on the growth of four species of native submersed macrophytes: *Najas guadalupensis* (Sprengel) Magnus., *Potamogeton illinoensis* Morong., *Vallisneria americana* Michaux and *Chara* sp. Objective two was to investigate the amount of photosynthetically active radiation (PAR) required for the survival and growth of the four study species. In Objective 1, plants were cultured outdoors in growth tanks in south Florida for three separate 9-week culture periods on sand sediments collected three different times from four littoral stations in Lake Hollingsworth. In Objective 2, shade cloth was used to establish four light treatment groups in an outdoor growth tank in south Florida. Mature macrophytes were cultured on artificial sediments during three separate culture periods, one approximately 4 weeks in length and the other two approximately 7 weeks in length. The light requirements of macrophyte propagules grown on artificial sediments were also evaluated during three separate

culture periods. This research will provide valuable information for use in the establishment of desirable native submerged macrophytes in lake restoration projects.

Hypotheses

Experiment 1: Effects of Sediment on Macrophyte Growth

The experimental procedure was designed to test several hypotheses based upon findings from the literature and previous experience. The first was that there would be spatial variation in the organic matter and nutrient content of the littoral sediments in Lake Hollingsworth. I also anticipated that *N. guadelupensis*, *P. illinoensis* and *Chara* sp., the canopy-forming, erect species, would produce greater biomass in sediments with higher nutrient levels while net growth of *V. americana*, a rosette species, would be inversely related to sediment nutrient content. Based upon my personal experience growing stock cultures of the study plants, I expected to observe differences in growth rate among the species such that *N. guadelupensis* would be the fastest grower followed by *P. illinoensis*, *V. americana* and lastly *Chara* sp. I further expected the growth rate of *Chara* sp to be positively related to increasing culture period length. I anticipated that *N. guadalupensis, V. americana and Chara* sp. would produce the greatest biomass during the summer months while growth of *P. illinoensis,* a species with a range distribution that stretches into more temperate regions, would be stimulated by the cooler temperatures of the winter months. Finally, I hypothesized that sediments collected from the littoral zone of Lake Hollingsworth would have sufficient quantities of sediment nutrients to support the growth of *N. guadalupensis, P. illinoensis, V. americana and Chara* sp. when grown in the experimental growth tanks used in this study.

Experiment 2: Effects of Light on Macrophyte Growth

The procedure used in the first part of Experiment 2 was designed to test several hypotheses reagrding the light requirements of mature plants of the study species. The first hypothesis was that *N. guadelupensis* and *Chara* sp. would exhibit the highest light requirements for net growth due to self-shading due to the habit and growth strategies of these species. I anticipated that *V. americana* would exhibit the lowest light requirement for net growth among the study species, approximately 4% incident irradiance. Based upon the literature, I expected the PPFD for no net growth of *P. illinoensis* and *N. guadelupensis* to be approximately 11% and 15% incident irradiance, respectively. I anticipated PPFD for no net growth of *Chara* sp. to range between 4 and 20% incident irradiance. I further expected to observe photoinhibition in those species that are either canopy-formers or have an erect habit, *N. guadelupensis*, *P. illinoensis* and *Chara* sp., at light levels > 26.2% incident irradiance.

The procedure used in the second part of Experiment 2 was designed to test several hypotheses regarding the light requirements of vegetative propagule plants of the study species. First, I hypothesized that plants grown from propagules would have higher light requirements than mature plants of the same species. I anticipated that *V. americana* would exhibit the greatest capability for growth in low levels of light. I further expected that plants grown from propagules would not become photoinhibited during the course of each culture period.

CHAPTER 2
LITERATURE REVIEW

Background Information

A review of the literature indicates that little is known about the macrophytes that are often an integral component in the ecosystem processes of shallow productive lakes and ponds concentrated in subtropical to tropical climates. With the exception of some limited studies (i.e. Denny 1972b, 1973, Finlayson et al. 1980, Geddes 1984, Roijackers and Verstraelen 1988, Limon et al. 1989, Mitchell 1989, Schriver et al. 1995), most limnological research has been conducted in deepwater oligotrophic to mesotrophic systems located in temperate climes. Much of the limited research in shallow systems has been conducted in Denmark (e.g. Finlayson et al. 1980, Roijackers and Verstraelen 1988, Schriver et al. 1995) and other temperate climes (Donnabaum et al. 1999). Most of the research to date on the autotrophic component of lacustrine systems has been conducted on the phytoplankton occurring in deepwater temperate lakes. The majority of the research that has been conducted on macrophytes occurring in subtropical to tropical climes has been focused either on emergent vegetation or on nuisance exotic species such as hydrilla (Pieterse 1981, Barko 1982, Steward 1984, Barko and Smart 1986, Sutton 1990, 1993, Sutton et al. 1992, Sutton and Portier 1995, Sutton and Latham 1996).

The emerging interest in using macrophytes in lake restoration projects has identified the need for more information on the growth requirements of submerged

aquatics especially native species occurring in subtropical to tropical climates (Nichols 1991, Smart 1996). This information will be very useful to lake managers conducting restoration projects which include a littoral zone enhancement component.

Lake Restoration Techniques

Restoration can be defined as the action of returning an ecosystem to a former condition following degradation caused by some kind of disturbance. A typical lake restoration project would begin with the elimination or significant reduction of anthropogenic nutrient loading and include a land restoration and management program (Cooke et al. 1993). It might also include the manipulation of the structure of the fish community, reestablishment of native plant communities, and restoration of wetland areas in the watershed. The objective of the typical lake restoration project is to return the system to a long-term steady state condition similar to its pre-disturbance condition and in accord with reasonably attainable conditions, as dictated by the characteristics of the ecological region (Cooke et al. 1993). Prior to the implementation of any lake restoration initiative, a diagnosis and feasibility study should be conducted. Cooke et al. (1993) provide a comprehensive discussion of the components and procedures for the development of diagnosis and feasibility studies. The purpose of such studies is to evaluate the current status of the lake, determine the severity of the problem, and use the results to propose the most suitable restoration technique. Proposed techniques should be cost efficient while still permitting attainment of water quality goals.

Lake restoration methods are divisible into four groups depending upon their primary objective: 1) to control algal biomass, 2) to control macrophyte biomass, 3)

to alleviate oxygen problems and 4) to remove sediment. These methods are described in the following.

Algal Biomass Control Methods

A variety of mechanical and chemical methods are used to control algal biomass in surface waters. The first step in the control of algal biomass is the reduction of nutrient loading to the system (Cooke et al. 1993, Hosper 1998, Anadotter et al. 1999, Jeppesen et al.1999, Phillips et al. 1999, Van der Molen and Portielje 1999, Robertson et al. 2000, Sondergaard et al. 2000b, Barbieri and Simona 2001). This can be done using a variety of Best Management Practices (BMPs) in the watershed (Cooke et al. 1993, Robertson et al. 2000), mechanical devices such as CDS units to screen out debris and large particulate matter in inflows, enhancement of wetlands in the watershed and/or physical diversion, or re-meandering of inflows (Annadotter et al. 1999, Jeppesen et al. 1999, Robertson et al. 2000). Chemical solutions include aluminum sulfate (alum) injection systems installed in stormwater pipes and improved treatment of wastewater inflows (Anadotter et al. 1999, Donnabaum et al. 1999). After ensuring the quality of the water entering the system, the next step is to minimize internal loading of sediment nutrients, especially phosphorus. Internal loading can be a significant source of nutrient input to a lake, especially in shallow lakes with a long fetch (Maciena and Soballe 1990). Various methods can be used to decrease internal loading including the more traditional approach of alum application (Cooke et al. 1993, Anon 1996, Robertson et al. 2000, Rydin et al. 2000) or sediment oxidation through enhanced nitrification (Donnabaum et al. 1999). Some more recent experiments have been conducted to investigate other

chemical alternatives for sediment phosphorus inactivation, including hypolimnetic nitrate dosing and gypsum application to sediments. Sondergaard et al. (2000a) concluded that hypolimnetic nitrate dosing is a potential restoration technique for use in deepwater lakes in which internal loading is a problem. Salonen and Varjo (2000) determined that the application of gypsum to sediments to control nutrient release had potential value as a restoration technique. In their evaluation of the most commonly used restoration techniques in Danish lakes, Sondergaard et al. (2000b) concluded that hypolimnetic oxidation can be used to reduce internal phosphorus loading. Copper sulfate, a traditional method used to lower the algal standing crop in lakes, is used infrequently in contemporary restoration projects due, in part, to detrimental environmental impacts (Cooke et al. 1993).

There are also a variety of mechanical methods for lake restoration. Dilution with low-nutrient water and subsequent flushing of nutrients and algal biomass is a method that can be applied in the absence of external control of inflows to the lake (Hosper 1999). Other mechanical restoration methods include withdrawal of nutrient-rich hypolimnetic waters and aeration (Lindenschmidt and Hamblin 1997). Biological control methods involving food web manipulations are also options for decreasing algal biomass in systems. Numerous studies have documented the successful use of biomanipulation as a lake restoration strategy (Shapiro 1990, Perrow et al. 1995, Drenner and Hambright 1999, Jeppesen et al 1999, Robertson et al. 2000, Sondergaard et al. 2000b). Kairesalo et al. (1999) discuss the necessary components of a successful biomanipulation plan for use in system restoration.

Each of the aforementioned methods has its associated advantages and disadvantages. Some methods are considerably more costly than others. Flushing, for example, is a very effective technique, but is highly dependent on the availability of water in the area (Cooke et al. 1993). Remediation time and sustainability are other factors that must be considered when choosing a restoration method. McQueen (1998) discusses some of the challenges managers face in the use of biomanipulation. Alum has a very rapid and dramatic effect on water quality that can last two to five years or more (Smeltzer 1990, Anon 1996). However, the longevity of the positive effects of alum application on water quality vary from system to system and in many systems alum reapplication is required on a yearly basis in order to maintain desired water quality levels (Dr. Harvey Harper, President of Environmental Research and Design, personal observation). Conversely, biomanipulation of systems is an inexpensive method that can yield sustainable results (Drenner and Hambright 1999). Positive impacts of this method may not become apparent for several years, however. Another very important consideration is the impact of each method on ecosystem health. For example, little is known to date about the long-term ecological effects of alum on lakes especially on invertebrate populations (Smeltzer 1990, Cooke et al. 1993). Other studies (e.g. Neville 1985, Ramamoorthy 1988) have documented the lethal effects of aluminum salts in fish. Copper sulfate addition also has a detrimental effect on the ecology of aquatic systems (Cooke et al. 1993). Moss (1999) made a comprehensive comparison of the ecological implications associated with some of the more commonly used lake restoration methods.

Macrophyte Biomass Control Methods

Managers currently have a variety of chemical, physical and biological methods available to them for the control of macrophyte biomass in lake systems. Chemical methods involve the use of herbicides. Smith (1995) successfully used Sonar in the restoration of Long Lake, Washington. The results of this study indicated that the active ingredient in Sonar, fluoridone, targets exotic species; native species exhibited less sensitivity to the herbicide. Physical methods for macrophyte control include preventative measures, hand and mechanical harvesting, water level drawdown, lake stage manipulations, sediment covers and surface shading. Cooke et al. (1993) discussed the limitations of the use of preventative measures as a macrophyte control technique. Carter et al. (1994) reported significant reductions in macrophyte biomass when they used plastic sheets in several manmade lakes in Australia.

The escalating problem with proliferation of exotic macrophyte species, especially in Florida and other southeastern states, in combination with a growing dissatisfaction with chemical and mechanical methods, has resulted in intense research into and development of biological controls for aquatic vegetation (Cooke et al. 1993). Biological controls currently being used in lake systems include phytophagous insects and fish, plant pathogens (e.g. fungi and viruses) and allelopathy. Painter and McCabe (1988) documented the successful use of the moth *Acentria nivea* to control milfoil in certain Ontario lakes. Crosson (1992) reported that a weevil, *Euhrychiopsis lecontei*, and *A. nivea* together were probably responsible for declines in milfoil biomass in several Vermont lakes. Other studies

(e.g. Haag and Habeck 1991) suggest that an integration of chemical and biological methods is the most effective means by which to control aquatic macrophytes. One of the most commonly used phytophagous fish is the grass carp, or white amur (*Ctenopharyngodon idella* (Val.)). The popularity of the use of grass carp is due to the relatively low cost and long term results associated with the use of this control agent (Cooke et al. 1993). However, introduction of carp into systems often results in total eradication of all submerged plants (e.g. Mitchell 1980, Shireman et al. 1985), suggesting that the viability of the use of carp in macrophyte control is highly dependent upon the management goal.

As with algal biomass control methods, the management techniques employed to control macrophyte biomass have associated advantages and disadvantages. Cooke et al. (1993) provide a thorough discussion of the positive and negative effects of the use of the various macrophyte control methods and include case studies.

Aeration and Sediment Removal

The two remaining categories of lake restoration methodologies are aeration and sediment removal. Cooke et al. (1993) refer to these two categories as "multiple benefit treatments." Aeration of lakes can be achieved through hypolimnetic aeration and artificial circulation. Ashley and Hall (1990) define hypolimnetic aeration as a management technique designed to prevent anoxic conditions in the hypolimnion and the problems associated with anoxic hypolimnea. Potential benefits associated with the use of this method include oxygenation of the hypolimnion without causing an increase in temperature or de-stratification, increased habitat for aerobic organisms, and a decrease in internal phosphorus loading, due to the establishment of aerobic

conditions at the sediment-water interface (Cooke et al. 1993). The objective of artificial circulation is complete turnover of the lake. Benefits associated with artificial circulation include an increase in habitat for aerobic organisms, a potential decrease in internal phosphorus loading, and a possible decrease in algal biomass by increasing the depth to which phytoplankton are mixed in the water column (Cooke et al. 1993).

Sediment removal is a multipurpose restoration method. Objectives for the use of sediment removal include increasing the mean depth of the system, reduction of internal loading, removal of toxic substances, and mechanical control of aquatic macrophytes. Disadvantages include sediment resuspension during removal, release of sediment-bound toxic substances, destruction and degradation of habitat of benthic organisms, and the need for a disposal location for dredged sediments (Cooke et al. 1993). There are two methods by which sediment can be removed from a lake: lake drawdown followed by excavation with a bulldozer and sediment removal using a dredge.

Sediment removal is a frequently used management technique that has recently been used in a variety of different systems (Bengtsson et al. 1975, Kelly et al. 1994, Annadotter et al. 1999). At the beginning of the present study, hydraulic dredging was being used in Lake Hollingsworth to remove 2.7 million cubic meters of flocculent organic sediment. A rapid dewatering process patented by the Florida Institute of Phosphate Research (FIPR) was used to facilitate disposal of dredged sediments (Patel 1995). The project in Lake Hollingsworth represents the first field-testing of this dewatering process. Dredging was halted at approximately 60%

completion to prevent an unplanned drawdown due to drought conditions in the region. Post-dredge measurements of water quality parameters, including chlorophyll a, indicate that the removal of sediment is reducing water column nutrients in the system (Dan Mahone, City of Lakeland Wastewater Chemist, personal communication).

Numerous researchers have concluded that successful restoration of their systems required a combination of restoration techniques (Hulon 1994, Kelly et al. 1994, Annadotter et al. 1999, Phillips et al. 1999, Robertson et al. 2000). Restoration of Delavan Lake, Wisconsin, was conducted in three phases: 1) limiting external loading using BMPs in the watershed, enhancing an existing wetland, and diverting inflows, 2) reducing internal P loading using alum inactivation and carp removal, and 3) biomanipulation of the fish population (Robertson et al. 2000). Phillips et al. (1999) discuss the variety of restoration techniques used in the Norfolk Broads over a 25-year period.

Revegetation Projects in Lake Restoration

The littoral zone habitat enhancement component of restoration projects in shallow subtropical to tropical eutrophic lakes often includes the establishment of diverse communities of native submerged macrophytes. As more research is conducted in shallow lake systems, researchers are beginning to recognize the importance of aquatic macrophytes in ecosystem processes. Macrophytes are a key component of most restoration projects involving shallow lakes due to their pivotal role in determining community dominance. Numerous studies have identified the existence of multiple alternative stable states in ecosystems (May 1977, 1981, Hosper

1994, Timms and Moss 1984, Scheffer 1990, Scheffer et al. 1993). Hosper (1994), Timms and Moss (1984), Scheffer et al. (1993) and Donnabaum et al. (1999) identify two alternative stable states in their study systems: a clear one dominated by aquatic macrophytes and a turbid one dominated by phytoplankton. Moss (1990) emphasized the importance of biomanipulation in the prevention of a shift to an undesired stable state following lake restoration. Scheffer (1990) discusses the use of simple models to identify the existence of alternate stable states in lakes.

Various studies have attempted to elucidate the mechanism(s) that control the alternation between dominance by macrophytes and phytoplankton. Some studies suggest that the stabilizing effect of submerged macrophytes on sediments reduces internal loading during sediment resuspension events (Carpenter and Lodge 1986, Sondergaard and Moss 1998) thereby reducing the nutrients available for phytoplankton uptake. Barko and James (1998) suggested that submerged macrophytes serve as a sink for phosphorus and thus exert positive effects on water quality in lacustrine systems. Zimba et al. (1995) used chemical composition data in conjunction with GIS areal estimates to determine the amount of bound nutrients within the submersed macrophyte communities occurring in Lake Okeechobee in 1990 and 1991. They concluded that 117 metric tons of phosphorus were bound in the tissues of submersed macrophytes present in the lake in 1990. This number increased to 125 metric tons in 1991. The results of their study indicated that nutrients were sequestered by the five species studied in the following order (from the greatest to the least extent): *Vallisneria americana*> *Hydrilla verticillata*>> *Potamogeton illinoenesis*>> *Chara* sp. > *Najas guadelupensis*. Several studies (e.g. Timms and

Moss 1984, Schriver et al. 1995, Jeppesen et al. 1997) have indicated that the effect of aquatic macrophytes on the food web in shallow lakes may reduce the turbidity of the whole system. Landers (1982), Wetzel (1983) and Carpenter and Lodge (1986) concluded that aquatic macrophytes and their associated epiphytes also play an important role in littoral and littoral:pelagic nutrient cycling. Moss (1998) discussed the role of macrophytes in limiting phytoplankton productivity via production of allelopathic substances. Carpenter and Lodge (1986) presented a thorough discussion of the effects of submerged macrophytes on ecosystem processes. Using Charisma, a simulation model, to describe the growth of *Chara aspera*, van Nes et al. (2002) concluded that the effect of water clarity on aquatic plant growth is the most important factor affecting the stable state equilibria of shallow lakes. Similarly, a positive relationship between water transparency and macrophyte percent volume infested (PVI) in excess of 30%, irregardless of water column nutrient concentrations has been identified in several empirical studies conducted in tropical and temperate systems (Canfield et al.1984, Jeppesen et al. 1990, Canfield and Hoyer 1992).

There are other additional benefits associated with native submerged macrophyte communities. Submerged macrophytes are a vital component of the aquatic ecosystem (Wetzel 1983). This is especially true of shallow highly productive subtropical to tropical systems. From an ecological point of view, these primary producers provide habitat for zooplankton (Soszka 1975, Timms and Moss 1984, Winfield 1986, Diehl 1988, Schriver et al. 1995), epiphytes (Cattaneo and Kalff 1980), fish (Wiley et al. 1984, Engel 1985, Killgore et al. 1989) and a variety of other aquatic organisms (van der Velde 1987). Schriver et al. (1995) concluded that fish

predation on zooplanktivores decreased with increasing PVI of macrophytes especially when PVI exceeds 15-20%. They discussed the positive implications of these refugia for zooplankton in the control of phytoplankton. From a management perspective, several studies (Smart and Barko 1989, McCreary et al. 1991, Barko et al. 1991 and Smart et al. 1994) have indicated that some native submerged species are highly competitive and may offer protection from invasion by weedy exotic species. *V. americana* outcompeted *Hydrilla* at low light levels in reciprocal replacement series studies conducted by Smart and Barko (1989). The results of the same study indicated that *Vallisneria* exhibits better growth on fertile sediments as compared to *Hydrilla*. The introduction of *Eleocharis coloradoensis* reduced new growth in a variety of submerged species including *Hydrilla verticillata* and *Myriophyllum spicatum* in an additive study conducted by Yeo and Thurston (1984). Diverse communities of native submersed macrophytes typically produce less biomass than monotypic stands of exotic weedy species. In many native species, the growth form concentrates the majority of the plant biomass beneath the water surface. Accordingly, native submersed aquatics provide enhanced habitat with fewer of the management problems such as interference with recreation, navigation, water supply and hydropower generation and access to the water typically associated with exotic macrophyte species. Smart and Doyle (1995), Van et al. (1976) and Haller and Sutton (1975) discuss the adaptations that often make exotics superior competitors, especially in disturbed areas, as compared to native species. Filling this niche with desirable native species is essential in order to avoid exchanging one management challenge for another (Smart et al. 1996).

Despite the rising popularity of littoral zone enhancement projects, there remains a need for the development of a systematic approach for establishing and maintaining submerged macrophyte communities that can be used with more widespread success in lacustrine systems (Nichols 1991, Phillips et al. 1999). The most common approach to littoral zone development is the introduction of monocultures of a submerged aquatic species planted on 1-m centers. For example, in Florida, *Vallisneria* is the plant most often used in revegetation projects due to its availability and relative ease in planting (personal observation: results of informal survey of lake managers around the state). Planting monocultures can often lead to frustrating results such as invasion by a faster-growing exotic species (Smart and Doyle 1995). For this reason, the common practice of planting only monospecific stands of *Vallisneria* should be avoided. Certainly, the positive characteristics of *Vallisneria* make it a highly desirable species for use in restoration efforts. Vallisneria provides forage for waterfowl and refuge for juvenile fish. Studies have also shown that it has relatively low light requirements as compared to other species (Carter and Rybicki 1985, Smart and Barko 1989, this study) thus potentially making it a good candidate for growth in turbid waters. The growth habit of this macrophyte also concentrates biomass below the water column. However, in their 1975 study, Haller and Sutton concluded that *Vallisneria* is not a strong competitor against *Hydrilla.* Although both species are members of the Hydrocharitaceae family, differences in their growth structure render *Hydrilla* the superior competitor in most cases. *Hydrilla* forms a dense canopy at the water surface and reduces light penetration by 95% in the first 0.3 m of the water column (Haller and Sutton 1975). *Vallisneria* communities

produce less biomass, which is concentrated below the water surface so that light penetration is similar to that of open water. *Hydrilla* exhibits faster growth than *Vallisneria*. Haller and Sutton (1975) determined that *Vallisneria* invests energy in the production of large quantities of nonphotosynthetic biomass that would appear to negate any advantage of its greater leaf area index as compared to *Hydrilla*. The difference in biomass production is also attributable to differences in reproductive strategy. Haller and Sutton (1975) determined that the production of millions of meristematic tissues per hectare by *Hydrilla* is a highly competitive strategy. McCreary (1991) characterized hydrilla and *Vallisneria americana* as exhibiting guerilla and phalanyx growth strategies, respectively. She proposed the use of *V. americana* in combination with an equally robust native canopy-forming phalanyx species in order to outcompete *Hydrilla*. In conclusion, use of a variety of species with a diverse array of reproductive and growth strategies appears to be the most likely way to avoid colonization of troublesome weedy exotics.

Research being conducted by the Army Corps of Engineers Aquatic Plant Control Research Program has led to the development of a new revegetation method that they refer to as the "founder colony" approach (sensu Smart et al. 1998). The key to this approach is to treat submerged macrophytes introduced into the system much as one would treat fish i.e., close monitoring, habitat modification and restocking are provided, on a continuing basis, as necessary. Use of the founder colony approach has yielded successful results in reservoirs and natural lakes in several states including Texas, Oklahoma, Kansas, Alabama and New York (Smart et al. 1996, Smart et al. 1998). Establishment of diverse communities of native submerged aquatic plants in

these systems resulted in improved water quality, enhanced fish habitat and prevention of invasion by weedy exotics. These results indicate the potential for the successful use of the founder colony approach in a wide variety of lake systems. Smart et al. (1996) suggested the use of preliminary investigations of selected environmental characteristics including sediment suitability to evaluate the viability of a location as submerged macrophyte habitat.

Macrophyte Species Selection

Establishment of diverse submersed macrophyte communities should be a fundamental goal of lake restoration projects. In order to accomplish this goal, species selected for lake restoration should include both annuals (pioneer species) and perennials and r- and K-selected species. In an ecological sense, lakes in which restoration methods have been recently used to improve water quality are disturbed environments. In addition, submersed aquatic plant communities are often absent from such systems for up to many years prior to restoration primarily due to insufficient light availability for sustained growth. Natural systems recover in a predictable ecological succession following a disturbance that destroys the plant community at a given site. The first colonizers are r-selected pioneer species that exhibit rapid increases in biomass and produce large quantities of widely dispersed propagule material (Stearns 1977). These characteristics allow pioneer species to rapidly colonize available niches. Pioneer species also modify the localized environment in a variety of ways. These species promote stability and conditions favorable for subsequent establishment of perennials including increased sedimentation that creates more shallow areas suitable for macrophyte growth

(Carpenter 1981), increased water clarity due to decreased turbidity (Carpenter and Lodge 1986), decreased algal populations due to reduction in water column nutrients (Kufel and Ozimek 1994) and/or modifications in the aquatic food web (Schriver et al. 1995). Eventually, the system enters a more advanced state of succession, and perennial species dominate the community composition. The life history of perennials includes a longer life span and increased accumulation of energy reserves which results in slower growth as compared to that of pioneer species. However, the growth strategy of perennials makes these species more resistant to subsequent disturbance as compared to pioneer species (Grime 1979, Sheldon 1986). An additional advantage associated with the establishment of pioneer species is that these plants produce extensive seed banks that permit rapid recolonization following any future disturbance that might set the community back to an earlier successional stage (van der Valk 1981, Sheldon 1986). The plasticity in response to environmental perturbation conferred to macrophyte communities by the combined life strategies of annual and perennial species in diverse communities is probably of pivotal importance in the maintenance of some shallow eutrophic systems in the macrophyte-dominated stable state. Community diversity is a fundamental aspect of the founder colony approach. Smart et al. (1996) discussed the importance of selecting a variety of species, both annuals and perennials, r-selected and K-selected species as an important initial step in the use of this method.

Several criteria were used to select the macrophyte species investigated in this study. Initially, selection was determined by the need to identify Florida native submersed macrophytes with a wide distribution and for which propagule material is

readily available. In addition, a mix of annual and perennial species was selected in order to maximize the potential for establishment of viable, self-sustaining communities of SAV capable of out-competing exotic species in restored lakes. Finally, plants were selected based on their relative value as habitat for fish and other aquatic organisms, resistance to herbivory and relative potential positive impact on the water quality of the lake.

Description of Study Macrophytes

Four species were selected using the aforementioned criteria – *Najas guadelupensis* (Spreng.) Magnus), *Potamogeton illinoensis* Merong, *Vallisneria americana* Michx.*)* and *Chara* sp.

Annuals – *Najas guadelupensis* and *Chara* sp., both summer annuals, are excellent pioneer species. *N. guadalupensis* is often the primary colonizer in areas of disturbance (i.e. abandoned fish nests, boat trails and in areas previously not colonized by submersed macrophytes (Lawson 1991, Hopson-Fernandes personal observation). *N. guadalupensis* expansion is due to prolific seed production, drought tolerance and ability to colonize by vegetative fragmentation and subsequent adventitious root formation. *N. guadalupensis* exhibits a decumbent habit with very slender, sparsely branched stems with numerous finely dissected (2 cm long and 1.2 mm wide) leaves (Westerdahl and Getsinger 1988) (Figure 2.1). In nature, *N. guadalupensis* forms monospecific stands (defined as those in which a single species contributes greater than 90% of the total biomass).

There are 35 species of *Chara* occurring in the United States (Figure 2.2). The majority of the species are dioecious. Reproduction also occurs via fragmentation.

Chara sp. typically occur at shallow depths but have been found at depths greater than 10 m. Some recent studies (e.g. Blindow 1991, Kufel and Ozimek 1994, Kufel and Kufel 2002) found that *Chara* may play an important role in improving water quality due to its storage capacity and ability to colonize shallow lakes previously dominated by phytoplankton. In a study conducted in Lake Okeechobee, Steinman et al. (2002) observed high *Chara* biomass over a wide range of light levels. Charophytes were also the first submerged macrophytes to re-establish following a managed recession of the lake. Kufel and Kufel (2002) discuss additional positive effects of *Chara* growth in systems including relatively slower decomposition rates and lower contributions of sediment-derived nitrogen and phosphorus to the water column upon decomposition as compared to rooted vascular plants.

Perennials-*P. illinoensis*, a native perennial in the Potamogetonaceae, is a common inhabitant of many Florida lakes. As compared to the other macrophytes included in this study, *P. illinoensis* has slightly thicker stems and broad, flat lanceolate leaves measuring 15 cm long by 6 cm wide (Westerdahl and Getsinger 1988) (Figure 2.3). The growth form of this plant involves the production of elongate stems which typically extend to the water surface. The majority of the elodeid (*sensu* Hutchinson 1975) leaves are concentrated in the photic zone. *P. illinoensis* exhibits low biomass density (cf. Duarte and Kalff 1990). Members of the Potamogetonaceae propogate vegetatively via budding from underground rhizomes. The formation of tubers, structures in which the plant sequesters carbohydrate reserves, is critical to the germination and early development of young plants (Hodgson 1966).

V. americana, a perennial native in the Hydrocharitaceae, exhibits a growth form that is dissimilar to that of the other study plants (Figure 2.4). *V. americana* grows as rosettes of elongate, ribbon-like leaves, measuring up to 2 m in length by ca. 3 cm in width (Westerdahl and Getsinger 1988) which arise from a stoloniferous rootstock. Each rosette usually gives rise to several stolons which in turn produce multiple rosettes. The root systems may allow *V. americana* to exploit sediment nutrient reserves that are unavailable to other rooted macrophyte species (Titus and Stephens 1983). Titus and Stephens (1983) also observed that *V. americana* is capable of altering its growth pattern in response to the presence of other plant species. *Vallisneria* propagates by seeds and by the production of vegetative propagules. *Vallisneria* concentrates biomass lower in the water column as compared to canopy-forming species and exhibits median biomass density (*sensu* Duarte and Kalff 1990).

Submerged Macrophyte Biology

According to the classification scheme for aquatic macrophytes developed by Arber (1920) and Sculthorpe (1967) submerged macrophytes are those aquatic plants which are rooted in the sediment. This is a heterogeneous group of plants composed of filamentous algae (e.g. Cladophora), certain macroalgae (e.g. *Chara* and *Nitella*) numerous mosses, a few nonvascular macrophytes and approximately 20 families of vascular macrophytes (Wetzel 2001). There is considerable evidence that aquatic angiosperms originated on land. Wetzel (2001) discusses the fact that adaptation and specialization in aquatic macrophytes lags behind colonization of the aquatic habitat.

He uses this phenomenon to explain the presence of nonfunctional vestigial structures such as cuticles and stomates in many submerged macrophyte groups.

Life in the aquatic environment has, however, resulted in the development of salient morphological and physiological features in submerged macrophytes. Submerged macrophytes do not require a dominant erect axis; there is a reduction in the lignification of plant tissues and sclerenchyma and collenchyma are rarely present even in vascular tissues. There is no evidence of secondary growth or cambial development (Sculthorpe 1967, Wetzel 2001). In many species the vascular strands are condensed resulting in central lacunae. Submerged macrophytes also exhibit adaptations to low light availability similar to those displayed by terrestrial shade plants including a very thin cuticle, thin leaves (1-3 cell layers thick) and high numbers of chloroplasts in epidermal tissue. Leaves tend to be much more divided, resulting in much greater surface-to-volume ratios, than in terrestrial plants. Leaf morphology maximizes exposure to light and to dissolved gases and nutrients in the water column (Wetzel 2001). Many submerged macrophytes exhibit extreme heterophylly, often on the same stem or petiole. Submerged macrophytes can be divided into two groups based upon their growth form as determined by differences in apical organization: an abbreviated axis producing a rosette of radical leaves or an elongated flexuous stem with an abundance of leaves and rooted from the nodes (Sculthorpe 1967). The different growth forms are determined by differences in apical organization.

Vegetative and clonal reproduction are the primary mechanisms for propagation and dispersal of submerged macrophytes. Although sexual reproduction

has been retained, vegetative reproduction plays a far more important role in the life history of hydrophytes. Submerged macrophytes propagate vegetatively via fragmentation, horizontal expansion by rhizomes, stolons, runners, and tubers and via the production of specialized organs such as turions (winter buds). Most species produce aerial, insect or wind-pollinated flowers, although there are some examples of hydrophily (water-pollinated flowers) (e.g. the Najadales and many genera in the Hydrocharitaceae) (Sculthorpe 1967). Cox (1993) determined that hydrophily occurs in < 5% of aquatic species.

Additional morphological and physiological adaptations are necessary to permit the flow of gases required to minimize the toxic effects of the byproducts of anaerobic fermentation in aquatic sediments and to sustain photosynthetic and respiratory metabolic processes (Wetzel 2001). Metabolically significant gases move throughout the plant body through large intercellular spaces and cortical gas spaces in roots and shoots. Internal lacunae make up a large portion of the total plant volume (often > 70%) (Wetzel 2001). A temporal difference in the production of oxygen during photosynthesis and subsequent release to the environment creates an internal positive pressure (Hartmann and Brown 1967, Sorrell 1991). This internal pressure plays a significant role in the internal mass flow of gases throughout the plant body. Sorrell and Dromgoole (1989) determined that large portions of the oxygen stored in the lacunae were used in respiratory metabolism without affecting the surrounding water. Moeslund et al. (1981) concluded that, although oxygen to water exchange increases with increasing current, oxygen concentrations in the surrounding water do not reflect the entire amount of oxygen produced during photosynthesis. This

tendency to "stockpile" oxygen prompted Wetzel (2001) to suggest caution in the use of oxygen evolution experiments as a quantitative indicator of macrophyte photosynthesis. Much of the oxygen produced during photosynthesis is transported to the roots and diffuses into the microrhizosphere (Sand-Jensen et al. 1982, Zhang et al. 1998) where it forms an oxidized layer immediately around the root and is utilized by microaerophyllic bacteria.

There is increasing evidence that inorganic carbon availability may be the most significant factor limiting aquatic macrophyte growth in some systems. The diffusion rate of gases in water is four orders of magnitude slower than in air. Submerged organs are also surrounded by a stagnant boundary layer that can be from several mm to cm thick (Wetzel 2001) that further impedes gas diffusion into the plant. In order to survive the challenges posed by carbon acquisition in the aquatic environment, submerged macrophytes have evolved several adaptations that facilitate uptake, conservation and recycling of carbon. Morphological modifications to stems, leaves and petioles that facilitate absorption of dissolved gases include an extremely thin cuticle, highly reduced mesophyll tissue, concentration of photosynthetic pigments in the epidermis and thin tissues (1 to 3 cells thick). Once inside the plant body, large intercellular lacunae facilitate internal diffusion of gases to all plant organs. Most submerged species exhibit C3-Calvin-type photosynthesis with high rates of photorespiration (Wetzel 2001). Additional carbon sources for hydrophytes include the CO_2 produced during photorespiration and cellular respiration. Some softwater species also take up CO_2 from sediments (Wetzel 2001). Kimber et al. (1999) concluded from isotope tests that the majority of the carbon fixed in

Vallisneria was sediment-derived. Some aquatic angiosperms occupying carbon-poor systems photosynthesize via crassulacean acid metabolism (CAM) (e.g. *Vallisneria*). These CAM plants assimilate CO_2 at night and store it as malate for use in photosynthesis during the light hours.

Submerged macrophytes have developed two basic strategies for carbon assimilation in the aquatic environment (cf. reviews of Maberly and Spence 1983, Madsen and Sand-Jensen 1991). Some species depend primarily to exclusively on CO_2 for their carbon source (Wetzel 1969, Van et al. 1976, Moeller 1978b, Kadono 1980) while others utilize bicarbonate (Raven 1970, Prins et al. 1980, Beer and Wetzel 1981, Lucas 1983). Obligate CO_2 species are restricted to locales with sufficient levels of carbon dioxide to support their growth. Since bicarbonate often occurs in higher concentrations in natural waters as compared to carbon dioxide, uptake of bicarbonate would appear to be an advantageous adaptation (Wetzel 2001). This mechanism has been documented in many macroalgae, and in mosses and submerged angiosperms (Ruttner 1947, 1948, 1960, Bain and Proctor 1980). Raven and Lucas (1985) and Eighmy et al. (1991) discuss the energetic costs associated with the use of bicarbonate as the carbon source for photosynthesis. Many submerged species exhibit low CO_2 compensation points and relatively high productivity (Wetzel 2001).

Submerged macrophytes occurring in lakes inhabit the littoral zone in the area extending from the shoreline to the depth at which light becomes limiting. Submerged macrophytes colonize all depths of the photic zone of lakes (Wetzel 2001). Vascular angiosperms can occur up to depths of approximately 10 m while nonvascular

macrophytes (e.g. macroalgae) can be found growing up to the littoral-pelagic interface. McCreary (1991) provides a thorough review of the competitive causes for the zonation of macrophyte distribution characteristic of littoral areas.

Environmental Factors Controlling Production

Studies that have addressed the dynamics of submerged macrophyte communities suggest that macrophyte production is influenced by a variety of abiotic and biotic environmental factors. Among these factors are light availability (Van et al. 1976, Barko and Smart 1981b, Duarte and Kalff 1986, Chambers 1987a, Chambers and Kalff 1987, Canfield and Hoyer 1988b, Hough and Fornwall 1988, Goldsborough and Kemp 1988, Chambers and Prepas 1990, Grimshaw et al. 2002), sediment composition and nutrients (Sculthorpe 1967, Barko and Smart 1983, Short 1987, Barko and Smart 1986, Barko et al. 1991), water transparency and depth (Spence 1967, Canfield et al. 1985, Scheffer et al. 1992), nutrient availability (Spence 1967, Chambers and Kalff 1987b, Canfield and Hoyer 1988b, Hough and Fornwall 1988, Lauridsen et al. 1993) and temperature (Barko and Smart 1981b). Wind events were also found to have a significant effect on macrophyte production in the shallow lake studied by Lauridsen et al. (1993). Sculthorpe (1967) identified light intensity and quality, the nature of the substrate and turbulence as the most significant environmental factors determining the distribution of communities, species and growth forms of submerged macrophytes.

The effects of various biotic factors on macrophyte growth have been the topic of additional studies. Sand-Jensen and Borum (1984) and Sand-Jensen (1990) observed a negative relationship between macrophyte growth and the degree of light

attenuation by the epiphytes. In his 1971 study, Allen described the "antagonism" (sensu Fitzgerald 1969) between macrophytes, epiphytes, and phytoplankton competing for available light and nutrients within aquatic systems. In many systems, grazing also plays an integral role in macrophyte community dynamics (Wetzel 1983, Cattaneo 1990, Lauridsen et al. 1993). Lodge (1991) reviewed the literature on the effects of herbivory on freshwater macrophytes.

A variety of studies have reported species-specific responses of macrophytes to their environment (Spence 1964, Van et al. 1976, Chambers and Kalff 1987b, Hough and Fornwall 1988, Chambers and Prepas 1990, Scheffer et al. 1992, Spencer et al. 2000, Cenzato and Ganf 2001). Scheffer et al. (1992), for example, documented a differential response in two species of *P. illinoensis* to changes in water depth in the study system. Spencer et al. (2000) reported species-specific differences in the number of degree-days required for emergence of vegetative propagules of three submerged species. Changing environmental conditions often result in shifts in dominance among competing species within macrophyte communities.

The Role of Light in Submerged Macrophyte Growth

Numerous studies have identified light availability as one of the most significant factors controlling macrophyte production in aquatic systems (Duarte and Kalff 1986, Canfield et al. 1985, Barko et al. 1986, Smith and Barko 1990). Various studies have investigated the impact of light attenuation on submerged macrophyte productivity (Carter and Rybicki 1990, Duarte 1991, Dunton 1994, Goodman et al. 1995, Masini et al. 1995, Zimmerman et al. 1995, Livingston et al. 1998). The classification scheme Gessner (1955) developed for submerged macrophytes based on

their physiological adaptations to light availability indicates the extreme plasticity and adaptability of submerged macrophytes to the highly variable underwater light environment. The adaptation types include strictly shade adapted requiring low light intensities, strictly light adapted and requiring high light intensities, shade adapted but exhibiting optimum photosynthesis at intermediate light levels, etc. Van et al. (1976) and Kenworthy and Fonseca (1996) reported species-specific differences in minimum light requirements. All submerged angiosperms are shade plants (Wetzel 2001). Spencer and Bowes (1990) determined that light saturation for photosynthesis ranges from 10-50% full sunlight. Van et al. (1976) reported photosynthetic rates less than 5% of those achieved by terrestrial plants at atmospheric levels of CO_2 for three species of submerged aquatic macrophytes. They attributed this to low activities of the carboxylation enzymes. Goldsborough and Kemp (1988) investigated the response and recovery of *Potamogeton perfoliatus* to experimentally induced shade during a 17-day treatment period followed by a 16-day "recovery" period. During the treatment period, plants responded by increasing photosynthetic pigments and producing elongate stems, thinning lower leaves and canopy formation at the surface. Plants showed significant recovery 10 days after removal of light treatments. The results of their study indicated that the study species requires a minimum of 11% full sunlight in order to survive and grow. Other investigations of photosynthetic capacity and compensation points indicated considerable variation among submerged macrophytes (Wetzel 2001). Light compensation points often occur at 1-3% full sunlight (Wilkinson 1963, Spence 1982, Bowes et al. 1977, Moeller 1980). Kimber et al. (1995) reported that tuber production in *Vallisneria americana* ceased at light

levels less than 5% of ambient sunlight. They discussed the implications of these results for vallisneria growth in relation to light attenuation by turbidity in their study system. Dennison (1987) discussed the use of photosynthesis vs. irradiance curves together with diurnal light curves to predict growth responses to changes in light regime (Dennison and Alberte 1985), seasonal growth patterns and the maximum depth of colonization for Zostera marina. Carter et al. (1996) reported an 11-fold increase and a 38-fold increase in total biomass of *V. americana* americana in lighted cages in the Chesapeake Bay and the Potomac River. Plants exposed to increased light levels were more robust and fewer in number as compared to controls.

The Role of Sediments in Macrophyte Nutritional Ecology

The function of roots in submersed macrophytes, especially in angiosperms, has been subject to debate (Wetzel 2001). After much research (reviewed in Gessner 1959, Wetzel 1964, Sculthorpe 1967, Hutchinson 1975), two theories were proposed: a) the function of roots was strictly to anchor the plant and b) the roots function in nutrient absorption from the substrate. Considerable recent research has now confirmed that the primary function of the root systems in aquatic macrophytes is to assimilate nutrients from the sediment (Barko and Smart 1986, Short 1987, Barko et al. 1991, Wetzel 2001). A positive absorptive capacity is present in the roots that facilitates uptake of sediment nutrients. Most submerged species obtain the majority of their nutrient requirements from the interstitial water of the sediments, where nutrients are present at much greater concentrations than in the water column. Shannon (1953) determined that absorptive capacity of the roots is enhanced by the presence of root hairs. Many species also exhibit symbiotic relationships with

vesicular-arbuscular micorrhyzae (Sondergaard and Laegaard 1977, Tanner and Clayton 1985, Wetzel and van der Valk 1996). The mechanism for assimilation of sediment nutrients – the solubilization and mobilization of soil nutrients by secreted organic acids- utilized by terrestrial plants is thought to be the same in submerged plants (Wetzel 2001).

Researchers first became aware of the influence of sediment composition on the productivity and distribution of submerged macrophytes almost one century ago (Pond 1905, Pearsall 1920, Misra 1938). Subsequent studies conducted in a wide variety of aquatic systems have confirmed that sediment composition exerts a significant effect on submerged macrophyte growth (Moeller 1975, Anderson 1978, Sand-Jensen and Sondergaard 1979, Kiorboe 1980, Danell and Sjoberg 1982, Wheeler and Giller 1982, Barko and Smart 1983, Barko and Smart 1986, Barko et al. 1991, Livingston et al. 1998). Barko et al. (1991) reported that nitrogen, phosphorus, iron, manganese and micronutrients are taken up from the sediment. Calcium, magnesium, sodium, potassium, sulfate and chloride are assimilated from the water column. The majority of research into the plant available sediment nutrients has been conducted on phosphorus and nitrogen. Of this literature, most has focussed on phosphorus (Barko et al. (1991). As a result of the large exchangeable pool of phosphorus in most lake sediments, phosphorus is rarely the limiting factor in hydrophyte growth (Wetzel 2001). Of the limited information available on the relative contributions of sediment and open water to the nitrogen economy of submerged aquatics, several studies indicate that nitrogen can be assimilated from both the sediment and the open-water (Nichols and Keeney 1976, Short and McRoy

1984). These isotope studies concluded that nitrogen uptake rates were positively correlated with concentration and that the study macrophytes preferred ammonium to nitrate for uptake. Nichols and Keeney (1976) reported that, since sediment ammonium concentrations are typically much greater than water column concentrations, sediment is probably the major source of nitrogen. There are relatively few documented cases of inorganic nutrient limitation in submerged aquatic macrophytes (Anderson and Kalff 1986, Barko et al. 1986). Barko and Smart (1983) concluded that sediment organic matter can greatly inhibit the growth of submerged macrophytes. Refractory organic matter appeared to have a longer-lasting inhibitory effect as compared to labile organic matter. Barko and Smart (1983) attributed this inhibition to the presence of high concentrations of soluble organic compounds in the interstitial water produced during anaerobic decomposition. In a later study involving the growth of *Hydrilla* and *Myriophyllum* on 40 different sediments collected from 17 North American lakes, Barko and Smart (1986) concluded that sediment density rather than organic matter content was most influential in regulating plant growth. They identified a significant relationship between macrophyte growth, nutrition and sediment density. Short (1987) reviewed the effects of sediment nutrients on seagrasses and concluded that seagrass production is strongly correlated with nutrient availability. He cites examples of how differences in the geochemistry of systems can result in either phosphorus or nitrogen limitation and concludes. He concludes that sediment geochemistry is the most significant factor controlling seagrass growth.

Rybicki and Carter (1986) reported better growth of *Vallisneria americana* on silty clay than on sand. Barko and Smart (1986) had similar results. They found that

Hydrilla and *Myriophyllum* exhibited poor growth on highly organic sediments and on sands. The results of fertilization studies indicated that macrophyte growth limitation on these substrates was due to nutrient deficiencies. Amendment of sand substrates with fertilizer has been used in many studies to circumvent problems with nutrient limitation when using sand substrates (c.f. Sutton 1985, 1993, 1996). Denny (1972b) reported species-specific responses to sediment composition and nutrient concentrations. Barko and Smart (1983) identified the difficulty in separating the effects of sediment nutrient availability from other sediment characteristics as one of the greatest challenges associated with investigations of the relationship between sediment composition and macrophyte growth.

Culturing Submerged Macrophytes

Early attempts to culture submerged macrophytes were complicated by the misconception that macrophyte nutrient requirements were water-derived (c.f. Bourn 1932, Sculthorpe 1967) and that roots functioned simply as organs of attachment (c.f. Brown 1913, Den Hartog and Segal 1964). Further complicating the effort to culture submerged macrophytes was the use of culture media developed for macrophytic algae (e.g. (Pringsheim and Pringsheim 1962, Forsberg 1965) and for hydroponic growth of terrestrial plants (Hoagland and Arnon 1938). The realization of the importance of sediment nutrient acquisition in submerged aquatics by researchers such as Denny (1980) has resulted in significant advances in the culture of submerged macrophytes. Smart and Barko (1985), and Smart et al. (1996, 1998) provide a thorough discussion of culturing submerged aquatic plant species native to subtropical climes. Smart and Barko (1985) discuss the nutrient requirements of

submerged aquatics relative to the uptake mechanism (i.e. sediment- versus water-derived).

One of the challenges to conducting investigations of submerged macrophytes is the autocorrelation of environmental factors. Spence (1991) discussed the difficulty separating the effects of light and sediment composition on macrophyte growth poses for researchers. A plausible solution to this dilemma is the use of growth chambers located in ambient conditions. The use of growth chambers allows researchers to manipulate one variable and determine its effect in an otherwise controlled environment. Many studies investigating submerged macrophytes have been conducted in outdoor growth tanks (Sutton 1985, Short 1987, Sutton et al. 1992, Sutton and Portier 1995, Kimber et al. 1999, Spencer et al. 2000, Grimshaw et al. 2002). Grimshaw et al.(2002) used differing numbers of shade cloth layers to determine the response of several species of submerged aquatic macrophytes to differing light availability in the aquatic environment. Short (1987) used experimental mesocosms to investigate sediment nutrient effects on seagrass growth. Barko and Smart (1986) used large outdoor growth tanks to investigate sediment-related mechanisms of growth limitation in submerged macrophytes. Macrophyte growth studies in this experiment were conducted in large outdoor tanks (6.2 m in length by 3-1 m in width by 0.9 m in height filled with pond water to a depth of 0.8 m). Pond water was the water source and was exchanged daily.

Epiphytic Algae

Aquatic epiphytes are defined as those organisms that attach to aquatic macrophytes (Wetzel 1983). The "epiphytic community" associated with aquatic

macrophytes is composed of autotrophs and heterotrophs cohabitating in a matrix of detritus and mineral elements (Sand-Jensen et al. 1989). The autotrophic component includes epiphytic algae. This study focussed on the algal component of the epiphytic community associated with the submerged macrophytes occurring in the experimental tanks. From this point forward, the term epiphyte will be used to refer to the algal component only.

Epiphytes are an integral component of the autotrophic community within many aquatic systems. Cattaneo and Kalff (1980) reported that epiphytes can play a significant role in the total primary production of macrophyte beds (up to 62%), a role which may have been underestimated in previous studies of other systems (Wetzel 1964, Adams et al. 1974). Burkholder and Wetzel (1989a) determined that, as much as 70-85% of the total carbon produced in littoral systems may be a direct by-product of epiphyte metabolism. Stable isotope analyses suggest the importance of attached algae as a source of carbon and nitrogen for higher trophic levels in some marine systems (Sullivan and Moncrieff 1990).

Epiphyte growth is influenced by a variety of environmental factors. The epiphytic microenvironment—the metabolizing macrophyte—is an unstable one responding rapidly to variations in the host due to grazing pressure, age, changing nutrient status and light availability (Allanson 1973). Several studies (Cattaneo and Kalff 1979, Cattaneo and Kalff 1980, Rogers and Breen 1983, Borum 1987, Burkholder and Wetzel 1989a and Lalonde and Downing 1991) have reported a correlation between macrophyte growth form and epiphyte colonization rates and maximum attainable biomass.

The literature also suggests that epiphyte biomass is affected by a variety of additional environmental factors. Wetzel (1964) reported a positive correlation between temperature and epiphyte biomass. Several studies (Fox et al 1969, Phillips et al 1978, Cattaneo and Kalff 1980) have identified a positive relationship between epiphyte biomass and increasing water depth. This relationship may be due to decreased wave activity at greater water depths (Fox et al. 1969), decreased grazing pressure (Cattaneo 1990) or perhaps to a combination of both. Cattaneo (1990) concluded that increased grazing pressure contributed to a sudden decline in epiphyte biomass in Lake Memphremagog, Canada. Epiphyte communities are highly susceptible to loss due to scouring caused by heavy precipitation and strong wind events (Sand-Jensen 1983, Wetzel 1983, Kairesalo 1984, Sand-Jensen et al. 1989.) Tete et al. (1978) presented a thorough discussion of the influence of water flow rates on attached organisms in their 1978 review paper. High epiphytic biomass has also been related to high light (Wetzel 1964, Pieczynska and Szczepanska 1966, Gons 1982) and nutrient availablity (Straskraba and Pieczynska 1970, Phillips et al. 1978, Cattaneo and Kalff 1980). In studies in different lacustrine systems, Shelden and Boylen (1975), Tai and Hodgkiss (1975) and Hooper-Reid and Robinson (1978) correlated decreased epiphyte biomass with nutrient deficiency. Cattaneo and Kalff (1980) reported a strong correlation between phosphorus and epiphyte production suggesting that phosphorus may be the growth-limiting nutrient for epiphytes. Raschke (1993) observed that increased phosphorus loading in the Everglades National Park stimulated diatom growth. Davis et al. (1990) defined a significant relationship between light availability and phosphorus uptake by epiphytes.

Within aquatic communities, competition between autotrophs for resources has a significant effect on the composition and dynamics of the epiphyte community. In their 1971 and 1984 studies respectively, Allen and Sand-Jensen and Borum reported the same phenomenon that Fitzgerald (1969) described as an "antagonistic" relationship between macrophytes, epiphytes and phytoplankton. Other researchers have investigated the effect of competition for light (Sand-Jensen and Borum 1984 , Sand-Jensen 1990) and nutrients (Sondergaard and Sand-Jensen 1978) upon the composition and dynamics of the epiphyte community. Rapid nutrient and CO_2 uptake rates make planktonic autotrophs more successful than their attached competitors for nutrients in the water column (Sondergaard and Sand-Jensen 1978). Competition for water column nutrients is typically greatest between pelagic and epiphytic autotrophs, as rooted submerged macrophytes derive most of their nutrient requirements from the sediment (Sondergaard and Sand-Jensen 1978, Barko and Smart 1981a, Barko et al. 1991). Several studies (Wetzel et al. 1985, Rattray et al. 1991, Kimber et al. 1999) have suggested that submerged macrophytes may also utilize sediment-derived CO_2 under certain field conditions.

The Macrophyte-Epiphyte Complex

Submerged aquatic macrophytes and associated epiphytic algal communities are keystone components of lacustrine ecosystems with significant macrophyte populations (Wetzel 1983, Wetzel 2001, Kalff 2002). Several studies (Wetzel 1983, Moeller et al. 1988, Burkholder and Wetzel 1989a) have concluded that the high rates of productivity within the littoral zone of macrophyte-dominated lakes are due primarily to the presence of this primary producer complex. Aquatic macrophytes

also provide critical habitat for a variety of organisms consumed by higher trophic levels including epiphytes, invertebrates and bacteria (Soszka 1975, Cattaneo and Kalff 1980, Wiley et al. 1984, van der Velde 1987). The surfaces available for attachment of microorganisms are considerable; submerged vegetation provided a 5-fold increase in colonizable surface area in the system investigated by Moeller et al. (1988). Howard-Williams and Allanson (1981) reported a 30-fold increase of surface area in *P. illinoensis* communities in their study system.

Submerged macrophytes and associated epiphytes play a critical role in the nutrient dynamics of macrophyte-dominated systems (Cattaneo and Kalff 1980, Carpenter and Lodge 1986, Burkholder and Wetzel 1989b). Various studies (Carignan and Kalff 1980, Barko and Smart 1981a, Canfield et al. 1983) have indicated the importance of aquatic macrophytes in improving water quality in nutrient-rich systems by a variety of mechanisms including sequestering nutrients (e.g. nitrogen and phosphorus) in tissues. [See section entitled Revegetation Projects in Lake Restoration for a discussion of the significance of macrophytes in the stable state equilibria in shallow lakes.] In his study in Lake Okeechobee, Zimba determined that over 110 metric tons of phosphorus were bound in macrophyte tissue during the study period. Landers (1982) and Carpenter and Lodge (1986) discussed the importance of the role that nutrient release by senescing macrophytes plays in the cycling of nutrients within aquatic systems.

Additional research has been done on the role of epiphytes in nutrient cycling. Several studies have documented the transfer of nutrients from living macrophyte tissue to the epiphyte community (Wetzel and Penhale 1979, Moeller et al. 1988).

Other investigators (Riber et al. 1983, Carlton and Wetzel 1988, Moeller et al. 1988, Burkholder et al. 1990) observed direct nutrient uptake from the water column by the epiphyte community. Zimba (1995) determined that approximately 84% of the total phosphorus removed form the water column by the macrophyte:epiphyte complex in Lake Okeechobee was bound in the epiphytes colonizing submerged macrophytes. Howard-Williams (1981) discussed the implications of subsequent sedimentation of senescent epiphytic biomass as a critical water-to-sediment vector in sediment-to-water nutrient exchange.

Despite the ecological importance of the macrophyte:epiphyte complex, the dynamics of the relationship between epiphytes and macrophyte hosts are poorly understood (Allen 1971, Cattaneo and Kalff 1980, Wetzel 1983, Burkholder and Wetzel 1989b).Some researchers describe this relationship as a mutual symbiosis in which nutrient exchange is on going between epiphytes and host (Brezonik et al. 1979). Other studies have concluded that the epiphyte:macrophyte relationship is best described as a type of "parasitism" in which the epiphytes sap nutrients being pumped from the sediments by the macrophyte host (Harlin 1975, Moeller et al. 1988, Burkholder and Wetzel 1990, Burkholder et al. 1990). Sand-Jensen and Borum (1984) and Sand-Jensen (1990) describe the role that epiphytes can play in host light exclusion. Rogers and Breen (1981) identified a "necrotrophic" relationship in which epiphytes played a critical role in the initial decomposition of leaf tissue in a *P. illinoensis* species. Other investigators have described the relationship as a neutral one in which the role of the macrophyte is primarily one of physical support (Cattaneo and Kalff 1978, Carignan and Kalff 1982). However, the results of these

latter studies indicated the presence of nutrient transfer. Investigations that have documented host-specific algal assemblages (Godward 1934, Prowse 1959, Blindow 1987) and differing epiphyte productivity levels as a function of host plant (Cattaneo and Kalff 1980) suggest that there is some degree of interaction between epiphytes and their hosts. In their study of phosphorus transfer between *N. guadalupensis flexilis* and associated epiphytes, Burkholder and Wetzel (1989b) concluded that there is a probable correlation between epiphytes and their microenvironment.

Methods for Investigating Epiphytic Algae

The relative lack of information on epiphytes and their relationship to their macrophyte hosts is due in large part to the degree of effort involved in the collection and preparation of these organisms for study (Hickman 1971, Main 1973). Several different processing methods are available for use by researchers investigating epiphyte community composition. The first method entails the use of artificial substrates. Many studies have been conducted on algal colonization of artificial substrates (Allen 1971, Brown 1976, Siver 1977, Hooper-Reid and Robinson 1978, Cattaneo and Kalff 1979, Fontaine and Nigh 1983, Fairchild and Lowe 1984, Burkholder and Wetzel 1989b). Other research has indicated that this attempt to simplify the experimental design of epiphyte studies often yields communities that are not comparable to those occurring on natural substrates (Castenholz 1960, Sladeckova 1962, Tippet 1970, Brown 1976).

Another approach involves examination of the plant surface using scanning electron microscopy (SEM) (Allanson 1973, Sieburth et al 1974, Burkholder and Wetzel 1989b). There are several disadvantages associated with the use of SEM as the

primary investigative tool. SEM is a relatively expensive technique. In addition, for many studies, sample storage time required for transport to the laboratory would render epiphyte samples physiologically nonviable.

A third possible technique for investigating epiphytes uses an acid digestion method to allow quantification of the total diatom component present on the natural substrate (Cattaneo and Kalff 1979). This method excludes nondiatomaceous components of the epiphytic flora. It also renders impossible comparison of the percent living versus dead component.

Many studies have identified mechanical removal of the attached algae from living macrophytes as the most effective way to address the community composition as well as the physiological state and activities of epiphytes (Sladeckova 1962, Gough and Woelkerling 1976, Cattaneo and Kalff 1980, Kairesalo 1980, Fairchild and Lowe 1984, Tanaka et al. 1984, Goldsborough and Robinson 1985, Moeller et al. 1988, Sand-Jensen

et al. 1989, Goldsborough and Hickman 1991, Zimba and Hopson 1997). Mechanical removal was the method used in this study.

Figure 2-1: *Najas guadelupensis* is a pioneer species in the Najadaceae.

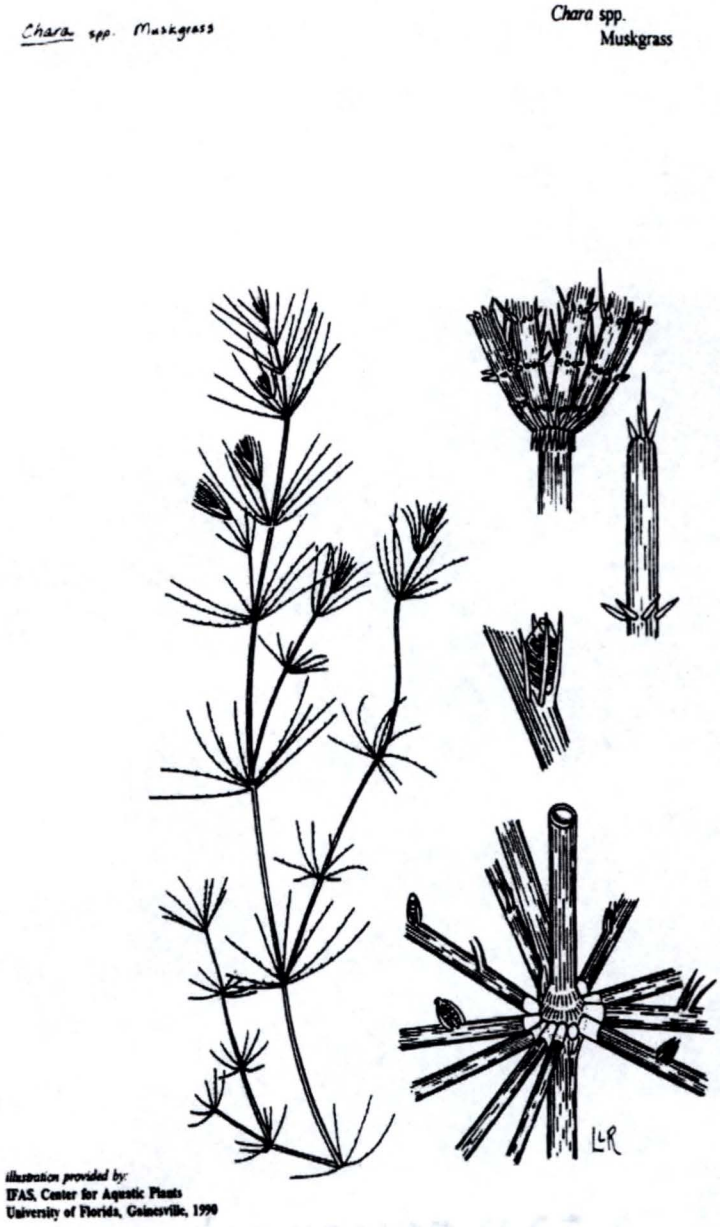


Figure 2-2: *Chara* spp. are macroalgae.

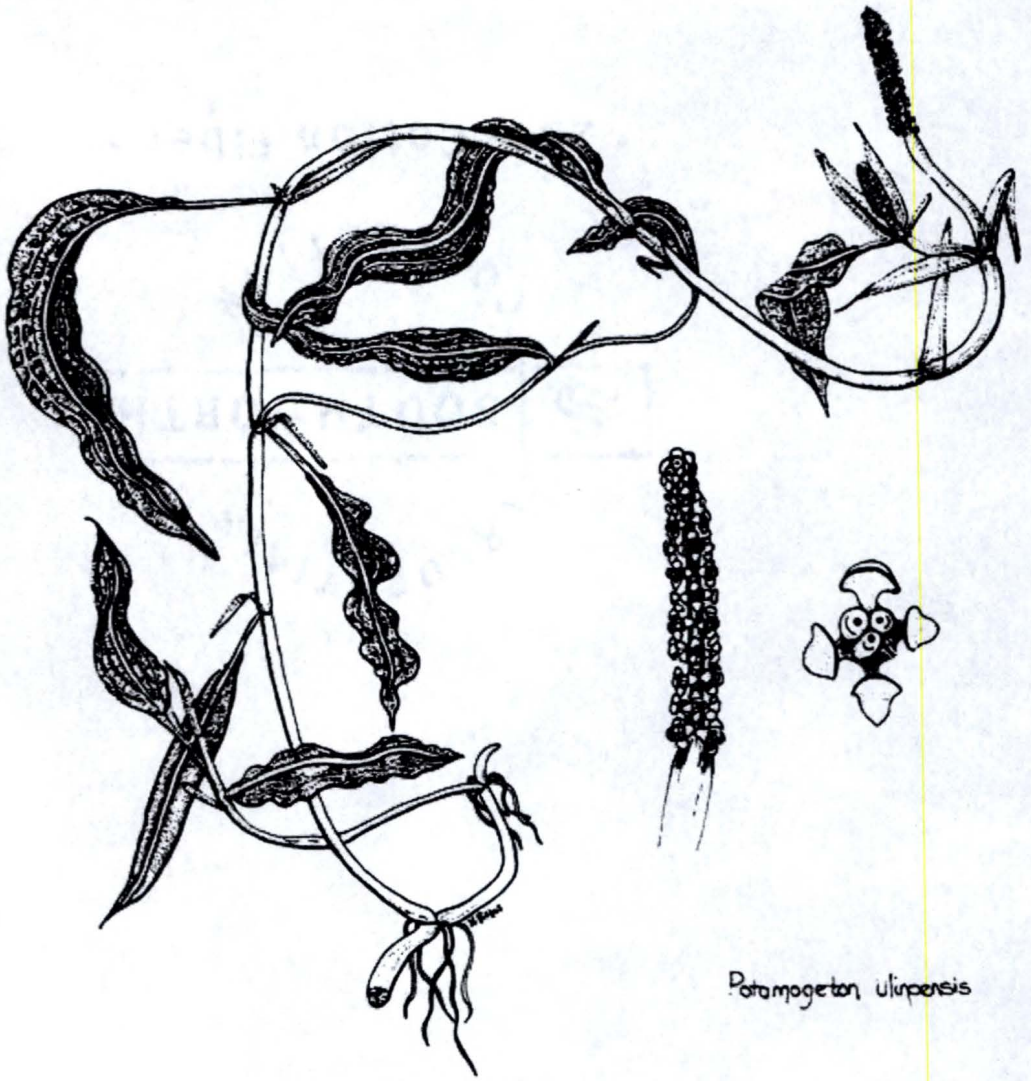


illustration provided by:
IFAS, Center for Aquatic Plants
University of Florida, Gainesville, 1993

Figure 2-3: *Potamogeton illinoensis* is a Florida native perennial with broad lanceolate leaves.

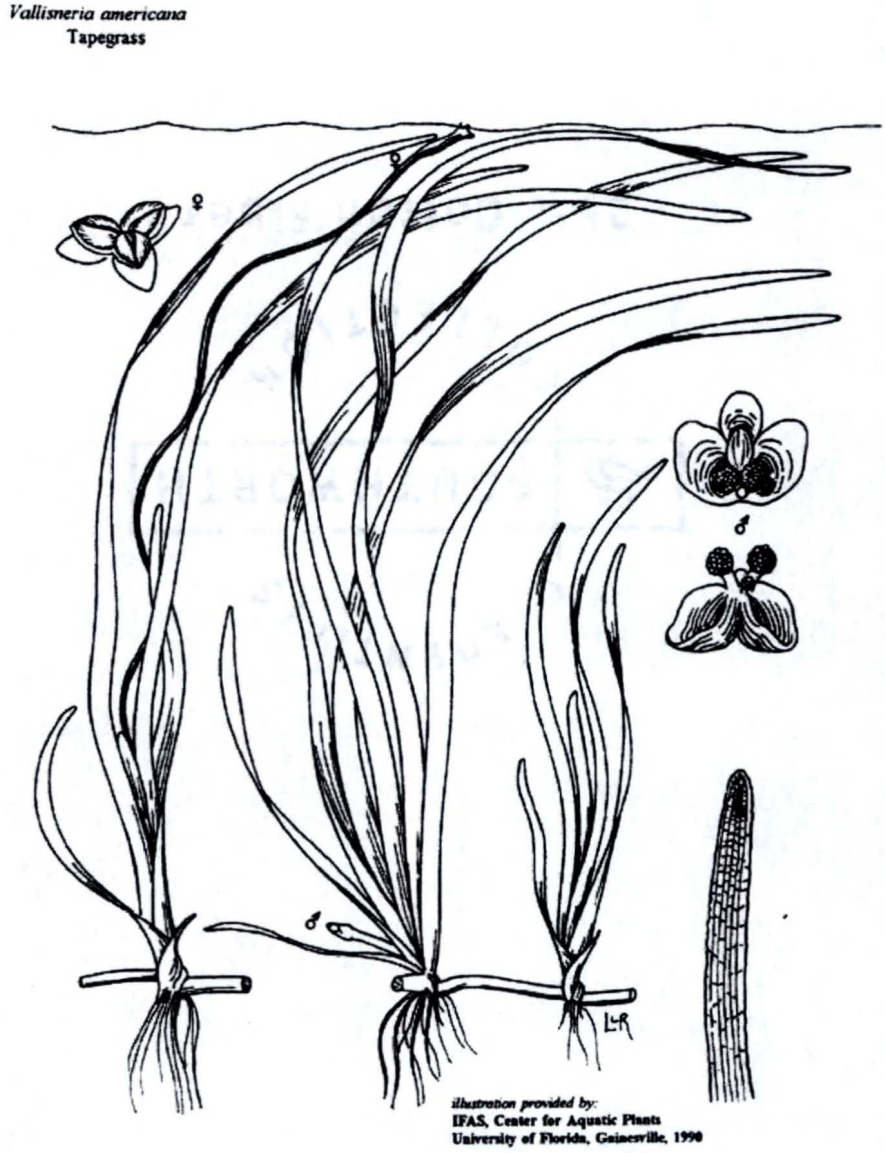


Figure 2-4: *Vallisneria americana* is a perennial with broad ribbonlike leaves.

CHAPTER 3
COMPARISON OF THE GROWTH OF FOUR SPECIES OF NATIVE SUBMERGED MACROPHYTES ON INORGANIC SEDIMENTS FROM A SHALLOW URBAN FLORIDA LAKE

Introduction and Literature Review

The role of submersed macrophyte roots as organs for physical attachment to the substrate versus absorption of sediment nutrients has been the subject of considerable historical debate (reviewed in Gessner 1959, Wetzel 1964, Sculthorpe 1967, Hutchinson 1975, Wetzel 2001). More recent research has confirmed that the primary function of the root systems is to assimilate nutrients from the sediment (Barko and Smart 1986, Short 1987, Barko and Smart 1991, Wetzel 2001). The roots take up nutrients primarily from the interstitial water of the sediments, where nutrients are present at much greater concentrations than in the water column. Shannon (1955) determined that the absorptive capacity of the roots is enhanced by the presence of root hairs. Many species also exhibit symbiotic relationships with vesicular-arbuscular micorrhyzae (Sondergaard and Laegaard 1977, Tanner and Clayton 1985, Wetzel and van der Valk 1996). The mechanism for assimilation of sediment nutrients is believed to be the same as that used by terrestrial plants – the solubilization and mobilization of soil nutrients by secreted organic acids (Wetzel 2001). Although macroalgae typically obtain nutrients through foliar absorption from the water column rather than by rhizoidal structures (Wetzel 1983), uptake of phosphorus from sediment has been documented in some Charophytes (Littlefield and

Forsberg 1965). Littlefield and Forsberg (1965) observed that *Chara* absorbed sediment phosphorus through the rhizoid from where it was translocated to other parts of the plant.

Researchers have been aware of the influence of sediment physical composition on the productivity and distribution of submerged macrophytes since the early 1900's (cf. Pond 1905, Pearsall 1920, Misra 1938). The results of numerous more recent studies conducted in a wide variety of aquatic systems have confirmed that sediment composition exerts a significant effect on submerged macrophyte growth (Moeller 1975, Anderson 1978, Sand-Jensen and Sondergaard 1979, Kiorboe 1980, Danell and Sjoberg 1982, Wheeler and Giller 1982, Barko and Smart 1983, Barko and Smart 1986, Barko et al. 1991, Livingston et al. 1998). Barko and Smart (1983) concluded that sediment organic matter can greatly inhibit the growth of submerged macrophytes. Their results further suggested that refractory organic matter appeared to have a longer-lasting inhibitory effect as compared to labile organic matter. They attributed this inhibition to the presence of high concentrations of soluble organic compounds in the interstitial water produced during anaerobic decomposition. Rybicki and Carter (1986) reported better growth of *Vallisneria americana* on silty clay than on sand. Barko and Smart (1986) found similarly that *Hydrilla* and *Myriophyllum* exhibited poor growth on sands and on highly organic sediments. They identified the effect of long diffusion distances associated with low densities as the cause of the growth response to organic sediments.

Other investigations have been focused on the effect of sediment chemical composition on rooted submersed macrophyte growth. Barko et al. (1991) reported

that nitrogen, phosphorus, iron, manganese and micronutrients are taken up from the sediment whereas calcium, magnesium, sodium, potassium, sulfate and chloride are assimilated from the water column. The majority of research into sediment macro- and micronutrients has been conducted on phosphorus and nitrogen. Of this literature, most has focused on phosphorus (Barko et al. 1991). As a result of the large exchangeable pool of phosphorus present in the sediments of most lakes, phosphorus is rarely the limiting factor in submersed macrophyte growth (Wetzel 2001). Literature available on the nitrogen economy of submerged aquatics is limited in comparison. Several isotope studies indicated that nitrogen can be assimilated from both the sediment and the open-water (Nichols and Keeney 1976, Short and McRoy 1984). The results of these studies suggested that nitrogen uptake rates were positively correlated with concentration and that ammonium was the preferred form of nitrogen for uptake. Nichols and Keeney (1976) suggested that, since sediment ammonium concentrations are typically much greater than water column concentrations, sediment is probably the major source of nitrogen for rooted submersed plants. Fertilization studies conducted in freshwater systems indicated that nitrogen limitation of submersed macrophyte growth may occur in some sediments (Anderson and Kalff 1986, Duarte and Kalff 1988, Moeller et al. 1988).

There are relatively few documented cases of inorganic nutrient limitation in submerged aquatic macrophytes (Anderson and Kalff 1986, Barko et al. 1986). Short (1987) reviewed the effects of sediment nutrients on seagrasses and concluded that seagrass production is strongly correlated with nutrient availability. He cites examples of how differences in the geochemistry of systems can result in either

phosphorus or nitrogen limitation. He concluded that sediment geochemistry is the most significant factor controlling nutrient limitation of seagrass growth. The results of fertilization studies conducted by Barko and Smart (1986) indicated that macrophyte growth limitation on both sand and organic substrates was due to nutrient deficiencies. Many studies have used fertilizer amendments to circumvent problems with nutrient limitation when using sand substrates (c.f. Moeller 1983, Sutton 1985, 1993, Sutton and Latham 1996, this study).

There is still considerable controversy, however, as to the relative importance of sediment chemical and physical composition in macrophyte nutrition (Sculthorpe 1967, Haslam 1978, Denny 1980, Barko and Smart 1986). Barko and Smart (1983) identified the difficulty in separating the effects of sediment nutrient availability from other sediment characteristics as one of the greatest challenges associated with investigations of the relationship between sediment composition and macrophyte growth. Additional studies have indicated that macrophyte growth is significantly impacted by the combined effects of sediment nutrient composition and physical characteristics. In a study involving the growth of *Hydrilla* and *Myriophyllum* on 40 different sediments collected from 17 North American lakes, Barko and Smart (1986) identified a significant relationship between macrophyte growth, nutrition and sediment density. Their results suggested that sediment density is more influential than organic matter content in regulating plant growth.

The results of several studies have suggested that differences in sediment composition may play a significant role in determining species composition within rooted submersed macrophyte communities. Denny (1972) reported species-specific

responses to sediment composition and nutrient concentrations in a growth study that he conducted in concrete ponds. Denny explained these variations in growth response in terms of differences in anatomy and morphology that in turn affected sediment nutrient uptake among the study species. Barko and Smart (1980, 1981, 1986) observed similar species-specific variations in growth response to sediment fertility in several laboratory studies. Chambers (1987) observed a shift in community composition along a natural gradient in Lake Memphremagog such that the percentage of erect and canopy-forming species decreased and rosette and bottom-dwelling species increased with decreasing sediment fertility. See Barko et al. (1991) for a comprehensive review of the ecological implications of variations in sediment composition on submersed macrophyte communities and littoral ecosystem processes. In their guide for establishing SAV, Smart and Barko (1996) suggested the importance of the use of preliminary investigations of sediment composition to evaluate the viability of a location as habitat for colonization by a diverse community of submerged macrophytes.

A review of the literature indicated a paucity of information on the relationship between sediment composition and submersed macrophyte growth. Of the limited body of research on the macrophyte-sediment relationship, most studies have been focussed on nuisance exotic species such as *Hydrilla* and *Myriophyllum*. Very little information has been published on the sediment requirements of desirable native submerged species. The objective of this study was to compare the growth of four species of Florida native submerged macrophytes with varied life histories in inorganic sediments from Lake Hollingsworth. The findings should contribute vital

information on the growth habits of native submerged macrophytes and will be of value to lake managers for use in the development of a systematic approach to the establishment of diverse communities of desirable aquatic vegetation in restored Florida lakes.

The experimental procedure was designed to test several hypotheses based upon findings from the literature and previous experience. The first was that there would be spatial variation in the organic matter and nutrient content of the littoral sediments in Lake Hollingsworth. I also anticipated that *N guadelupensis*, *P. illinoensis* and *Chara* sp., the canopy-forming, erect species, would produce greater biomass in sediments with higher nutrient levels while net growth of *V. americana*, a rosette species, would be inversely related to sediment nutrient content. Based upon my personal experience growing stock cultures of the study plants, I expected to observe differences in growth rate among the species such that *N. guadelupensis* would be the fastest grower followed by *P. illinoensis*, *V. americana* and lastly *Chara* sp. I further expected the growth rate of *Chara* sp to be positively related to increasing culture period length. I anticipated that *N. guadalupensis, V. americana and Chara* sp. would produce the greatest biomass during the summer months while growth of *P. illinoensis,* a species with a range distribution that stretches into more temperate regions, would be stimulated by the cooler temperatures of the winter months. Finally, I hypothesized that sediments collected from the littoral zone of Lake Hollingsworth would have sufficient quantities of sediment nutrients to support the growth of *N. guadalupensis, P. illinoensis, V. americana and Chara* sp. when grown in the experimental growth tanks used in this study.

The study was limited to sediments with low organic matter content to avoid growth inhibition previously associated with high organic matter concentrations (c.f. Barko and Smart 1983, 1986, 1991). Submerged macrophyte species were selected on the basis of several criteria. Initially, species selection was determined by the need to identify Florida native submersed macrophytes for which propagule material is widely available. In addition, a mix of annual and perennial species was selected in order to maximize the potential for establishing viable, self-sustaining communities of SAV capable of out-competing exotic species in restored lakes. Finally, macrophytes were selected based on their relative value as habitat for fish and other aquatic organisms, resistance to herbivory and relative potential positive impact on the water quality of the lake.

Four species were identified using the aforementioned selection criteria – *Najas guadelupensis* (Spreng.) Magnus, *Potamogeton illinoensis* Merong., *Vallisneria americana* Michaux*)* and *Chara* sp. (Refer to the Description of Study Macrophytes section in Chapter 2 for a detailed description of the study species.) *N. guadalupensis* and *Chara* sp. were selected for inclusion in this study for several reasons. Both *N. guadalupensis* and *Chara* are pioneer annual species that expand and rapidly cover the sediment with a carpet of vegetation hence filling a niche that would otherwise be ideal habitat for invasion by an exotic weedy species such as *Hydrilla*. In addition, the increased surface area provided by the finely dissected "leaf" architecture characteristic of both of these native plants makes these macrophytes excellent habitat for fish-food organisms and refugia for juvenile fishes (Engel 1985). *V. americana* and *P. illinoensis* are perennials.

Managed introduction of these four species should result in the establishment of diverse communities that can maintain restored lakes in a macrophyte-dominated stable state. The combination of growth strategies exhibited by these annuals and perennials should make the community relatively resistant to short-term environmental perturbations. Plasticity in the response of the community to changes in water depth and light regime is essential in shallow eutrophic lakes that experience frequent resuspension events and are susceptible to water level fluctuations caused by seasons of heavy rainfall or drought. A combination of growth strategies also improves the chances of the managed community to out-compete exotic species by eliminating available niche space.

Materials and Methods

Site Description

Lake Hollingsworth is a 144 ha shallow (z_{mean}= 1.2 m) (City of Lakeland 1988-2000) urban lake located in central Florida (28°01'30", 81° 56'45") (Figure 3-1). The lake is a solution basin roughly circular in shape with a mean depth of 1.2 m (Lake Hollingsworth Diagnostic Feasibility Study 1994). Water inputs to the lake are in the form of rainfall, groundwater seepage, stormwater runoff and inputs from Lakes Morton and Horney. Groundwater recharge contributes up to 85% of the total water budget for the lake (SWFWMD 1994 Section II). Surface drainage flows southeast into Lake Bentley. Paleolimnological study indicated that Lake Hollingsworth is naturally eutrophic (Brenner et al., 1995). This is probably due to the fact that the lake lies in the Bartow Embayment division of the Central Lakes District where the underlying bedrock consists of the phosphatic sands and clays of

the Bone Valley region of Central Florida (Canfield and Hoyer 1992). In the early 1990's, the results of a Diagnostic Feasibility Study conducted on the lake indicated that Lake Hollingsworth was in an advanced state of aging with estimated sedimentation rates of approximately 30 cm in 20 years. The result of years of sedimentation was a layer of flocculent organic sediments measuring up to 4.6 m in thickness in some areas of the lake. The recommendation of the study was to restore Lake Hollingsworth using the method of whole lake hydraulic dredging. Dredging was commenced in 1999 and was halted before completion due to complications from drought conditions during the dredging period. Post-dredge monitoring efforts indicate a dramatic improvement in water quality in the lake. The lake is a highly valued resource, serving as the site for a variety of social, educational and recreational activities for the City of Lakeland and the surrounding community. The lake also supports sustenance, commercial and sport fisheries.

Sediment Survey

A survey study was conducted to screen the littoral sediments of Lake Hollingsworth in order to identify any possible locations at which sediment characteristics, such as flocculance or relatively high organic matter content, could inhibit submersed macrophyte growth. Lake Hollingsworth is a shallow, highly productive lake with the potential for highly variable organic sediment distribution. In their 1996 study of the variability of sediment distribution in shallow Florida lakes, Whitmore et al. (1996) indicated the importance of using systematic mapping to locate optimal coring sites. A grid was superimposed on the bathymetric map of the lake to identify sampling stations distributed to ensure equal area coverage of the

littoral region (Hakanson 1981). The bathymetric map was drawn on April 13. The convention, when using the grid method, is that stations can either be located within the space between the lines or at the intersection of lines. In this study, stations were located within the spaces between the lines. The sampling grid identified twelve littoral stations occurring along the 2 ft (0.8 m) contour line. Four of the grid stations did not cover the 2 ft (0.8 m) contour line and were disregarded. Eighty centimeters was selected as the optimum planting depth based on several considerations. It has been previously determined that maximum depth of colonization for most submerged macrophyte species in shallow eutrophic lakes similar to Lake Hollingsworth is 1 m. I observed a significant decrease in the biomass of the study species at lake levels above 1 m in Lake Okeechobee (Hopson 1995). Final lake stage in Lake Hollingsworth at completion of the restoration project was subject to fluctuation. In addition, increased turbidity in the system was expected to accompany the whole-lake dredging being conducted at the time of this study. The goal of this study was to provide practical information for use by managers in the lake. Therefore, I decided that planting at 0.8 m would allow for fluctuations in lake stage while ensuring that submerged macrophytes would have sufficient depth for growth while still having sufficient available light. The eight grid stations included in the survey were lettered from A to H, proceeding in a counterclockwise direction (Figure 3-1). Stations on the grid were 0.45 km apart, on the average. Coordinates of station locations were determined using a Trimble CDS1 GPS unit.

Triplicate sediment samples were collected from each station using the sediment coring device described by Sutton (1982). Samples were stored in Ziplock

bags and transported in a dark cooler. Samples were air-dried for at least 72 hours and then dried to constant weight in a forced-air drying oven at 60 °C (Hakanson and Jansson 1983). Dried samples were homogenized and deflocculated in a hammer mill. Samples were then further homogenized using a clockwise layering method, weighed and separated into subsamples for nutrient, pH and organic matter analysis. The pH of the samples was determined by scooping 20 cm^3 of the dried, homogenized sediments into plastic cups. Samples were hydrated by adding 40 mL of deionized water. Samples were then stirred thoroughly and allowed to settle for a minimum of 30 minutes. All samples were processed within 2 hours. A calibrated Orion Research model 601A/digital IONALYZER was used to measure the sample pH. A standard was used as the last sample in each batch to verify the accuracy of the ionalyzer. Organic matter content was determined as weight loss on ignition (LOI) at 550° C in a muffle furnace (Hakanson and Jansson 1983). The results of this survey study were used to select four stations that appeared to provide the most suitable habitat for submersed macrophytes among the eight survey stations. Sediment samples - from the stations selected for the growth study (B, D, F and H) - were analyzed for the plant macro- and micronutrients P, K, Ca, Mg, Zn, Mn, Cu, Al, Na, Fe, Cl^-, NH_4 and NO_3. All samples were extracted using Mehlich-1 extractant and analyzed by inductively coupled argon plasma (ICAP) spectroscopy (Southern Region Information and Exchange Group on Soil Testing and Plant Analyses 1983).

Sediment Collection and Processing

Three sediment cores from each of the four selected stations were collected and loaded into twelve 7.6 L polyethylene nursery containers. Containers were

covered with plastic wrap to prevent drying of sediments during transport. Holes were punched in the plastic wrap to allow for oxygen transfer. Sediments were transported to the University of Florida Fort Lauderdale Research and Education Center (FLREC) (26° 05'N and 80° 14'W) and submerged within 24 hours of collection. The results of the analyses performed on samples collected during the preliminary survey were used to describe the sediment pH, organic matter content and concentration of selected macro- and micronutrients.

Experimental Environment and Procedures

Plants were grown outdoors at the FLREC in concrete tanks (6.2 m L x 31 m W x 0.9 m H) on natural and artificial sediments. Pond water (Steward 1984) flows into the tanks at the surface of one end and out from a drainpipe at the other at a rate providing one volume exchange every 24 hr. Plants were exposed to natural daylight. Water temperature was measured using maximum/minimum thermometers placed 30 cm below the surface of the water. Readings were taken 5 days a week at approximately 3:00 P.M. each day.

Total irradiance (400-1100 nm) (W m^{-2}) was measured by a LICOR LI200SZ pyrometer. These data were sampled every 3.75 min and reported as 15-min averages by a data logger. Total irradiance was converted to photosynthetically active radiation (PAR) (400-700 nm) assuming that PAR is approximately 45% of total irradiance (Baker and Froiun 1987). PAR in W m^{-2} was converted to photosynthetic photon flux density (PPFD) units (μ mol photons s^{-1} m^{-2}) using a multiplier of 4.6 (see Table 3 in Thimijan and Heins 1983). The mean incident PPFD for the photic portion of an experimental period was found by averaging the nonzero fluxes. Accumulated PAR

was found by multiplying the mean incident PPFD by the length of the photic period for the experiment.

A total of 20 treatment groups (4 species x 4 sediment samples plus the control) were tested in each culture period. Three 7.6 L containers were planted for each treatment group, with four propagules per culture container, for a total n = 12 plants per treatment group and an overall total of 60 containers. Plant propagules were obtained from stock cultures maintained at the FLREC. Propagules were harvested from similarly aged, actively growing stock plants. Apical cuttings of *N. guadalupensis* 20 cm in length were planted to a depth of ≥ 3 nodes. Apical cuttings of *P. illinoensis* were planted to a depth of ≥ 2 nodes. Propagules of *V. americana* growing on rhizomes were planted to a depth such that the rhizome was submerged just beneath the sediment surface but the basal rosette was not covered. Apical cuttings of *Chara sp.* 20 cm in length were planted to a depth of ≥ 3 cm. Apical cuttings and rosettes were collected at the beginning of each culture period to give at least ten representative initial samples for each species. Each sample was dried at 60 °C to constant weight (Hakanson 1981). Mean g dry weight at the time of planting was calculated for each species.

The total of 60 culture containers was divided equally into two groups of 30. Each group, containing representatives of each treatment group and plant type, was placed into a separate tank. A random numbers table was used to arrange culture containers in four rows within each tank, parallel to the flow of water (Figure 3-2.) All species were submerged in 0.8 m of water. The study was conducted over three culture periods from 30 April to 29 June 2001 (Spring Culture Period), 20 July to 21

September 2001 (Summer Culture Period) and 19 December 01 to 22 February 02 (Winter Culture Period). Culture period length in each experiment (approximately 9 weeks) was adequate for the development of treatment-related differences in growth but minimized tissue deterioration associated with senescence. In a similar study conducted at the FLREC using sand media amended with controlled-release fertilizers, including Osmocote, to culture *Hydrilla verticillata*, a submersed macrophyte, Sutton and Latham (1996) documented statistical differences in growth after 8 weeks. (See Figure 1.1 of Sutton and Latham (1996) for a presentation of biomass data.)

The control group consisted of plants grown in a medium of coarse builders' sand amended with 15-9-12 (N:P:K) Osmocote Southern Formula, a commercially available fertilizer. Osmocote is manufactured by Grace Sierra Horticulture Products Company, Milpitas, CA. This fertilizer is formulated to slow-release in soil over an 8-9 month period with increased rates of nutrient release at temperatures ≥ 21°C (Harbaugh and Wilfret 1981). Fertilizer was added at a rate of 40 g per pot for *N. guadalupensis* and *P. illinoensis* and 15 g for *V. americana* and *Chara* (Dr. David Sutton, UF FLREC, personal communication).

In the spring culture period, the activity of the herbivorous moth *Paraponyx diminutalis* Snellen apparently caused a significant reduction in the above-sediment biomass of *N. guadalupensis* and *P. illinoensis*. The total loss in *N. guadalupensis* and *P. illinoensis* above-sediment biomass due to herbivore damage was estimated to be approximately 50% (Figure 3-3). This estimate was made based upon field estimates of biomass loss in conjunction with trends in root:shoot ratios for *N.*

guadalupensis observed in the summer and winter culture periods. The estimated loss fits within the range of loss reported in the literature. Estimated total biomass values for *N. guadalupensis* and *P. illinoensis* were then obtained by adding the calculated loss to the actual total biomass values measured for each species. These estimated values were used in subsequent statistical analyses involving the growth of *N. guadalupensis* and *P. illinoensis* in the spring culture period. An emulsifiable concentrate of malathion (0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate) was used to achieve a dosing concentration of 1.0 mg/L in order to control herbivore activity during the summer and winter culture periods.

Tanks were dosed biweekly with a concentrate of Malathion (*O,O*-dimethyl dithiophosphate of diethyl mercaptosuccinate) to an initial concentration of 1.0 ppm in the summer and winter culture periods. Dosing was initiated when plant shoots reached the water surface in order to control the feeding activity of the herbivorous moth, *Parapoynix diminutalis* Snellen.

Plants were harvested and separated into above-sediment biomass (shoots) and below-sediment biomass (roots) at the end of each culture period. The length of each experiment (9 weeks) was adequate for the development of treatment – related differences in growth, while minimizing tissue deterioration associated with senescence. Total number of plants harvested per pot was recorded for all species in the spring culture period. (It was determined during this first harvest that the brittle nature of *N. guadalupensis* and *Chara* stems made it very difficult to get a reproducible assessment of the number of plants of these two genera. Therefore, the average number of harvested plants per pot was quantified only for *V. americana* and

P. illinoensis in the summer and winter culture periods.) Plant material was dried to constant weight (Hakanson 1981) at 60° C in a forced-air drying oven and dry weights were measured. Macrophyte growth was measured as the change in dry weight of plant biomass, after correction for epiphyte biomass (explained below), since planting of propagule material. From this point forward, the term biomass will be used to refer to total plant biomass (shoots + roots).

Epiphyte biomass was removed from the macrophytes at the time of harvest. In the spring culture period, macrophyte material was washed gently with pond water in order to remove sediment, debris and epiphytic algae. In the summer and winter culture period, the mechanical removal technique described by Zimba and Hopson (1997) was used to separate epiphytic biomass from macrophyte biomass. (See Appendix A for a more detailed discussion of the method used.) A subsample of the resultant suspension containing the epiphytes was concentrated onto a glass fiber filter (0.7-μm porosity) and chlorophyll *a* and phaeophytin *a* were determined in accordance with *Standard Methods* (SM 10200 H) (A.P.H.A. 1995). All epiphyte data collected were normalized to host plant dry weight. Chlorophyll *a* values corrected for phaeophytin *a* were used as a correction factor to determine total macrophyte yield. More rigorous efforts were also made in the summer and winter culture periods to use mechanical removal methods to maintain the tanks relatively free of both epiphytic and floating algae.

Statistical Analysis of Results

Sediment chemical composition, macrophyte biomass and ratios of root biomass to shoot biomass were analyzed using the general linear model (GLM)

procedure of Statistical Analysis System (SAS) for personal computers (SAS Institute 1999-2001). Tukey's studentized range distribution test (HSD) procedure was used for means separation to investigate differences in the pH, organic matter and nutrient contents of the sediment and among and within species differences in macrophyte biomass and root:shoot ratios.

Regression analysis (SAS Institute, 1999-2001) was used to identify those sediment characteristics that had a significant effect on macrophyte growth. Sediment nutrients, pH and organic matter content and light and water temperature were used as the independent variables and macrophyte biomass was used as the dependent variable Stepwise multiple regression analysis was used to further evaluate the association between macrophyte biomass production and those environmental factors identified as significant. In an effort to improve the models, sediment calcium, magnesium, potassium and sodium were excluded from analysis on the basis that numerous studies have shown that the water column is the primary source of these nutrients for submersed macrophytes. Data transformation was used to reduce variance as necessary.

Results

Sediment Survey

The results of the preliminary sediment survey study of Lake Hollingsworth littoral sediments revealed significant differences in sediment organic matter content and pH among the survey stations (Table 3-1). (See Figure 3-1 for a map of the survey study stations). Organic matter content (%) ranged from 0.38% at station B to

39.18% at station E. Station G sediments also had a high organic matter content (34.96%). The pH varied from a low of 5.4 at station B to a high of 8.0 at station F.

Four stations were selected that appeared to provide the most suitable habitat for submersed macrophytes among the eight survey stations (Figure 3-1). Study station selection criteria included low organic matter content, absence of flocculent sediment layer and protection from boat traffic. Comparison among the survey stations indicated differences in the relative amount of boat traffic in the different lake regions. Stations A and E were located in the path of two water ski courses in the lake. Consequently, these stations were eliminated due to heavy boat traffic that would be damaging to macrophyte communities. B, C, D, F and H were relatively protected from boat traffic. Stations B,D,F and H appeared to have the optimum combination of low organic matter, absence of flocculent layer and minimal perturbation. In my opinion, these locations had the best potential, dependent upon chemical composition, to be sites for the successful establishment of founder colonies of the study species.

Chemical Composition of Study Sediments

Differences in pH, percent organic matter and macro- and micronutrient contents were apparent among the sediments (Table 3-2). The lake sediments were more alkaline than the control sediments with the exception of sediment from Station B, which exhibited the lowest mean pH, 5.4 (Table 3-2). Organic matter contents of the natural sediments ranged from 0.42 to 1.08%, with Station D having the lowest percentage and Station F the highest percentage. Sediment from Station F also contained significantly higher calcium contents (5617 $mgKg^{-1}$) than the other

sediments. Magnesium, phosphorus, manganese and sodium levels were significantly greater at Station F and in the controls than in the other sediments. There were no significant differences in the content of these elements in the other sediments. Zinc was present at significantly highest levels in sediments from Station F and lowest in sediments from Station D. Control sediments dosed with 40 g of fertilizer (controls for *N. guadalupensis* and *P. illinoensis*) contained significantly greater levels of copper, potassium and chloride ion as compared with the other sediments. There were no significant differences in copper, potassium and chloride ion among the remaining sediments. No significant differences in manganese content were observed among the sediments. Aluminum content was lowest in sediments from Site D. Iron, ammonium and nitrate were detected in significantly greater amounts in the control sediments as compared with the lake sediments. A comparison of the relative iron contents observed in the control sediments to the amounts contributed by the Osmocote indicated the presence of iron in the sand used to create the control sediments. The iron content measured in the control sediments amended with 15 g of Osmocote were unexpectedly high relative to the those controls amended with 40 g of fertilizer. This may have resulted in levels of iron that were inhibitory to some species.

Temperature and Irradiance during the Culture Periods

As shown in Table 3-3, the highest average daily water temperature was recorded during the summer culture period (29.6 °C) while the lowest average daily water temperature was recorded during the winter culture period (21.2°C). The mean incident PAR were greatest during the spring culture period (806 $\mu mol\ s^{-1}\ m^{-2}$) and least during the winter culture period (624 $\mu mol\ s^{-1}\ m^{-2}$). Photoperiod was longest

during the spring (13.8L:10.2D) and shortest during the winter culture period (11L:13D).

Effects of Sediment on Macrophyte Production

Spring culture period (30 April to 29 June 2001)

Analysis of variance of pooled total macrophyte biomass (n = 60) produced during the spring culture period using GLM procedures (SAS Institute 1999-2001) indicated highly significant differences due to plant type (Table 3-4). (Note that total biomass values for *N. guadalupensis* and *P. illinoensis* were corrected for losses due to herbivory. Refer to the Materials and Methods section for a description of the method used to estimate the corrected values.) There were no significant differences in total biomass due to sediment source or to the interaction between sediment source and plant type. Means separation using Tukey's HSD method indicated that there were no significant differences in biomass production among species in Site D, Site F, and control sediments ($\alpha = 0.05$). *V. americana* produced significantly less biomass than the other species in Site B and Site H sediments. *N. guadalupensis* and *Chara* sp. produced greater biomass in the control sediments and most of the natural sediments than *P. illinoensis* and *V. americana* (Table 3-5). *V. americana* exhibited the slowest growth of all species in all but one sediment. Within species comparisons indicated that *N. guadalupensis* and *Chara* sp. produced produced greatest mean total biomass in control sediments (16.4 and 16.9 g DWT, respectively) (Figure 3-34). *N. guadalupensis* growth was slowest in Site H sediments (11.0 g DWT). *Chara* sp. accrued the least biomass in Site B sediments (4.1 g DWT). *P. illinoensis* produced greatest mean biomass in Site F sediments (9.9 g DWT) and least biomass in Site D

sediments (6.0 g DWT). *V. americana* production was greatest (6.8 g DWT) on Site D sediments and lowest in Site B (2.3 g DWT). Analysis of variance using the GLM Procedure (SAS Institute 1999-2001) indicated that there were no statistically significant differences in macrophyte growth within species in response to sediment type (Figure 3-4a).

Comparison of the mean number of individual plants produced by each species indicated that *P. illinoensis* produced more plants than the other species in most of the sediments (Figure 3-5a). As a general trend, all species produced the least number of individual plants on Site B sediments. Note that herbivory had a negative effect on the total number of plants counted and probably resulted in a significant underestimate of the total number of plants of *N. guadalupensis* and *P. illinoensis*.

Simple regression analysis used to investigate the relationship between macrophyte biomass production and sediment chemical composition suggested that sediment pH, OM and macro- and micro-nutrients had relatively little effect on macrophyte growth in the spring culture period (Table 3-6). The only significant relationship was between aluminum and *N. guadalupensis* biomass. There were no other significant relationships.

Root: shoot ratios produced by all species in response to all sediments were less than 1 (Figure 3-6a). Analysis of variance of macrophyte root:shoot ratios using GLM procedures (SAS Institute 1999-2001) indicated highly significant differences in total root:shoot ratios due to plant type ($P < .0001$) (Table 3-7). There were no significant differences due to sediment source or to the interaction between sediment source and plant type. There were differences among and within the study species. *V.*

americana allocated energy for the production of greater relative amounts of root biomass in all sediment types as compared with the other species. These differences were statistically significant in Site B, Site D and Site H sediments. *N. guadalupensis* ratios were significantly lower than that of the other species in Station D sediments. Within species comparisons suggested that *N. guadalupensis* and *V. americana* allocated relatively more resources to root production in Station B sediments as compared with the other sediments. In the case of *N. guadalupensis*, the difference was statistically significant. *P. illinoensis* and *V. americana* root:shoot ratios were lowest in response to the control sediments.

Summer culture period (20 July to 21 September 2001)

Analysis of variance of pooled total macrophyte biomass (n = 60) produced during the summer culture period using GLM procedures (SAS Institute 1999-2001) indicated that sediment and plant type had a highly significant effect on macrophyte biomass production ($P < .0001$) (Table 3-8). The interaction between these two factors was not significant. Means separation using Tukey's HSD Procedure suggested that there were significant differences among and within macrophyte species in response to sediment type ($\alpha = 0.05$). *N. guadalupensis* produced significantly higher biomass on all natural sediments as compared to the other species (Table 3-9). *Chara* sp. exhibited the slowest relative growth in response to all sediments. This difference was statistically significant for growth in Site B and Site D sediments. *N. guadalupensis* and *P. illinoensis* exhibited more rapid growth in all sediments as compared to *V. americana* and *Chara sp.*. There were no significant differences in growth among macrophytes in response to the control sediments. Within species comparisons

suggested that all species exhibited the slowest growth in Site B sediments. This difference was statistically significant for *V. americana* (18.6 g DWT) and *Chara sp.* (3.8 g DWT) (Figure 3-4b). *V. americana* produced statistically greatest biomass in control, Site D and Site F sediments (44.4, 41 and 28 g DWT). *N. guadalupensis* and *P. illinoensis* produced greatest biomass in control sediments, however, these differences were not found to be statistically significant (56.5 and 21 g DWT, respectively). Although *Chara sp.* growth was best in control sediments, there were no statistical differences in the biomass produced in control, Site F, Site H and Site D sediments.

V. americana produced a greater number of individual plants per pot on all sediments as compared with *P. illinoensis* (Figure 3-5b). *V. americana* exhibited the greatest increase in total number of plants in response to Site D sediments while the greatest increase in number of *P. illinoensis* plants was observed on control sediments.

The results of simple regression analyses of macrophyte biomass and sediment chemical components indicated that there were species-specific differences in response to sediment pH, OM, macro- and micro-nutrients and epiphyte biomass in this culture period (Table 3-6). Sediment phosphorus, copper, manganese, iron, ammonium, nitrate, magnesium, potassium, sodium, chloride and epiphyte biomass had highly significant ($P < .0001$) effects on total macrophyte biomass ($n = 60$) while aluminum had a significant effect on total biomass. Models with *P. illinoensis* biomass and phosphorus, copper, manganese, iron, ammonium, nitrate, potassium , sodium and chloride were all significant. Iron, ammonium, nitrate, and potassium

were all found to have significant effects on *V. americana* biomass. Aluminum had a highly significant effect ($P < .0001$) on biomass while copper was only slightly significant. A significant negative relationship was observed between *Chara sp.* biomass and epiphyte biomass. None of the models with *N. guadalupensis* biomass gave significant results.

The results of stepwise multiple regression analyses using a composite of the biomasses of all species (n=60) and the biomass of each individual species (n=15) as the response and the significant factors as the predictors also indicated species-related differences (Table 3-6). Total biomass was most significantly affected by copper ($P < 0.0002$) and ammonium ($P < 0.0149$). Biomass and ammonium were negatively related. Epiphyte biomass was the most significant predictor of *Chara sp.* biomass accumulation. Epiphyte biomass had a negative effect on *Chara sp.* biomass.

Root: shoot ratios produced by all species in response to all sediment types were less than 1 (Figure 3-6b). Analysis of variance of macrophyte root:shoot ratios using GLM procedures (SAS Institute 1999-2001) indicated differences in total pooled (n = 60) root:shoot ratios due to plant type ($P <.0033$) (Table 3-10). There were no significant differences due to sediment source or to the interaction between sediment source and plant type. There were differences among and within the study species (Figure 3-6b). *V. americana* and *P. illinoensis* produced significantly greater root:shoot ratios on Site B and Site H sediments versus *N. guadalupensis*. There were no other species-specific responses to any of the other sediment types. Within species comparisons suggested that root:shoot ratios for *N. guadalupensis* were slightly greater on Site H and Site C sediments. *P. illinoensis* ratios were greatest on Site B,

Site H and control sediments while *V. americana* produced the greatest mean ratio in response to Site B sediments.

GLM analysis of epiphyte biomass (g/g host DWT) indicated significant differences due to sediment source ($P < 0.01$) and highly significant host-specific differences ($P < .0001$). Epiphyte biomass on *Chara sp.* was greatest in Site B sediments. There were no other host-specific differences. Comparisons among species indicated that in control and Site D sediments, *Chara sp.* and *P. illinoensis* exhibited higher epiphyte biomass than *V. americana* and *N. guadalupensis*. There were no differences among the macrophytes in Site B, Site F or Site H sediments.

Winter culture period (19 December 2001 to 22 February 2002)

Analysis of variance of the composite macrophyte biomass ($n = 60$) produced during the winter culture period using GLM procedures (SAS Institute 1999-2001) suggested that plant type had a highly significant while sediment type had a significant effect on macrophyte biomass production ($P < .0001$ and $P < 0.0004$, respectively) (Table 3-11). The interaction between the two factors was slightly significant. Means separation using Tukey's HSD Procedure suggested that there were significant differences among and within macrophyte species in response to sediment type ($\alpha = 0.05$) (Figure 3-4c).

P. illinoensis and *N. guadalupensis* exhibited greater growth in all sediments as compared with *V. americana* and *Chara* sp. (Table 3-12). *V. americana* biomass was lower than that produced by the other species in all sediments. *P. illinoensis* biomass was significantly greater than that of the other species in Site B, Site D, Site H and control sediments. Within species comparisons indicated that *N. guadalupensis*

growth was significantly best in Sites F, C and H sediments. *P. illinoensis* accrued greatest biomass in control and Site H sediments, however, there were no significant differences in the biomass produced in any sediment type. Although *V. americana* exhibited the greatest growth on Site H sediments (12.9 g DWT), differences in response to the different sediment types was minimal. *Chara* sp. accrued significantly greatest biomass on control sediments (21.3 g DWT) and lowest biomass in Site B (8.2 g DWT) and Site D sediments (7.3 g DWT) (Table 3-12).

There were species-specific differences in macrophyte biomass production in response to sediment chemical composition. The results of simple regression analyses of macrophyte biomass using sediment pH, OM and macro- and micro-nutrients as predictors indicated a highly significant relationship ($p < .0001$) between phosphorus, copper, manganese, ammonium, nitrate, magnesium, potassium, sodium and chloride and composite macrophyte biomass of all species ($n = 60$) (Table 3-6). Iron had a significant effect on composite biomass. Models with *N. guadalupensis* biomass indicated highly significant relationships with organic matter, zinc and magnesium, significant relationships with phosphorus and manganese and slightly significant relationships with calcium and sodium. None of the models with *V. americana* biomass gave significant results. *Chara* sp. biomass was highly related ($p < .0001$) to phosphorus, copper, manganese, iron, ammonium, nitrate, magnesium, potassium and sodium.

P. illinoensis produced a greater average number of individual plants per pot than *V. americana* in all sediments (Figure 3-6c). *P. illinoensis* exhibited the greatest increase in mean number of plants per pot in control sediments. There were no

obvious differences in the number of plants produced by *V. americana* in response to any of the sediments.

Root: shoot ratios produced by all species in response to all sediment types were less than 1 (Figure 3-6c). Graphical analyses indicated that *P. illinoensis* and *V. americana* produced greatest root:shoot biomass on all sediments. Comparisons within species suggested that *N. guadalupensis* ratios were greatest on Site D sediments. *P. illinoensis* produced greatest ratios in response to Site B and Site F sediments. *V. americana* produced considerably more root biomass in comparison to shoot biomass in Site H sediments. Analysis of variance of macrophyte root:shoot ratios using GLM procedures (SAS Institute 1999-2001) indicated differences in total root:shoot ratios due to plant type($P < 0.0004$) (Table 3-13). There were no significant differences due to sediment source or to the interaction between sediment source and plant type. Among species comparisons indicated that *P. illinoensis* and *V. americana* produced significantly greater ratios than *N. guadalupensis* on Site B and Site F sediments. There were no significant differences among species on any of the other sediment types. Within species comparisons indicated that there were no statistical differences in macrophyte root:shoot response to sediment type.

The results of GLM analysis of the data indicated that there were no significant host-specific differences in epiphytic biomass (g/g host dry weight) during the winter culture period. There were no differences in epiphyte biomass due to sediment source or the interaction between sediment source and plant type.

Identification of General Trends

Graphical analysis of the data indicated differences in macrophyte biomass production among the three culture periods (Figure 3-4). Overall, greatest plant growth occurred during the summer culture period. Comparisons among species indicated that *N. guadalupensis* produced the greatest biomass of all study species when grown in lake sediments in spring and in all sediments in summer. *P. illinoensis* exhibited the greatest growth among the species on four of the five sediment types during the winter growing period. Differences in biomass production within macrophyte species were also observed. The results suggest that *N. guadalupensis*, *V. americana* and *Chara sp.* grew best during the summer culture period. *P. illinoensis* produced greatest biomass during and grew equally well in the summer and winter culture periods. With the exception of *V. americana* at station H, there was relatively little difference in the biomass production of *V. americana* and *Chara sp.* during the spring and winter culture periods.

Overall trends in macrophyte growth in response to different sediment types were also observed. Generally, greatest macrophyte biomass accumulation during all culture periods occurred in control sediments. Site F and Site H were apparently the most suitable natural substrates for macrophyte growth. Station B sediments supported the least biomass production among the species over the three culture periods.

Macrophyte biomass data collected during the summer and winter culture period were pooled and statistically analyzed as a randomized complete block design where culture periods were considered blocks. Data collected during the spring

culture period were not included in this statistical analysis. (Refer to the Materials and Methods section for an explanation.) Analysis of the data using GLM procedures (SAS Institute 1999-2001) indicated highly significant differences in total pooled macrophyte biomass due to culture period, sediment source, plant type and the interaction between culture period and plant type ($P < .0001$ for all) (Table 3-14). A weak interaction was also noted for culture period and sediment source ($P < 0.0142$). No interaction was observed for sediment source and plant type or for all three effects together. There were significant differences in biomass among and between the study species ($\alpha = 0.05$). Means separation using Tukey's HSD Method indicated that *N. guadalupensis* produced significantly greater biomass on F sediments as compared to the other species (Table 3-15). *N. guadalupensis* and *P. illinoensis* biomass was significantly greater on Station B and Station H sediments as compared to that of *V. americana* and *Chara* sp.. *V. americana* response on control sediments was significantly less than that of the other species. There were no significant differences in growth among the species when grown on Station D sediments. Within species analysis of the variance in biomass indicated that *Chara* sp. produced significantly greatest biomass in the control sediments Table 3-16). However, it should be noted that there was only a slight difference between *Chara* sp. biomass produced in control and Site F, Site H and Site D sediments. *P. illinoensis* produced greatest biomass in control and Site H sediments. No significant differences were noted in the response of *N. guadalupensis* and *V. americana* biomass production to different sediments. Further investigation of the effect of culture period on macrophyte biomass indicated that *N. guadalupensis* produced significantly greater biomass during the summer

culture period followed by *P. illinoensis* and *V. americana* with statistically equal summer biomass. *Chara sp.* produced the least summer biomass. *P. illinoensis* produced the greatest biomass during the winter culture period while *Chara sp.* and *V. americana* exhibited the least amount of growth.

A comparison of the mean number of individual plants produced per pot by each plant type indicated differences among species and within species during different culture periods (Figure 3-5). *P. illinoensis* produced greater numbers of plants in the winter than in the spring and summer culture periods. A considerably greater number of *V. americana* plants per pot was measured for the summer culture period than for the other culture periods.

Comparison of the root:shoot biomass produced indicated values < 1 for all species during all culture periods (Figure 3-6). *V. americana* produced considerably greater root:shoot ratios in the spring as compared to the summer and winter culture periods when ratios were fairly similar. These findings may have been due to reduction of shoot material by herbivores. Ratios for *P. illinoensis* were higher in the winter and spring than in the summer culture period. Similar root:shoot ratios were observed for *N. guadalupensis* in the summer and winter culture periods. Slightly higher ratios in the spring culture period were probably skewed by an underestimate of shoot biomass due to herbivore damage.

Statistical analysis of pooled root:shoot ratios (n = 120) observed during the summer and winter culture periods using GLM procedures (SAS Institute 1999-2001) suggested highly significant species-specific differences in root:shoot biomass ($P < .0001$) and a weak interaction between culture period and plant type (Table 3-17).

Further analysis indicated significant differences among plant types such that root:shoot biomass ratios for *P. illinoensis* and *V. americana* were greater on Station B and Station F sediments as compared to *N. guadalupensis* (Table 3-18). There were no significant differences on any of the other sediments. *V. americana* root:shoot ratios were greater during the summer culture period as compared to the other species ($P < 0.0032$) while *V. americana* and *P. illinoensis* root:shoot biomass was significantly greater than that of *N. guadalupensis* during the winter culture period ($P < 0.0004$). Within species comparisons indicated that *P. illinoensis* produced significantly greater root biomass on Site B sediments and lowest root:shoot ratios on control sediments (Table 3-19). Sediment source did not appear to have a significant effect on the amount of below-sediment biomass produced by *N. guadalupensis* and *V. americana* during the summer and winter culture periods.

Results of analysis of pooled epiphyte data from the summer and winter culture periods (n=120) using the GLM procedure indicated that culture period had a highly significant effect ($P < .0001$) on epiphyte biomass (Table 3-20). Neither sediment source, host macrophyte species or any interactions among the factors were significant. Means separation using Tukey's HSD Test indicated that the epiphytic biomass occurring on all four species was significantly greater in the winter than in the summer culture period (Table 3-21). Generally, greater epiphyte biomass was associated with *Chara* sp. and *N. guadalupensis* on all sediments, however none of these differences were statistically significant (Table 3-22). Similarly, there were no significant differences in the epiphyte biomass occurring within host species on any sediment type (Table 3-23). There was no apparent relationship between epiphyte

biomass and sediment source during either the summer or the winter culture period (Table 3-24).

Effects of Sediment, Temperature, Light and Epiphytes on Macrophyte Production

Simple regression models revealed differences in macrophyte growth response to sediment characteristics, light, water temperature and epiphyte biomass during the summer and winter culture periods. Results from the spring culture period were not included due to the unquantifiable effects of herbivory on shoot biomass in the study species. The results suggested that sediment ammonium and nitrate had a highly significant effect on total pooled (summer and winter culture period) macrophyte biomass ($n = 120$) ($p < .0001$) (Table 3-25). Magnesium had a significant effect on total pooled ($n = 120$) and pooled *N. guadalupensis* biomass ($n = 30$). No other models with sediment nutrients and *N. guadalupensis* biomass were significant. Pooled *P. illinoensis* ($n = 30$) biomass was significantly related to phosphorus, copper, manganese, aluminum, iron, ammonium, nitrate, magnesium, potassium, sodium, and chloride. None of the models with pooled *V. americana* biomass ($n = 30$) and sediment nutrients were significant. Sediment phosphorus, copper, manganese, iron, ammonium, nitrate, magnesium, potassium and sodium were all significantly related to pooled *Chara* sp. biomass ($n = 30$).

Additional simple regression models using pooled summer and winter biomass also indicated that light, water temperature and epiphyte biomass had significant effects on the growth of the study species (Table 3-26). Models relating pooled total biomass ($n = 120$) and biomass of *N. guadalupensis* and *V. americana*

separately (n =30) suggested that total PAR (μmol photons m^{-2} per CP), mean instantaneous PAR (μmol photons m^{-2} s-1) and water temperature had a highly significant ($p < .0001$) effect on biomass production in all cases. None of the models with pooled *Chara sp.* biomass (n = 30) and pooled *P. illinoensis* biomass (n = 30) were significant.

Stepwise multiple regression analyses conducted using pooled summer and winter macrophyte biomass as the dependent variable and the significant sediment nutrients and climatic factors identified using simple regression analyses as the independent variables indicated macrophyte growth in this study was affected by a combination of factors. Total pooled biomass (n = 120) was most significantly affected by nitrate, total PAR per culture period and ammonium (Table 3-27). The data indicated species-related differences in response to the independent variables. *N. guadalupensis* and *V. americana* biomass exhibited a strong positive relationship with water temperature. Water temperature explained almost 50% of the variation in *N. guadalupensis* biomass and over 64% of the variation in *V. americana* production. The results suggested that *P. illinoensis* growth was positively correlated with sediment copper content and negatively correlated with sediment iron content. Collectively, these two predictors explained over 44% of the variation in the biomass of this species. There was a significant positive relationship between *Chara sp.* growth and sediment phosphorus content.

Discussion

The results of the sediment survey conducted at the beginning of this study confirmed the hypothesis that that there would be spatial variation in the organic

matter content of the littoral sediments in Lake Hollingsworth. Organic matter content in the sediment samples ranged from 0.38% to 39.18% (Table 3-1). Sediment pH values also ranged from 5.4 to 8.0. In their 1996 study of seven shallow productive Florida lakes including Lake Hollingsworth, Whitmore et al. identified the presence of highly variable sediment organic matter content. They attributed the lack of uniform organic buildup in their study lakes to frequent mixing, lack of thermal stratification and warm temperatures resulting in the breakdown of organic material. They identified three types of organic sediment distribution patterns: uniform distribution, distribution to deeper areas, and distribution to peripheral areas and embayments where the type of distribution pattern greatly depends on lake bathymetry. The highly variable nature of organic sediment composition in their study lakes led these researchers to conclude that systematic sediment mapping surveys are necessary to characterize the sediments in shallow "wind-stressed" productive lakes such as Lake Hollingsworth. The findings suggested the importance of beginning revegetation projects in Florida lakes with a preliminary investigation of sediment chemical composition.

The findings of the sediment survey conducted in this study made it possible to identify sites around the lake that were not only relatively protected from anthropogenic disturbance but that had organic matter contents that were not inhibitory to plant growth. Having this type of "background information" on a system should facilitate site selection for planting in revegetation projects. I believe that the study stations selected in this study should provide highly suitable habitat for the

establishment of founder colonies (*sensu* Smart et al. 1996) of the study species in Lake Hollingsworth.

Analyses of sediment chemical composition corroborated the hypothesis that there would be spatial variation in the nutrient content of the littoral sediments in Lake Hollingsworth. The study sediments varied in pH, organic matter and nutrient content (Table 3-2). Natural sediments, with the exception of Site B sediments, were more alkaline than control sediments. Although organic matter contents were not available for the control sediments, low organic matter contents are typical for sand sediments. Therefore, even though the organic content of Site F sediments (1.08%) was significantly greater than that of the other natural sediments, the limited range of values made it difficult to identify any effects on macrophyte growth due to sediment organic content. Calcium content in Site F sediments was significantly greater than that of the other sediments (5617 $mgKg^{-1}$). In my opinion, this value was high probably due to the presence of shell or pebble material in the analytical sample that was not thoroughly homogenized during processing. Control, Site F and Site H sediments all exhibited relatively high levels of phosphorus, zinc, copper, manganese and iron as compared with Site B and Site D sediments. Ammonium and nitrate were present in significantly greater amounts in the control sediments. Although there were significant differences in the content of some other nutrients in the sediments, i.e. potassium and magnesium, the literature indicates that these nutrients are primarily derived by submersed macrophytes from the water column (reviewed in Barko et al. 1991). Sutton (1990) observed similar chemical composition in sediments collected

from Lake Okeechobee. Sediment nutrient analyses in his study were conducted using the same procedure as was used in this study.

Light and temperature data gathered throughout the study indicated climatic variations among the culture periods. Highest average daily water temperature occurred during the summer culture period. Lowest water temperatures were observed during the winter. Despite low winter temperatures, average water temperatures were sufficient to meet the minimum requirement during each culture period for nutrient release by the Osmocote fertilizer used to amend the control sediments. Harbaugh and Wilfret (1981) determined that release rates of Osmocote are temperature-dependent and increase at temperatures above 21° C. Sutton and Latham (1996) measured minimum average water temperatures equal to or greater than 21°C during a growth study conducted at the FLREC using sand media amended with Osmocote. Based upon these water temperatures, they concluded that release rates remained high enough to maintain sufficient sediment concentrations of phosphorus, ammonium and nitrate during the winter culture period. Photoperiod was longest in spring and shortest in winter. Average PAR values showed a similar trend. These findings are probably best explained by the fact that partly cloudy days are the norm in South Florida. Relatively lower average PAR values in summer compared to spring are probably due in part to afternoon thunderstorms that result in 100% cloud cover during parts of most summer afternoons.

Biomass data collected in this study indicted that macrophyte growth and sediment nutrient content were positively related. Greatest biomass production occurred on control sediments probably due to the presence of significantly greater

levels of ammonium and nitrate. Ammonium and nitrate were those sediment nutrients found to have the most significant impact on total macrophyte production in this study (Table 3-16). There are multiple examples in the literature in which nitrogen has been identified as one of if not the most probable factor controlling submersed macrophyte growth. For example, in their comparative growth study of *Myriophyllum spicatum* and *V. americana americana*, Titus and Adams (1979) reported that sediment nitrate content appeared to have a significant effect on the biomass production of *Vallisneria*. Studies conducted by Barko et al. (1988) and Barko et al. (1991) suggested that, although there is little evidence for limitation of SAV growth by sediment P, sediment nitrogen content may limit the growth of some submersed species. Squires and Lesack (2003) observed a positive relationship between macrophyte biomass and total sediment nitrogen in their study in several lakes in the Mackenzie Delta. The negative relationship observed in this study between ammonium and macrophyte biomass may indicate that ammonium was present in the control sediments at inhibitory levels for some or all of the study species. Another possible explanation is that sediment biochemistry in flooded soils affects the relationship between ammonium and macrophyte biomass production. Walstad (2003) observed cases in which ammonium levels were toxic to plant roots. She attributed this toxicity to bacterial metabolic processes that rapidly convert nitrates to toxic nitrites.

Initially, superior growth on higher density sand than on the fine-textured lake sediments would appear to contradict the findings of several studies that have documented poor macrophyte growth on sand and highly organic sediments (Sand-

Jensen and Sondergaard 1979, Moeller 1983, Barko and Smart 1986). Barko and Smart (1986) concluded that sediment density or related factors regulate nutrient uptake by affecting nutrient diffusion distances. They concluded that nutrient uptake on low-density, high-porosity organic sediments was limited by long diffusion distances. They observed an increase in *Hydrilla* growth with increased sediment density. In the same study, they determined that *Hydrilla* and *Myriophyllum* grew best on fine-textured inorganic sediment. They observed that additions of fine-textured inorganic sediments to sediments previously determined to be unfavorable for SAV growth (i.e. organic and sandy sediments) resulted in improved macrophyte growth. Apparently, nutrient addition in the form of fertilizer was sufficient to meet the nutrient requirements of the study macrophytes. The control sediments had significantly higher levels of nutrients that encourage plant growth such as phosphorus and nitrogen as compared with the other sediments (Table 3-2). Sutton and Latham (1996) investigated soil porewater concentrations of sediment nutrients in sand media amended with Osmocote. They observed the following mean summer and winter phosphorus, nitrate and ammonium values in the amended sediments after curing for 8 weeks: phosphorus – 80 mg/L, 45 mg/L; nitrate – 2125 mg/L, 1725 mg/L; ammonium –700 mg/L, 575 mg/L. These values are approximations of the data presented in Figure 1 of Sutton and Latham (1996).

Growth on natural sediments also reflected the importance of sediment nutrient content. F and H were the best natural sediments for biomass production. These sediments contained relatively greater amounts of copper, zinc and phosphorus, those nutrients found to be most significant to the growth of *P. illinoensis* and *Chara*

sp. in this study (Table 3-16). Barko and Smart (1986) concluded that the diminished macrophyte growth that they observed on organic sediments was due to multiple nutrient deficiency. In the same study, they observed that additions of phosphorus and a combination of phosphorus and iron to organic sediments had a significant positive effect on the growth of *Hydrilla.* Intuitively, we would expect that phosphorus would not be limiting in the phosphatic soils typical of the region, however, in his literature review of the effects of sediment nutrients on seagrasses, Short (1987) discussed the importance of the role of sediment geochemistry in plant nutrient-uptake from sediments. He cited the example of phosphorus limitation of plant growth in tropical environments and carbonate sediments due to binding of phosphorus in sediments.

Biomass accumulation data collected in this study indicated that Station B sediments were the least suitable substrate for macrophyte growth. This trend was markedly more pronounced for the spring and summer culture periods while plants exhibited relatively improved growth on Station B sediments during the winter culture period. Initially, these results appear difficult to interpret given the fact that the chemical composition of Station B sediments was very similar to that of the other study sediments (Table 3-2). The major difference between the sediments was that Station B sediments were exposed due to drought conditions at the time of collection and subsequent subsample analysis. Perhaps the most plausible explanation is that oxidation of the sediments, in combination with low acidity (5.4), resulted in nutrient binding in the sediments making nutrients less available for plant uptake. Barko et al. (1988) reported a similar negative correlation between sediment oxidation and nutrient availability and plant growth. In contrast, Sutton (1990) observed improved

growth of *Hydrilla verticillata* on dried sediments as compared with sediments maintained in the moist state prior to planting. The reasons for this discrepancy are not known. However, the observed increase in plant growth in Site B sediments in the winter culture period after sediments had been flooded appeared to confirm the explanation that soil nutrients were not as readily available for plant uptake in the oxidized sediments.

The positive relationship between increasing sediment fertility and growth observed for all species contradicted my hypothesis that *N guadelupensis*, *P. illinoensis* and *Chara* sp., the canopy-forming, erect species, would produce greater biomass in sediments with higher nutrient levels while net growth of *V. americana*, a rosette species, would be inversely related to sediment nutrient content. The results obtained by pooling the biomass produced by the study plants during the summer and winter culture periods (n = 120) indicated that *N. guadalupensis* and *Chara* sp. grew best on control, Station F and Station H sediments. *P. illinoensis* and *V. americana* produced greatest biomass on control and Station H sediments and control and Station D sediments, respectively. There is considerable documentation of the relationship between macrophyte life history and response to sediment fertility. Chambers (1987) observed a relationship between macrophyte growth form and sediment nutrient availability along a natural gradient in Lake Memphremagog. According to his findings, rosette and bottom-dwelling species grew better on less fertile soils while increasing fertility was associated with an increase in the growth of canopy-forming and erect species. In a competition study among seven aquatic macrophyte species, Wilson and Keddy (1986) observed that species with low competitive ability were

typically found on nutrient-poor sites, while species with high competitive ability flourished on nutrient-rich sites. Perhaps the limited range of values investigated obscured the trends in this study that are documented in the literature.

Species-related responses to differences in sediment nutrient content are well-documented in the literature. Denny (1972) observed a wide-range of responses in growth rate to sediment fertility among submersed macrophyte species. He concluded that these variations were due to anatomical and morphological differences among species investigated. Similar differences in species-specific responses to sediment fertility were observed in several studies by Barko and Smart (1980, 1981, 1986). Barko and Smart (1986) observed that *Hydrilla* was more sensitive to sediment nutrient composition as compared with *Myriophyllum*. Zimba et al. (1993) observed among species differences in the chemical composition of *N. guadalupensis*, *V. americana*, *P. illinoensis* and *Chara* sp. among the species. They concluded in the same study that plant tissue micronutrient concentrations, especially iron, copper and magnesium, were the most significant in the separation of *N. guadalupensis*, *V. americana*, *P. illinoensis* and *Chara* sp. using discriminant analysis. Duarte and Kalff (1988) also reported species-specific differences in response to sediment nutrients in fertilization mesocosm studies. Barko et al. (1991) discussed the importance of species-specific macrophyte-sediment interactions in determining compositional changes in community composition over time.

Evaluation of the root:shoot ratios supported the hypothesis that sediments collected from the littoral zone of Lake Hollingsworth would have sufficient quantities of sediment nutrients to support the growth of *N. guadalupensis, P.*

illinoensis, V. americana and Chara sp. when grown in the experimental growth tanks used in this study. The root:shoot ratios of less than one observed for all species in response to all sediment types indicated that there was no sediment nutrient limitation of macrophyte growth in this study. High root:shoot ratios (≥ 1) are typically associated with submersed macrophytes growing in infertile environments (Aung 1974, Chapin 1980). Clarkson and Hanson (1980) identified this mechanism as a strategy for maximizing the volume of soil in contact with plant roots. Barko and Smart (1986) observed increasing root to shoot ratios with decreasing sediment fertility in *Hydrilla* and *Myriophyllum*. Denny (1972), Sand-Jensen and Sondergaard (1979) and Aioi (1980) observed a similar trend in other submersed species.

Species-specific differences in root to shoot biomass production were consistent with the different life histories of the study species. As one might expect, the perennial species, *P. illinoensis* and *V. americana*, were characterized by significantly greater root:shoot ratios especially during the winter culture period as compared to the ruderal species, *N. guadalupensis*. Resource allocation for the production of increased below-sediment biomass is a mechanism used by perennials to increase resistance to harsh environmental conditions i.e. those associated with winter conditions.

The differences in macrophyte biomass accumulation observed among the culture periods confirmed the hypothesis that *N. guadalupensis, V. americana and Chara* sp. would produce the greatest biomass during the summer months while growth of *P. illinoensis,* a species with a range distribution that stretches into more temperate regions, would be stimulated by the cooler temperatures of the winter

months. Comparisons of the relative amounts of biomass produced by each species during each of the culture periods also indicated that, as hypothesized, *N. guadalupensis* was the fastest grower followed by *P. illinoensis, V. americana and lastly, Chara* sp. *N. guadalupensis* produced greatest biomass among the study species in the spring and summer culture periods (Figure 3-4). *P. illinoensis* was the fastest growing species during the winter culture period. Within species comparisons indicated that *N. guadalupensis* and *Chara* sp. exhibited greatest summer growth. *P. illinoensis* grew equally well in the summer and winter culture periods. *V. americana* growth was maximal in the summer culture period while spring and winter growth was similar. These results suggest that *V. americana* competed more successfully with *N. guadalupensis* and *P. illinoensis* during the summer culture period. Titus and Adams (1976) described *V. americana* as a "summer specialist". I observed that *Chara* sp. was a slow starter when I was growing stock cultures of the study species. However, in time, *Chara* sp. produced luxuriant growth and stable populations that, once established, have survived for several growing seasons. These observations would imply that, perhaps if culture periods had been longer, *Chara* sp. might have exhibited more competitive biomass production.

Investigations of the effect of environmental factors on biomass accumulation in this study indicated that macrophyte growth in this study was significantly affected by a combination of climatic factors. Light had one of the most significant effects on pooled composite summer and winter biomass (n = 120) while water temperature explained almost 50% and 64.5% of the variation in *N. guadalupensis* and *V. americana* biomass, respectively (Table 3-27). It should be noted that temperature

effects in this study were probably more pronounced than they would be in a natural environment due to the small volume of water in the growth tank as compared to that of a lake. There are, however, numerous cases in the literature where a similar relationship between light and temperature and macrophyte production has been identified. In his literature review, Short (1987) cited the findings of multiple studies conducted over a wide range of geographical locations that suggested that the seasonal growth cycle of submersed macrophytes is determined by a combination of climatic factors including irradiance, photoperiod and temperature. Many studies have identified light as one of, if not the most, significant factors affecting aquatic macrophyte growth (Canfield et al. 1985, Duarte and Kalff 1986, Barko et al. 1986, Smith and Barko 1990, Strand 1999). In their comparative study of *Myriophyllum spicatum* and *Vallisneria americana,* Titus and Adams (1979) reported that, although both species exhibited similar carbon uptake rates and coexisted in the same Wisconsin lakes, low light availability resulted in morphological adaptations in *M. spicatum* as a competitive mechanism. In a sediment fertilization study using sand rooting media amended with Osmocote and a second type of fertilizer, Sutton (1993) observed seasonal differences in *Hydrilla* growth. He attributed these differences in biomass produced during the fall and winter culture periods to the effects of water temperature on plant metabolic processes rather than on fertilizer nutrient release rates. Dale (1986) observed that in the absence of a growth limiting sediment nutrient, species adaptations more commonly reflected the influences of light and temperature than the effects of sediment nutrient composition. He reported that, even in cases

where there was sufficient water transparency, maximum depth of colonization was limited by water temperatures below the thermocline.

Macrophyte growth was also affected by epiphytic colonization. Generally greatest epiphyte biomass was observed on *Chara sp.* and *N. guadalupensis*, the study species with a finely dissected leaf architecture. The most probable explanation for the fact that many of the graphical trends in the epiphyte data were not statistically different was due to the sampling protocol used. Since the epiphyte biomass data was to be used to calculate a correction factor for final macrophyte biomass, the sampling protocol was designed to ensure that a representative estimate was made. Accordingly, epiphyte biomass samples were collected from a more mature, a mature and a young plant. This sampling regime resulted in high internal variation. This high variation may have masked relevant statistical trends.

The sediment nutrient needs of the macrophytes also appeared to vary seasonally. Whereas no sediment nutrients were significant to *N. guadalupensis* biomass accumulation in the spring and summer culture periods, zinc and phosphorus explained over 58% of the variation in macrophyte biomass in the winter culture period (Table 3-6). *Chara sp.* followed a similar pattern such that sediment nutrients only appeared to play a significant role in the winter culture period where sediment phosphorus explained almost 66% of the variation in biomass. Nutrient composition was also a more significant factor in *V. americana* growth in the summer as compared to the other culture periods, while sediment nutrients played a more significant role in *P. illinoensis* growth in the summer and winter culture periods. Copper was the most significant sediment nutrient for *P. illinoensis* growth explaining 41 and 36% of the

variation in biomass during the summer and winter culture periods, respectively (Table 3-6).

Field results indicated that there was a significant difference in the susceptibility of the study species to grazing by the larvae of *Parapoynix diminutalis* Snellen, an aquatic moth. Herbivore damage eliminated > 50% of above-sediment *N. guadalupensis* and *P. illinoensis* biomass while *V. americana* and *Chara sp.* appeared relatively unaffected. Painter and McCabe (1988) reported that herbivory by insect larvae can result in reductions of macrophyte root or shoot biomass ranging up to 100%. In his comprehensive literature review on herbivory on freshwater macrophytes, Lodge (1991) observed that grazing preference was negatively correlated with the phenolic content in plant tissues. The yield of *N. guadalupensis* and *P. illinoensis* in all sediments during the spring culture period was probably underestimated due to the conservative approach taken to estimate the reduction in above-sediment biomass due to herbivory. (See the Materials and Methods section for a description of the method used to estimate.)

Conclusions

The results of this study provide initial evidence that the sediments occurring at stations B, D, F and H in Lake Hollingsworth had sufficient quantities of sediment nutrients to support the growth of *N. guadalupensis guadelupensis*, *Potamogeton illinoensis*, *Vallisneria americana* and *Chara* sp. Field-testing of these results is necessary in order to determine the effect of such factors as sediment quantity on the applicability of these findings to the natural environment. The results of this investigation further indicate that, although all species should grow year-round, late

spring appears to be the optimum time in which to introduce propagules of *N. guadalupensis*, *P. illinoensis*, *V. americana* and *Chara* sp. into restored systems. Submersed macrophyte growth in this study appeared to be significantly affected by a combination of factors including water temperature and sediment macro- and micronutrients. Macrophyte growth response to climatic and sediment factors was species-related. However, additional investigation over a greater range of sediment organic matter contents, nutrient levels and physical characteristics is necessary in order to further elucidate the growth requirements of submersed aquatic macrophytes.

Table 3-1: Organic matter content and pH of sediments (n=3) from the preliminary survey. Values presented are means followed by standard error. Analysis of variance using GLM procedures followed by Tukey's HSD procedure was used to identify differences among stations. Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Procedure.

Station	Organic Matter (%)	pH
A	0.68 ± 0.07 b	5.8 ± 0.1 e
B	0.42 ± 0.09 b	5.4 ± 0.1 f
C	0.45 ± 0.05 b	6.3 ± 0.1 cd
D	0.38 ± 0.05 b	6.5 ± 0.1 c
E	39.18 ± 1.7 a	6.1 ± 0.1 de
F	1.08 ± 0.10 b	8.0 ± 0.0 a
G	34.96 ± 4.9 a	6.3 ± 0.1 cd
H	0.59 ± 0.02 b	7 ± 0 b

Table 3-2: Organic matter, pH and selected macro- and micronutrient composition of sediments from the four study stations and control sediments. Each value is the mean of three 100g sub-samples of sediment from each sediment source followed bv the standard error. Analysis of variance using GLM procedures followed by Tukey's HSD procedure was used to identify differences among stations. Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Procedure.

Sediment Source	**PH**	**OM**	**P**	**NH_4**	**NO_3**
		(%)	(mg Kg^{-1})	(mg Kg^{-1})	(mg Kg^{-1})
Station B	5.37 ± 0.09 e	0.42 ± 0.09 b	6.5 ± 0.12 b	7.00 ± 0.44 b	18.46 ± 2.32 b
Station D	6.53 ± 0.13 bc	0.39 ± 0.05 b	3.73 ± 0.35 b	6.19 ± 0.20 b	16.24 ± 0.64 b
Station F	8.03 ± 0.03 a	1.08 ± 0.10 a	79.43 ± 28.67ab	5.72 ± 0.50 b	15.21 ± 1.85 b
Station H	7.00 ± 0 b	0.59 ± 0.02 b	11.9 ± 1.12 b	5.46 ± 0.60 b	13.44 ± 2.07 b
Control 15g	6.00 ± 0 cd	NA	105.9 ± ab	145.00 ± 47.23 ab	558.92 ± 186.26 ab
Control 40g	5.77 ± 0.23 de	NA	246.33 ± 50.04 a	393.02 ± 150.14 a	1600.71 ± 648.15 a
Sediment Source	**Fe**	**Cu**	**Mn**	**Zn**	**K**
	(mg Kg^{-1})	(mg Kg^{-1})	(mg Kg^{-1})	(mg Kg^{-1})	(mg Kg^{-1})
Station B	10.82 ± 1.13 b	0.07 ± 0.003 b	0.23 ± 0.02 b	0.76 ± 0.10 c	7.55 ± 0.61 b
Station D	5.74 ± 0.79 b	0.04 ± 0.003 b	0.20 ± 0.04 b	0.40 ± 0.02 c	3.63 ± 0.27 b
Station F	10.92 ± 2.49 b	0.21 ± 0.01 b	1.62 ± 0.10 ab	7.77 ± 0.19 a	10.47 ± 0.75 b
Station H	5.69 ± 0.40 b	0.19 ± 0.003 b	0.24 ± 0.02 b	1.45 ± 0.05 bc	5.03 ± 0.13 b
Control 15g	58.53 ± 8.08 a	0.90 ± 0.28 b	2.57 ± 1.38 ab	1.70 ± 0.47 bc	350.33 ± 197.94 ab
Control 40g	72.5 ± 10.01 a	2.64 ± 0.68 .a	4.23 ± 1.05 a	3.08 ± 0.86 b	626.67 ± 144.73 a
Sediment Source	**Mg**	**Ca**	**Na**	**Al**	**Cl^-**
	(mg Kg^{-1})	(mg Kg^{-1})	(mg Kg^{-1})	(mg Kg^{-1})	(mg Kg^{-1})
Station B	15.5 ± 0.70 b	73.93 ± 2.20 b	11.0 ± 0.56 b	36.03 ± 3.51 ab	7.03 ± 0.81 ab
Station D	5.63 ± 0.35 b	73.63 ± 3.53 b	5.9 ± 0.12 b	16.6 ± 1.81 b	2.83 ± 0.23 b
Station F	52.3 ± 3.36 ab	5616.67 ± 259.63 a	14.5 ± 0.31 ab	44.5 ± 13.17 a	3.30 ± 0.40 b
Station H	10.93 ± 0.49 b	126.1 ± 24.49 b	7.13 ± 0.22 b	31.63 ± 0.63 ab	3.97 ± 0.38 b
Control 15g	46.97 ± 24.18 ab	154.67 ± 15.59 b	18.67 ± 6.59 ab	18.1 ± 0.79 ab	6.80 ± 1.92 ab
Control 40g	77.63 ± 19.23 a	206 ± 30.14 b	29.6 ± 6.01 a	21.67 ± 2.22 ab	18.23 ± 0.75 a

Table 3-3: Temperature and irradiance during the three culture periods. Temperature values are average daily temperatures followed by the standard error. Values shown in parentheses are the lowest and highest measured temperatures for each culture period.

Culture Period (CP)	Water Temperature (°C)	Total PAR[a] (μmol photons m^{-2} per CP)	Mean Instantaneous PAR[a] (μmol photons $s^{-1}m^{2}$)	Mean Photoperiod (hours)	Period (days)
Spring 4/30 to 6/29/01[b] 5/14 to 6/29/01[c]	26.6 ± 0.3[b] (20-33.5)[b] 27.3 ± 0.3[c] (20-33.5)[c]	2.44 x 10^{9b} 1.83 x 10^{9c}	806[b] (2-2306) 783[c] (2-2302)	13.8L:10.2D	61[b] 47[c]
Summer 7/20/ to 9/21/01	29.6 ± 0.2 (20-34)	2.24 x 10^{9}	742 (2-2153)	13.1L:10.9D	64
Winter 12/19/01 to 2/22/02	21.2 ± 0.3 (11-30)	1.63 x 10^{9}	624 (2-1863)	11.0L:13.0D	66

[a]Photosynthetically active radiation.

[b] These values apply to *Najas*, *Vallisneria* and *Chara*. The discrepancy in PAR values and length of culture periods among the study species was caused by the lack of availability of viable propagule material at the initial time of planting. This resulted in a two-week delay in planting time for *Potamogeton* as compared with the other species.

[c] These values apply to *Potamogeton*.

Table 3-4: Spring Culture Period – 30 April to 29 June 2001. Results of analysis of variance using GLM Procedure (SAS Institute 1999-2001) to investigate the effect of sediment source, plant type and the interaction between these two factors on macrophyte growth.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Sediment source (S)	4	122.3145657	30.5786414	1.70	0.1708
Plant type (PT)	3	577.13334529	192.3778176	10.68	<.0001
S*PT	12	221.6270488	18.4689207	1.03	0.4467

Table 3-5. Spring Culture Period – 30 April to 29 June 2001. Dry weight of study macrophytes cultured on sediments collected from the four study stations in Lake Hollingsworth and on artificial sediments. Values for each species are the mean biomass for three pots. The standard error follows the mean. Shoot biomass represents all above-sediment biomass including some rhizome and adventitious root material. Root biomass represents all below-sediment biomass and includes some rhizome material. Means for a given sediment source followed by the same lowercase letters are not significantly different ($\alpha = 0.05$).

Sediment source	Plant Type	Dry Weight (g)			
		Shoot	Root	Shoot+Root	Mean Root:Shoot
Station B	*Najas*	9.6 ± 1.2	2.7 ± 0.7	12.3 ± 1.8 a	0.28 ± 0.04
	Potamogeton	8.0 ± 2.0	1.4 ± 0.3	9.4 ± 2.3 ab	0.18 ± 0.01
	Vallisneria	1.6 ± 0.4	0.7 ± 0.2	2.3 ± 0.5 b	0.44 ± 0.07
	Chara	4.1 ± 0.5	NA	4.1 ± 0.5 ab	NA
Station D	*Najas*	13.2 ± 3.2	1.1 ± 0.5	14.3 ± 3.7 a	0.08 ± 0.01
	Potamogeton	5.0 ± 0.5	1.0 ± 0.7	6.0 ± 0.1 a	0.20 ± 0.02
	Vallisneria	5.1 ± 2.4	1.7 ± 0.6	6.8 ± 3.0 a	0.37 ± 0.04
	Chara	9.7 ± 2.0	NA	9.7 ± 2.0 a	NA
Station F	*Najas*	11.3 ± 1.9	1.1 ± 0.3	12.4 ± 2.2 a	0.10 ± 0.02
	Potamogeton	8.5 ± 1.5	1.4 ± 0.3	9.9 ± 1.5 a	0.16 ± 0.05
	Vallisneria	4.3 ± 0.4	1.5 ± 1.0	5.8 ± 1.4 a	0.35 ± 0.20
	Chara	12.4 ± 4.5	NA	12.4 ± 4.5 a	NA
Station H	*Najas*	9.7 ± 0.6	1.3 ± 0.1	11.0 ± 0.8 a	0.13 ± 0.003
	Potamogeton	6.9 ± 0.03	1.4 ± 0.1	8.3 ± 0.1 ab	0.20 ± 0.02
	Vallisneria	3.3 ± 0.4	1.2 ± 0.1	4.5 ± 0.6 b	0.36 ± 0.03
	Chara	12.2 ±	NA	12.2 ± a	NA
Control 40[a]	*Najas*	14.2 ± 4.2	2.2 ± 0.6	16.4 ± 4.7 a	0.15 ± 0.01
	Potamogeton	7.4 ± 0.8	0.8 ± 0.1	8.2 ± 0.8 a	0.11 ± 0.02
Control 15[b]	*Vallisneria*	3.8 ± 1.6	0.7 ± 0.1	4.5 ± 1.6 a	0.18 ± 0.12
	Chara	16.9 ± 4.5	NA	16.9 ± 4.5a	NA

[a] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus.

[b] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.

Table 3-6: Stepwise multiple regression analysis results using macrophyte biomass for each species during each culture period as the dependent variable and sediment macro- and micronutrient concentrations ($mgKg^{-1}$) and epiphyte biomass as the independent variables ($\alpha = 0.15$).

Culture Period	Dependent variables	Independent variables found to be significant in simple regression analyses ($\alpha = 0.05$)[a]	Significant independent variables	Cumulative R^2	F value	Prob. > F
Spring	Composite biomass of all species	NS[b]				
	Najas biomass	NS[b]	--	--	--	--
	Potamogeton biomass	Al	0.1214 * Al	29.10	5.33[c]	0.0380[d]
	Vallisneria biomass	NS[b]	--	--	--	--
	Chara biomass	NS[b]	--	--	--	--
Summer	Composite biomass of all species	P, Cu, Mn, Al, Fe, NH_4, NO_3, epiphyte biomass	68.0585 * Cu -0.3892 * NH_4	22.14 30.03	16.20 6.31	0.0002 0.0149
	Najas biomass	NS[b]	--	--	--	--
	Potamogeton biomass	P, Cu, Mn, Fe, NH_4, NO_3,	9.3042 * Cu	41.15	9.09	0.0100
	Vallisneria biomass	Cu, Al, Fe, NH_4, NO_3	-0.7420 * Al	47.55	11.79	0.0044
	Chara biomass	Epiphyte biomass	-1.3205 * Epiphyte biomass	41.44	8.06[c]	0.0130[d]

Table 3-6: Continued

Culture Period	Dependent variables	Independent variables found to be significant in simple regression analyses ($\alpha = 0.05$)[a]	Significant independent variables	Cumulative R^2	F value	Prob. > F
Winter	Composite biomass of all species	P, Cu, Mn, Fe, NH_4, NO_3	68.0585 * Cu -0.3892 * NH_4	22.14 30.03	16.20 6.31	0.0002 0.0149
	Najas biomass	P, Zn, Mn	2.5794 * Zn 0.0457 * P	47.80 58.11	10.99 2.71	0.0062 0.1280
	Potamogeton biomass	P, Cu, Fe, NH_4, NO_3	6.9493 * Cu	35.75	7.23	0.0186
	Vallisneria biomass	NS[b]	--	--	--	--
	Chara biomass	P, Cu, Mn, Fe, NH_4, NO_3	0.0749 * P	65.53	24.71	0.0003

[a] These independent variables were identified as significant in simple regression models with macrophyte biomass. Although other nutrients may have been significant factors, only those sediment nutrients known from the literature to be derived from the sediment were included in these multiple regression analyses. Values for sediment nutrients were mean values measured in subsamples of each sediment type taken during the sediment survey study.

[b] No independent variables were identified in initial investigations using simple regression models.

[c] t-value

[d] $P > |t|$

Table 3-7: Spring Culture Period – 30 April to 29 June 2001. Results of analysis of variance using GLM Procedure (SAS Institute 1999-2001) to investigate the effect of sediment source, plant type and the interaction between tthese two factors on macrophyte root:shoot ratios.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Sediment source (S)	4	0.07920971	0.01980243	1.79	0.1574
Plant type (PT)	2	0.41140822	0.20570411	18.62	<.0001
S*PT	8	0.06583398	0.00822925	0.74	0.6522

Table 3-8: Summer Culture Period – 20 July to 21 September 2001. Results of analysis of variance using GLM Procedure (SAS Institute 1999-2001) to investigate the effect of sediment source, plant type and the interaction between these two factors on macrophyte biomass.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Sediment source (S)	4	3377.848571	844.462143	9.33	<.0001
Plant type (PT)	3	7062.072762	2354.024254	26.02	<.0001
S*PT	12	980.133679	81.677807	0.90	0.5525

Table 3-9. Summer Culture Period –20 July to 21 September 2001. Dry weight of study macrophytes cultured on sediments collected from the four study stations in Lake Hollingsworth and on artificial sediments. Values presented for each species are the mean biomass for three pots. The standard error follows the mean. Shoot biomass represents all above-sediment biomass including some rhizome and adventitious root material. Root biomass represents all below-sediment biomass and includes some rhizome material. Means for a given sediment source followed by the same lowercase letters are not significantly different ($\alpha = 0.05$).

Sediment Source	Plant Type	Dry Weight (g)			
		Shoot	Root	Shoot+Root	Mean Root:Shoot
Station B	*Najas*	32.9 ± 4.0	2.9 ± 0.3	35.8 ± 3.7 a	0.09 ± 0.02
	Potamogeton	17.1 ± 1.2	3.5 ± 0.3	20.6 ± 0.9 ab	0.21 ± 0.03
	Vallisneria	15.0 ± 1.9	3.6 ± 0.2	18.6 ± 2.1 b	0.25 ± 0.02
	Chara	3.8 ± 1.6	NA	3.8 ± 1.6 c	NA
Station D	*Najas*	47.0 ± 9.5	5.1 ± 0.7	52.1 ± 9.0 a	0.12 ± 0.04
	Potamogeton	27.5 ± 4.1	3.5 ± 0.3	31.0 ± 4.3 ab	0.13 ± 0.01
	Vallisneria	35.4 ± 6.6	5.6 ± 1.1	41.0 ± 6.6 ab	0.17 ± 0.04
	Chara	17.4 ± 4.5	NA	17.4 ± 4.5 b	NA
Station F	*Najas*	48.9 ± 3.4	4.0 ± 0.4	52.9 ± 3.3 a	0.08 ± 0.01
	Potamogeton	25.4 ± 4.5	3.4 ± 0.7	28.8 ± 4.3 b	0.14 ± 0.01
	Vallisneria	23.9 ± 1.9	4.1 ± 0.4	28.0 ± 1.9 b	0.17 ± 0.02
	Chara	20.0 ± 2.3	NA	20.0 ± 2.3 b	NA
Station H	*Najas*	34.8 ± 4.2	6.1 ± 1.4	40.9 ± 2.8 a	0.19 ± 0.07
	Potamogeton	28.8 ± 4.1	3.3 ± 0.2	32.1 ± 4.2 ab	0.12 ± 0.02
	Vallisneria	19.5 ± 3.8	4.7 ± 0.8	24.2 ± 4.6 b	0.24 ± 0.01
	Chara	19.0 ± 2.0	NA	19.0 ± 2.0 b	NA
Control	*Najas*	49.8 ± 7.7	6.7 ± 0.3	56.5 ± 7.9 a	0.14 ± 0.02
40[a]	*Potamogeton*	46.0 ± 13.7	5.2 ± 1.4	51.2 ± 15.0 a	0.12 ± 0.01
Control	*Vallisneria*	37.4 ± 5.3	7.0 ± 1.9	44.4 ± 3.5 a	0.21 ± 0.09
15[b]	*Chara*	21.0 ± 4.0	NA	21.0 ± 4.0 a	NA

[a] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus.

[b] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.

Table 3-10: Summer Culture Period –20 July to 21 September 2001. Results of analysis of variance using GLM Procedure (SAS Institute 1999-2001) to investigate the effect of sediment source, plant type and the interaction of these two factors on macrophyte root:shoot ratios.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Sediment source (S)	4	0.020748	0.005187	1.26	0.3084
Plant type (PT)	2	0.057243	0.028622	6.94	0.0033
S*PT	8	0.036890	0.004611	1.12	0.3797

Table 3-11: Winter Culture Period –19 December 2001 to 22 February 2002. Results of analysis of variance using GLM Procedure (SAS Institute 1999-2001) to investigate the effect of sediment source, plant type and the interaction between these two factors on macrophyte biomass.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Sediment source (S)	4	1457.814725	3 64.453681	6.57	0.0004
Plant type (PT)	3	6163.120948	2054.373649	37.04	<.0001
S*PT	12	1398.410282	116.534190	2.10	0.0402

Table 3-12. Winter Culture Period –19 December 2001 to 22 February 2002. Dry weight of study macrophytes cultured on sediments collected from the four study stations in Lake Hollingsworth and on artificial sediments. Values presented for each species are the mean biomass for three pots. The standard error follows the mean. Shoot biomass represents all above-sediment biomass including some rhizome and adventitious root material. Root biomass represents all below-sediment biomass and includes some rhizome material. Means for a given sediment source followed by the same lowercase letters are not significantly different ($\alpha = 0.05$).

Sediment Source	Plant Type	Dry Weight (g)			
		Shoot	Root	Shoot+Root	Mean Root:Shoot
Station B	*Najas*	12.6 ± 1.8	1.2 ± 0.2	13.8 ± 1.9 b	0.10 ± 0.005
	Potamogeton	21.3 ± 1.4	6.1 ± 0.8	27.4 ± 2.0 a	0.29 ± 0.03
	Vallisneria	6.0 ± 0.9	1.3 ± 0.2	7.3 ± 1.0 b	0.22 ± 0.04
	Chara	8.2 ± 0.8	NA	8.2 ± 0.8 b	NA
Station D	*Najas*	9.8 ± 3.1	1.1 ± 0.1	10.9 ± 3.2 b	0.11 ± 0.05
	Potamogeton	25.7 ± 0.3	5.4 ± 0.2	31.1 ± 0.4 a	0.21 ± 0.004
	Vallisneria	6.1 ± 1.3	1.3 ± 0.3	7.4 ± 1.6 b	0.21 ± 0.02
	Chara	7.3 ± 1.2	NA	7.3 ± 1.2 b	NA
Station F	*Najas*	33.4 ± 8.8	2.6 ± 0.7	36.0 ± 9.5 a	0.08 ± 0.009
	Potamogeton	21.2 ± 4.1	6.1 ± 1.7	27.3 ± 5.7 a	0.29 ± 0.03
	Vallisneria	7.3 ± 1.3	1.1 ± 0.6	8.4 ± 1.9 a	0.15 ± 0.05
	Chara	13.2 ± 4.7	NA	13.2 ± 4.7 a	NA
Station H	*Najas*	23.8 ± 7.0	1.6 ± 0.1	25.4 ± 6.9 ab	0.07 ± 0.03
	Potamogeton	32.3 ± 4.8	5.4 ± 0.4	37.7 ± 4.4 a	0.17 ± 0.05
	Vallisneria	9.7 ± 1.1	3.2 ± 1.2	12.9 ± 1.4 b	0.33 ± 0.14
	Chara	10.5 ± 1.9	NA	10.5 ± 1.9	NA
Control 40[a]	*Najas*	29.7 ± 1.8	2.6 ± 0.8	32.3 ± 2.5 ab	0.09 ± 0.02
	Potamogeton	41.9 ± 10.4	6.3 ± 1.1	48.2 ± 11.6 a	0.15 ± 0.01
Control 15[b]	*Vallisneria*	5.8 ± 1.8	0.9 ± 0.03	6.7 ± 1.9 c	0.16 ± 0.06
	Chara	21.3 ± 1.5	NA	21.3 ± 1.5 ab	NA

[a] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus.

[b] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.

Table 3-13: Winter Culture Period –19 December 2001 to 22 February 2002. Results of analysis of variance using GLM Procedure (SAS Institute 1999-2001) to investigate the effect of sediment source, plant type and the interaction between these two factors on macrophyte root:shoot ratios.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Sediment Source (SS)	4	0.021118	0.005279	0.73	0.5772
Plant type (PT)	2	0.148970	0.074485	10.33	0.0004
SS*PT	8	0.091293	0.011412	1.58	0.1731

Table 3-14: Results of analysis of variance using GLM Procedure (SAS Institute 1999-2001) of pooled total macrophyte biomass of all species combined produced during the summer and winter culture periods (n = 120) to investigate the effect of culture period, sediment source, plant type and the interaction between the factors.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Culture period (CP)	1	4368.353429	4368.353429	59.97	<.0001
Sediment source (S)	4	3935.090475	983.772619	13.50	<.0001
Plant type (PT)	3	9420.311571	3140.103857	43.11	<.0001
CP*S	4	971.075191	242.768798	3.33	0.0142
CP*PT	3	3762.750644	1254.250215	17.22	<.0001
S*PT	12	1483.280431	123.606703	1.70	0.0835
CP*S*PT	12	877.638647	73.136554	1.00	0.4535

Table 3-15: Differences among the study species in the pooled mean biomass produced during the summer and winter culture periods (n=24) determined using Tukey's HSD Test.

Sediment Source	Macrophyte	Mean Biomass (g DWT)	Tukey Grouping
Station B	*N. guadelupensis*	24.80	A
	P. illinoensis	24.04	A
	V. americana	12.93	AB
	Chara sp.	6.42	B
Station D	*N. guadelupensis*	31.47	A
	P. illinoensis	31.05	A
	V. americana	24.23	A
	Chara sp.	12.37	A
Station F	*N. guadelupensis*	44.43	A
	P. illinoensis	27.25	B
	V. americana	18.18	B
	Chara sp.	16.58	B
Station H	*P. illinoensis*	34.92	A
	N. guadelupensis	34.71	A
	V. americana	18.57	B
	Chara sp.	14.75	B
Control 40[a]	*P. illinoensis*	49.7	A
	N. guadelupensis	44.4	AB
Control 15[b]	*V. americana*	25.5	AB
	Chara sp.	21.1	B

[a] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus.

[b] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.

Table 3-16: Differences within the study species in the pooled mean biomass produced during the summer and winter culture periods (n=30) determined using Tukey's HSD Test.

Macrophyte	Sediment source	Mean Biomass (g DWT)	Tukey Grouping
N. guadelupensis	Station F	44.43	A
	Control 40[a]	44.40	A
	Station H	34.71	A
	Station D	31.47	A
	Station B	24.80	A
P. illinoensis	Control 40[a]	49.70	A
	Station H	34.91	AB
	Station D	31.05	B
	Station F	27.25	B
	Station B	24.06	B
V. americana	Control 15[b]	25.52	A
	Station D	24.23	A
	Station H	18.57	A
	Station F	18.18	A
	Station B	12.93	A
Chara sp.	Control 15[b]	21.14	A
	Station F	16.58	AB
	Station H	14.75	AB
	Station D	12.37	AB
	Station B	6.42	B

[a] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus.

[b] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.

Table 3-17: Results of analysis of variance using GLM Procedure (SAS Institute 1999-2001) of pooled macrophyte root:shoot ratios from the summer and winter culture periods (n = 90) to investigate the effect of culture period, sediment source, plant type and the interaction between the factors.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Culture period (CP)	1	0.009589	0.009589	1.70	0.1973
Sediment source (S)	4	0.032664	0.008166	1.45	0.2297
Plant type (PT)	2	0.165277	0.082638	14.65	<.0001
CP*S	4	0.008985	0.002246	0.40	0.8091
CP*PT	2	0.044910	0.022455	3.98	0.0239
S*PT	8	0.091688	0.011461	2.03	0.0579
CP*S*PT	8	0.036163	0.004520	0.80	0.6037

Table 3-18: Differences in the pooled mean root:shoot ratios (n=18) among the study species determined using Tukey's HSD Test.

Sediment Source	Macrophyte	Mean Root:Shoot Ratio	Tukey Grouping
Station B	*P. illinoensis*	0.2491	A
	V. americana	0.2338	A
	N. guadelupensis	0.0956	B
Station D	*V. americana*	0.1947	A
	P. illinoensis	0.1704	A
	N. guadelupensis	0.1307	A
Station F	*P. illinoensis*	0.2094	A
	V. americana	0.1527	AB
	N. guadelupensis	0.0809	B
Station H	*V. americana*	0.2912	A
	P. illinoensis	0.1499	A
	N. guadelupensis	0.1454	A
Control 15[a]	*V. americana*	0.2006	A
Control 40[b]	*P. illinoensis*	0.1404	A
	N. guadelupensis	0.1134	A

[a] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.

[b] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus

Table 3-19: Differences within macrophyte species in the pooled mean root:shoot ratios produced during the summer and winter culture periods (n=30) determined using Tukey's HSD Test.

Macrophyte	Sediment source	Mean Root:Shoot Ratio	Tukey Grouping
N. guadelupensis	Station H	0.1454	A
	Station D	0.1307	A
	Control 40[a]	0.1134	A
	Station B	0.0956	A
	Station F	0.0809	A
P. illinoensis	Station B	0.2491	A
	Station F	0.2094	AB
	Station D	0.1704	AB
	Station H	0.1499	AB
	Control 40[a]	0.1404	B
V. americana	Station H	0.2912	A
	Station B	0.2338	A
	Control 15[b]	0.2006	A
	Station D	0.1947	A
	Station F	0.1527	A

[a] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus.
[b] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.

Table 3-20: Results of analysis of variance using GLM Procedure (SAS Institute 1999-2001) of pooled epiphyte biomass from the summer and winter culture periods (n = 120) to investigate the effect of culture period, sediment source, plant type and the interaction between the factors.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Culture period (CP)	1	8948.2605	8948.2605	36.78	<.0001
Sediment source (S)	4	1096.6994	274.1748	1.13	0.3501
Plant type (PT)	3	655.1598	218.3866	0.90	0.4464
CP*S	4	1402.4206	350.6052	1.44	0.2286
CP*PT	3	239.8873	79.9624	0.33	0.8046
S*PT	12	4006.0660	333.8388	1.37	0.1979
CP*S*PT	12	4760.6347	396.7196	1.63	0.1008

Table 3-21: Differences in pooled mean epiphyte biomass occurring in the summer and winter culture periods (n=30). Means separation was performed using Tukey's HSD Test.

Macrophyte	Culture period	Epiphyte biomass (mg/g host DWT)	Tukey Group	Pr > F
Chara sp	Winter	23.540	A	0.0067
	Summer	5.822	B	
N. guadelupensis	Winter	25.175	A	<.0001
	Summer	2.485	B	
P. illinoensis	Winter	19.654	A	0.0320
	Summer	3.427	B	
V. americana	Winter	15.983	A	0.0236
	Summer	1.280	B	

Table 3-22: Differences in pooled mean epiphyte biomass occurring on each host macrophyte species in each sediment type (n=18) in the summer and winter culture period. Means were separated using Tukey's HSD Test.

Sediment Source	Macrophyte	Epiphyte Biomass (mg/g DWT)	Tukey Grouping
Station B	*Chara* sp.	17.84	A
	P. illinoensis	12.43	A
	N. guadelupensis	11.13	A
	V. americana	3.86	A
Station D	*Chara* sp.	22.18	A
	N. guadelupensis	14.90	A
	V. americana	8.98	A
	P. illinoensis	4.59	A
Station F	*Chara* sp.	10.08	A
	N. guadelupensis	8.88	A
	V. americana	7.40	A
	P. illinoensis	7.00	A
Station H	*P. illinoensis*	28.06	A
	N. guadelupensis	11.07	A
	Chara sp.	8.15	A
	V. americana	4.77	A
Control 40[a]	*N. guadelupensis*	23.31	A
	P. illinoensis	5.62	A
Control 15[b]	*V. americana*	21.12	A
	Chara sp.	17.15	A

[a] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus.

[b] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.

Table 3-23: Differences in mean pooled epiphyte biomass (n=30) occurring on each host macrophyte species in the summer and winter culture periods at the study stations. Means were separated using Tukey's HSD Test.

Macrophyte	Station	Epiphyte Biomass (mg/g host DWT)	Tukey Group	Pr > F
Chara sp.	Station D	22.18	A	0.6925
	Station B	17.84	A	
	Control 15[a]	17.15	A	
	Station F	10.08	A	
	Station H	8.15	A	
N. guadelupensis	Control 40[b]	23.31	A	0.6615
	Station D	14.90	A	
	Station B	11.13	A	
	Station H	11.07	A	
	Station F	8.88	A	
P. illinoensis	Station H	28.06	A	0.2824
	Station B	12.43	A	
	Station F	7.00	A	
	Control 40[b]	5.62	A	
	Station D	4.59	A	
V. americana	Control 15[a]	21.12	A	0.4478
	Station D	8.98	A	
	Station H	4.77	A	
	Station F	4.44	A	
	Station B	3.86	A	

[a] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.

[b] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus.

Table 3-24: Differences in mean epiphyte biomass (n=60) occurring at each station during the summer and winter culture periods. Means separation was done using Tukey's HSD Test.

Culture period	Sediment source	Epiphyte Biomass (mg/g host DWT)	Tukey Group	Pr > F
Summer	Station B	5.39	A	0.1715
	Control 15[a] and Control 40[b]	3.17	A	
	Station F	2.85	A	
	Station H	2.76	A	
	Station D	2.12	A	
Winter	Control 15[a] and Control 40[b]	30.43	A	0.3166
	Station H	23.61	A	
	Station D	23.21	A	
	Station B	16.21	A	
	Station F	11.85	A	

[a] Control 15 sediments were composed of washed builder's sand amended with 15 g of 15-9-12 Osmocote Plus.
[b] Control 40 sediments were composed of washed builder's sand amended with 40 g of 15-9-12 Osmocote Plus.

Table 3-25: Results of the use of simple regression analysis to evaluate the relative significance of each sediment characteristic on the pooled mean biomass of all species combined (total) (n = 120) and each individual species (n = 30) during the summer and winter culture periods. Those characteristics that were found to have a significant effect are indicated with X. Highly significant characteristics (P <.0001) are denoted by X+.

Plant	pH	OM	P	Zn	Cu	Mn	Al	Fe	NH4	NO3	Ca	Mg	K	Na	Cl
Composite									X+	X+		X			
Najas												X			
Pot			X		X	X	X	X	X	X		X	X	X	X
Val															
Chara			X		X	X		X	X	X		X	X	X	

Table 3-26: Results of the use of simple regression analysis to evaluate the relative significance of light and water temperature on the pooled mean biomass of all species (n = 120) and each individual species (n = 30) during the summer and winter culture periods. Those characteristics that were found to have a significant effect are indicated with X. Highly significant characteristics (P <.0001) are denoted by X+.

Plant	Epiphyte Biomass (mg/g host DWT)	Total PAR	Mean Instantaneous PAR	Water Temperature
		(μmol photons m^{-2} CP^{-1})	(μmol photons s^{-1} m^{-2})	(°C)
Composite biomass of all species	X	X+	X+	X+
Najas	X	X+	X+	X+
Potamogeton				
Vallisneria		X+	X+	X+
Chara				

Table 3-27: Stepwise multiple regression analysis results using pooled macrophyte biomass produced during the summer and winter culture periods (n = 120) for composite biomass of all species and n = 30 for each individual species). Macrophyte biomass was used as the dependent variable and sediment macro- and micronutrient concentrations, light, water temperature and epiphyte biomass (mg/g host DWT) as the independent variables ($\alpha = 0.15$).

Dependent variables	Independent variables found to be significant in simple regression analyses ($\alpha = 0.05$)[a]	Significant independent variables	Cumulative R^2	F value	Prob. > F
Composite biomass of all species	NH_4, NO_3, total PAR[b], PAR[c], water temperature, epiphyte biomass	0.3540 * NO_3 2.001E-8 * total PAR -1.3880 * NH_4	18.36 32.83 35.48	25.63 24.35 4.60	<.0001 <.0001 0.0340
Najas biomass	PAR, total PAR, water temperature, epiphyte biomass	2.8953 * Water temperature	48.69	23.73	<.0001
Potamogeton biomass	P, Cu, Mn, Al, Fe, NH_4, NO_3	39.0962 * Cu -1.2241 * Fe	38.04 44.44	17.19 3.11	0.0003 0.0891
Vallisneria biomass	PAR, total PAR, water temperature	2.7015 * Water temperature	64.52	50.91	<.0001
Chara biomass	P, Cu, Mn, Fe, NH_4, NO_3,	0.0597 * P	31.17	12.23	0.0016

[a] These independent variables were identified as significant in simple regression models with macrophyte biomass. Although other nutrients may have been significant factors, only those sediment nutrients known from the literature to be derived from the sediment were included in these multiple regression analyses. These independent variables were previously identified as significant in simple regression models with macrophyte biomass. Values for sediment nutrients were mean values measured in subsamples of each sediment type taken during the sediment survey study.

[b] total PAR per culture period (μmol photons m^{-2} per CP)

[c] mean instantaneous PAR during each culture period (μmol photons s^{-1} m^{-2})

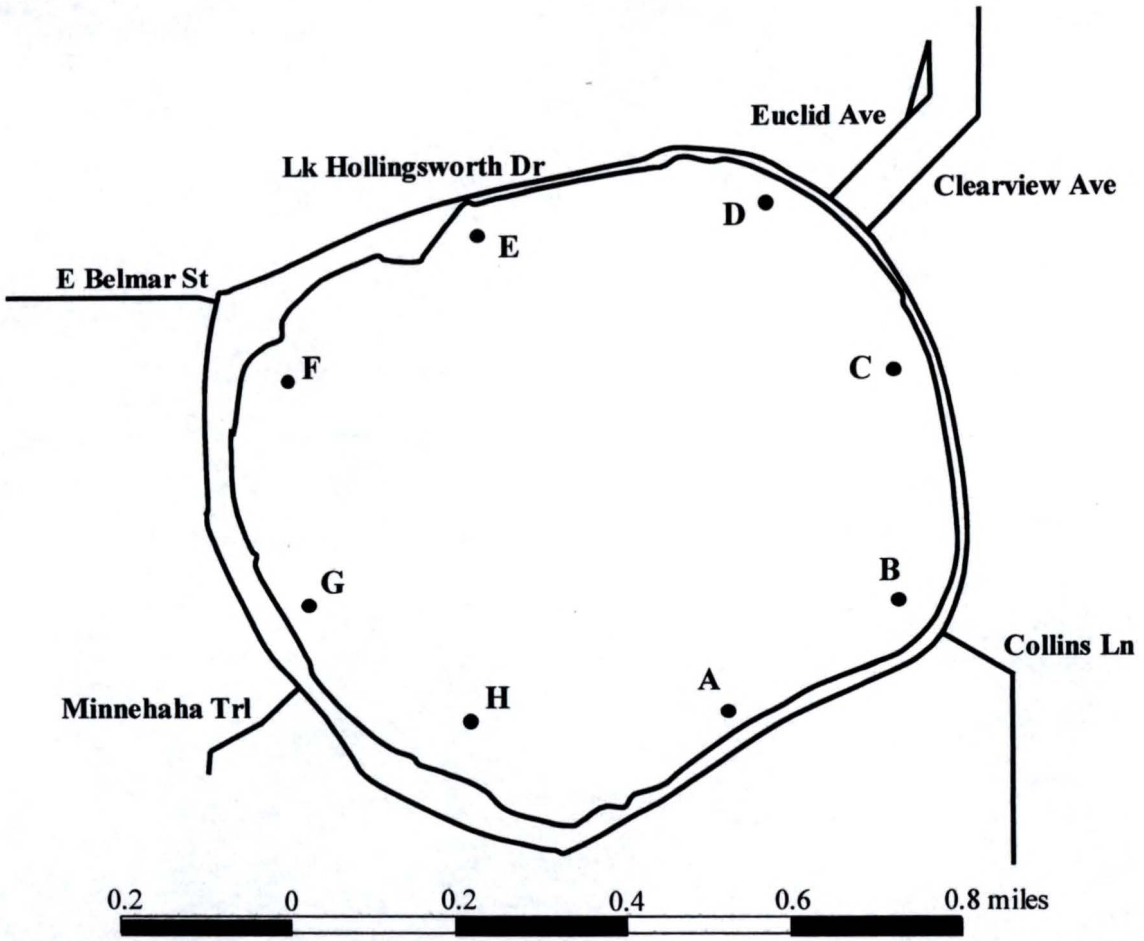


Figure 3-1: Map of Lake Hollingsworth showing the location of the preliminary sediment survey stations. Stations B, D, F and H were selected as the study stations for further investigation.

Sediment Suitability Study

6 sediment types: B, D, F, H, Control 40 and Control 15

4 plant types* triplicate samples per sediment type = 12 pots

60 pots divided equally between 2 tanks

3 culture periods

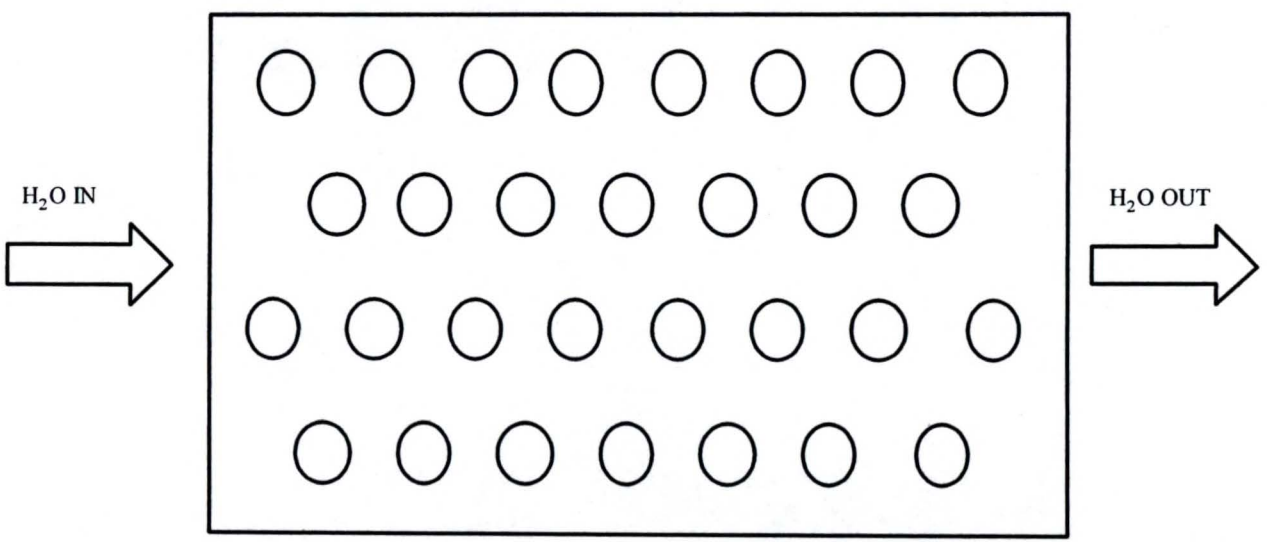


Figure 3-2: A diagrammatic sketch of the distribution of 7.6-L nursery containers in the experimental tanks. Containers were arranged at random. Pots were divided to ensure representative distribution of combinations of macrophyte species and sediments between the tanks.

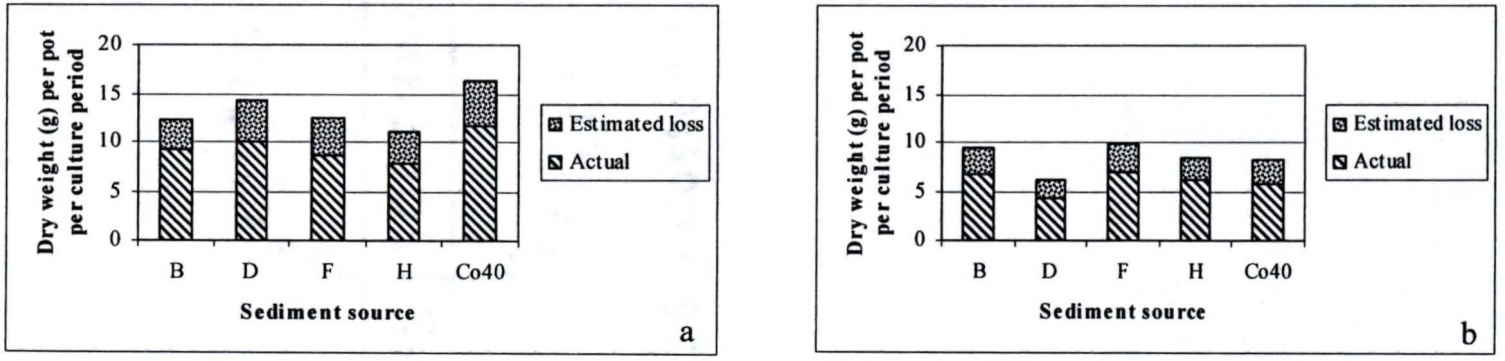


Figure 3-3: Biomass produced by *Najas* and *Potamogeton* during the spring culture period. Bars represent the mean value determined for three culture containers per plant type. The lower portion of each bar represents the measured values. The upper portion of each bar represents the calculated biomass lost to herbivory.

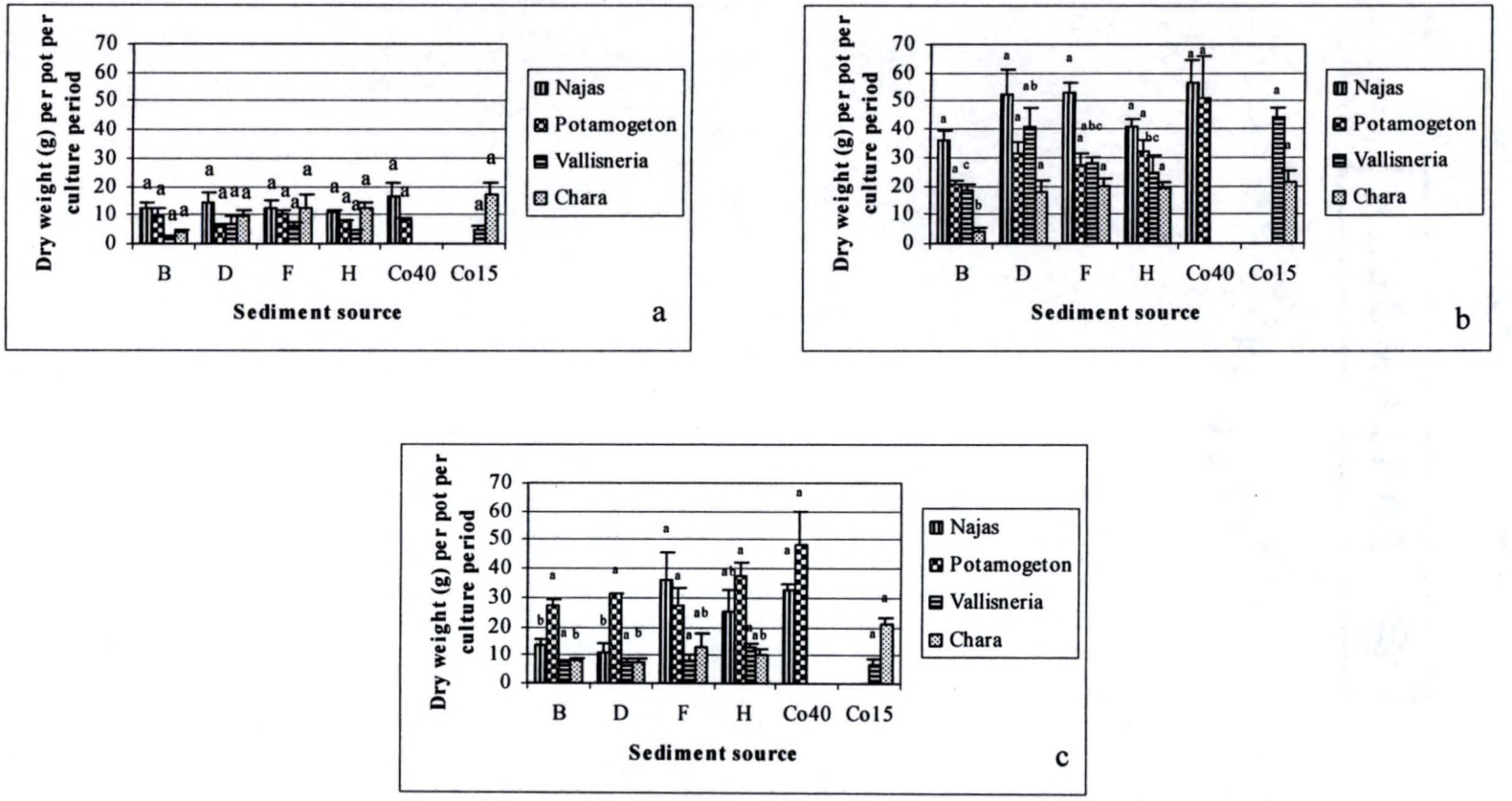
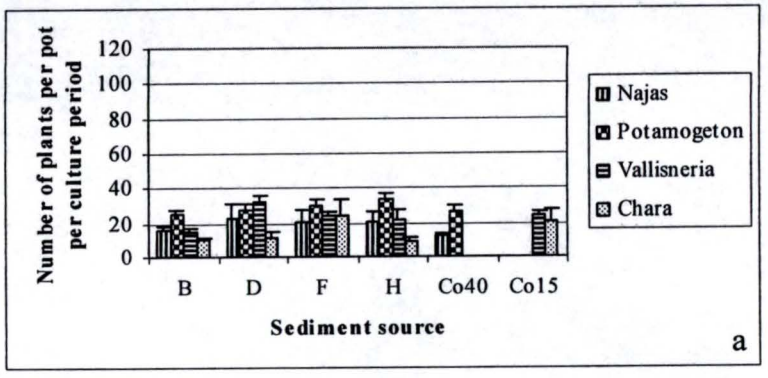
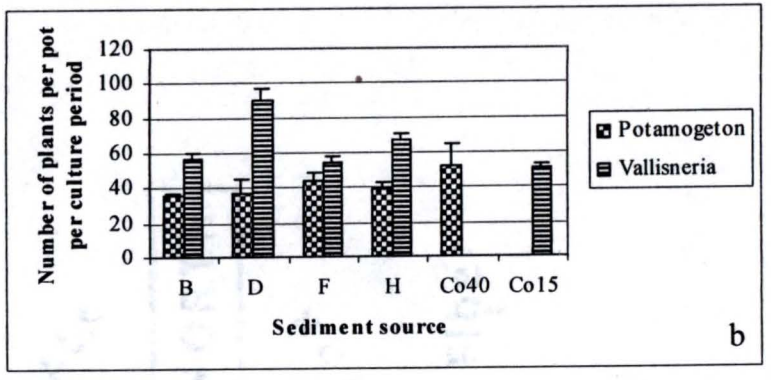


Figure 3-4: A comparison of the mean biomass produced by each plant species when grown in sediments from stations B, D, F and H and controls for each of the three culture periods. Control sediments, designated as Co40 and Co15, were amended with 40 g and 15 g of Osmocote fertilizer, respectively. Biomass values represent the mean value determined for three culture containers per plant type. Bars are the standard error. Means for a given species with the same lowercase letters above them are not significantly different. a) Spring culture period - 4/30/01 to 6/29/01. Biomass for *Najas* and *Potamogeton* are estimated values corrected for loss to herbivory. b) Summer culture period - 7/20/01 to 9/21/01. c) Winter culture period - 12/19/01 to 2/22/02.





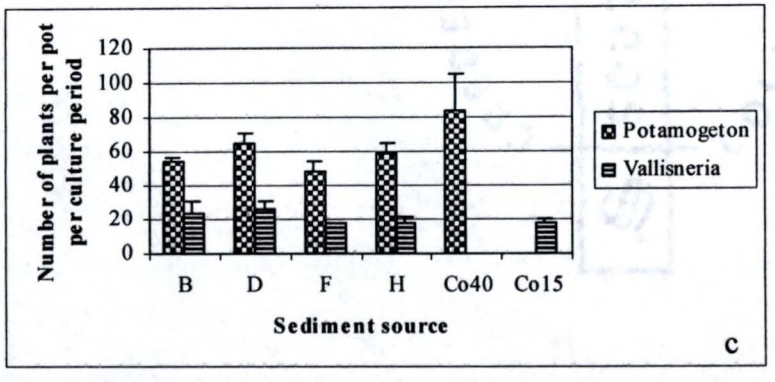


Figure 3-5: A comparison of the average number of individual plants produced by each species when grown in the six sediments. Control sediments, designated as Co40 and Co15, were amended with 40 g and 15 g of Osmocote fertilizer, respectively. Values are means of three culture containers per macrophyte species per sediment type. Bars are the standard error. Means for a given species with the same lowercase letters above them are not significantly different. a) Spring culture period - 4/30/01 to 6/29/01. Average Najas and Potamogeton values may be underestimated due to stem loss resulting from herbivore activity. b) Summer culture period - 7/20/01 to 9/21/01. c) Winter culture period - 12/19/01 to 2/22/02.

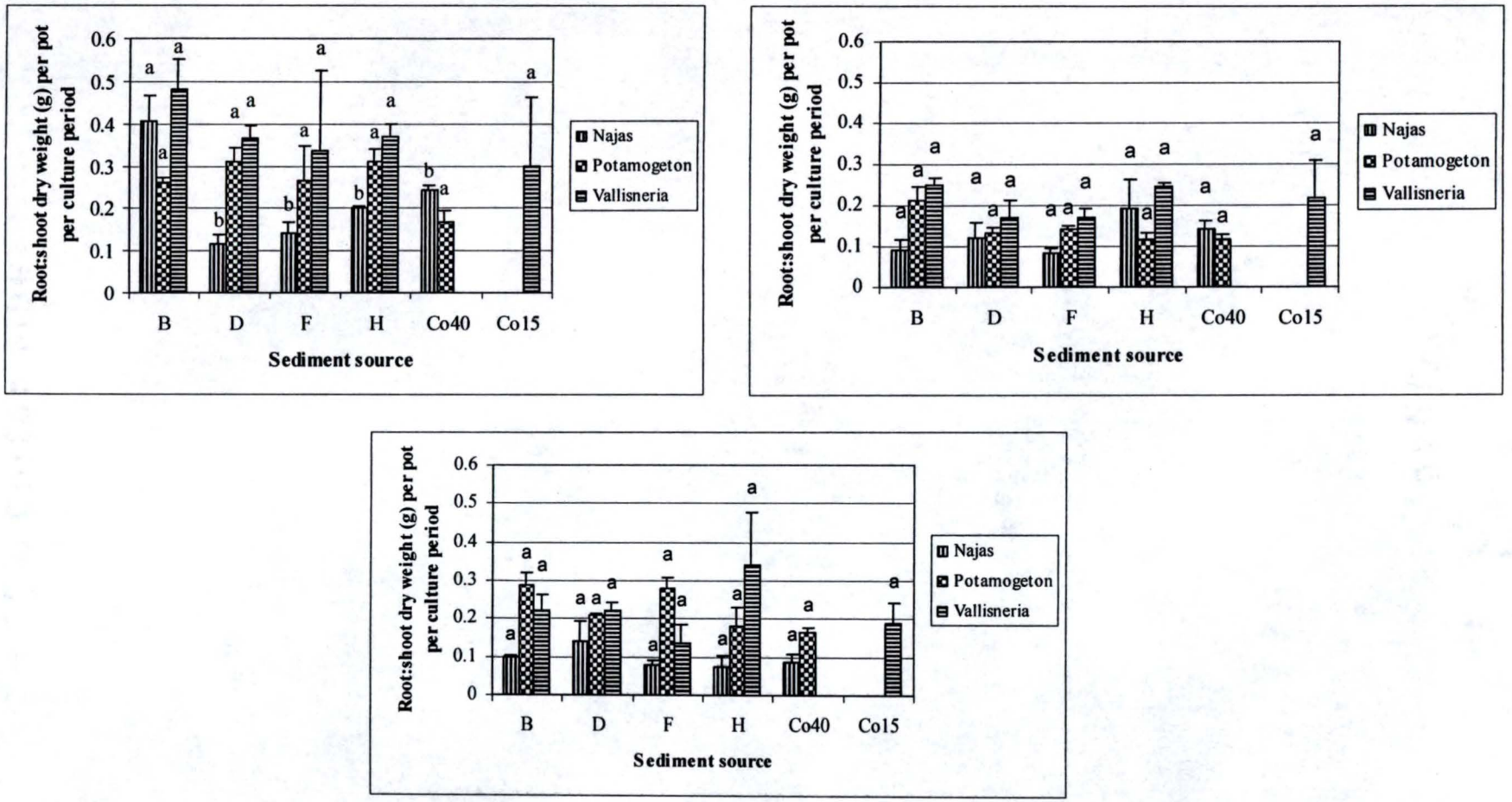


Figure 3-6: Ratio of root:shoot biomass for the four study species in the spring, summer and winter culture periods. Ratios represent the mean value determined for three culture containers per plant type. Control sediments designated as Co40 and Co15 were amended with 40 g and 15 g of Osmocote fertilizer, respectively. Bars are the standard error. Means for a given species with the same lowercase letters above them are not significantly different. a) Spring culture period - 4/30/01 to 6/29/01. Biomass for *Najas* and *Potamogeton* are estimated values corrected for loss to herbivory. b) Summer culture period - 7/20/01 to 9/21/01. c) Winter culture period - 12/19/01 to 2/22/02.

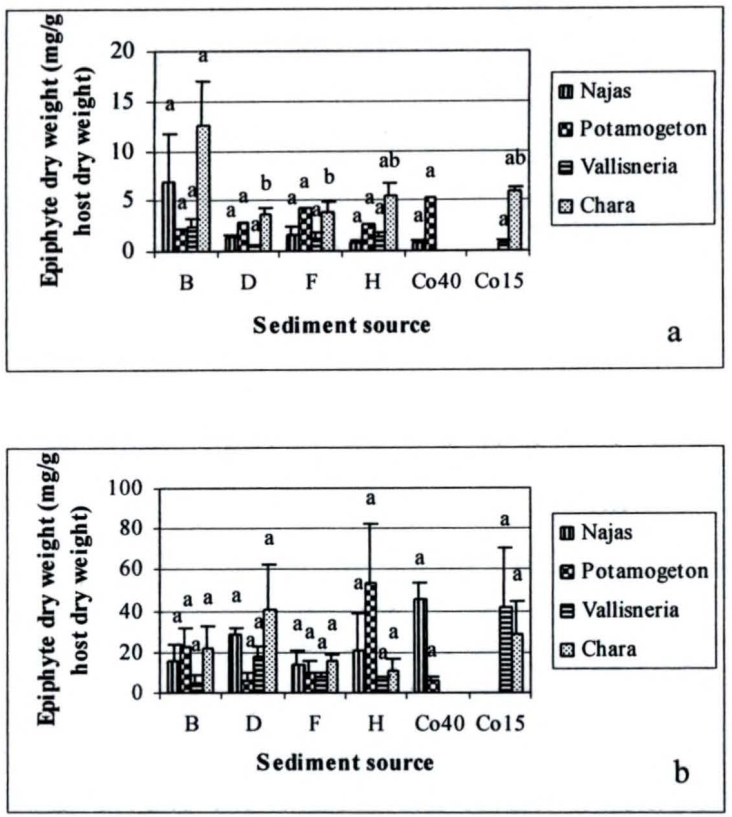


Figure 3-7: Epiphyte biomass occurring on the study plants. Values indicated are the mean of the biomass determined at the time of harvest for three individual representative plants of each host species. Control sediments designated as Co40 and Co15 were amended with 40 g and 15 g of Osmocote fertilizer, respectively. Bars are the standard error. Means with the same lowercase letters above them are not significantly different. a) Summer culture period (7/20 - 9/21/01). b) Winter culture period (12/19/01 - 2/22/02).

CHAPTER 4
LIGHT REQUIREMENTS FOR FOUR SPECIES OF NATIVE SUBMERSED MACROPHYTES: IMPLICATIONS FOR THE RESTORATION OF SHALLOW EUTROPHIC LAKES 1. ASSESSMENT OF THE LIGHT REQUIREMENTS OF MATURE PLANTS

Introduction

Numerous studies have identified light availability as one of the most significant factors controlling macrophyte production in aquatic systems (Duarte and Kalff 1986, Canfield et al. 1985, Barko et al. 1986, Smith and Barko 1990, Strand 1999). Additional studies have investigated the impact of light attenuation on submerged macrophyte productivity and growth (Carter and Rybicki 1990, Duarte 1991, Dunton 1994, Goodman et al. 1995, Masini et al. 1995, Zimmerman et al. 1995, Livingston et al. 1998, Grimshaw et al. 2002). Dennison (1987) discussed the use of photosynthesis vs. irradiance curves together with diurnal light curves to predict growth responses to changes in light regime (Dennison and Alberte 1985), seasonal growth patterns and the maximum depth of colonization for *Zostera marina*. All submerged angiosperms are shade plants (Wetzel 2001). Spencer and Bowes (1990) determined that light saturation for photosynthesis ranges from 10-50% full sunlight.

The classification scheme Gessner (1955) developed for submerged macrophytes based on their physiological adaptations to light availability reflects the extreme plasticity and adaptability of submerged macrophytes to the highly variable underwater light environment. The adaptation types identified include strictly shade adapted requiring low light intensities, strictly light adapted requiring high light

intensities, shade adapted but exhibiting optimum photosynthesis at intermediate light levels, etc. Some aquatic macrophytes are capable of adapting to lower light environments via changes at the ecvllular and the whole-plant level (Wetzel 2001). Cellular adaptations include changes in pigment and enzyme concentrations and composition (Dennison and Alberte 1982, Barko and Filbin 1983). Morphological responses to increasing shade include changes in length and biomass proportions (i.e. of leaves and stems) (cf. Barko et al. 1982). Goldsborough and Kemp (1988) investigated the response and recovery of *Potamogeton perfoliatus* to experimentally induced shade during a 17-day treatment period followed by a 16-day "recovery" period. During the treatment period, plants responded by increasing photosynthetic pigments and producing elongate stems, thinning lower leaves and canopy formation at the surface. Plants showed significant recovery 10 days after removal of light treatments.

The findings of more recent research have indicated that there is considerable variation in photosynthetic capacity and compensation points among submerged macrophyte species (Van et al. 1976, Kenworthy and Fonseca 1996, Wetzel 2001). Light compensation points often occur at 1-3% full sunlight (Wilkinson 1963, Spence 1982, Bowes et al. 1977, Moeller 1980). Kimber et al. (1995) reported that tuber production in *Vallisneria americana* ceased at light levels less than 5% of ambient sunlight. They discussed the implications of these results for *V. americana* growth in relation to light attenuation by turbidity in their study system.

The purpose of this study was to investigate the amount of photosynthetically active radiation (PAR) at which there was no net growth of *Najas guadelupensis*,

Potamogeton illinoensis, *Vallisneria americana* and *Chara* sp. The underwater light environment of shallow productive systems is highly variable due to changes in light regime resulting primarily from resuspension events and fluctuations in phytoplankton standing crop. A review of the literature indicates that data available on the light requirements of submersed macrophytes is sparse and highly variable (Kimber et al. 1995). Most of the studies that have been conducted have investigated the light requirements of weedy exotic species. The four species selected for investigation in this study, *Najas guadalupensis*, *Potamogeton illinoensis, Vallisneria americana* and *Chara* sp., are native species commonly occurring in Florida lakes. *N. guadalupensis and Chara* sp. are annuals; *.P. illinoensis* and *V. americana* are perennials. Of the limited information available, at the time of this study, essentially no quantitative data was available for *Najas guadalupensis*, *Potamogeton illinoensis* and *Chara.* Although numerous studies have indicated that *Vallisneria americana* may have a low light affinity (cf. Titus and Adams 1979, Davis and Brinson 1980, Kimber et al. 1995, Grimshaw et al. 2002), the high suseptibility of the light capability to the effects of other environmental factors can often result in high light requirements for this species (cf. Barko et al. 1982, Carter and Rybicki 1990, Doyle and Smart 2001).Further study is required in order to elucidate some of these apparent contradictions. In an attempt to address the significant differences in the light microenvironments of mature shoots and actively growing emergent propagule plant material, this study was divided into two parts such that three of the six experiments were designed to investigate the light requirements of mature plants while the experiments conducted in the second phase addressed propagule light requirements.

The experiments in this first part were designed to test several hypotheses. The first hypothesis was that *N. guadelupensis* and *Chara* sp. would exhibit the highest light requirements for net growth due to self-shading due to the habit and growth strategies of these species. I anticipated that *V. americana* would exhibit the lowest light requirement for net growth among the study species, approximately 4% incident irradiance. Based upon the literature, I expected the PPFD for no net growth of *P. illinoensis* and *N. guadelupensis* to be approximately 11% and 15% incident irradiance, respectively. I anticipated PPFD for no net growth of *Chara* sp. to range between 4 and 20% incident irradiance. I further expected to observe photoinhibition in those species that are either canopy-formers or have an erect habit, *N. guadelupensis*, *P. illinoensis* and *Chara* sp., at light levels > 26.2% incident irradiance

Additional information on the growth response of macrophyte species to decreased light availability should be of value to lake managers whose goal it is to determine which species to plant and maximum planting depths in revegetation projects.

Materials and Methods

Experimental Environment and Procedures

Najas guadelupensis and *Chara* sp. are fast-growing pioneer plants with a finely dissected leaf architecture. *Potamogeton illinoensis* and *Vallisneria americana* are perennials with flat, lanceolate and elongate, ribbon-like leaves, respectively. (See Chapter 3 for a more detailed description of the study plants). Propagules (apical cuttings of *N. guadalupensis*, *P. illinoensis* and *Chara* sp. and suckers of *V.*

americana) were harvested from stock cultures of approximately the same age and in a state of active growth maintained at the FLREC from plants originally collected in Lake Okeechobee, Florida (Dr. David L. Sutton, UF FLREC, personal communication). Sixty 7.6-L black plastic nursery pots 22.5 cm in top diameter and 20 cm deep were loaded to 11 cm from the top with air-dried coarse builders' sand. Osmocote Plus Southern Formula 15-9-12 (N:P:K), a commercially available fertilizer, was added as a layer to each container and mixed into the sand at a rate of 40 g per pot for *N. guadalupensis* and *P. illinoensis*, and 15 g for *V. americana* and *Chara sp.* (Dr. David Sutton, UF FLREC, personal communication). This fertilizer is formulated to slow-release in soil over an 8-9 month period with increased rates of nutrient release at temperatures ≥ 21°C (Harbaugh and Wilfret 1981). Each container was then filled with sand to within 2.5 cm of the top. Containers were submerged in concrete tanks measuring 6.2 m in length by 3-1 m in width by 0.9 m in height filled with pond water to a depth of 0.8 m. Twenty-centimeter apical cuttings of *N. guadalupensis*, *Chara sp.* and *P. illinoensis* and single rosettes of *V. americana* were then planted at a frequency of four propagules per pot. *N. guadalupensis* and *Chara sp.* were planted to a depth of ≥ 3 nodes. *P. illinoensis* was planted to a depth of at least 2 nodes. Individual sucker plants of *V. americana* were cut from the rhizome, roots were gently washed to remove organic sediments and each rosette was planted deep enough to submerge the rhizome beneath the sediment surface. Care was taken to avoid covering the basal rosette. Two (Experiment 1) and three containers (Experiment 2 and 3) were planted per treatment group for each macrophyte species. Pond water from a groundwater fed pond (as described by Steward 1984) located on-

site at the UF FLREC flowed into the tanks at the surface of one end and out from bottom drains at the other end at a rate that allowed for an exchange of water every 24 hr. After planting, the experimental propagules were allowed to grow to maturity in full sunlight for at least a 4-week acclimation period. The light variable was introduced after the macrophytes reached maturity. Maturity was defined as growth that was "topped out" (e.g. shoots had reached the water surface). Pots were arranged to allow at least 1 foot of space between containers in order to prevent competitive shading. Containers were placed in rows parallel to the flow of water in the tanks (see Figure 4-1). Growing all plants in the same water prevented skewing of the data due to variability in the nutrient composition of the water column.

A wooden frame 19.22 m^2 in area was placed over and positioned on the tank walls. Cross beams were used to create five rectangles each 3.7 m^2 in area. Shade cloth was used in differing numbers of layers to adjust experimental shade levels to 1%, 2.6%, 5.8%, 26.2% and 100% incident PAR at the surface below the treatment groups. The control group was not shaded and was exposed to full sunlight.

The experiment was conducted over three culture periods: August 22 to September 16, 2001, February 25 to April 13, 2002 and July 16 to August 31, 2002. Each of the three experiments was a random design with four light treatments with two replicates per species per treatment in Experiment 1 and three replicates per plant species per treatment in Experiments 2 and 3. Data are presented on a per container basis. Culture period length in each experiment was adequate for the development of treatment-related differences but minimized tissue deterioration associated with senescence.

Water temperature was measured using maximum/minimum thermometers placed 30 cm below the surface of the water. Readings were taken 5 days a week at approximately 3:00 P.M. each day. Water temperature was calculated as the mean of the maximum and minimum values from the thermometers for a recording period.

Two recently certified 2-pi quantum sensors attached to a LI-COR datalogger were used to measure instantaneous photosynthetic photon flux density. Multiple simultaneous measurements taken above and one centimeter below the shade cloth in each treatment group throughout the day from 0700 to 1700h on three separate days during the culture period were used to calculate the percent transmittance for each treatment. Sheets of Weed Block were used between the treatment groups in Experiments 2 and 3 to prevent light transfer among the groups while still permitting water flow through the tank.

A long-term incident PPFD mean was calculated using short-term incident means of total irradiance (400-1100 nm) (W m^{-2}) measured by a LICOR LI200SZ pyrometer located on-site and maintained by the University of Florida Florida Automated Weather Network (FAWN). Short-term incident means were based on data collected every 3.75 minutes and averaged and reported every 15 minutes. Total irradiance was converted to photosynthetically active radiation to (PAR) (400-700 nm) assuming that PAR is approximately 45% of total irradiance (Baker and Froiun 1987). PAR in W m^{-2} was converted to photosynthetic photon flux density (PPFD) units (μ mol photons s^{-1} m^{-2}) using a multiplier of 4.6 (see Table 3 in Thimijan and Heins 1983). The long-term mean incident PPFD for the photic portion of an experimental period was found by averaging the nonzero fluxes. Accumulated PAR was found by multiplying the

mean incident PPFD by the length of the photic period for the experiment. The long-term incident PPFD mean was multiplied by the mean percent transmittance discussed above to obtain mean estimates of the incident PPFD at the air-water interface in each treatment.

Pot level incident PPFD in each treatment was estimated using Equation 4.1.

$I_z = I_o \cdot (\%\ \text{light attenuation by shade cloth}) \cdot e^{-kz}$ Equation 4.1

where:
I_z = incident irradiance above each treatment group,
I_o =irradiance at depth, z,
k = light extinction coefficient,
z = depth, in this case, 60 cm.

The light extinction coefficient was calculated from simultaneous measurements of incident irradiance and irradiance at a given depth (depth varied from to 32 to 47 cm). The light extinction coefficient was calculated using Equation 4.2.

Equation 4.2

$$\frac{\ln I_o - \ln I_z}{z} = k$$

Light measurements were taken on several sampling dates throughout the course of the entire study: 27 April 2002, 2 and 3 August 2003 and 7 October 2003 in order to ensure representative sampling of seasonal variation in the value of the coefficient. Actual measurements of the pot-level irradiance were taken on several sample dates in order to field-test the accuracy of the calculated values. Irradiance values reported in this study were not corrected for reflectance.

At the end of each study period, plants were harvested and separated into above- and below-sediment biomass. The above-sediment portions of three individual

representative plants of each species were separated and placed into individual Ziplock bags. The bags were placed into a dark cooler and processed within six hours of collection. The mechanical separation technique described by Zimba and Hopson (1997) was used to separate epiphytic biomass from macrophyte biomass. A subsample of the resultant suspension was concentrated onto a glass fiber filter (0.7-μm porosity) and chlorophyll *a* and phaeophytin *a* were determined in accordance with Standard Methods (SM 10200 H) (A.P.H.A. 1995). All epiphyte data collected were normalized to host plant dry weight. Chlorophyll *a* values corrected for phaeophytin *a* were used as a correction factor to determine total macrophyte yield. Total above- and below-sediment biomass was then dried to constant weight (Hakanson 1981) at 60°C in a forced-air drying oven and dry weights were measured.

Macrophyte success was defined in terms of total (above and below-sediment) biomass accumulation. Growth in each container was determined as the difference in the g DWT from the start to the end of each experiment. The initial g DWT was estimated from the weights of at least ten representative plants collected and processed at the time that the light variable was introduced.

The absence of Weed Block curtains between the treatment groups in Experiment 1 allowed transfer of light through the treatment groups. Field observations of the diurnal change in the angle of the sun were used in conjunction with the percent shade measured for each treatment group to estimate the total amount of light received in each treatment group during the study period. These estimates were based on the assumptions that

1) light was passing through the treatments in an east to west direction from 6 to 11 AM, in a west to east direction from 1 to 7 PM and that light contamination between treatments was negligible between the hours of 11AM to 1 PM, 2) light contamination between the groups was only significant at the pot and mid-water levels.

A Weed Block curtain was installed between each treatment group to prevent light transfer between treatments in subsequent experiments. Weed Block was chosen in order to allow water flow through the treatment groups. This modified design was used for Mature Plant Light Requirements Experiments 2 and 3.

Data Analysis

All macrophyte biomass data were statistically analyzed using GLM procedures (SAS Institute, Inc. 1999-2001). Significant means ($p \leq 0.05$) were separated using Tukey's HSD Test. Best-fit curves based on Michaelis-Menten kinetics were used to investigate the the relationship between photosynthetic photon flux density and macrophyte biomass. The photosynthetic photon flux density for no net growth of each of the submersed macrophyte species was calculated as the x – intercept. Data were transformed to reduce the variance as necessary (Ricker 1973). Data points greater than two standard deviations away from the mean were considered outliers and were excluded from further analysis.

Results

Temperature and Irradiance

Average daily water temperature during the study period was relatively constant with highest temperatures occurring during Experiment 1 (29.8 °C) while lowest temperatures were measured during Experiment 2 (23.2 °C) (see Table 4-1).

The mean photic instantaneous photosynthetic photon flux density (PPFD) was greatest during Experiment 2 (831 μmol s^{-1} m^{-2}) and least during Experiment 1 (696 μmol s^{-1} m^{-2}). Photoperiod was shortest during Experiment 2 (12.4 hr) (Table 4-1). Partly cloudy days are the norm in South Florida. Relatively lower average PAR values in summer as compared to spring are probably due in part to afternoon thunderstorms that result in 100% cloud cover during parts of most summer afternoons.

Effects of Light Availability on Macrophyte Production

Experiment 1 (8/22/01 to 9/16/01)

Mean incident PPFD measured at the air-water interface of the treatment groups ranged from 696 μmol s^{-1} m^{-2} or 100% of incident PAR with no shade in the control group to 5.1 μmol s^{-1} m^{-2} or 0.7 % incident PAR in the 99% shade group. Mean incident PPFD at the pot level (z = 60 cm) was approximately 30% of the incident PAR available at the air-water interface ranging from 174 μmol s^{-1} m^{-2} in the control group to 1.5 μmol s^{-1} m^{-2} in the 99% shade group (Table 4-2).

Comparison of the final dry weights to the mean dry weight measured for the samples collected at the time of introduction of the light exclusion device to establish an initial biomass indicated a problem with the values estimated for *Chara* sp.. Accordingly, net growth values determined for *Chara* sp. were not included in subsequent analyses.

There were significant treatment effects among and within the macrophyte species. *N. guadalupensis* produced significantly more biomass than the other species in the 73.8% shade group (Table 4-3). *V. americana* and *P. illinoensis* growth was

statistically greater than that of *N. guadalupensis* in the 99% shade group. There were no differences among the species in the other treatment groups. Comparisons within species indicated that *N. guadalupensis* produced significantly lower biomass at shade levels ≥ 94.2%. *P. illinoensis* growth was greatest with 73.8% shade and least in the 99% shade treatment. There were no significant treatment effects on *V. americana* biomass production in this experiment.

Comparison of the estimated irradiance at which there was no net growth indicated species-specific differences in the amount of PAR required for macrophyte growth. Macrophyte biomass production increased with increasing light availability up to 26.2% incident PAR. There was a slight decrease in the growth of *N. guadalupensis* and *P. illinoensis* at light levels > 26.2% incident PAR Growth rates of *V. americana* appeared to saturate and level out at light levels ≥ 26.2% incident PAR (Figure 4-4). The apparent photosynthetic photon flux density for no net *N. guadalupensis* growth was estimated to be 34.8 μmol s^{-1} m^{-2} at a depth of 1 cm below the water surface or 5% incident PAR (Fig. 4-2). The apparent photosynthetic photon flux density for no net *P. illinoensis* growth was estimated to be 27.8 μmol s^{-1} m^{-2} at a depth of 1 cm below the water surface or 4% incident PAR (Figure 4-3). The estimated photosynthetic photon flux density for no net *V. americana* growth was estimated to be 13.9 μmol s^{-1} m^{-2} at a depth of 1 cm below the water surface or 2% incident PAR (Figure 4-4).

Experiment 2: 2/25/01 to 4/13/01

PAR measured at the air-water interface of the treatment groups ranged from 831 μmol s^{-1} m^{-2} or 100% of incident PAR with no shade in the control group to 6.0

μmol s^{-1} m^{-2} or 0.73 % incident PAR in the 99% shade group (Table 4-4). PAR available at the pot level (z = 60 cm) was approximately 30% of the PAR available at the air-water interface ranging from 243 μmol s^{-1} m^{-2} in the control group to 1.7 μmol s^{-1} m^{-2} in the 99% shade group (Table 4-4).

Comparison of the final dry weights to the mean dry weight measured for the samples collected at the time of introduction of the light exclusion device to establish an initial biomass indicated a problem with the values estimated for *V. americana*. Accordingly, net growth values determined for *V. americana* were not included in subsequent analyses.

Analysis of variance using GLM procedures (SAS Institute 1999-2001) indicated that treatment and plant species had a highly significant effect on macrophyte yield in this experiment ($P < .0001$).

There were significant treatment effects among and within the macrophyte species. *Chara sp.* produced significantly more biomass than the other species in the 5.8, 2.6 and 0.7% shade groups (Table 4-5). *N. guadalupensis* exhibited the lowest relative growth in the 26.2, 2.6 and 0.7% light groups. There were no differences among the species in the control group. Comparisons within species indicated that *N. guadalupensis* produced significantly lower biomass at light levels ≥ 100%. *P. illinoensis* growth decreased significantly at light levels 5.8% full sun and below. *Chara* sp. biomass production declined significantly when available light at the water surface was 26.2% full sun and below.

Comparison of the estimated irradiance at which there was no net growth indicated species-specific differences in the amount of PAR required for macrophyte

growth. Biomass production of *N. guadalupensis* and *V. americana* increased linearly with increasing light availability (Figures 4-5 and 4-7, respectively). *P. illinoensis* net growth appeared to depend on light in accordance with the Michaelis-Menten relation (Figure 4-6) with growth leveling out at irradiances > 26.2% incident PAR. The apparent photosynthetic photon flux density for no net *N. guadalupensis* growth was estimated to be 416 μmol s^{-1} m^{-2} at a depth of 1 cm below the water surface or 50.3% incident PAR (Figure 4-5). The estimated PPFD for no net growth of both *P. illinoensis* and *Chara sp* was 191 μmol s^{-1} m^{-2} at a depth of 1 cm below the water surface or 23% incident PAR (Figures 4-6 and 4-7).

Analysis of the root to shoot ratios for the study species using GLM Procedures (SAS Institute 1999-2001) indicated significant differences within and among plant types in response to decreasing light availability. The ratio of root biomass to shoot biomass was significantly greater in *P. illinoensis* and *V. americana* in all treatment groups (α=0.05). The trend to produce more root biomass in response to decreasing light availability was significantly greater in *P. illinoensis* and *V. americana* (Table 4-6).

Experiment 3: 7/16/02 to 8/31/02

PAR measured at the air-water interface of the treatment groups ranged from 821 μmol s^{-1} m^{-2} or 100% of incident PAR with no shade in the control group to 6 μmol s^{-1} m^{-2} or 0.7 % incident PAR in the 99% shade group (Table 4-2). PAR available at the pot level (z = 60 cm) was approximately 30% of the PAR available at the air-water interface ranging from 240 μmol s^{-1} m^{-2} in the control group to 1.8 μmol s^{-1} m^{-2} in the 99% shade group (Table 4-7).

Analysis of variance using GLM procedures (SAS Institute 1999-2001) indicated that treatment and plant species had a highly significant effect on macrophyte yield in this experiment ($P < .0001$, $P < 0.0007$ respectively). A significant interaction between these two factors was also observed ($P < .0001$).

There were significant treatment effects among and within the macrophyte species. *N. guadalupensis* and *P. illinoensis* produced significantly more biomass than the other species in the control and 26.2% light treatment groups. (Table 4-8). *V. americana* and *Chara sp.* exhibited the lowest greatest growth in the 1% light group while *N. guadalupensis* produced the least biomass in this treatment. There were no differences among the species in the 5.8 and 2.6% groups. Comparisons within species indicated that *N. guadalupensis* produced significantly lower biomass at light levels ≤ 5.8% (Table 4-8). *P. illinoensis* growth decreased significantly at light levels below 26.2% and again at levels 5.8% full sun and less. *Chara sp.* biomass production declined significantly when available light at the water surface was 5.8% full sun and below. There was a slightly significant decline in *V. americana* growth at light levels below 26.2% and a significant decline at light levels below 2.6% full sun. The results from treatment C for *V. americana* did not fit the trend observed in the other four treatment groups and were excluded from statistical analysis.

Comparison of the estimated irradiance at which there was no net growth indicated species-specific differences in the amount of PAR required for macrophyte growth. *N. guadalupensis* biomass production increased with increasing light availability (Figure 4-8). Growth rates of *P. illinoensis* , *V. americana* and Chara sp. appeared to saturate and level out at light levels ≥ 26.2% incident PAR (Figures 4-9

to 4.11). The apparent PPFD for no net *N. guadalupensis* growth was estimated to be 57.5 μmol s^{-1} m^{-2} at the water surface or 7% incident PAR (Figure 4-8). The estimated PPFD for no net *P. illinoensis* growth was 82.1 μmol s^{-1} m^{-2} at the water surface or 10% incident PAR (Figure 4-9). The apparent PPFD for no net *V. americana* growth was estimated to be 49.3 μmol s^{-1} m^{-2} at the water surface or 6% incident PAR (Figure 4-10). The estimated PPFD for no net *Chara sp.* growth was 98.5 μmol s^{-1} m^{-2} at the water surface or 12% incident PAR (Figure 4-11).

There were also significant differences in the root:shoot ratios within and between macrophyte species. Statistical analyses of the data using GLM Procedures (SAS Institute 1999-2001) indicated species-specific responses in root to shoot ratios due to treatment group (Table 4-9). *P. illinoensis* and *V. americana* root biomass increased with decreasing light availability. Comparisons between plant types indicated that *P. illinoensis* and *V. americana* produced greater root to shoot biomass at stations A, B and C as compared with the other species. *P. illinoensis* root to shoot ratios were greater than those of the other macrophytes in treatments D and E.

Effects of Light Availability on Macrophyte Chlorophyll *a*

The data indicate that there were differences in the amount of chlorophyll *a* produced by the study plants among and within the study species. Analysis of variance using GLM procedures (SAS Institute 1999-2001) indicated that plant species had a highly significant effect on macrophyte chlorophyll *a* (mg/g macrophyte dry weight) in all three experiments. Significant differences due to shade level were observed in Experiments 1 and 2.

Among-species comparisons of the effect of decreasing light availability on the amount of macrophyte chlorophyll *a* measured per g macrophyte DWT indicated that *V. americana* frequently produced significantly greater chlorophyll *a* than the other study species. This trend was observed in all three experiments (Figure 4-12).

Differences in macrophyte chlorophyll *a* were also observed among the three experiments. *N. guadalupensis* and *V. americana* exhibited considerably greater chlorophyll a contents in Experiment 1 as compared with the other experiments. *P. illinoensis* chlorophyll *a* content was also greater during Experiment 1 while greatest values for *Chara sp.* were measured during Experiment 3.

Discussion

Light is recognized as one of, if not the most, important factors affecting the growth of submersed aquatic macrophytes. This relationship is particularly well-defined in highly turbid, eutrophic lakes (van Dijk et al. 1992, Lauridsen et al.1994, Strand 1999). Light availability drives a variety of population dynamics within natural plant communities including species composition, distribution and maximum depth of colonization. However, despite the fact that the importance of light to SAV growth is so well-accepted, there remains considerable uncertainty as to the specific light requirements of submersed macrophytes. Studies conducted to date vary widely and few have distinguished between the requirements for plant survival and reproduction (Kimber et al. 1995). Dennison et al. (1993) observed that, at present, there is no consensus concerning the light environment required for the growth and survival of *V. americana* in shallow water bodies. A review of the literature indicates that minimum light requirements vary from species to species and are affected by the

length of the growing season (Table 4-10). In his investigation of 8 species of seagrasses, Dennison (1987) observed light compensation points that varied from 9 to 26 μmol m^{-2} s^{-1}. Goldsborough and Kemp (1988) concluded from their treatment and "recovery" studies that 11% ambient irradiance was required for the survival of *Potamogeton perfoliatus*. Sand-Jensen and Madsen (1991) observed among-species differences in their comparative study of the light requirements of charophytes, bryophytes and angiosperms. Van et al. (1976) used photosynthetic rate measurement studies to calculate the light compensation points of two native and two exotic species of submersed macrophytes: *Hydrilla verticillata, Ceratophyllum demersum, Myriophyllum spicatum* and *Cabomba caroliniana*. They concluded that *H. verticillata* had the lowest LCP – 15 μmol m^{-2} s^{-1} followed by *C. demersum* and *M. spicatum* – both 35 μmol m^{-2} s^{-1}. *C. caroliniana* exhibited the greatest light requirement with an estimated LCP of 55 μmol m^{-2} s^{-1}. They also observed that *Hydrilla* had the lowest light requirement to achieve half-maximal photosynthetic rate. They inferred that the superior photosynthetic efficiency conferred by these adaptations probably explains the competitive advantage that *H. verticillata* has over many submersed macrophyte species. Carter et al. (1996) reported an 11-fold increase and a 38-fold increase in total biomass of *Vallisneria americana* in lighted cages in the Chesapeake Bay and the Potomac River. Plants exposed to higher light levels were more robust and fewer in number as compared to controls. In their 1998 study, Blanch et al. concluded that recruitment of above-sediment biomass in Vallisneria may be completely restricted when irradiance is less than 35 μmole m^{-2} s^{-1}. Canfield et al. (1985) estimated LCP's for hydrilla equivalent to less than 1% full sun at the

maximum depth of colonization in several study lakes. (See Table 2 in Canfield et al. 1985). Spence and Chrystal (1970) reported LCP's less than 1 $\mu E\ m^{-2}\ s^{-1}$ in their study of the light requirements of submersed macrophytes.

The results indicated that decreasing light had a significant effect on macrophyte growth in this study. The plants responded to decreasing light availability by reducing total, above and below-ground dry weight . There was a concommitant decrease in the number of *V. americana* and *P. illinoensis* plants per pot with increasing shade. (No data are available for *N. guadalupensis* and *Chara sp.* - see Materials and Methods.) In a laboratory study of the effects of turbidity on *V. americana*, Doyle and Smart (2001) observed similar decreases in total AFDM and plant number per pot. Grimshaw et al. (2002) also reported a decrease in the AFDM of *V. americana* with decreasing light. In this study, *N. guadalupensis*, *P. illinoensis* and *Chara* sp. exhibited elongation of stems, thinning of lower leaves and increased canopy formation in response to increasing shade. Goldsborough and Kemp (1988) observed similar shade responses in *P. perfoliatus*.

The amount of light required for the net growth of the submersed aquatic macrophytes in this study varied among species ranging from 13.9 to 415.5 $\mu mol\ m^{-2}\ s^{-1}$ or 2 to 50% incident irradiance (Table 4-11). There was no apparent relationship between light requirements of and *N. guadalupensis* and *Chara* sp. and their growth habit, however, higher *N. guadalupensis* light requirements may have been due to self-shading by the extensive canopy formation charateristic of this species

Vallisneria americana exhibited the lowest minimum light requirement for net growth of all of the study species. During Experiment 1, *V. americana* produced new

vegetative growth at less than 2% of surface incident light or 13.9 μmol photons m^{-2} s^{-} (Table 4-11). Many studies have suggested that *V. americana* is well-adapted to low light environments (cf. Titus and Adams 1979, Davis and Brinson 1980, Kimber et al. 1995, Grimshaw et al. 2002). Titus and Adams (1979) described *V. americana* as a shade-adapted summer specialist. Davis and Brinson (1980) concluded that *V. americana* is a turbidity-tolerant native species. Kimber et al. (1995) observed tuber production at light levels as low as 5% surface light. Grimshaw et al. (2002) determined that no net growth of *V. americana* occurred at 4.09% surface irradiance or 29 μmol photons m^{-2} s^{-1} (measured 27 cm above the sediment surface).

Macrophyte light requirements also varied on a seasonal basis (Table 4-11). Light contamination in Experiment 1 precluded the use of the results from Experiment 1 in a quantitative seasonal comparison accordingly, only the results from Experiments 2 and 3 were used. *N. guadalupensis*, *P. illinoensis and Chara* sp. exhibited a considerably greater minimum light requirement for growth in the winter/spring than in the summer. Other environmental factors such as greater total PPFD in summer as compared to winter/spring (1822 versus 1781 Mol photons m^{-2}) and differences in photoperiod, solar angle and water temperature probably contributed to this phenomenon. No data was available to make a seasonal comparison of the light requirements for *V. americana*.

Despite the characteristics *V. americana* possesses that make it a very attractive candidate for use in restoration projects, caution should be used when considering planting monocultures of this species. There is considerable implication in the literature that environmental factors other than light have a highly significant

effect on *V. americana* growth, hence possibly explaining the seemingly contradictory findings that *V. americana* often exhibits high light requirements (cf. Barko et al. 1982, Carter and Rybicki 1990, Doyle and Smart 2001). In addition, due in part to the concentration of biomass close to the sediment, Vallisneria is often out-competed by canopy-forming plants such as Hydrilla (Haller and Sutton 1975).

There was no apparent effect of decreased light availability on macrophyte chlorophyll *a* concentration. The sample size (n = 3) used in this study was not large enough to ensure representative sampling of shoot materials of differing ages. The high internal variation obscured any observable trends in macrophyte chlorophyll a production in response to increasing shade in this study. Goldsborough and Kemp (1988) observed significant increases in chlorophyll *a* content in response to decreasing light within 3 days of introduction of the light variable. Possible explanations for this contradiction include differences in the length of the experiment – 17 days in their experiment versus 25, 48 and 46 days respectively in Experiments 1, 2 and 3 of this study. *V. americana* exhibited significantly greater chlorophyll *a* content per g macrophyte dry weight than the other study species.

Decreasing PAR resulted in significant increases in root to shoot ratios within species in both of the experiments in which root:shoot ratios were calculated. *P. illinoensis* and *V. americana* exhibited significantly greater root:shoot ratios as compared with *N. guadalupensis* in all experiments. The typical life strategy of perennial species involves investment in organs such as roots that can allow the plant to survive periods of harsh environmental stress. Haller and Sutton (1975) discuss the implications of significantly greater energy allocation to below-ground biomass by *V.*

americana on its relative competitiveness with Hydrilla. Doyle and Smart (2001) measured higher above:below-ground AFDM at higher shade levels. Grimshaw et al. (2002) did not observe a significant effect of light on above to below-ground ratios. The explanation for these discrepancies in the effect of shading on the above:below ground biomass of *V. americana* is unknown at present.

Conclusions

There was a decrease in total biomass produced by *N. guadalupensis guadelupensis*, *Potamogeton illinoensis*, *Vallisneria americana* and *Chara* sp. in response to decreasing PAR. There was a decrease in above to below-ground biomass ratios for all species as available light decreased. The perennial species, *P. illinoensis* and *V. americana*, produced significantly greater below-ground biomass as compared to the annual, *N. guadalupensis*.

V. americana appeared to be the most well-adapted study species for survival in low light environments. The combination of low light compensation point, high concentration of chlorophyll *a* and extensive root structure possibly confer an advantage to *V. americana* in shallow turbid systems. However, additional study is required in order to clarify some of the contradictory findings regarding the light requirements of *V. americana* before managers should rely on monocultures of *V. americana*.

In summary, establishment of viable SAV communities composed of a combination of several species exhibiting diverse life strategies is the most advisable goal for revegetation projects especially in shallow eutrophic systems. The findings of this study suggest that the optimum time to plant *N. guadalupensis*, *P. illinoensis* and

V. americana is during the summer when the light requirements for these species is minimal. The data suggest that minimum PPFD levels approximately 12% ambient light would be required to allow for the summertime growth of diverse macrophyte communities consisting of mature plants of all of the study species. The results of this study indicated that summer appeared to be the optimum planting time for the study species. Additional research is needed in order to quantify the amount of light required by submersed macrophytes in all stages of their life cycles. A better understanding of the role of light in inter-specific competition is also required.

Table 4-1: Temperature and irradiance during the three culture periods. Temperature values are average daily temperatures followed by the standard deviation. Values shown in parentheses are the lowest and highest measured temperatures for each culture period.

Culture Period	Water Temperature (°C)	Mean Instantaneous PAR[a] ($\mu mol\ m^{-2}\ s^{-1}$)	Photoperiod
Experiment 1 8/22-9/16/01	29.8 ± 1.3 (27 to 32)	696 (2 - 2130)	(12.8L:11.2D)
Experiment 2 2/25 –4/13/02	23.2 ± 1.4 (18 to 31)	831 (2 - 2343)	(12.4L:11.6D)
Experiment 3 7/16 – 8/31/02	29.5 ± 0.5 (27.5 to 32.0)	821 (2 – 2327)	(13.4L:10.6D)

[a]Photosynthetically active radiation. Values shown are means of the daily average PAR measured over the course of each respective culture period.

Table 4-2: Experiment 1: Estimated average instantaneous, daily and total photosynthetic photon flux density (PPFD) received over the period 22 August to 16 September 2001 (25 days) assuming 12.8-hour daylength. Values shown have been corrected for light contamination between treatment groups. See materials and methods section for a description of the procedure used to estimate the correction factor.

Treatment	Light level (% light)		Total PPFD For CP[a] Mol*m^{-2}		Daily PPFD mol*d^{-1}*m^{-2}		Instantaneous PPFD umol*m^{-2}* s^{-1}	
	Surface	Pot[b]	Surface	Pot[b]	Surface	Pot[b]	Surface	Pot[b]
A	100.0	25.0	801.8	200.8	32.1	8.0	696.0	174.3
B	26.2	11.5	210.4	92.71	8.4	3.7	182.6	80.0
C	5.8	2.9	46.3	23.2	1.9	0.9	40.2	20.1
D	2.6	0.9	20.78	7.0	0.8	0.3	18.0	6.1
E	0.7	0.3	5.9	2.7	0.2	0.1	5.1	2.4

[a]Culture period.

[b]Pot level estimates are not corrected for self-shading by the above-sediment portions of the individual macrophytes. Quantification of these values is beyond the scope of this research.
The degree of self-shading should vary with species differences in leaf architecture and growth strategy.

Table 4-3: Results of analysis of net growth (g dry weight per pot per culture period) of above-ground macrophyte biomass produced in Experiment 1 using GLM procedures (SAS Institute 1999-2001) Values are the averages calculated for two culture containers per macrophyte species per treatment group followed by the standard error. Averages within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

	Macrophyte Species			
Treatment[a]	Najas	Potamogeton	Vallisneria	Chara
A	22.3 ± 12.1 ab	7.0 ± 1.7 ab	7.0 ± 3.0 a	-3.8 ± 0.5
B	38.5 ± 6.9 a	13.5 ± 1.8 a	4.7 ± 2.7 a	1.0 ± 4.3
C	-1.6 ± 3.0 b	1.6 ± 2.3 bc	0.3 ± 0.03 a	-8.4 ± 1.3
D	-13.0 ± 3.6 b	-1.4 ± 0.3 bc	-0.7 ± 1 a	-2.4 ± 0.9
E	-15.0 ± 1.7 b	-1.9 ± 0.4 c	0.8 ± 1.5a	-7.3 ± 0.2

[a]Treatments are the same as indicated in Table 4.2.

Table 4-4: Experiment 2: Estimated average instantaneous, daily and total PPFD received over the period 25 February to 13 April 2002 (48 days). Assumes 12.4-hour daylength.

Treatment	Light level (%|light)		Total PPFD For CP $Mol*m^{-2}$		Daily PPFD $mol*m^{-2}*d^{-1}$		Instantaneous PPFD $umol*m^{-2}*s^{-1}$	
	Surface	Pot[a]	Surface	Pot[a]	Surface	Pot[a]	Surface	Pot[a]
A	100.0	29.2	1780.6	520.3	37.1	10.8	831.0	242.8
B	26.2	7.7	467.2	136.6	9.7	2.9	218.1	63.7
C	5.8	1.7	102.7	30.1	2.1	0.6	47.9	14.0
D	2.6	0.8	46.1	13.5	1.0	0.3	21.5	6.3
E	0.7	0.2	13.0	3.7	0.3	0.1	6.1	1.7

[a]Pot level estimates are not corrected for self-shading by the above-sediment portions of the individual macrophytes. Quantification of these values is beyond the scope of this research. The degree of self-shading should vary with species differences in leaf architecture and growth strategy.

Table 4-5: Results of analysis of net growth (g dry weight per pot per culture) of total macrophyte biomass (above plus below ground biomass) in Experiment 2 using GLM procedures (SAS Institute 1999-2000) Values are the means calculated for three culture containers per macrophyte species per treatment group followed by the standard error. Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

	Macrophyte Species			
Treatment[a]	*N. guadalupensis*	*P. illinoensis*	*V. americana*	*Chara* sp.
A	60.6 ± 16.5 a	28.4 ± 13.5 a	-13.8 ± 0.9 NA	78.5 ± 15.3 a
B	-35.5 ± 2.6 b	3.5 ± 2.3 a	-16.4 ± 1.5 NA	3.7 ± 10.9 b
C	-54.0 ± 1.0 b	-47.0 ± 1.5 b	.9 ± 0.9 NA	-6.9 ± 0.8 b
D	-50.2 ± 3.2 b	-43.5 ± 1.7 b	-4.5 ± 0.3 NA	-15.1 ± 4.8 b
E	-54.3 ± 0.6 b	-44.7 ± 0.6 b	-1.8 ± 0.1 NA	-20.9 ± 0.1 b

[a]Treatments are the same as indicated in Table 4.4.

Table 4-6: Root:shoot ratios for Experiment 2. Values are the means calculated for three culture containers per macrophyte species per treatment group followed by the standard deviation. All data were log(y+1) transformed prior to analysis by GLM procedures (SAS Institute 1999-2001). The nontransformed values are presented here. Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

	Macrophyte Species		
Treatment[a]	*N. guadalupensis*	*P. illinoensis*	*V. americana*
A	0.03 ± 0.01 b	0.12 ± 0.01 c	0.17 ± 0.05 c
B	0.04 ± 0.00 b	0.11 ± 0.01 c	0.28 ± 0.16 bc
C	0.17 ± 0.01 ab	0.90 ± 0.18 b	0.51 ± 0.41 bc
D	0.27 ± 0.19 a	1.61 ± 0.59 ab	0.76±0.18 ab
E	0.48 ±0.24 a	2.93 ± 1.87 a	1.90 ± 0.62 a

[a]Treatments are the same as indicated in Table 4.4.

Table 4-7: Experiment 3: Estimated average instantaneous, daily and total PPFD received over the culture period (CP) 16 July to 31 August 2002 (46 days). Assumes 13.4-hour daylength.

Treatment	Light level (% light)		Total PPFD for CP $mol*m^{-2}$		Daily PPFD $mol*d^{-1}*m^{-2}$		Instantaneous PPFD $umol\ s^{-1}*m^{-2}*$	
	Surface	Pot[a]	Surface	Pot[a]	Surface	Pot[a]	Surface	Pot[a]
A	100.0	29.2	1821.8	532.3	39.61	11.6	821.0	239.9
B	26.2	7.7	478.1	140.0	10.39	3.0	215.4	63.0
C	5.8	1.7	105.1	30.7	2.29	0.7	47.4	13.8
D	2.6	0.8	47.2	13.8	1.03	0.3	21.3	6.2
E	0.7	0.2	13.3	3.9	0.3	0.1	6.0	1.8

[a]Pot level estimates are not corrected for self-shading by the above-sediment portions of the individual macrophytes. Quantification of these values is beyond the scope of this research.
The degree of self-shading should vary with species differences in leaf architecture and growth strategy.

Table 4-8: Results of analysis of net growth (g dry weight per pot per culture)of total macrophyte biomass (above plus below ground biomass) in Experiment 3 using GLM procedures (SAS Institute 1999-2000) Values are the means calculated for three culture containers per macrophyte species per treatment group followed by the standard error. Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

	Macrophyte Species			
Treatment[a]	Najas	Potamogeton	Vallisneria	Chara
A	173.1 ± 69.2 a	88.7 ± 10.2 a	10.8 ± 3.9 a	23.3 ± 9.3 a
B	71.2 ± 14.20 ab	6.7 ± 9.2 b	7.7 ± 2.6 ab	12.9 ± 12.5 a
C	-18.5 ± 19.4 b	-19.0 ± 0.5 c	10.3 ± 7.0 NA	-26.7 ± 0.3b
D	-17.0 ± 17.0 b	-31.7± 1.3 c	-1.2 ± 0.5 b	-26.1 ± 1.6 b
E	-58.0 ± 12.2 b	-34.8± 1.4 c	-4.0 ± 1.1 b	-27.3 ± 0.4 b

[a]Treatments are the same as indicated in Table 4.7.

Table 4-9: Root:shoot ratios for Experiment 3. Values are the means calculated for three culture containers per macrophyte species per treatment group followed by the standard deviation. Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

	Macrophyte Species		
Treatment Group	Najas	Potamogeton	Vallisneria
A	0.04 ± 0.01 a	0.05 ± 0.01 c	0.16 ± 0.03 c
B	0.03 ± 0.00 a	0.07 ± 0.02 bc	0.13 ± 0.04 bc
C	0.05 ± 0.02 a	0.19 ± 0.06 bc	0.11 ± 0.03 bc
D	0.04 ± 0.01 a	0.52 ± 0.17 ab	0.20 ± 0.04 ab
E	0.07 ± 0.04 a	0.72 ± 0.23 a	0.27 ± 0.03 a

Table 4-10: Selected field observations and experimental conclusions concerning the light requirements of several species of submersed macrophytes.

Observed an inter-specific variation in LCP's among four species of SAV ranging from 55 μmole photons m^{-2} s^{-1} to 15 μmole photons m^{-2} s^{-1}	VAN ET AL. 1976
Estimated that an average midday irradiance of at least 250 μmole photons m^{-2} s^{-1} would be necessary for seed production	AGAMI and AGAMI 1980
Estimated that the light level that determined the lower depth limit of plant colonization could be as much as 21% of surface light	CHAMBERS and KALFF 1985
Estimated that *Potamogeton perfoliatus* required > 11% of ambient irradiance for survival	GOLDSBOROUGH and KEMP 1988
Observed that an average of 100 umol*m^{-2}*s^{-1} was necessary at the sediment-water interface for submersed macrophytes to survive in the tidal Potomac River	CARTER and RYBICKI 1990
Estimated that 7% of surface light or 505 mol*m^{-2} per year was needed for rooted aquatic plants to grow.	SAND-JENSEN and MADSEN 1991
Concluded that the light requirements for submersed plant growth vary widely and few of these [studies] have distinguished between requirements for plant survival and plant reproduction.	KIMBER ET AL. 1995
Determined that the PPFD for no net growth of *Vallisneria americana*, measured approximately a quarter meter from the sediment surface, was 29 μmole photons m^{-2} s^{-1} or 4.09% surface irradiance.	GRIMSHAW ET AL. 2002
Estimated that the minimum PPFD for no net growth of *N. guadelupensis* was 34.8 μmole photons m^{-2} s^{-1} or 5% ambient light, for *P. illinoensis* was 27.8 μmole photons m^{-2} s^{-1} or 4% ambient light, for *V. americana* was 13.9 μmole photons m^{-2} s^{-1} or 2% ambient light and for *Chara* sp. was 377.7 μmole photons m^{-2} s^{-1} or 12% ambient light.	THIS STUDY

Table 4-11: A comparison of the seasonal variation in light levels at which there is zero net growth of the study species.

	Najas		Potamogeton		Vallisneria		Chara	
	LCP (%)	PPFD (μmol photons m^{-2} s^{-1})	LCP (%)	PPFD (μmol photons m^{-2} s^{-1})	LCP (%)	PPFD (μmol photons m^{-2} s^{-1})	LCP (%)	PPFD (μmol photons m^{-2} s^{-1})
Experiment 1 8/22 to 9/16/01	5.0	34.8	4.0	27.8	2.0	13.9	NA	NA
Experiment 2 2/25 to 4/13/02	50.0	415.5	23.0	191.1	NA	NA	23.0	191.1
Experiment 3 7/16 to 8/31/02	7.0	57.5	10.0	82.1	6.0	49.3	12.0	377.7

Mature Light Requirement Experiments 1, 2 and 3 and Propagule Plant Light Requirement Experiment 1

5 Treatment Groups

Triplicate samples of 4 plant types = 12 pots per group

Pots randomly arranged

3 culture periods

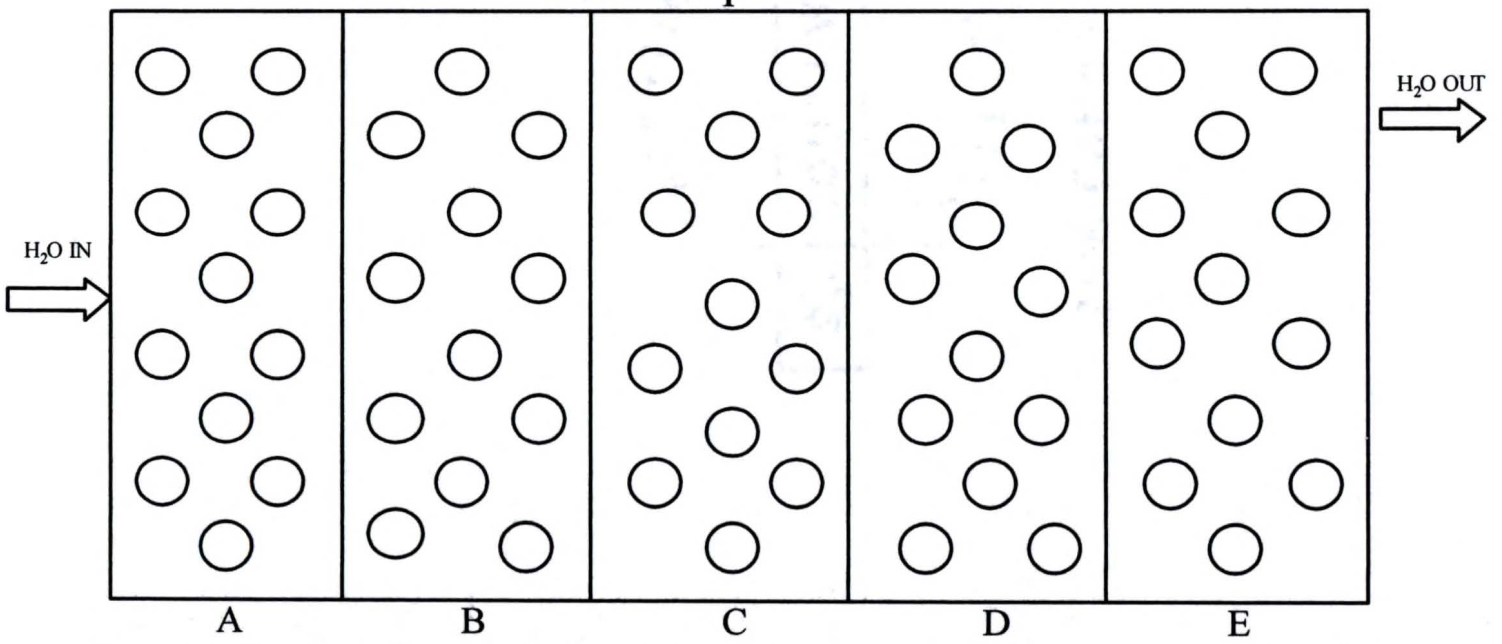


Figure 4-1: Experimental set-up for mature plant light requirement Experiments 1, 2 and 3 and propagule plant light requirement Experiment 1. Containers were arranged randomly within each treatment group. Note: In mature plant light requirement Experiment 1, only two containers per species were used in each treatment group (n=2).

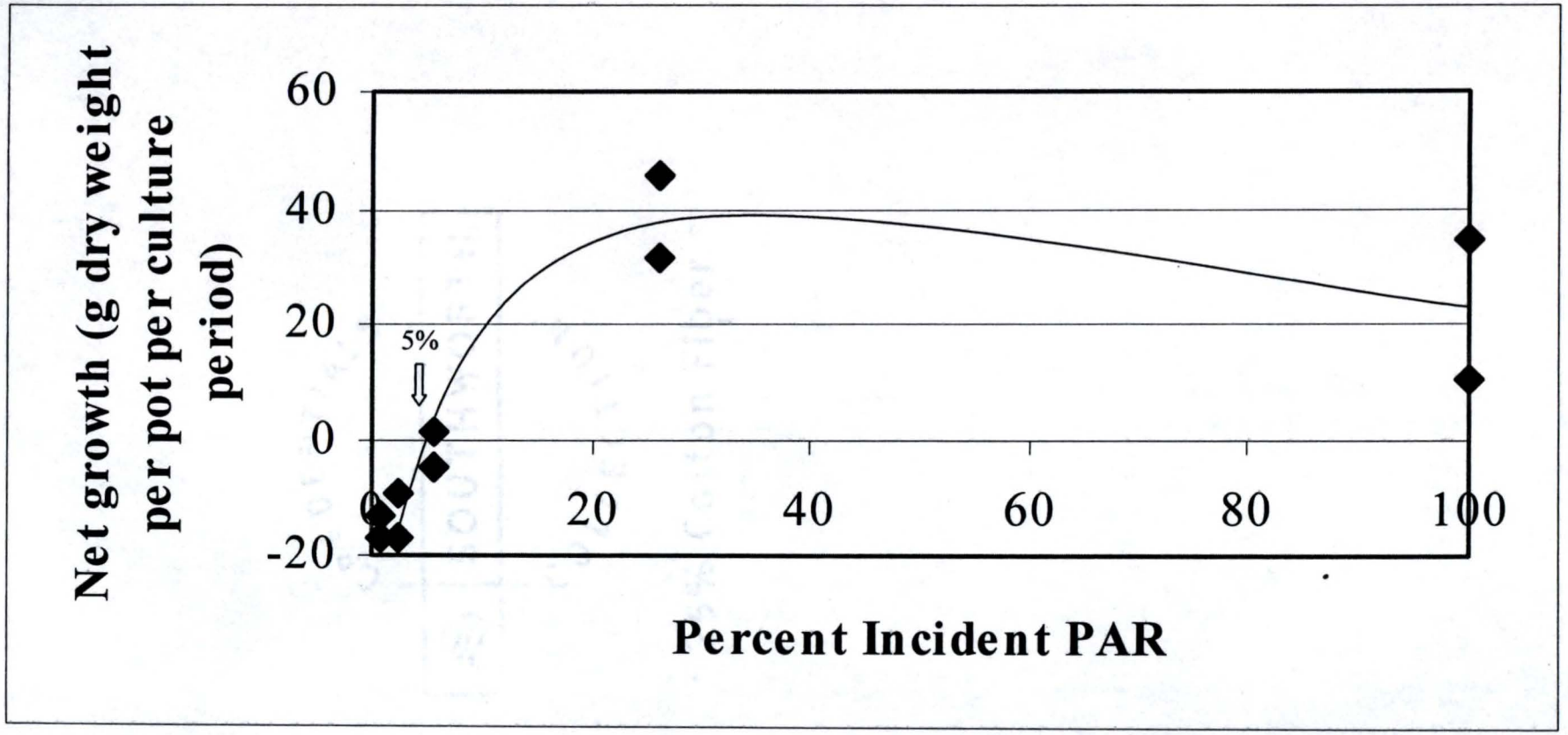


Figure 4-2: Experiment 1: Graphical interpretation of the net growth (g dry weight per pot per culture period) of *Najas guadelupensis* in response to the percent incident PAR at the water surface. A best-fit curve, based upon Michaelis-Menten kinetics, was used to estimate the x-intercept, indicated by the downward facing arrow. The x-intercept which represents that percent incident PAR at which there was no net growth.

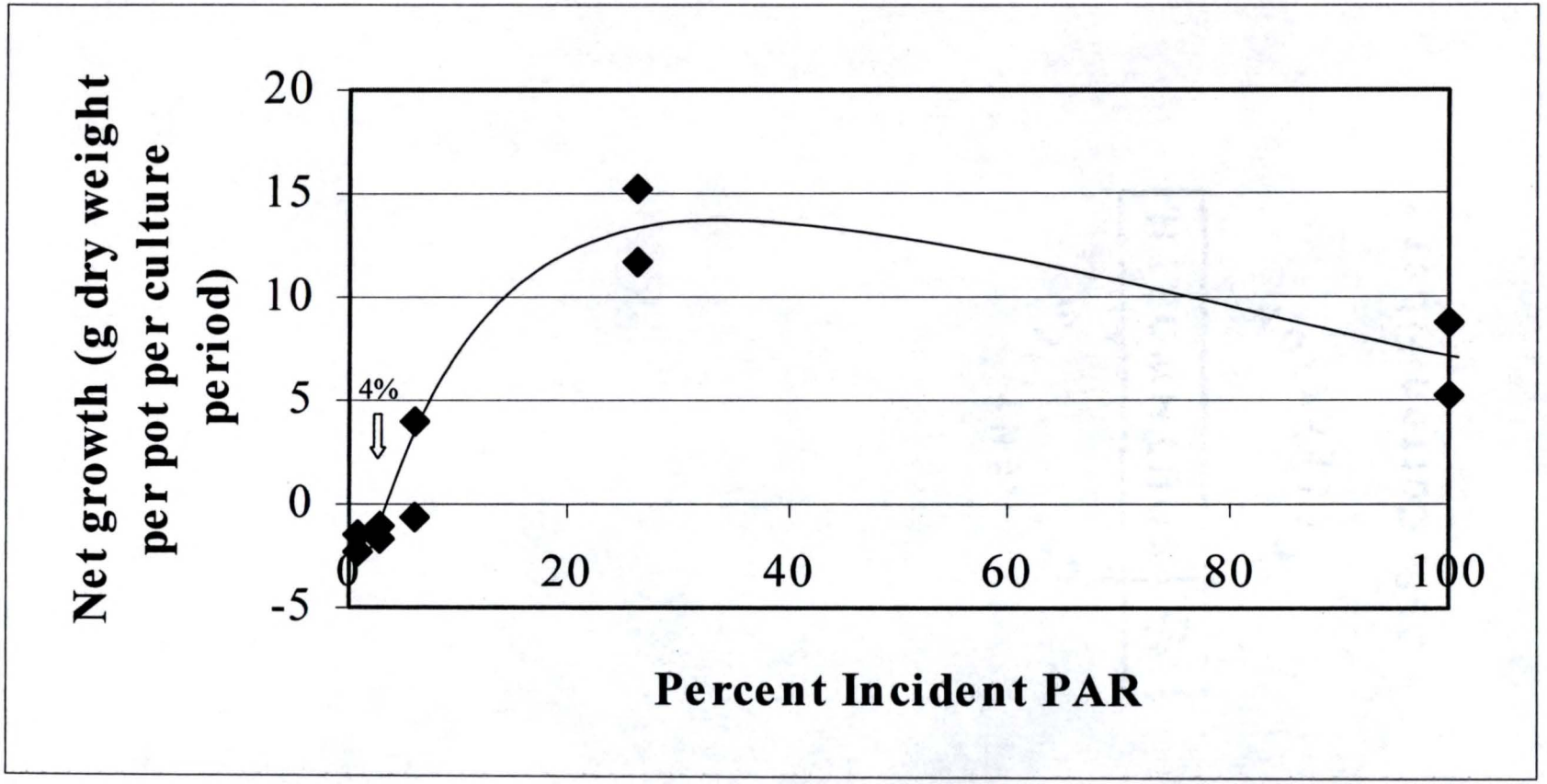


Figure 4-3: Experiment 1: Graphical interpretation of the net growth (g dry weight per pot per culture period) of *Potamogeton illinoensis* in response to the percent incident PAR at the water surface. A best-fit curve, based upon Michaelis-Menten kinetics, was used to estimate the x-intercept, indicated by the downward facing arrow The x-intercept represents that percent incident PAR at which there was no net growth.

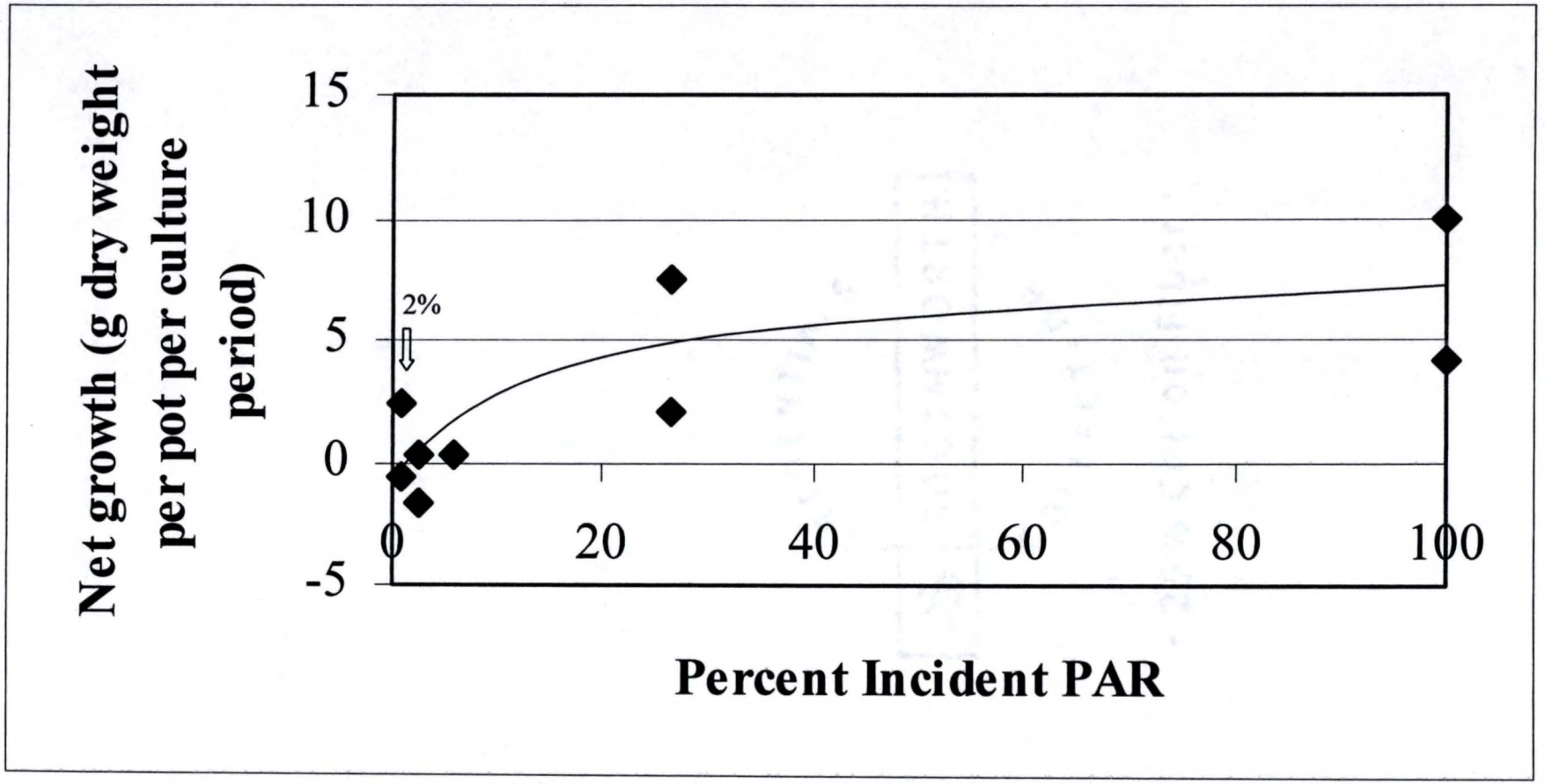


Figure 4-4: Experiment 1: Graphical interpretation of the net growth (g dry weight per pot per culture period) of *Vallisneria americana* in response to the percent incident PAR at the water surface. A best-fit curve, based upon Michaelis-Menten kinetics, was used to estimate the x-intercept, indicated by the downward facing arrow. The x-intercept represents that percent incident PAR at which there was no net growth.

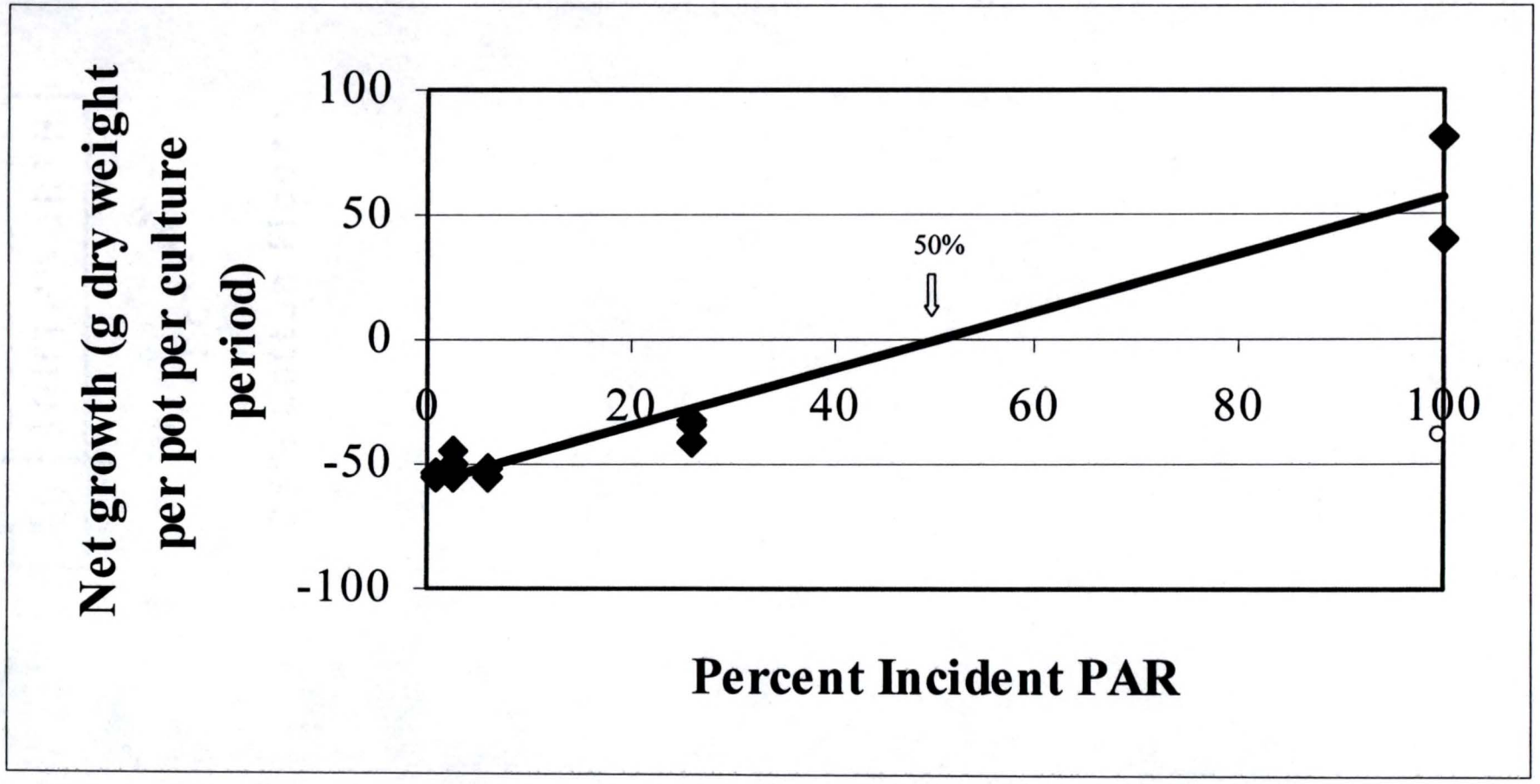


Figure 4-5: Experiment 2: Graphical interpretation of the net growth (g dry weight per pot per culture period) of *Najas guadelupensis* in response to the percent incident PAR at the water surface. A best-fit curve was used to estimate the x-intercept, indicated by the downward facing arrow The x-intercept represents that percent incident PAR at which there was no net growth. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

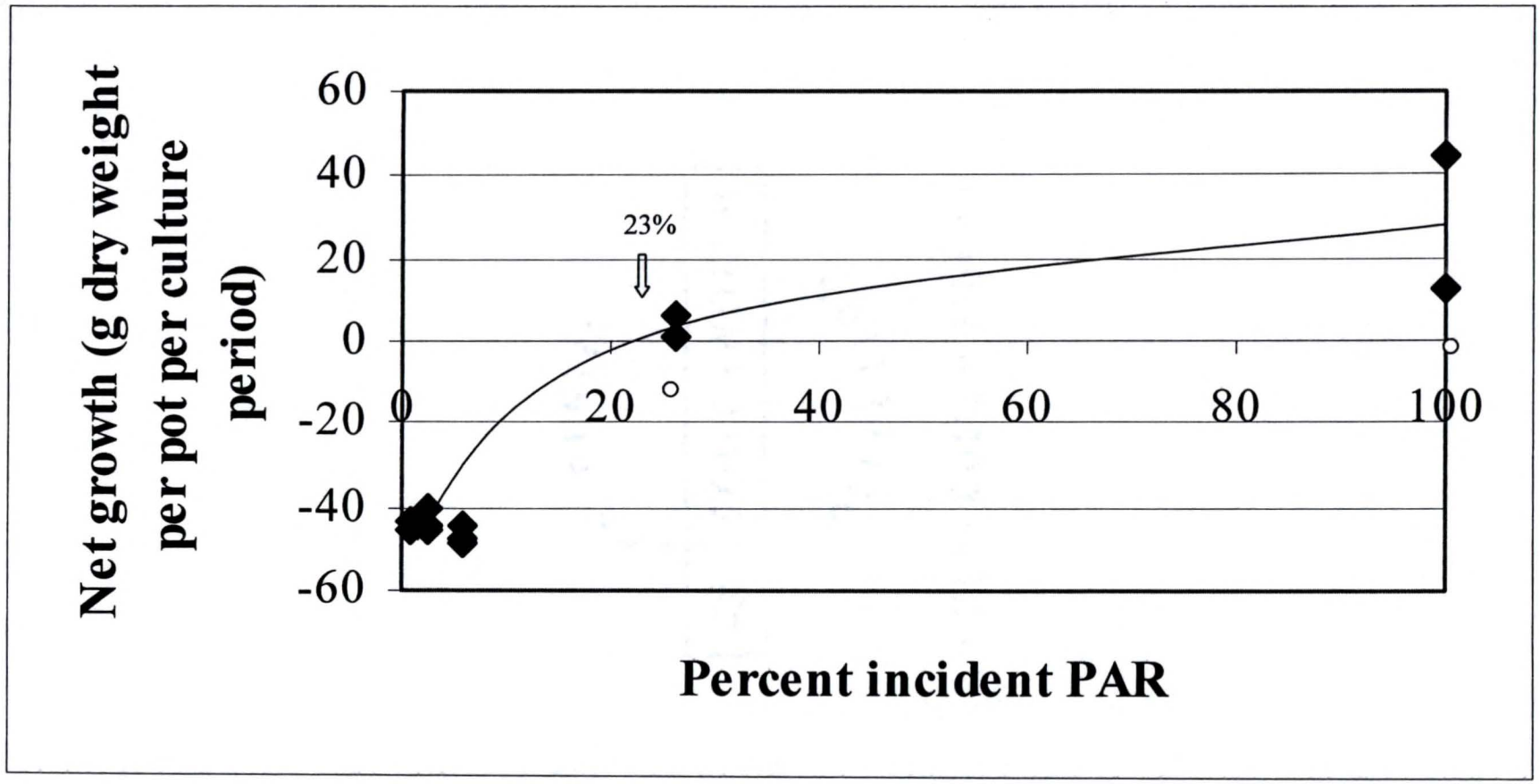


Figure 4-6: Experiment 2: Graphical interpretation of the net growth (g dry weight per pot per culture period) of *Potamogeton illinoensis* in response to the percent incident PAR at the water surface. A best-fit curve, based on Michaelis-Menten kinetics, was used to estimate the x-intercept, indicated by the downward facing arrow. The x-intercept represents that percent incident PAR at which there was no net growth. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

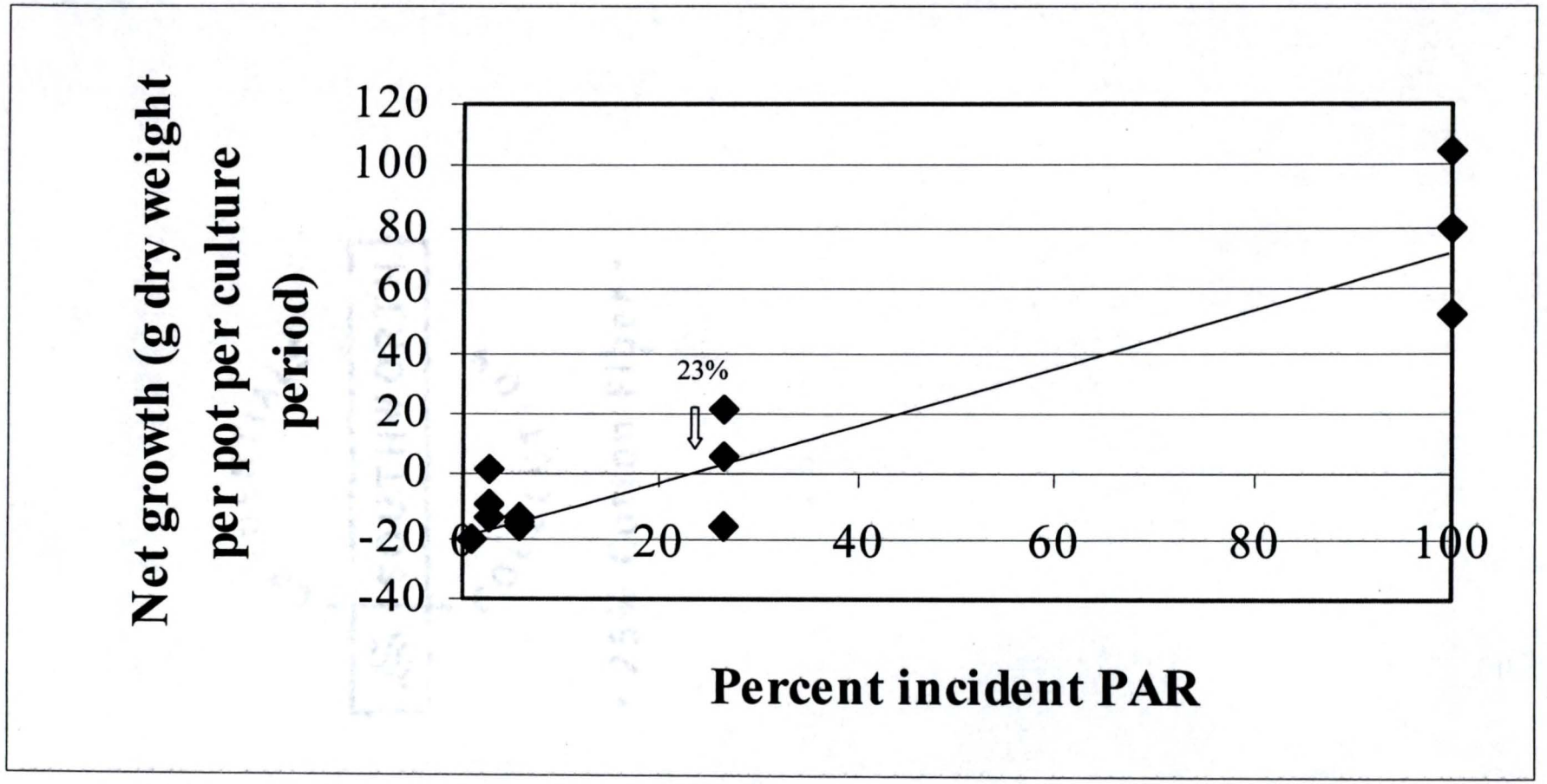


Figure 4-7: Experiment 2: Graphical interpretation of the net growth (g dry weight per pot per culture period) of *Chara* sp. in response to the percent incident PAR at the water surface. A best-fit curve was used to estimate the x-intercept, indicated by the downward facing arrow The x-intercept represents that percent incident PAR at which there was no net growth.

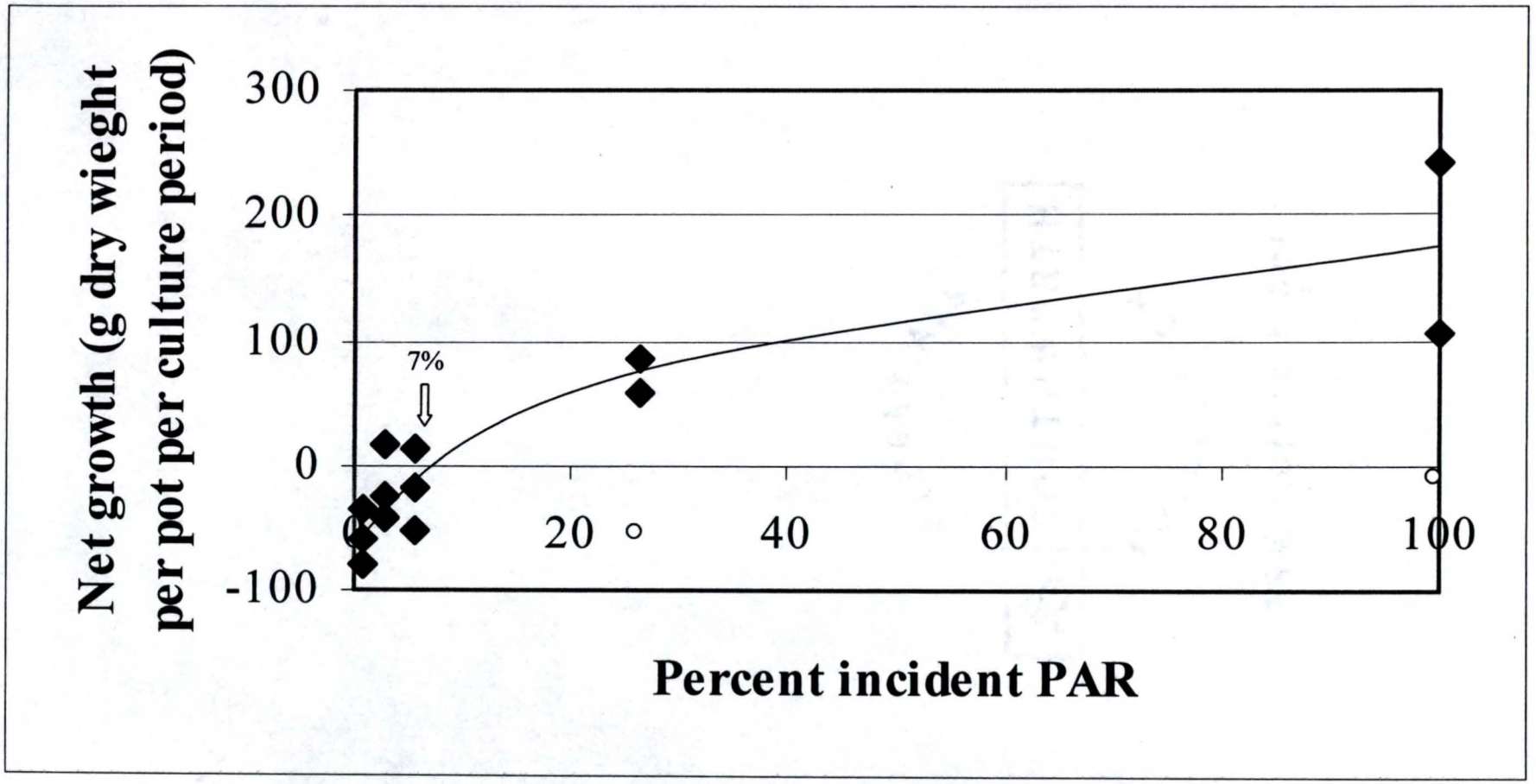


Figure 4-8: Experiment 3: Graphical interpretation of the net growth (g dry weight per pot per culture period) of *Najas guadelupensis* in response to the percent incident PAR at the water surface. A best-fit curve, based on Michaelis-Menten kinetics, was used to estimate the x-intercept, indicated by the downward facing arrow. The x-intercept represents that percent incident PAR at which there was no net growth. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

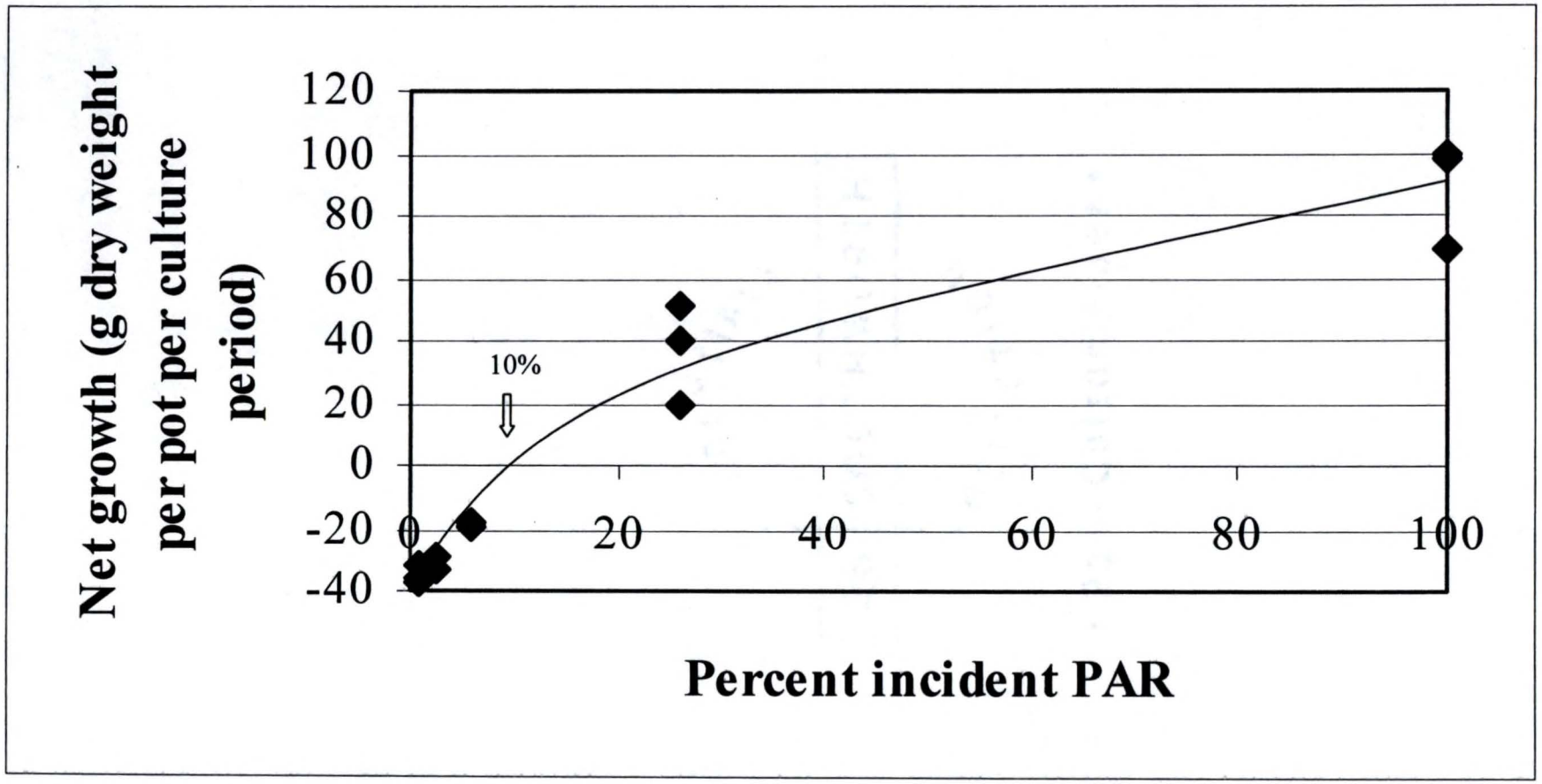


Figure 4-9: Experiment 3: Graphical interpretation of the net growth (g dry weight per pot per culture period) of *Potamogeton illinoensis* in response to the percent incident PAR at the water surface. A best-fit curve, based on Michaelis-Menten kinetics, was used to estimate the x-intercept, indicated by the downward facing arrow. The x-intercept represents that percent incident PAR at which there was no net growth.

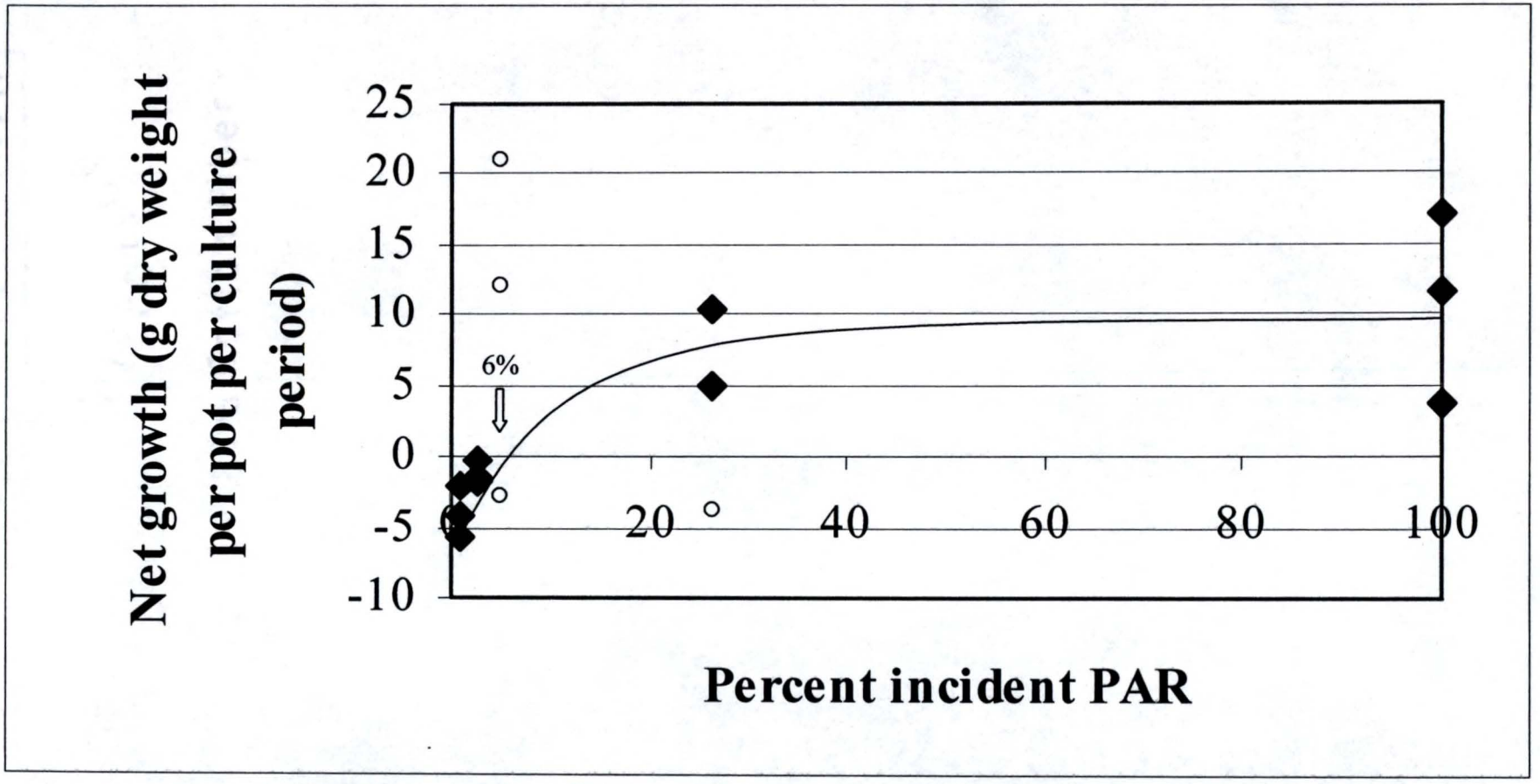


Figure 4-10: Experiment 3: Graphical interpretation of the net growth (g dry weight per pot per culture period) of *Vallisneria americana* in response to the percent incident PAR at the water surface. A best-fit curve, based on Michaelis-Menten kinetics, was used to estimate the x-intercept, indicated by the downward facing arrow. The x-intercept represents that percent incident PAR at which there was no net growth. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

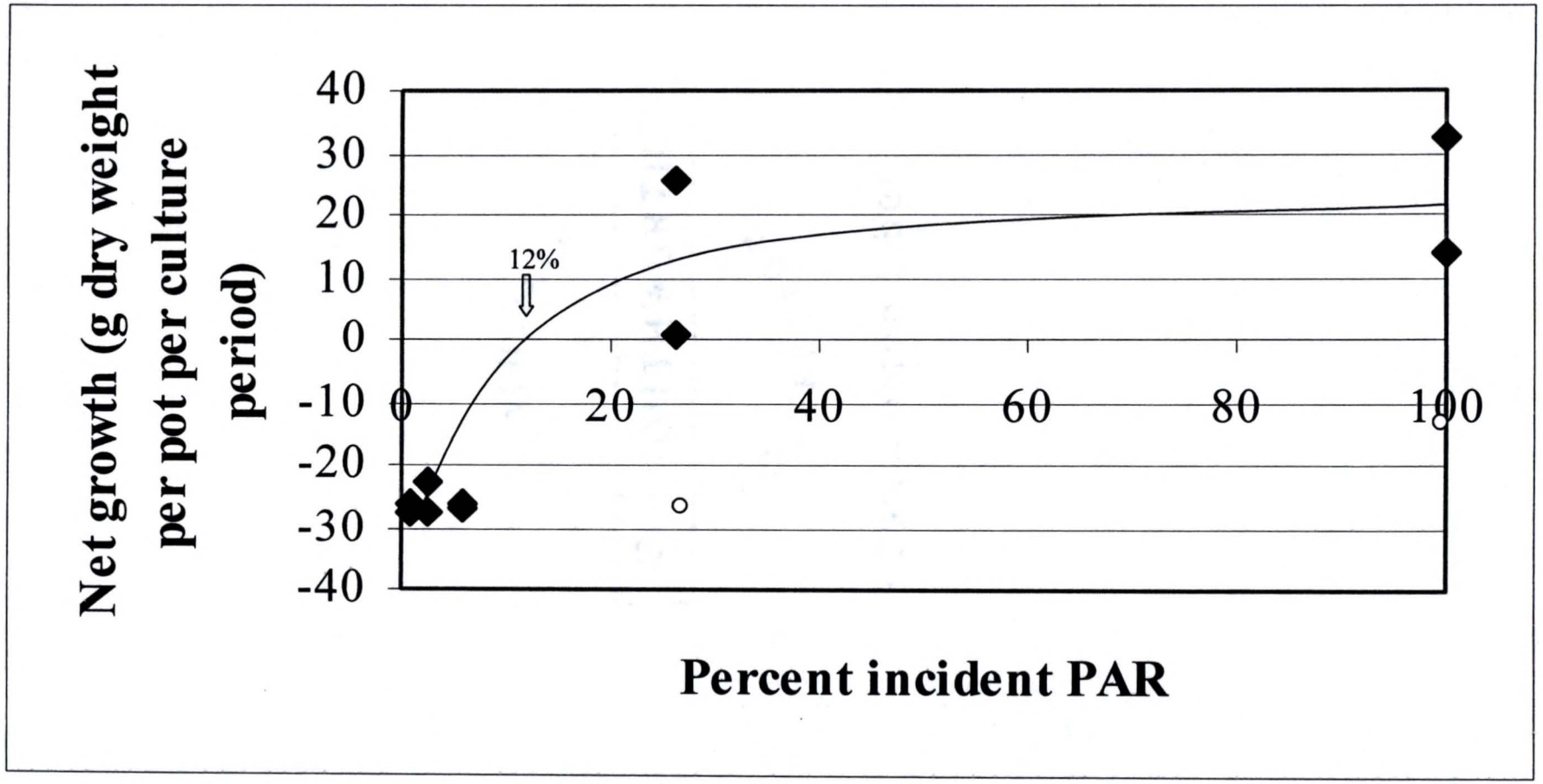


Figure 4-11: Experiment 3: Graphical interpretation of the net growth (g dry weight per pot per culture period) of. *Chara* sp. in response to the percent incident PAR at the water surface. A best-fit curve, based on Michaelis-Menten kinetics, was used to estimate the x-intercept, indicated by the downward facing arrow. The x-intercept represents that percent incident PAR at which there was no net growth. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

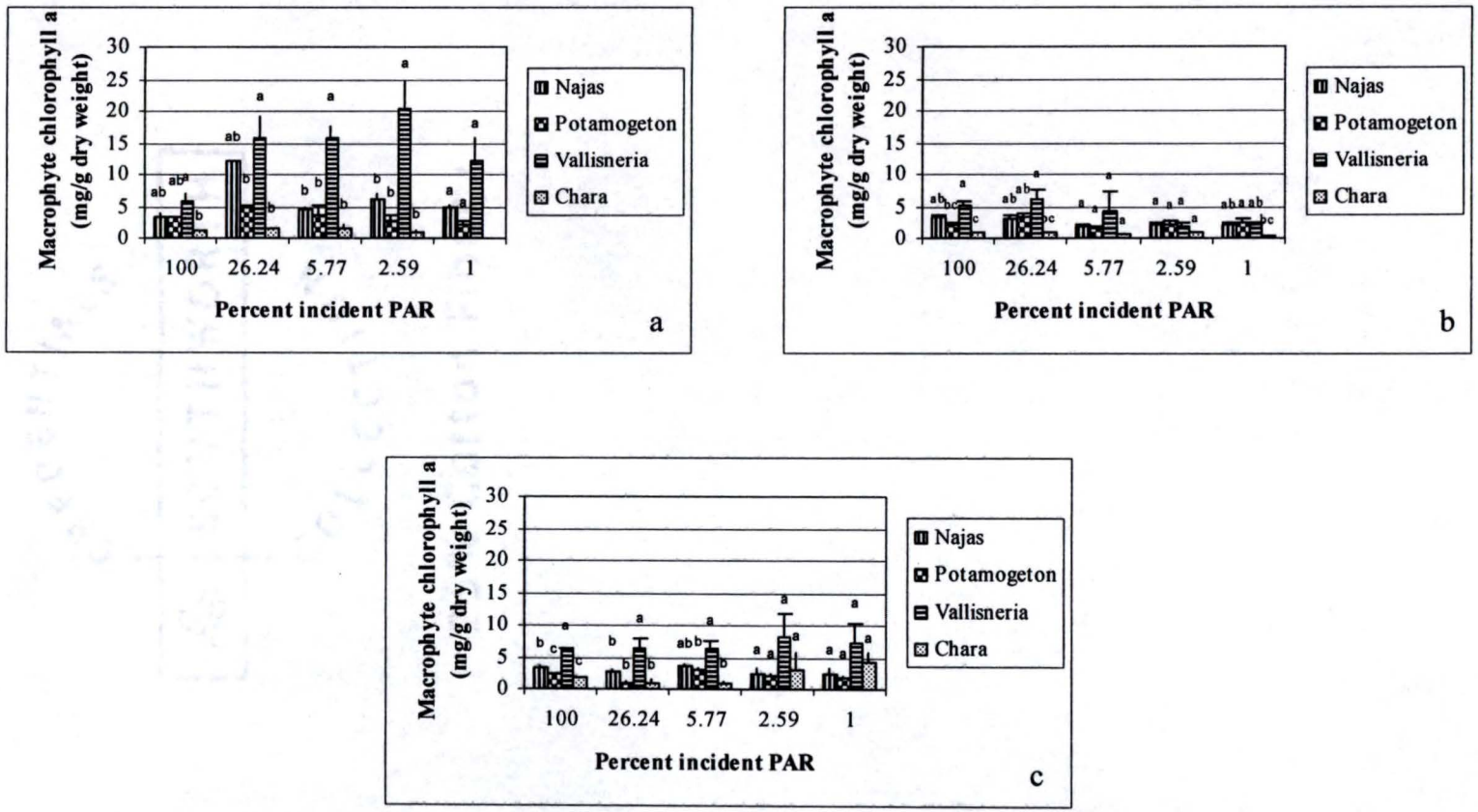


Figure 4-12: Mature plant light requirement experiments: Among species comparisons of macrophyte chlorophyll *a* measured for each of the study plants during a) Experiment 1 (8/22-9/16/01), b) Experiment 2 (2/25-4/13/02) and c) Experiment 3 (7/16-8/31/02). Bars represent the means of three samples (n=3) per macrophyte species. Error bars represent the standard error. Means with the same letter are not significantly different.

CHAPTER 5
LIGHT REQUIREMENTS FOR FOUR SPECIES OF NATIVE SUBMERSED MACROPHYTES: IMPLICATIONS FOR THE RESTORATION OF SHALLOW EUTROPHIC LAKES 2. ASSESSMENT OF THE LIGHT REQUIREMENTS OF VEGETATIVE PROPAGULES

Introduction

Numerous studies have identified light availability as one of the most significant factors controlling macrophyte production in aquatic systems (Duarte and Kalff 1986, Canfield et al. 1985, Barko et al. 1986, Smith and Barko 1990, Strand 1999). Additional studies have investigated the impact of light attenuation on submerged macrophyte productivity and growth (Carter and Rybicki 1990, Duarte 1991, Dunton 1994, Goodman et al. 1995, Masini et al. 1995, Zimmerman et al. 1995, Livingston et al. 1998, Grimshaw et al. 2002). Dennison (1987) discussed the use of photosynthesis vs. irradiance curves together with diurnal light curves to predict growth responses to changes in light regime (Dennison and Alberte 1985), seasonal growth patterns and the maximum depth of colonization for *Zostera marina*. All submerged angiosperms are shade plants (Wetzel 2001). Spencer and Bowes (1990) determined that light saturation for photosynthesis ranges from 10-50% full sunlight.

The classification scheme Gessner (1955) developed for submerged macrophytes based on their physiological adaptations to light availability reflects the extreme plasticity and adaptability of submerged macrophytes to the highly variable underwater light environment. The adaptation types identified include strictly shade adapted requiring low light intensities, strictly light adapted requiring high light

intensities, shade adapted but exhibiting optimum photosynthesis at intermediate light levels, etc. Some aquatic macrophytes are capable of adapting to lower light environments via changes at the ecvllular and the whole-plant level (Wetzel 2001). Cellular adaptations include changes in pigment and enzyme concentrations and composition (Dennison and Alberte 1982, Barko and Filbin 1983). Morphological responses to increasing shade include changes in length and biomass proportions (i.e. of leaves and stems) (cf. Barko et al. 1982). Goldsborough and Kemp (1988) investigated the response and recovery of *Potamogeton perfoliatus* to experimentally induced shade during a 17-day treatment period followed by a 16-day "recovery" period. During the treatment period, plants responded by increasing photosynthetic pigments and producing elongate stems, thinning lower leaves and canopy formation at the surface. Plants showed significant recovery 10 days after removal of light treatments.

The findings of more recent research have indicated that there is considerable variation in photosynthetic capacity and compensation points among submerged macrophyte species (Van et al. 1976, Kenworthy and Fonseca 1996, Wetzel 2001). Light compensation points often occur at 1-3% full sunlight (Wilkinson 1963, Spence 1982, Bowes et al. 1977, Moeller 1980). Kimber et al. (1995) reported that tuber production in *Vallisneria americana* ceased at light levels less than 5% of ambient sunlight. They discussed the implications of these results for *V. americana* growth in relation to light attenuation by turbidity in their study system.

The purpose of this study was to investigate the amount of photosynthetically active radiation (PAR) required for the survival and growth of vegetative propagules

of *Najas guadelupensis*, *Potamogeton illinoensis*, *Vallisneria americana* and *Chara* sp. The underwater light environment of shallow productive systems is highly variable due to changes in light regime resulting primarily from resuspension events and fluctuations in phytoplankton standing crop. A review of the literature indicates a need for further investigation of the light requirements for plant reproduction. Information currently available submersed macrophytes is sparse and highly variable. Most of the studies that have been conducted have investigated the light requirements of weedy exotic species. The experiments in this phase of the study were designed to investigated the growth response of vegetative propagules of the four study species to decreasing light availability.

The experiments in this study were designed to test several hypotheses. First, I hypothesized that plants grown from propagules would have higher light requirements than mature plants of the same species. I anticipated that *V. americana* would exhibit the greatest capability for growth in low levels of light. I further expected that plants grown from propagules would not become photoinhibited during the course of each culture period.

Additional information on the growth response of vegetative propagules to decreased light availability should be of value to lake managers interested in the selection of species for use in revegetation projects and in the calculation of maximum planting depths.

Materials and Methods

Experimental Environment and Procedures

Propagules (apical stem cuttings of *N. guadalupensis*, *P. illinoensis* and *Chara* sp and suckers of *V. americana*) were harvested from stock cultures of approximately the same age and in a state of active growth maintained at the FLREC from plants originally collected in Lake Okeechobee, Florida (Dr. David L. Sutton, UF FLREC, personal communication). In Experiment 1, sixty 7.6-L black plastic nursery pots 22.5 cm in top diameter and 20 cm deep were loaded to 11 cm from the top with air-dried coarse builders' sand. Osmocote Southern Formula 15-9-12 (N:P:K), a commercially available fertilizer, was added as a layer to each container and mixed into the sand at a rate of 40 g per pot for *N. guadalupensis* and *P. illinoensis*, and 15 g for *V. americana* and *Chara* (Dr. David Sutton, UF FLREC, personal communication). This fertilizer is formulated to slow-release in soil over an 8-9 month period with increased rates of nutrient release at temperatures ≥ 21°C (Harbaugh and Wilfret 1981). In Experiments 2 and 3, 135 4-L black plastic nursery containers 16.5 cm in top diameter by 16.5 cm deep were loaded to 8 cm from the top with air-dried coarse builders' sand. A layer of Osmocote Southern Formula 15-9-12 (N:P:K) was added to each pot at a rate of 30 g for *P. illinoensis* and 11.3 g for *V. americana* and *Chara sp.* (Dr. David Sutton, UF FLREC, personal communication). Each container in all three Experiments was then filled with sand to within 2.5 cm of the top. Containers were submerged in concrete tanks measuring 6.2 m in length by 3-1 m in width by 0.9 m in height filled with pond water to a depth of 0.8 m. Twenty-centimeter apical cuttings of *N. guadalupensis*, *Chara sp.* and *P. illinoensis* and single

rosettes of *V. americana* were then planted at a frequency of four propagules per pot. *N. guadalupensis* and *Chara sp.* were planted to a depth of ≥ 3 nodes. *P. illinoensis* was planted to a depth of at least 2 nodes. Individual sucker plants of *V. americana* were cut from the rhizome, roots were gently washed to remove organic sediments and each rosette was planted deep enough to submerge the rhizome beneath the sediment surface. Care was taken to avoid covering the basal rosette. Pond water from a groundwater fed pond located on-site at the UF FLREC flowed into the tanks at the surface of one end and out from bottom drains at the other end at a rate that allowed for an exchange of water every 24 hr. Containers were placed in rows parallel to the flow of water in the tanks (Figure 4-1). Growing all plants in the same water prevented skewing of the data due to variability in the nutrient composition of the water column.

A wooden frame 19.22 m^2 in area was placed over and positioned on the tank walls. Cross beams were used to create five rectangles each 3.7 m^2 in area in Experiment 1 and fifteen rectangles each 0.87 m^2 in area in Experiments 2 and 3. Shade cloth was used in differing numbers of layers to adjust experimental light levels to 0.96%, 3.57%, 7.29%, and 27% full sun in Experiment 1, 0.6%, 1.8%, 5.1% and 6.5% full sun in Experiment 2 and 2.8%, 8.3%, 11.4%, and 26.2% full sun in Experiment 3 at the air-water interface below the treatment groups. The control group in all experiments was not shaded and was exposed to full sunlight. Sheets of Weed Block were used between the treatment groups in all experiments to prevent light transfer among the groups while still permitting water flow through the tank. In

Experiments 2 and 3, shade cloth was attached to the peripheral beams of the frame and draped over the outer walls of the tank.

The experiment was repeated over three culture periods: 27 April to 14 July 2002, 21 April to 7 June 2003 and 28 June to 2 August 2003. Experiment 1 was a random design with four light treatments with three replicates per plant species per treatment. Experiments 2 and 3 were complete randomized block designs such that each row of treatment groups was considered a block and n = 9 pots per plant species per light level. Data are presented on a per container basis. Culture period length in each experiment was adequate for the development of treatment-related differences but minimized tissue deterioration associated with senescence.

Water temperature was measured using maximum/minimum thermometers placed 30 cm below the surface of the water. Readings were taken 5 days a week at approximately 3:00 P.M. each day. Water temperature was calculated as the mean of the maximum and minimum values from the thermometers for a recording period.

Two recently certified 2-pi quantum sensors attached to a LI-COR datalogger were used to measure instantaneous photosynthetic photon flux density. Multiple simultaneous measurements taken above and one centimeter below the shade cloth in each treatment group throughout the day from 0700 to 1700h on three separate days during the culture period were used to calculate the percent transmittance for each treatment. Sheets of Weed Block were used between the treatment groups in Experiments 2 and 3 to prevent light transfer among the groups while still permitting water flow through the tank.

A long-term incident PPFD mean was calculated using short-term incident means of total irradiance (400-1100 nm) (W m^{-2}) measured by a LICOR LI200SZ pyrometer located on-site and maintained by the University of Florida Florida Automated Weather Network (FAWN). Short-term incident means were based on data collected every 3.75 minutes and averaged and reported every 15 minutes. Total irradiance was converted to photosynthetically active radiation to (PAR) (400-700 nm) assuming that PAR is approximately 45% of total irradiance (Baker and Froiun 1987). PAR in W m^{-2} was converted to photosynthetic photon flux density (PPFD) units (μ mol photons s^{-1} m^{-2}) using a multiplier of 4.6 (see Table 3 in Thimijan and Heins 1983). The long-term mean incident PPFD for the photic portion of an experimental period was found by averaging the nonzero fluxes. Accumulated PAR was found by multiplying the mean incident PPFD by the length of the photic period for the experiment. The long-term incident PPFD mean was multiplied by the mean percent transmittance discussed above to obtain mean estimates of the incident PPFD at the air-water interface in each treatment.

Pot level incident PPFD in each treatment was estimated using Equation 5.1.

$$I_z = I_o \cdot (\%\ \text{light attenuation by shade cloth}) \cdot e^{-kz} \qquad \text{Equation 5.1}$$

where:
I_z = incident irradiance above each treatment group,
I_o =irradiance at depth, z,
k = light extinction coefficient,
z = depth, in this case, 60 cm for Experiments 1 and 2 and 47 cm for Experiment 3.

The light extinction coefficient was calculated from simultaneous measurements of incident irradiance and irradiance at a given depth (depth varied from to 32 to 47 cm). The light extinction coefficient was calculated using Equation 5.2.

Equation 5.2

$$\frac{\ln I_o - \ln I_z}{z} = k$$

Light measurements were taken on several sampling dates throughout the course of the entire study: 27 April 2002, 2 and 3 August 2003 and 7 October 2003 in order to ensure representative sampling of seasonal variation in the value of the coefficient. Actual measurements of the pot-level irradiance were taken on several sample dates in order to field-test the accuracy of the calculated values. Irradiance values reported in this study were not corrected for reflectance.

Procedures for sample collection and processing during and following harvest were the same as those described in the Materials and Methods section of Chapter 3.

Macrophyte success was defined in terms of growth. Growth in each container was determined as the difference in the gram dry weight from the start to the end of each experiment. The initial gram dry weight was estimated from the weights of at least ten representative plants collected and processed at the time that the light variable was introduced.

Data Analysis

All macrophyte biomass data were statistically analyzed using GLM procedures (SAS Institute, Inc. 1999-2001). Significant means ($P \leq 0.05$) were separated using Tukey's HSD Test. Regression analysis was used to investigate the

relationship between photosynthetic photon flux density and macrophyte biomass. Data were transformed to reduce the variance as necessary (Ricker 1973). Data points greater than two standard deviations away from the mean were considered outliers and were excluded from further analysis.

The photosynthetic photon flux density for no net growth of each of the submersed macrophyte species was calculated using Equation 5.1. Equation 5.1 is a modification by Grimshaw et al. (2002) of Equation 16.30 developed by Zar (1996).

$$Xi = \frac{Yi - a}{b}$$

Equation 5.1

where:
X_i is the estimated photosynthetic photon flux density mean,
Y_i is set to zero,
a is the Y intercept and
b is the slope of the regression line.

Results

Temperature and Irradiance

Water temperature during the study period was relatively constant with highest measured mean temperatures occurring during Experiment 2 (26.2 °C) while lowest average temperatures were measured during Experiment 1 (24.8°C) (Table 5-1). Mean photosynthetic photon flux density (PPFD) was greatest during Experiment 2 (824 μmol $s^{-1}m^{-2}$) and least during Experiment 1 (777 μmol m^{-2} s^{-1}). Relatively lower average PAR values in summer as compared to spring are probably due in part to afternoon thunderstorms that result in 100% cloud cover during parts of most

summer afternoons. Photoperiod ranged from 13.5 hours in Experiment 2 to 13.8 hours in Experiments 1 and 3 (Table 5-1).

Effects of Light Availability on Propagule Macrophyte Production

Experiment 1 (4/27/02 to 7/14/02)

PAR measured at the air-water interface of the treatment groups ranged from 777 μmol m^{-2} s^{-1} or 100% incident PAR with no shade in the control group to 5.67 μmol m^{-2} s^{-1}or 0.73 % incident PAR in the 99% shade group. PAR available at the pot level (z = 60 cm) was approximately 30% of the PAR available at the air-water interface ranging from 227 μmol m^{-2} s^{-1} in the control group to 1.7 μmol m^{-2} s^{-1} in the 99% shade group (Table 5-2).

Analysis of variance using GLM procedures (SAS Institute 1999-2001) indicated that treatment, plant species and the interaction between treatment and plant species had a highly significant effect on macrophyte yield in this experiment ($P < .0001$).

There were significant treatment effects among and within the macrophyte species. *N. guadalupensis* produced significantly greater yield than the other species in the control group and in the 0.7% light group. *Chara sp.* exhibited statistically less growth in the control than the other species. There were no significant differences in growth among the species at 5.8% incident light. Comparisons within species indicated that all species produced significantly lower biomass at shade levels ≥ 74% (Table 5-3). There was a second significant decrease in *V. americana* biomass at shade levels ≥ 94%.

Comparison of the light compensation point (LCP) estimated using regression models indicated species-specific differences in the amount of PAR required for macrophyte growth. The results suggested that macrophyte biomass production decreased linearly with decreasing light availability. There was a strong regression relationship (R^2 = 90.1%) between PAR and *N. guadalupensis* yield (Figures 5-1 and 5-2). Using the equations from Figures 5-1 and 5-2, the apparent photosynthetic photon flux density for no net *N. guadalupensis* growth was estimated to be 59.8 μmol m^{-2} s^{-1} (7.7 % incident PAR) and 17.5 μmol m^{-2} s^{-1} (2.3% incident PAR) at the air-water and sediment-water interface, respectively. PAR explained over 94% of the variance in *P. illinoensis* yield (Figure 5-.3). Using the equations from Figures 5-3 and 5-4, the apparent photosynthetic photon flux density for no net *P. illinoensis* growth was estimated to be 63.4 μmol m^{-2} s^{-1} (8.3% incident PAR) and 18.6 μmol m^{-2} s^{-1} (2.4% incident PAR) at the air-water and sediment-water interface, respectively. PAR explained nearly 70% of the variance in *V. americana* biomass (Figure 5-5). Using the equations from Figures 5-5 and 5-6, the apparent photosynthetic photon flux density for no net *V. americana* growth was estimated to be 64.1 μmol m^{-2} s^{-1} (8.3% incident PAR) and 18.7 μmol m^{-2} s^{-1} (2.4% incident PAR) at the air-water and sediment-water interface, respectively. There was a strong relationship (R^2=81.12%) between *Chara sp.* biomass production and PAR (Figure 5-7). Using the equations from Figures 5.7 and 5.8, the apparent photosynthetic photon flux density for no net *Chara sp.* growth was 159.9 μmol m^{-2} s^{-1} (20.6% incident PAR) and 46.7 μmol m^{-2} s^{-1} (6% incident PAR) at the air-water and sediment-water interface, respectively.

Experiment 2: 4/21/03 to 6/7/03

PAR measured at the air-water interface of the treatment groups ranged from 824 μmol m^{-2} s^{-1} or 100% incident PAR with no shade in the control group to 5.1 μmol m^{-2} s^{-1}or 0.6 % incident PAR in the 99% shade group. PAR available at the pot level (z = 60 cm) was approximately 30% of the PAR available at the air-water interface ranging from 240.8 μmol m^{-2} s^{-1} in the control group to 1.5 μmol m^{-2} s^{-1} in the 99% shade group (Table 5-6).

Analysis of variance using GLM procedures (SAS Institute 1999-2001) indicated that light, plant type and the interaction between these two factors had a highly significant effect on macrophyte yield in this experiment ($P < .0001$).

There were significant treatment effects among and within the macrophyte species. *Chara sp.* produced significantly greater yield than the other species in the control group. *V. americana* produced statistically greatest biomass as compared to the other study species in all the light treatment groups. Comparisons within species indicated a significant decrease in the growth of all species at light levels ≤ 6.5% incident PAR (Table 5-7).

Comparison of the light compensation point (LCP) estimated using regression models indicated species-specific differences in the amount of PAR required for macrophyte growth. The results suggested that macrophyte biomass production decreased linearly with decreasing light availability. There was a very strong regression relationship (R^2 = 93.9%) between PAR and *V. americana* yield (Figure 5-9). Using the equations from Figures 5-9 and 5-10, the apparent photosynthetic photon flux density for no net *V. americana* growth was estimated to

be 150.5 μmol m^{-2} s^{-1} (18.3% incident PAR) and 43.9 μmol m^{-2} s^{-1} (5.3% incident PAR) at the air-water and sediment-water interfaces, respectively. PAR explained over 90% of the variance in *P. illinoensis* yield (Figure 5-11). Using the equation from Figures 5-11 and 5-12, the apparent photosynthetic photon flux density for no net *P. illinoensis* growth was estimated to be 182.5 μmol m^{-2} s^{-1} (22.2% incident PAR) and 53.3 μmol m^{-2} s^{-1} (6.5% incident PAR) at the air-water and sediment-water interfaces, respectively. *Chara sp.* biomass and PAR were strongly related (R^2 = 78.77%) (Figure 5-13). Using the equation from Figures 5-13 and 5-14, the apparent photosynthetic photon flux density for no net *Chara sp.* growth was estimated to be 94.4 μmol m^{-2} s^{-1} (11.5% incident PAR) and 27.5 μmol m^{-2} s^{-1} (3.3% incident PAR) at the air-water and sediment-water interfaces, respectively.

Experiment 3: 6/28 to 8/2/03

PAR measured at the air-water interface of the treatment groups ranged from 821 μmol m^{-2} s^{-1} or 100% of incident PAR with no shade in the control group to 22.9 μmol m^{-2} s^{-1}or 2.8 % incident PAR in the 97% shade group. PAR available at the sediment-water interface (z = 60 cm) was approximately 40% of the PAR available at the air-water interface ranging from 328.4 μmol m^{-2} s^{-1} in the control group to 9.2 μmol m^{-2} s^{-1} in the 97% shade group (Table 5-8).

Analysis of variance using GLM procedures (SAS Institute 1999-2001) indicated that light, plant type and the interaction between these two factors had a highly significant effect on macrophyte yield in this experiment ($P < .0001$).

There were significant treatment effects among and within the macrophyte species. *Chara sp.* exhibited greater growth than the other species in all treatment

groups except the 2.8% light group in which *V. americana* biomass was greatest. Comparisons within species indicated that all species produced greatest biomass in the control group. *Chara sp.* and *V. americana* growth decreased at light levels ≤ 26.2% and again at light levels ≤ 2.8%. There were no statistical differences in *P. illinoensis* growth at light levels ≤ 26.2% (Table 5-9).

Comparison of the light compensation point (LCP) estimated using regression models indicated species-specific differences in the amount of PAR required for macrophyte growth. The results suggested that macrophyte biomass production decreased linearly with decreasing light availability. There was a strong regression relationship (R^2 = 77.83%) between PAR and *V. americana* yield (Figure 5-15). Using the equations from Figures 5-15 and 5-16 , the apparent photosynthetic photon flux density for no net *V. americana* growth was estimated to be 134.9 μmol m^{-2} s^{-1} (16.4% incident PAR) and 53.8 μmol m^{-2} s^{-1} (6.6% incident PAR) at the air-water and sediment-water interfaces, respectively. PAR explained over 75% of the variance in *P. illinoensis* yield (Figure 5-17). Using the equations from Figures 5-17 and 5-18, the apparent photosynthetic photon flux density for no net *P. illinoensis* growth was estimated to be 350.2 μmol m^{-2} s^{-1} (42.7% incident PAR) and 140.1 μmol m^{-2} s^{-1} (17.1% incident PAR) at the air-water and sediment-water interfaces, respectively. *Chara sp.* biomass and PAR were strongly related (R^2= 80.36%) (Figure 5-19). Using the equations from Figures 5-19 and 5-20, the apparent photosynthetic photon flux density for no net *Chara sp.* growth was estimated to be 25 μmol m^{-2} s^{-1} (3% incident PAR) and 10 μmol m^{-2} s^{-1} (1.2% incident PAR) at the air-water and sediment-water interfaces, respectively.

Field observations at the time of harvest indicated the presence of the herbivorous moth, *Parapoynx diminutalis* Snellen, in all treatment groups other than the controls in Experiment 3. Although Malathion was added to each of the control groups in order to control herbivory, concentrations in the light treatment groups were apparently insufficient to eliminate this herbivore. Accordingly, we believe that herbivory had a significant negative effect on *P. illinoensis* production in all replicates of treatments B through E that led to a subsequent overestimate of the PPFD at which there was no net growth. *Parapoynx diminutalis* does not appear to feed on *V. americana* and *Chara sp.* (Hopson-Fernandes, personal observation).

Effects of Light Availability on Macrophyte Chlorophyll *a* in Propagules

Analysis of variance using GLM procedures (SAS Institute 1999-2001) indicated that treatment and plant species had a highly significant effect on macrophyte chlorophyll *a* (mg/g macrophyte DWT) in Experiment 1. The interaction between these two factors was slightly significant ($P<0.0139$). Macrophyte chlorophyll *a* content was not measured in Experiments 2 and 3.

The data indicate that there were differences in the amount of chlorophyll *a* produced among and within the study species. Among-species comparisons of the effect of decreasing light availability on the amount of macrophyte chlorophyll *a* measured per g macrophyte DWT indicated that *V. americana* produced significantly greater chlorophyll *a* than the other study species in all of the experimental light environments (Figure 5-21). Within species comparisons suggested that there was no relationship between *Chara sp.* chlorophyll *a* content and decreasing light availability. *N. guadalupensis* produced statistically greatest chlorophyll *a* in the

26.2% light group. *N. guadalupensis* chlorophyll *a* content decreased significantly in the 5.8% light group and again in the 0.7% light group. *P. illinoensis* chlorophyll *a* content was greatest in the 26.2% and decreased significantly in the 2.6% light group. *V. americana* produced the greatest amounts of chlorophyll *a* in the 26.2% light group while a significant decline in chlorophyll *a* was observed in the 0.7% group.

Discussion

Light is recognized as one of, if not the most, important factors affecting the growth of submersed aquatic macrophytes. This relationship is particularly well-defined in highly turbid, eutrophic lakes (van Dijk et al. 1992, Lauridsen et al.1994, Strand 1999). Light availability drives a variety of population dynamics within natural plant communities including species composition, distribution and maximum depth of colonization. However, despite the fact that the general significance of light to SAV growth is so well-accepted, there remains considerable uncertainty as to the specific light requirements of submersed macrophytes. Studies conducted to date vary widely and few have distinguished between the requirements for plant survival and reproduction (Kimber et al. 1995). Dennison et al. (1993) observed that, at present, there is no consensus concerning the light environment required for the growth and survival of *V. americana* in shallow water bodies. A review of the literature indicates that minimum light requirements vary from species to species and are affected by the length of the growing season (Table 5-10). In his investigation of 8 species of seagrasses, Dennison (1987) observed light compensation points that varied from 9 to 26 $\mu mol\ m^{-2}\ s^{-1}$. Goldsborough and Kemp (1988) concluded from their treatment and "recovery" studies that 11% ambient irradiance was required for the survival of

Potamogeton perfoliatus. Sand-Jensen and Madsen (1991) observed among-species differences in their comparative study of the light requirements of charophytes, bryophytes and angiosperms. Van et al. (1976) used photosynthetic rate measurement studies to calculate the light compensation points of two native and two exotic species of submersed macrophytes: *Hydrilla verticillata, Ceratophyllum demersum, Myriophyllum spicatum* and *Cabomba caroliniana*. They concluded that *H. verticillata* had the lowest LCP – 15 μmol s^{-1} m^{-2} followed by *C. demersum* and *M. spicatum* – both 35 μmol s^{-1} m^{-2}. *C. caroliniana* exhibited the greatest light requirement with an estimated LCP of 55 μmol s^{-1} m^{-2}. They also observed that Hydrilla had the lowest light requirement to achieve half-maximal photosynthetic rate. They inferred that the superior photosynthetic efficiency conferred by these adaptations probably explains Hydrilla's competitive advantage over many submersed macrophyte species. Canfield et al. (1985) estimated LCP's for hydrilla equivalent to less than 1% full sun at the maximum depth of colonization in several study lakes. (See Table 2 in Canfield et al. 1985). Spence and Chrystal (1970) reported LCP's less than 1 μE m^{-2} s^{-1} in their study of the light requirements of submersed macrophytes.

The findings of this research suggest that light had a statistically significant effect on macrophyte growth. Light explained over 90% of the variance in *N. guadalupensis* yield in Experiment 1, from 75 to 95% of the variation in *P. illinoensis* biomass, from 70 to 94% of the variance in *V. americana* yield and greater than 78% of the variation in *Chara sp.* biomass production in all three experiments. The plants responded to decreasing light availability by reducing total, above and below-ground

dry weight. In a laboratory study of the effects of turbidity on vallisneria, Doyle and Smart (2001) observed similar decreases in total AFDM in response to decreasing light availability. Grimshaw et al. (2002) also reported a decrease in the AFDM of *V. americana* with decreasing light. *N. guadalupensis*, *P. illinoensis* and *Chara* sp. exhibited elongated stems, relatively lesser numbers of lower leaves and increased canopy formation in response to increasing shade. Goldsborough and Kemp (1988) observed similar shade responses in *P. perfoliatus*.

The results indicated that changes in chlorophyll *a* content in relation to light availability varied according to plant species. *V. americana* exhibited significantly greater chlorophyll *a* content per g macrophyte DWT than the other study species in all treatment groups. Comparisons within macrophyte species indicated that there was no apparent effect of decreased light availability on *Chara sp.* chlorophyll *a* concentration. However, greatest chlorophyll *a* concentrations were measured in the 26.2% light group for all of the other species. This result was possibly due to the absence of sufficient energy at lower light levels to produce increased chlorophyll. Goldsborough and Kemp (1988) observed significant increases in chlorophyll *a* content in response to decreasing light within 3 days of introduction of the light variable. Chlorophyll concentrations returned to normal levels following removal of the light variable. The sample size (n = 3) used in this study was not large enough to ensure representative sampling of shoot materials of differing ages. The high internal variation obscured any observable trends in macrophyte chlorophyll a production in response to increasing shade in this study.

The results also suggested relationship between length of growing period and total PPFD for the culture period and light requirements. *N. guadalupensis*, *P. illinoensis* and *V. americana* exhibited lowest light requirements during the spring/summer culture period (Experiment 1) (Table 5-11). The length of this experiment was approximately twice that of the other two experiments. The plants also received considerably greater total PPFD in Experiment 1, 3011 mol photons m^{-2}, than in the other two experiments, 1922 and 1427 mol photons m^{-2}. It seems intuitive that immature plants might have greater light requirements when first established in order to ensure sufficient light levels at the sediment-water interface..

The amount of light required for the growth of propagules of the submersed aquatic macrophytes in this study also varied seasonally (Table 5-11). *N. guadalupensis*, *P. illinoensis* and *V. americana* exhibited lowest minimum light requirements in the late spring to early summer (59.8, 63.4 and 64.1 μmol photons m^{-2}, respectively). *Chara sp.* exhibited lowest light requirements in summer and highest light requirements in the spring/summer. These results would tend to suggest that other factors besides light may have a more significant on *Chara sp.* growth.

Conclusions

There was a decrease in total biomass produced by *Najas guadelupensis*, *Potamogeton illinoensis*, *Vallisneria americana* and *Chara* sp. in response to decreasing PAR. Macrophyte chlorophyll *a* content appeared to be unaffected by decreasing PAR. There was a decrease in above to below-ground biomass ratios for all species as available light decreased. The perennial species, *P. illinoensis* and *V.*

americana, produced significantly greater below-ground biomass as compared to the annual, *N. guadalupensis*.

Vallisneria appeared to be the most well-adapted study species for survival in low light environments. The combination of low light compensation point, high concentration of chlorophyll *a* and extensive root structure possibly confer an advantage to vallisneria in shallow turbid systems. However, additional study is required in order to clarify some of the contradictory findings regarding the light requirements of Vallisneria before managers should rely on monocultures of Vallisneria.

In summary, a combination of several or all of the study species should be considered in order to ensure the maximum possibility for the successful establishment of a healthy sustaining communities of desirable native submerged plants in shallow eutrophic lakes. Establishment of propagules of *N. guadalupensis*, *P. illinoensis* and *Chara sp.* during the late spring/early summer will require PPFD levels greater than 8% ambient light. The results suggested that summer is the optimum time to plant *Chara sp.*. Additional research is needed in order to quantify the amount of light required by submersed macrophytes in all stages of their life cycles. A better understanding of the role of light in inter-specific competition is also required.

Table 5-1: Temperature and irradiance during the three culture periods. Temperature values are average daily temperatures followed by the standard deviation. Values shown in parentheses are the lowest and the highest measured temperatures for each culture period.

Culture Period	Water Temperature (°C)	Mean Instantaneous PPFD[a] ($\mu mol\ s^{-1}\ m^{-2}$)	Photoperiod (hr)
Experiment 1 4/27 – 7/14/02	24.8 ± 2.2 (23.4 ± 26.8)	777 (0-2240)	(13.8L:10.2D)
Experiment 2 4/21 – 6/7/03	26.2 ± 1.7 (22.6 to 30.4)	824 (0-2283)	(13.5L:10.5D)
Experiment 3 6/28 – 8/2/03	28.2 ± 0.9 (22.3 to 33.4)	821 (0-2337)	(13.8L:10.2D)

[a]Photosynthetic photon flux density (PAR). Values shown are means of the daily average PAR measured over the course of each respective culture period.

Table 5-2: Experiment 1: Estimated average instantaneous, daily and total photosynthetic photon flux density (PPFD) received over the period 27 April to 14 July (78 days) assuming 13.8-hour daylength.

Treatment	Light level (% light)		Total PPFD For CP[a] Mol*m^{-2}		Daily PPFD mol*m^{-2}*d^{-1}		Instantaneous PPFD umol* s^{-1} m^{-2}*	
	Surface	Pot[b]	Surface	Pot[b]	Surface	Pot[b]	Surface	Pot[b]
A	100.0	29.2	3010.9	879.8	38.6	11.3	777.0	227.0
B	26.2	7.7	790.5	231.0	10.1	3.0	204.0	59.6
C	5.8	1.7	173.7	50.8	2.2	0.7	44.8	13.10
D	2.6	0.8	78.0	22.8	1.0	0.3	20.1	5.9
E	0.7	0.2	22.0	6.4	0.3	0.1	5.7	1.7

[a]Culture period.
[b]Pot level estimates are not corrected for self-shading by the above-sediment portions of the individual macrophytes. Quantification of these values is beyond the scope of this research.
The degree of self-shading should vary with species differences in leaf architecture and growth strategy.

Table 5-3: Results of within species analysis of net yield of total macrophyte biomass (above plus below ground biomass) in Experiment 1 using GLM procedures (SAS Institute 1999-2001) Values are the means calculated for three culture containers per macrophyte species per treatment group followed by the standard error. Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

	Macrophyte Species			
Treatment[a]	Najas	Potamogeton	Vallisneria	Chara
A	105.8 ± 16.6a	52.4 ± 6.2 a	4.9 ± 3.5 a	3.0 ± 1.0 a
B	1.8 ± 0.2 b	5.9 ± 1.1 b	1.1 ± 0.2 b	-0.2 ± 0.2 b
C	-0.2 ± 0.0 b	-0.6 ± 0.0 b	-0.2 ± 0.2 c	-0.5 ± 0.1 b
D	-0.3 ± 0 b (No survival)	-0.6 ± 0 b (No survival)	-0.3 ± 0.0 c	-0.6 ± 0.0 b
E	-0.3 ± 0 b	-0.6 ± 0 b (No survival)	-0.6 ± 0.0 c	-0.6 ± 0 b (No survival)

[a]Treatments are the same as indicated in Table 5.2.

Table 5-4: Within species comparisons of root:shoot ratios for Experiment 1. Values are the means calculated for three culture containers per macrophyte species per treatment group followed by the standard error. All data were analyzed using GLM procedures (SAS Institute 1999-2001). Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

	Macrophyte Species		
Treatment[a]	Najas	Potamogeton	Vallisneria
A	0.03± 0.00a	0.09 ± 0.00a	0.12 ± 0.04 b
B	0.03 ± 0.01 a	0.08 ± 0.00 a	0.28 ± 0.04 b
C	0.31 ± 0.24 a	0.09 ± 0.09 a	2.38± 0.01 ab
D	0.00 a	0.00 a	2.40 ± 0.49a
E	0.05 ± 0.02 a	0.00 a	0.28 ± 0.00 b

Table 5-5: Comparisons among species of root:shoot ratios for Experiment 1. Values are the means calculated for three culture containers per macrophyte species per treatment group followed by the standard error. All data were analyzed using GLM procedures (SAS Institute 1999-2001). Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

Treatment	Macrophyte	Root to Shoot Ratio	Tukey Group
A	*V. americana*	0.12 ± 0.04	A
	P. illinoensis	0.09 ± 0.00	A
	N. guadalupensis	0.03 ±0.00	A
B	*V. americana*	0.28 ± 0.04	A
	P. illinoensis	0.08 ± 0.00	B
	N. guadalupensis	0.03 ± 0.01	B
C	*V. americana*	2.38 ± 0.01	A
	N. guadalupensis	0.31 ± 0.24	B
	P. illinoensis	0.09 ± 0.09	B
D	*V. americana*	2.40 ± 0.49	A
	P. illinoensis	0	B
	Najas	0	B
E	*V. americana*	0.28 ± 0.00	A
	N. guadalupensis	0.05 ± 0.02	B
	P. illinoensis	0	B

Table 5-6: Experiment 2: Estimated average instantaneous, daily and total photosynthetic photon flux density (PPFD) received over the period 21 April to 7 June (7 weeks) assuming 13.5-hour daylength.

Treatment	Light level (% light)		Total PPFD For CP[a] Mol*m^{-2}		Daily PPFD mol*m^{-2}*d^{-1}		Instantaneous PPFD umol* s^{-1} m^{-2}*	
	Surface	Pot[b]	Surface	Pot[b]	Surface	Pot[b]	Surface	Pot[b]
A	100.0	29.2	1922.2	561.7	40.1	11.7	824.0	240.8
B	6.5	1.9	125.3	36.6	2.6	0.8	53.7	15.7
C	5.1	1.5	98.8	28.9	2.1	0.6	42.4	12.4
D	1.8	0.5	34.4	10.1	0.7	0.2	14.8	4.3
E	0.6	0.2	11.9	3.5	0.3	0.1	5.1	1.5

[a]Culture period.

[b]Pot level estimates are not corrected for self-shading by the above-sediment portions of the individual macrophytes. Quantification of these values is beyond the scope of this research. The degree of self-shading should vary with species differences in leaf architecture and growth strategy.

Table 5-7: Results of within- species analysis of net growth of total macrophyte biomass (above plus below ground biomass) in Experiment 2 using GLM procedures (SAS Institute 1999-2001) Values are the means calculated for three culture containers per macrophyte species per treatment group followed by the standard error. Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

Treatment[a]	Macrophyte Species		
	P. illinoensis	*V. americana*	*Chara* sp.
A	4.83 ± 0.96 a	2.53 ± 0.36 a	13.11 ± 2.31 a
B	-0.87 ± 0.20 b	-0.21 ± 0.09 b	-0.70 ± 0.10 b
C	-1.02 ± 0.05 b	-0.28 ± 0.13 b	-1.03 ± 0.05 b
D	-1.08 ± 0 b (No survival)	-0.49 ± 0.02 b	-1.13 ± 0 b (No survival)
E	-1.08 ± 0 b (No survival)	-0.50 ± 0.04 b	-1.13 ± 0 b (No survival)

[a]Treatments are the same as indicated in Table 5-4.

Table 5-8: Experiment 3: Estimated average instantaneous, daily and total photosynthetic photon flux density (PPFD) received over the period 28 June to 02 August (5 weeks) assuming 13.8-hour daylength.

Treatment	Light level (% light)		Total PPFD For CP[a] Mol*m^{-2}		Daily PPFD mol*m^{-2}*d^{-1}		Instantaneous PPFD umol*m^{-2}*s^{-1}	
	Surface	Pot[b]	Surface	Pot[b]	Surface	Pot[b]	Surface	Pot[b]
A	100.0	40.0	1427.6	571.0	40.8	16.3	821	328.4
B	26.2	10.5	374.6	149.8	10.7	4.3	215.4	86.2
C	11.4	4.6	163.0	65.2	4.7	1.9	93.8	37.5
D	8.3	3.3	118.3	47.3	3.4	1.4	68.1	27.2
E	2.8	1.1	39.8	15.9	1.1	0.5	22.9	9.2

[a]Culture period.

[b]Pot level estimates are not corrected for self-shading by the above-sediment portions of the individual macrophytes. Quantification of these values is beyond the scope of this research. The degree of self-shading should vary with species differences in leaf architecture and growth strategy.

Table 5-9: Results of analysis of net growth of total macrophyte biomass (above plus below ground biomass) in Experiment 3 using GLM procedures (SAS Institute 1999-2001) Values are the means calculated for three culture containers per macrophyte species per treatment group followed by the standard error. Means within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

Treatment[a]	Macrophyte Species		
	Potamogeton	Vallisneria	Chara
A	1.04 ± 0.26 a	1.44 ± 0.16 a	5.75 ± 0.92 a
B	-0.38 ± 0.10 b	0.27 ± 0.06 b	2.58 ± 0.66 b
C	-0.43 ± 0.03 b	-0.13 ± 0.15 bc	0.87 ± 0.30 bc
D	-0.58 ± 0.02 b	-0.08 ± 0.15 bc	0.56 ± 0.21 bc
E	-0.64 ± 0 b (No survival)	-0.38 ± 0.05 c	-0.45 ± 0.06 c

[a]Treatments are the same as indicated in Table 5-6.

Table 5-10: Selected field observations and experimental conclusions concerning the light requirements of several species of submersed macrophytes.

Observed an inter-specific variation in LCP's among four species of SAV ranging from 55 μmole photons m^{-2} s^{-1} to 15 μmole photons m^{-2} s^{-1}	VAN ET AL. 1976
Estimated that an average midday irradiance of at least 250 μmole photons m^{-2} s^{-1} would be necessary for seed production	AGAMI and AGAMI 1980
Estimated that the light level that determined the lower depth limit of plant colonization could be as much as 21% of surface light	CHAMBERS and KALFF 1985
Estimated that *Potamogeton perfoliatus* required > 11% of ambient irradiance for survival	GOLDSBOROUGH and KEMP 1988
Observed that an average of 100 umol*m^{-2}*s^{-1} was necessary at the sediment-water interface for submersed macrophytes to survive in the tidal Potomac River	CARTER and RYBICKI 1990
Estimated that 7% of surface light or 505 mol*m^{-2} per year was needed for rooted aquatic plants to grow.	SAND-JENSEN and MADSEN 1991
Concluded that the light requirements for submersed plant growth vary widely and few of these [studies] have distinguished between requirements for plant survival and plant reproduction.	KIMBER ET AL. 1995
Determined that the PPFD for no net growth of *Vallisneria americana*, measured approximately a quarter meter from the sediment surface, was 29 μmole photons m^{-2} s^{-1} or 4.09% surface irradiance.	GRIMSHAW ET AL. 2002
Calculated that the minimum PPFD at the sediment-water interface for no net growth of propagules of *N. guadalupensis* was 60 μmole photons m^{-2} s^{-1} or 7.7% incident irradiance, for *P. illinoensis* was 63 μmole photons m^{-2} s^{-1} or 8.2% incident irradiance, for *V. americana* was 64.1 μmole photons m^{-2} s^{-1} or 8.3% incident irradiance and for *Chara* sp. was 25 μmole photons m^{-2} s^{-1} or 3% incident irradiance..	THIS STUDY

Table 5-11: A comparison of the seasonal variation in light levels at which there was zero net growth of the study species. The percentages and PPFD values listed in the first row for each experiment are values measured at the air-water interface while values listed in the second row occurred at the sediment-water interface.

	Najas		Potamogeton		Vallisneria		Chara	
	LCP (%)	PPFD (μmol photons s^{-1} m^{-2})	LCP (%)	PPFD (μmol photons s^{-1} m^{-2})	LCP (%)	PPFD (μmol photons s^{-1} m^{-2})	LCP (%)	PPFD (μmol photons s^{-1} m^{-2})
Propagule	7.7	59.8	8.2	63.4	8.3	64.1	20.6	159.9
Experiment 1	2.3	17.9	2.4	18.6	2.4	18.7	6.0	46.7
4/27 to 7/14/02								
Propagule	--	--	22.2	182.5	18.3	150.5	11.5	94.4
Experiment 2			6.5	53.3	5.3	43.9	3.3	27.5
4/21 to 6/7/03								
Propagule	--	--	42.7[a]	350.2	16.4	134.9	3.0	25.0
Experiment 3			17.1[a]	140.1	6.6	53.8	1.2	10.0
6/28 to 8/2/03								

[a]Values not considered representative due to herbivory and/or competitive shading by other study species.

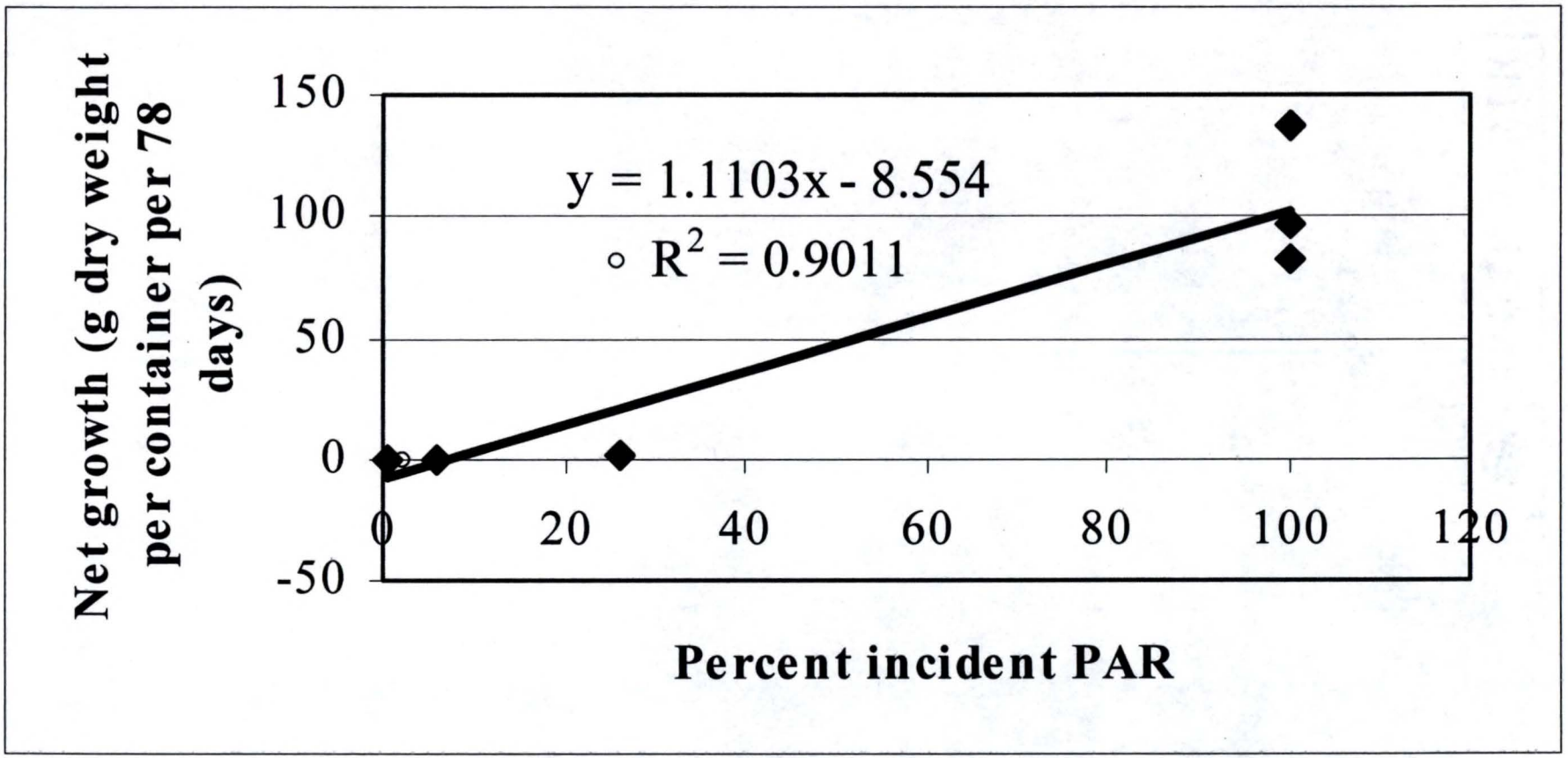


Figure 5-1: Propagule light requirements Experiment 1: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 78 days) of *Najas guadalupensis*. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

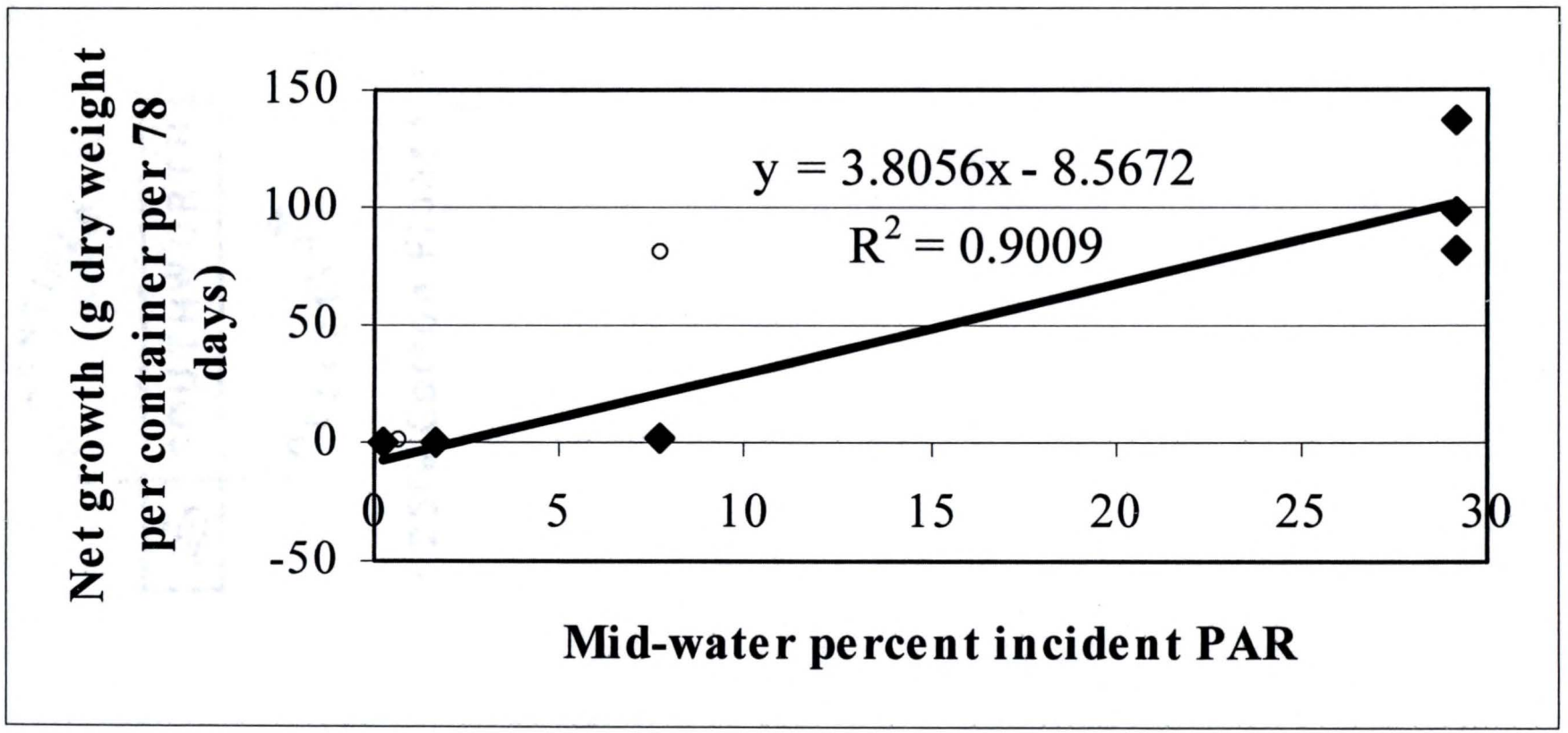


Figure 5-2: Propagule light requirements Experiment 1: Linear regression of mid-water PAR, measured at the sediment-water interface, versus the net growth (g dry weight per container per 78 days) of *Najas guadelupensis*. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

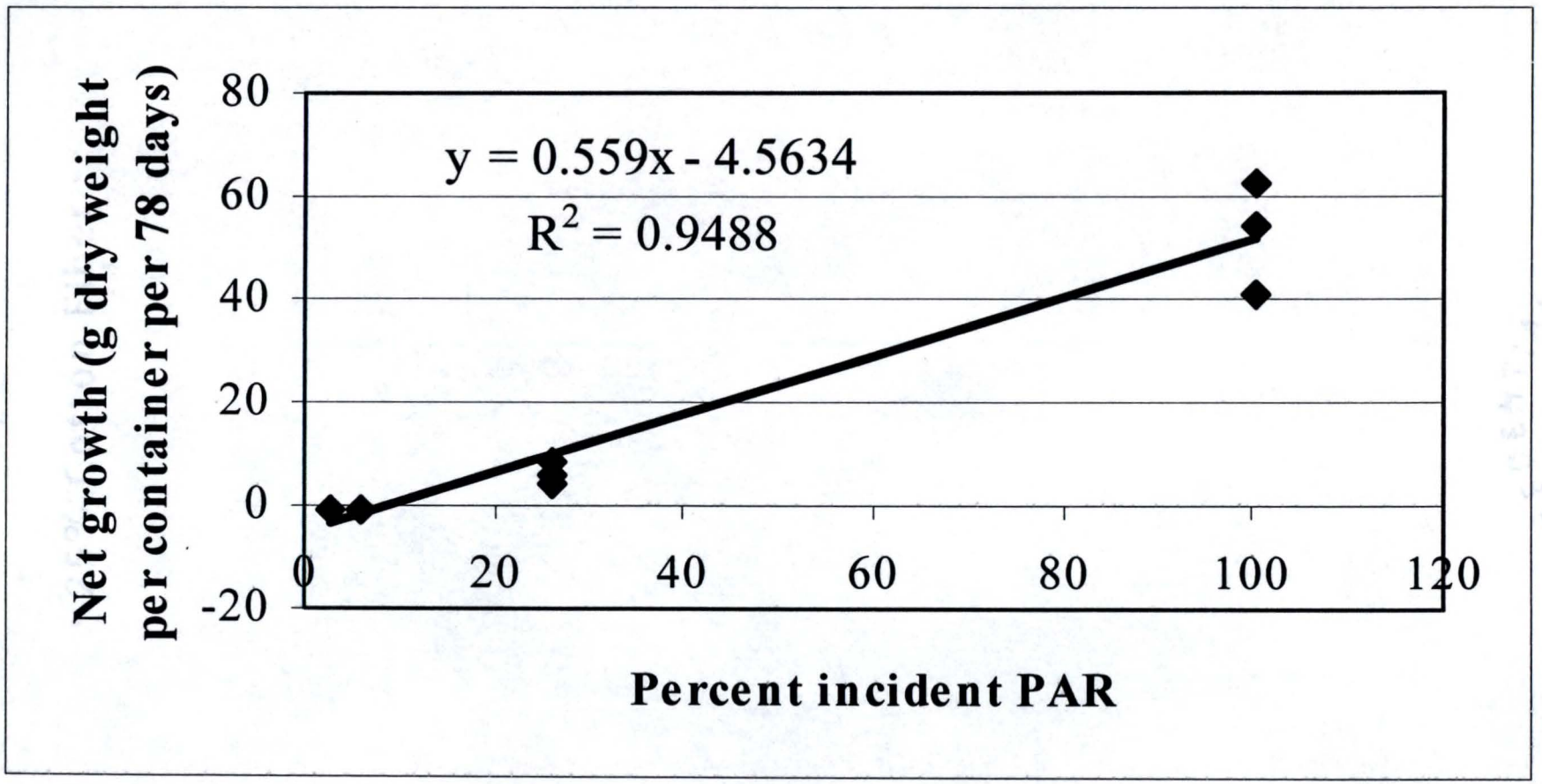


Figure 5-3: Propagule light requirements Experiment 1: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 78 days) of *P. illinoensis*. Since there was no survival in the two lowest light treatment groups, the 1% light group was excluded from the model.

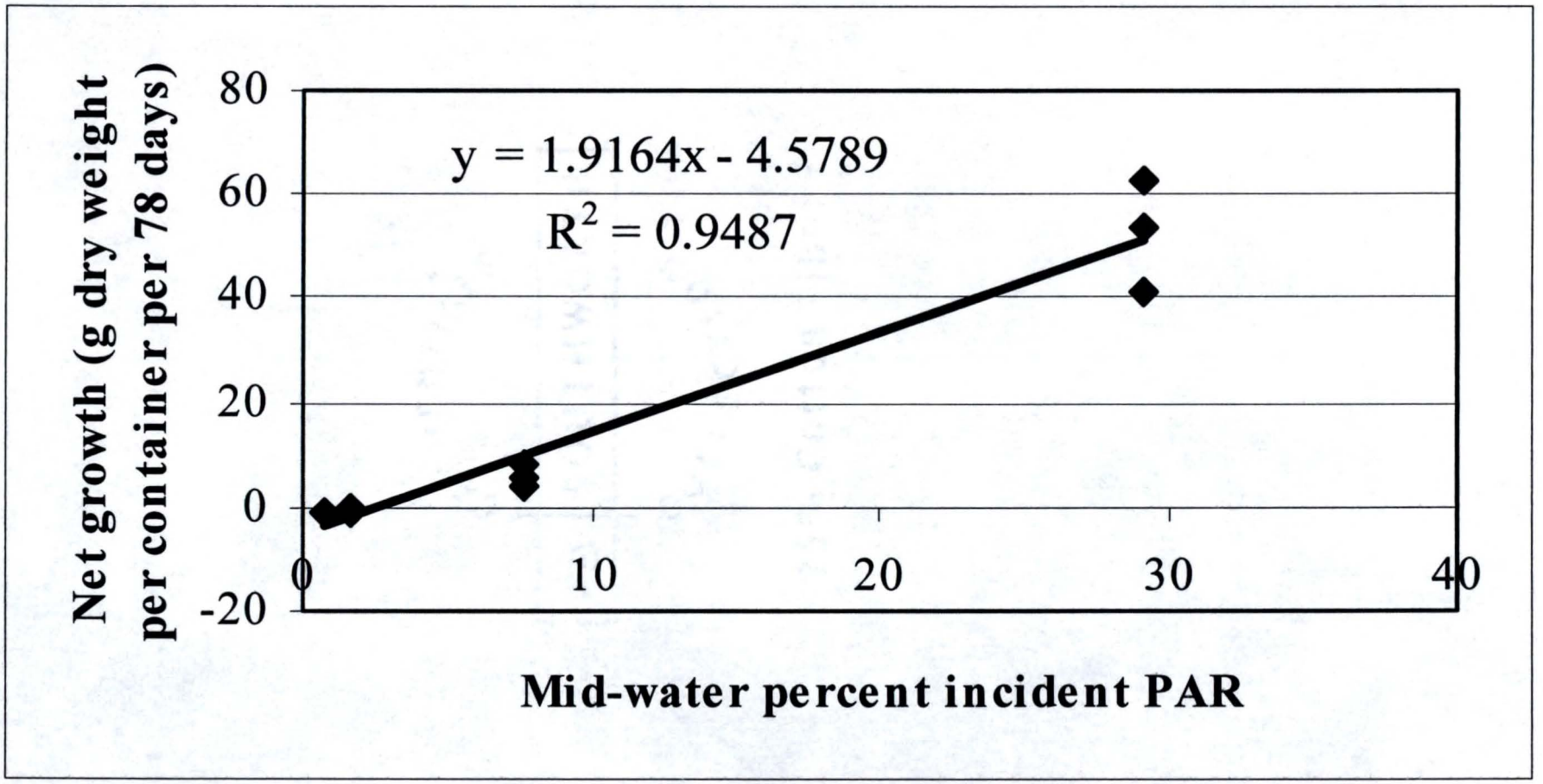


Figure 5-4: Propagule light requirements Experiment 1: Linear regression of mid-water PAR measured at the sediment-water interface versus the net growth (g dry weight per container per 78 days) of *P. illinoensis*. Since there was no survival in the two lowest light treatment groups, the 1% light group was excluded from the model.

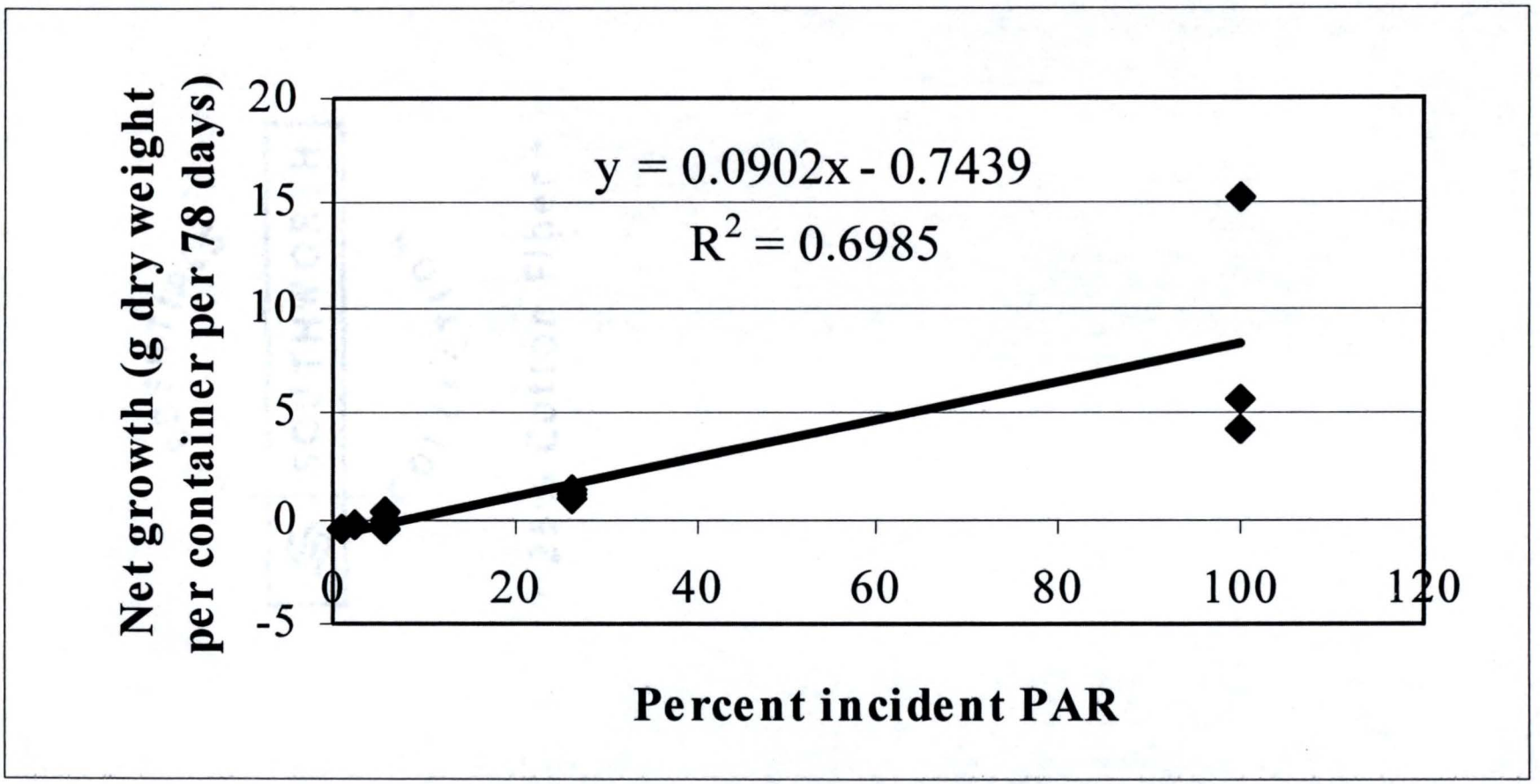


Figure 5-5: Propagule light requirements Experiment 1: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 78 days) of *V. americana*.

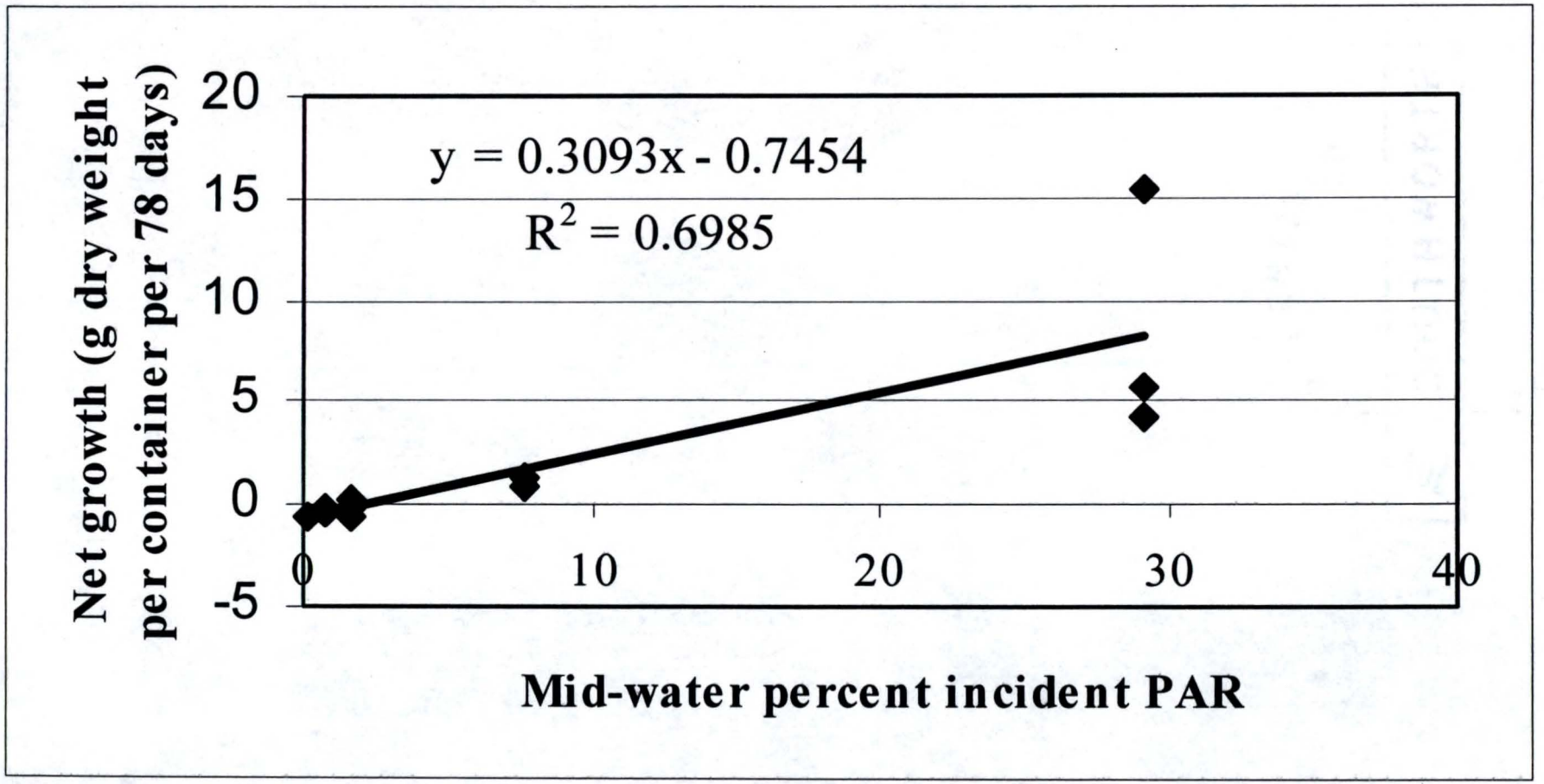


Figure 5-6: Propagule light requirements Experiment 1: Linear regression of mid-water PAR, measured at the sediment-water interface, versus the net growth (g dry weight per container per 78 days) of *V. americana*.

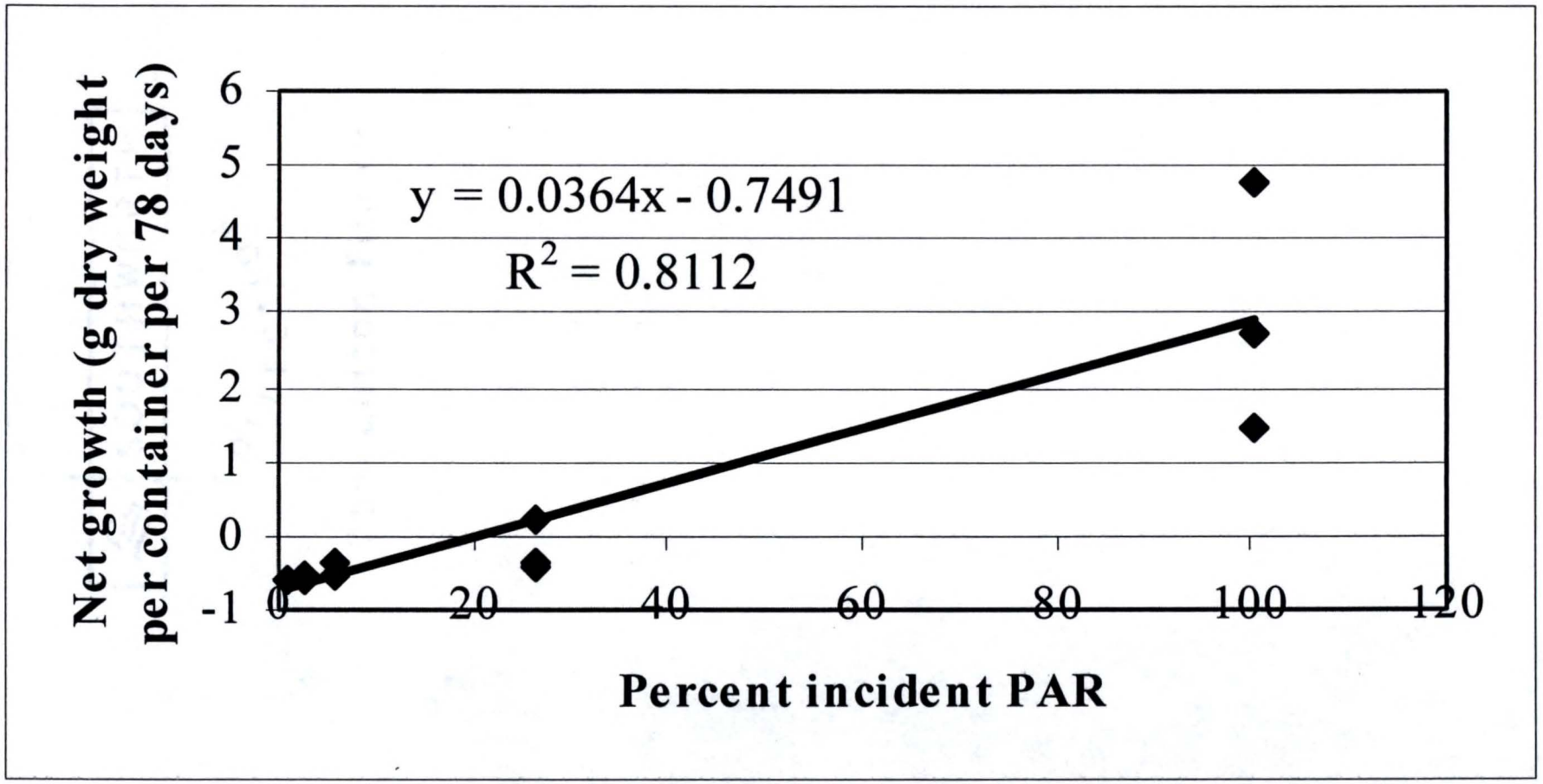


Figure 5-7: Propagule light requirements Experiment 1: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 78 days) of *Chara* sp.

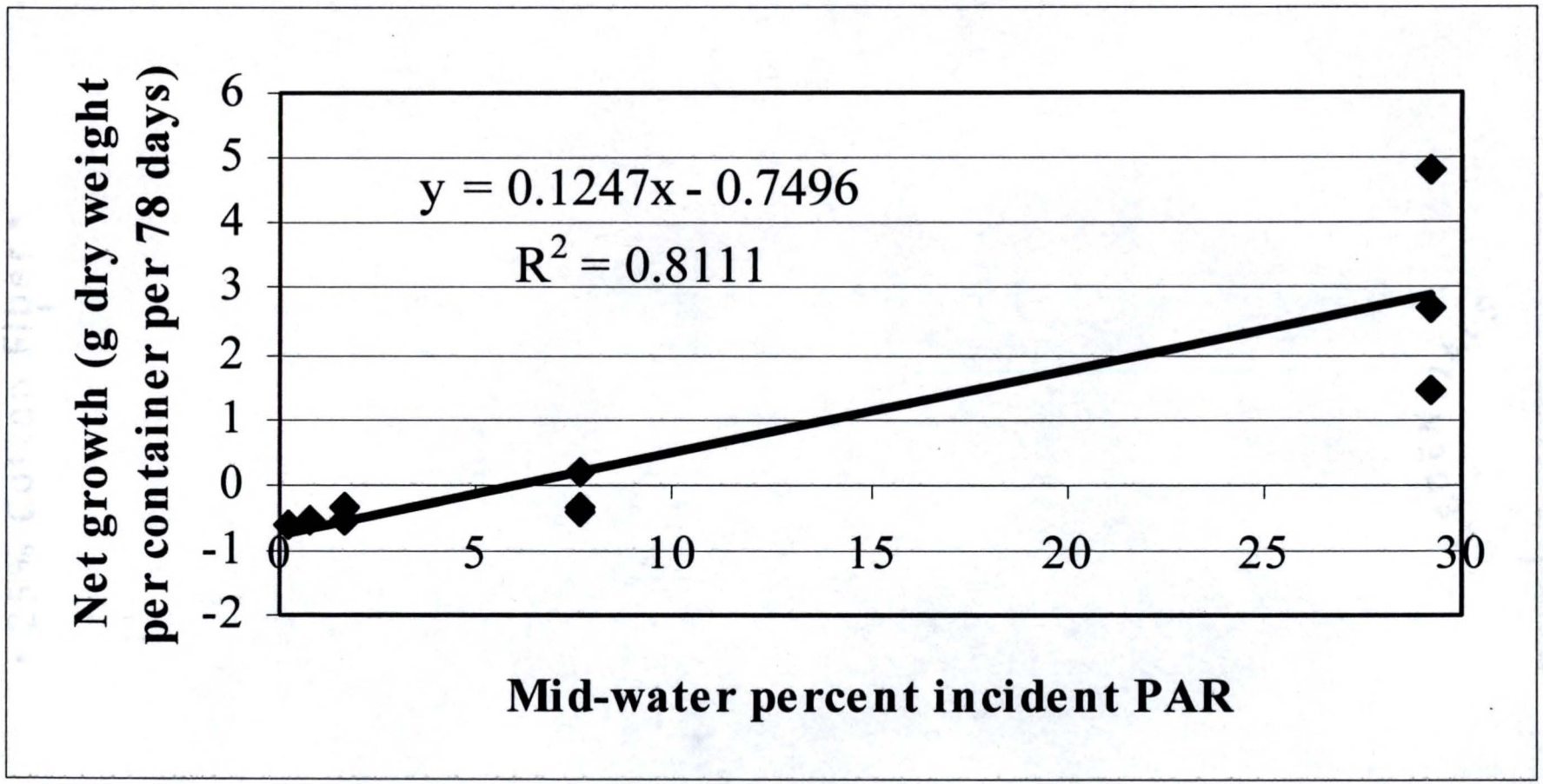


Figure 5-8: Propagule light requirements Experiment 1: Linear regression of mid-water PAR, measured at the sediment-water interface, versus the net growth (g dry weight per container per 78 days) of *Chara* sp.

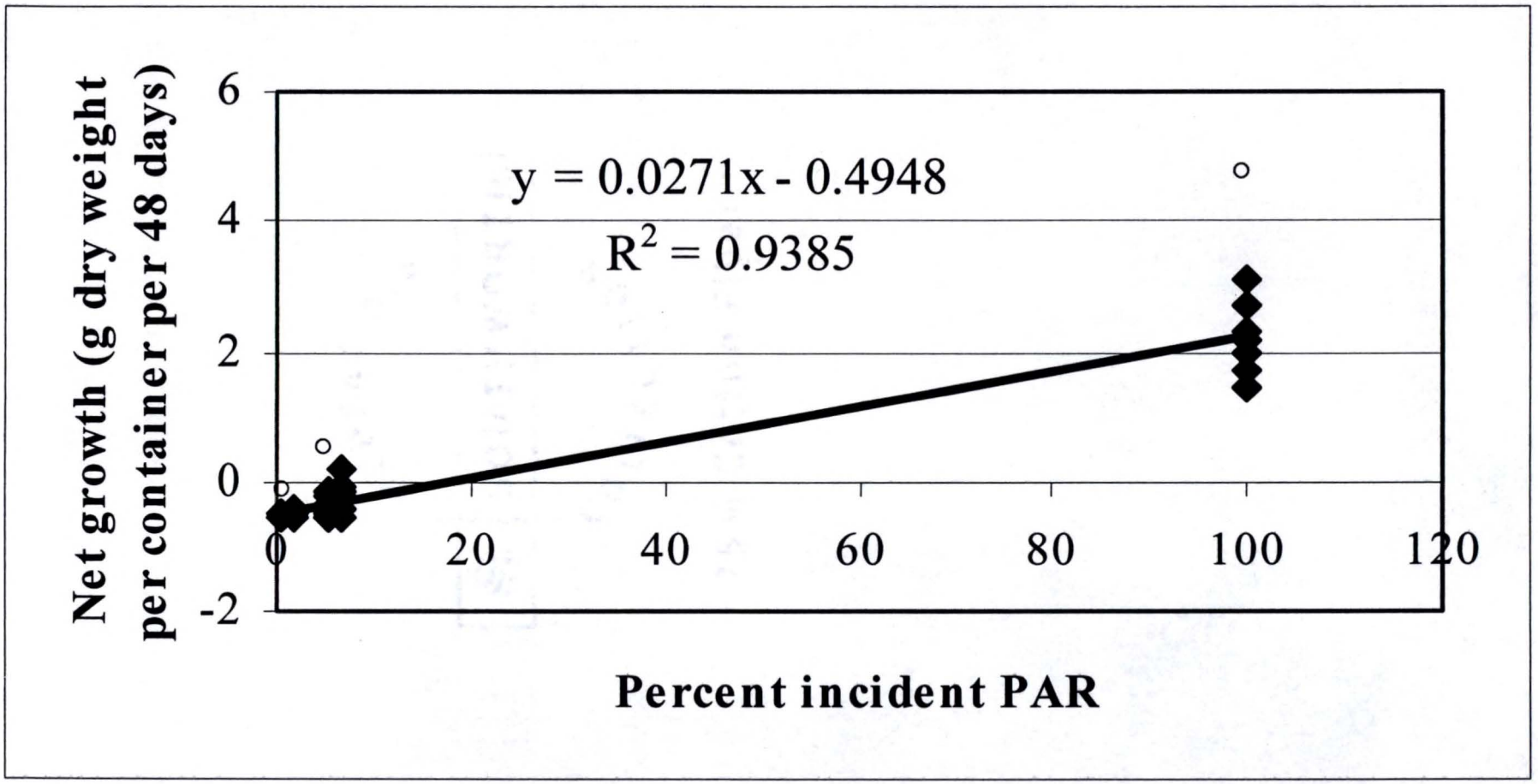


Figure 5-9. Propagule light requirements Experiment 2: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 48 days) of *V. americana*. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

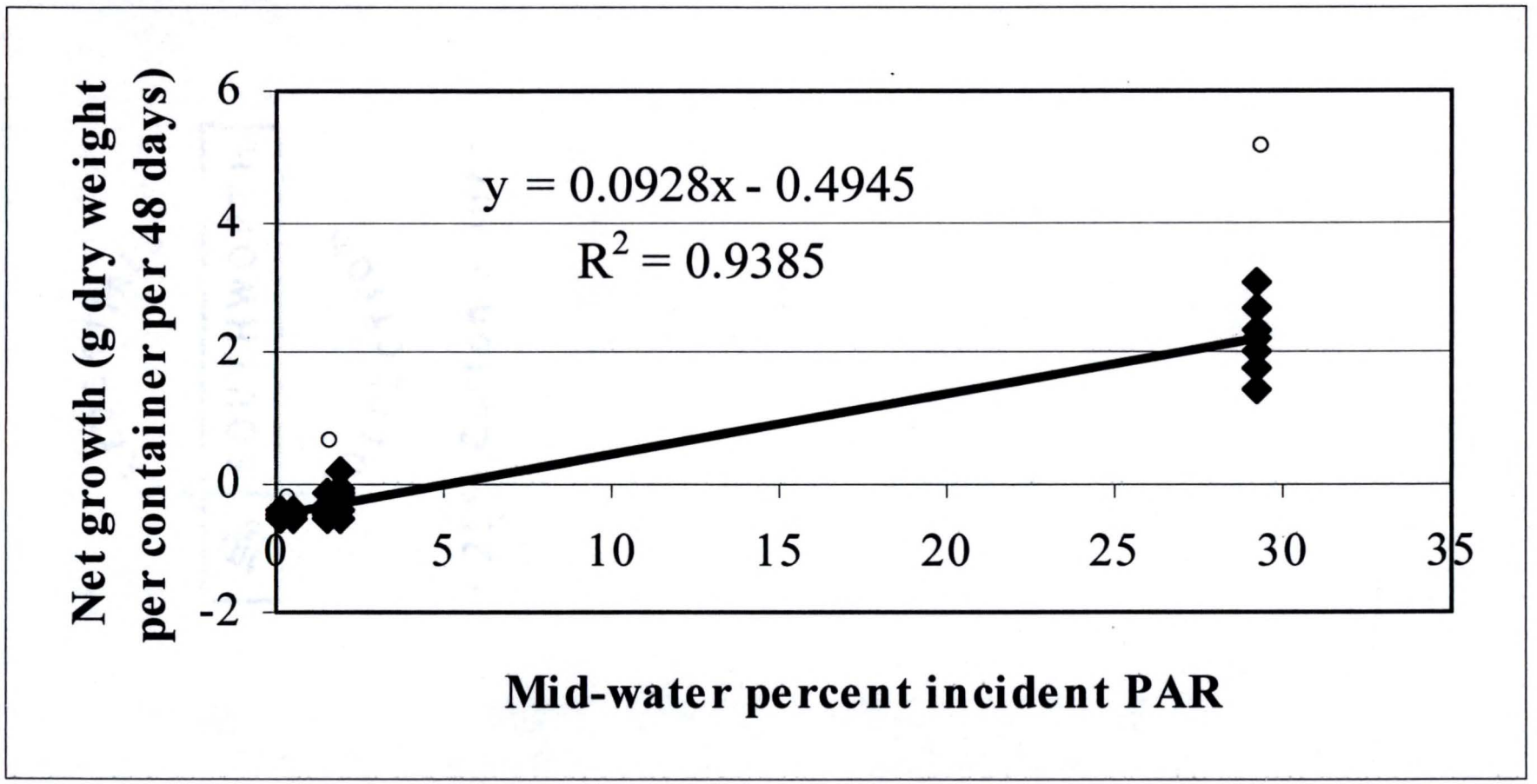


Figure 5-10. Propagule light requirements Experiment 2: Linear regression of mid-water PAR, measured at the sediment-water interface, versus the net growth (g dry weight per container per 48 days) of *V. americana*. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

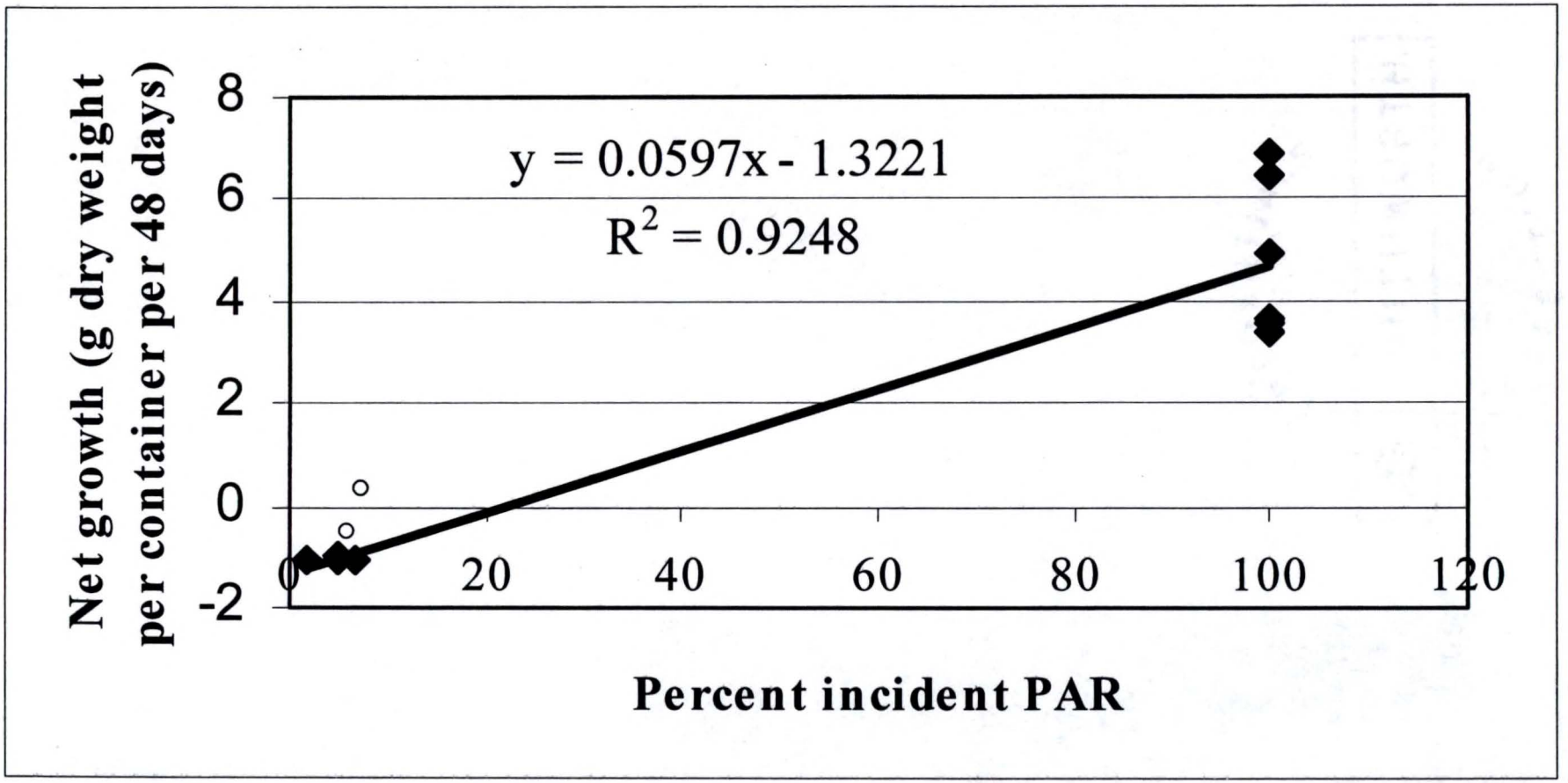


Figure 5-11. Propagule light requirements Experiment 2: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 48 days) of *P. illinoensis*. Outlying data points were excluded from analysis and are indicated in the figure as open circles. Since there was no survival in the two lowest light treatment groups, the 1% light group was excluded from the model.

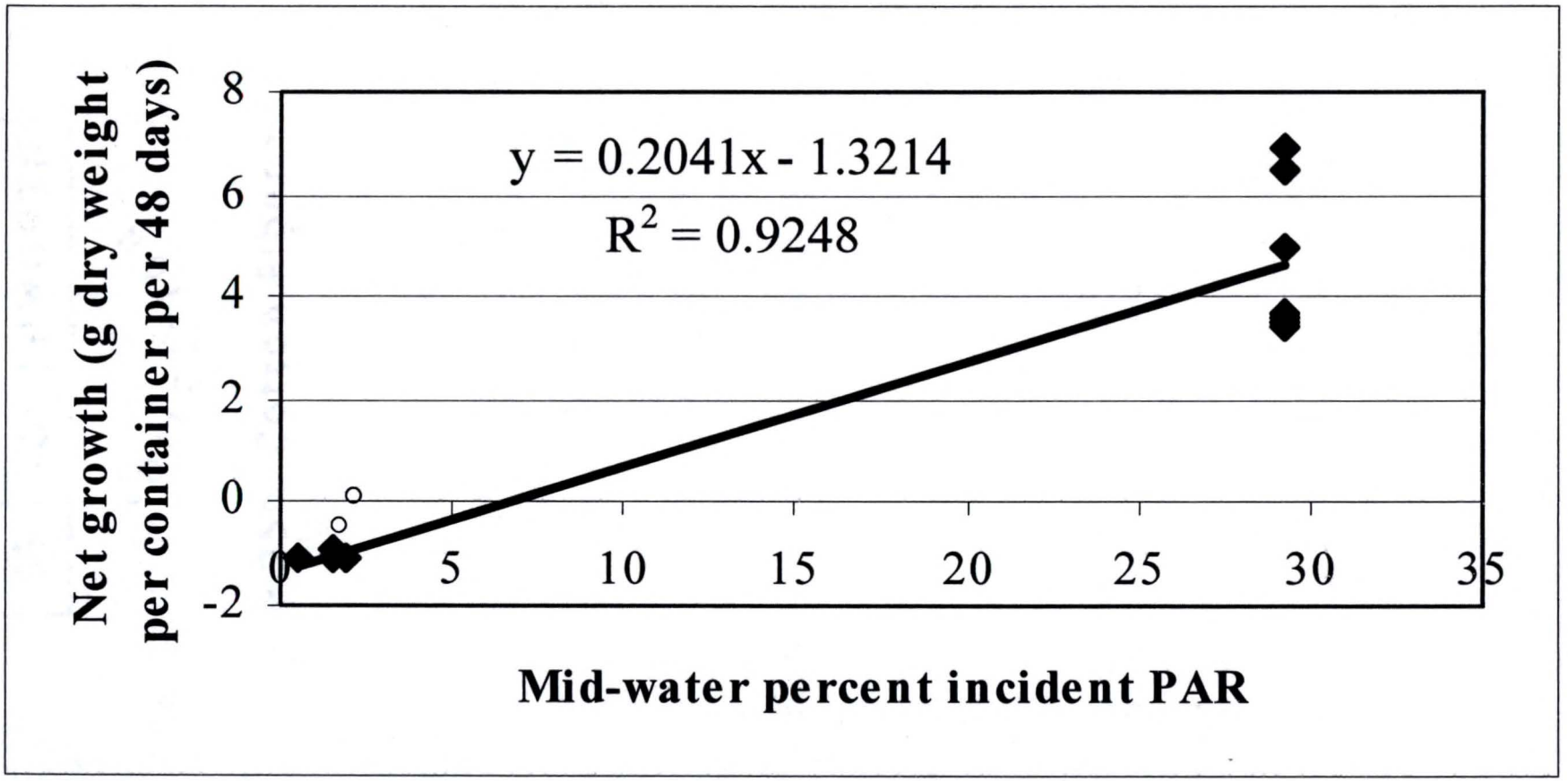


Figure 5-12. Propagule light requirements Experiment 2: Linear regression of mid-water PAR, measured at the sediment-water interface, versus the net growth (g dry weight per container per 48 days) of *P. illinoensis*. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

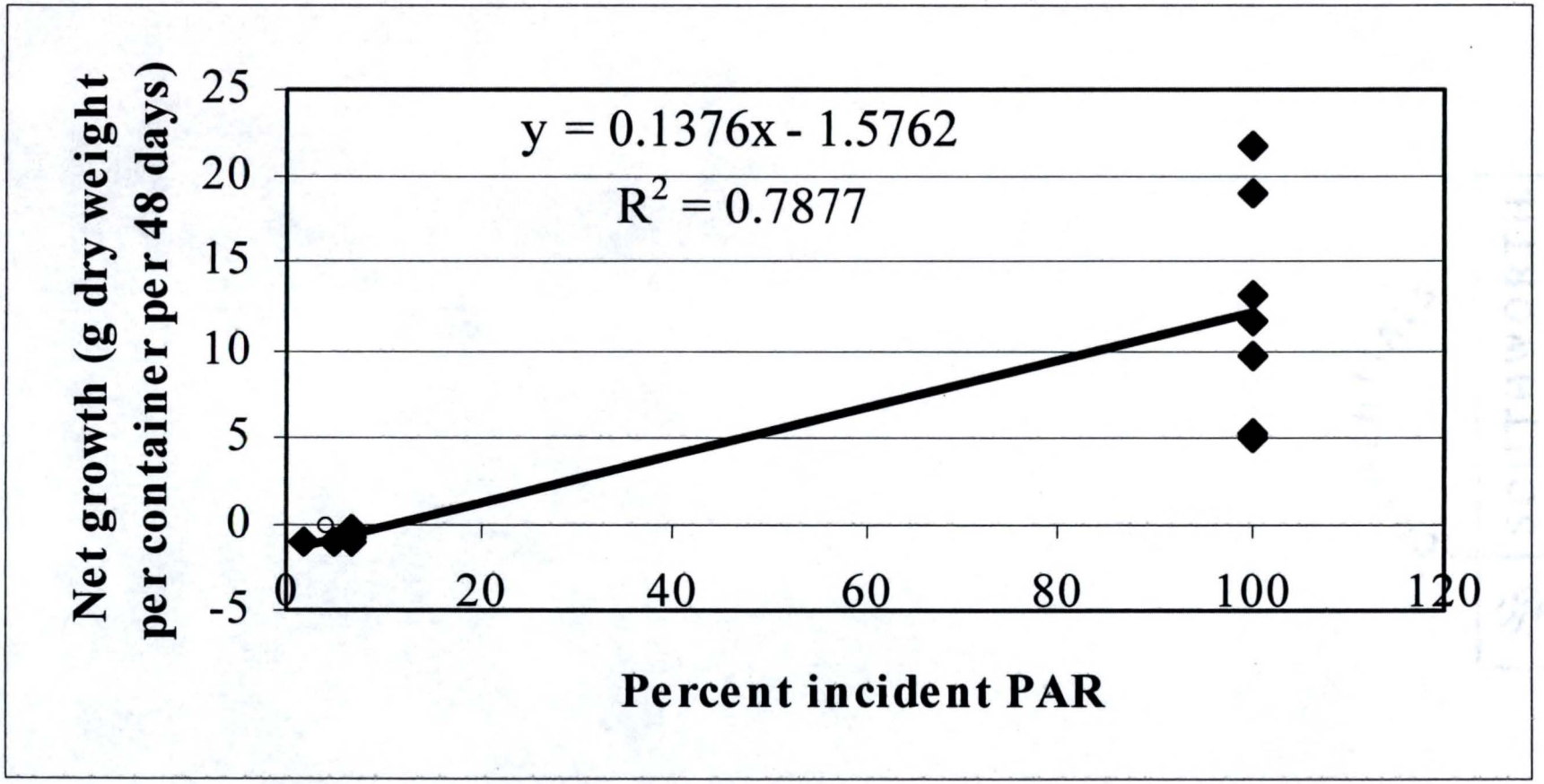


Figure 5-13. Propagule light requirements Experiment 2: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 48 days) of *Chara* sp. Outlying data points were excluded from analysis and are indicated in the figure as open circles. Since there was no survival in the two lowest light treatment groups, the 1% light group was excluded from the model.

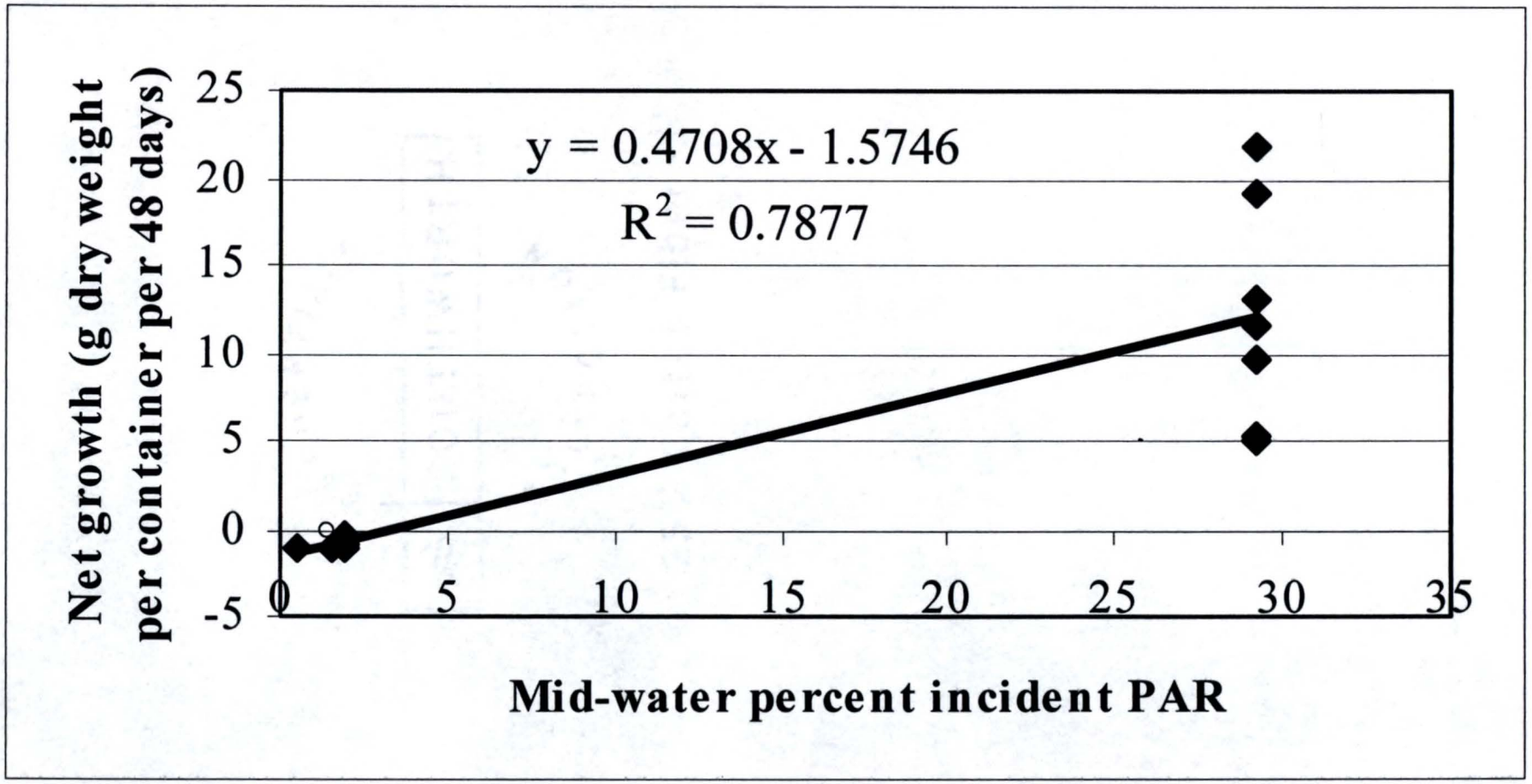


Figure 5.14. Propagule light requirements Experiment 2: Linear regression of mid-water PAR, measured at the sediment-water interface, versus the net growth (g dry weight per container per 48 days) of *Chara* sp. Outlying data points were excluded from analysis and are indicated in the figure as open circles. Since there was no survival in the two lowest light treatment groups, the 1% light group was excluded from the model.

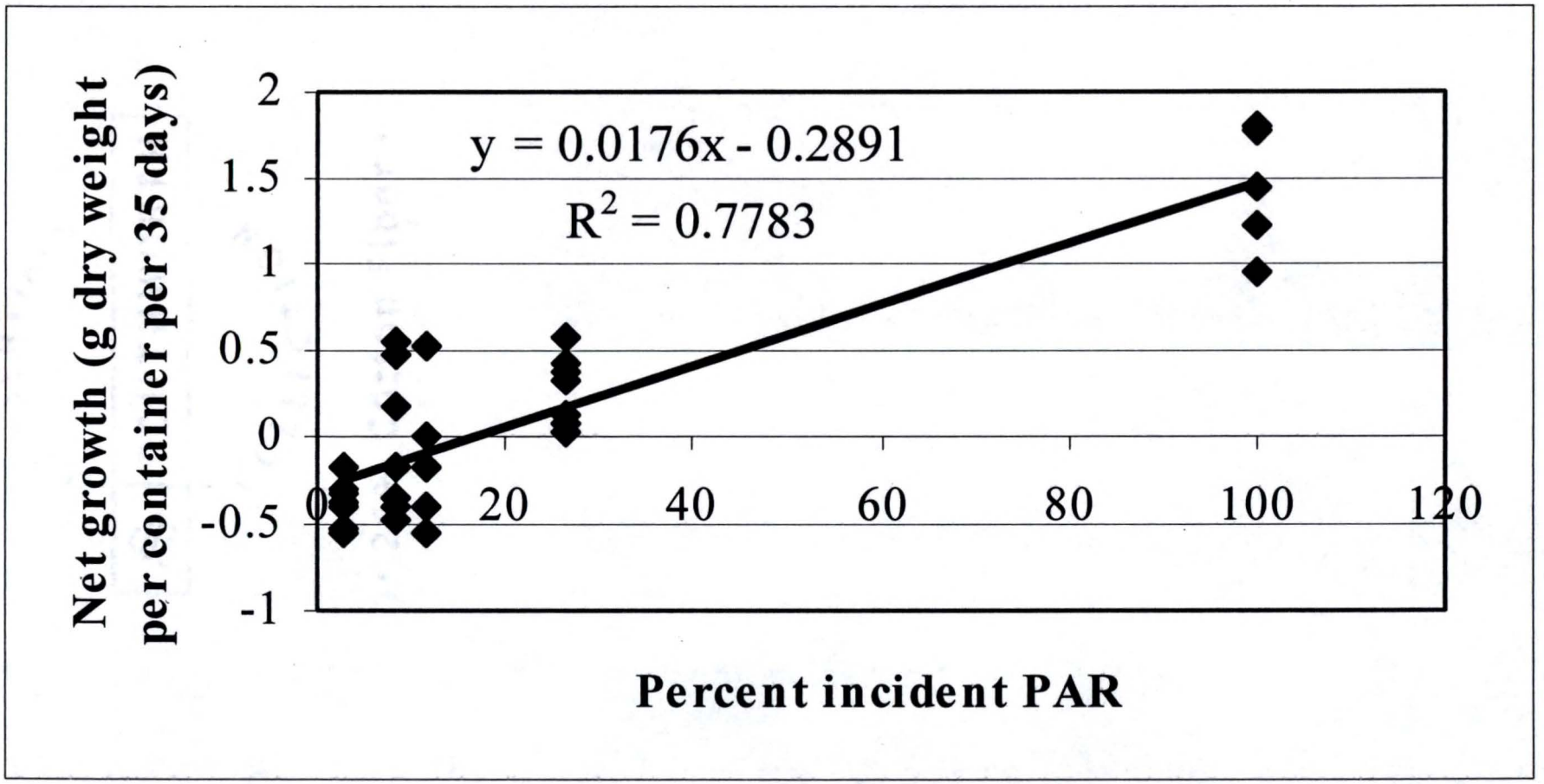


Figure 5-15: Propagule light requirements Experiment 3: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 48 days) of *V. americana*.

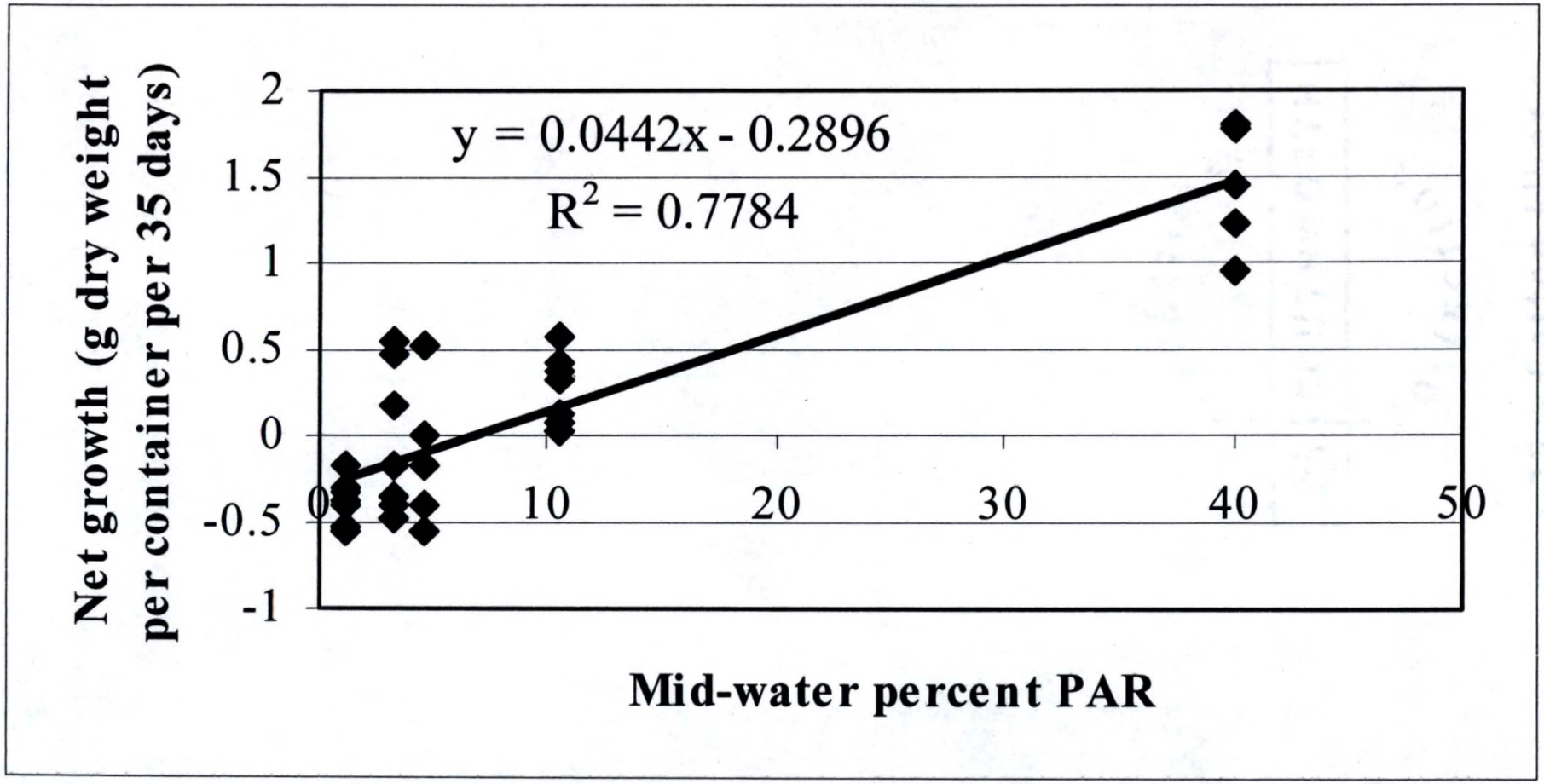


Figure 5-16: Propagule light requirements Experiment 3: Linear regression of mid-water PAR, measured at the sediment-water interface, versus the net growth (g dry weight per container per 35 days) of *V. americana*.

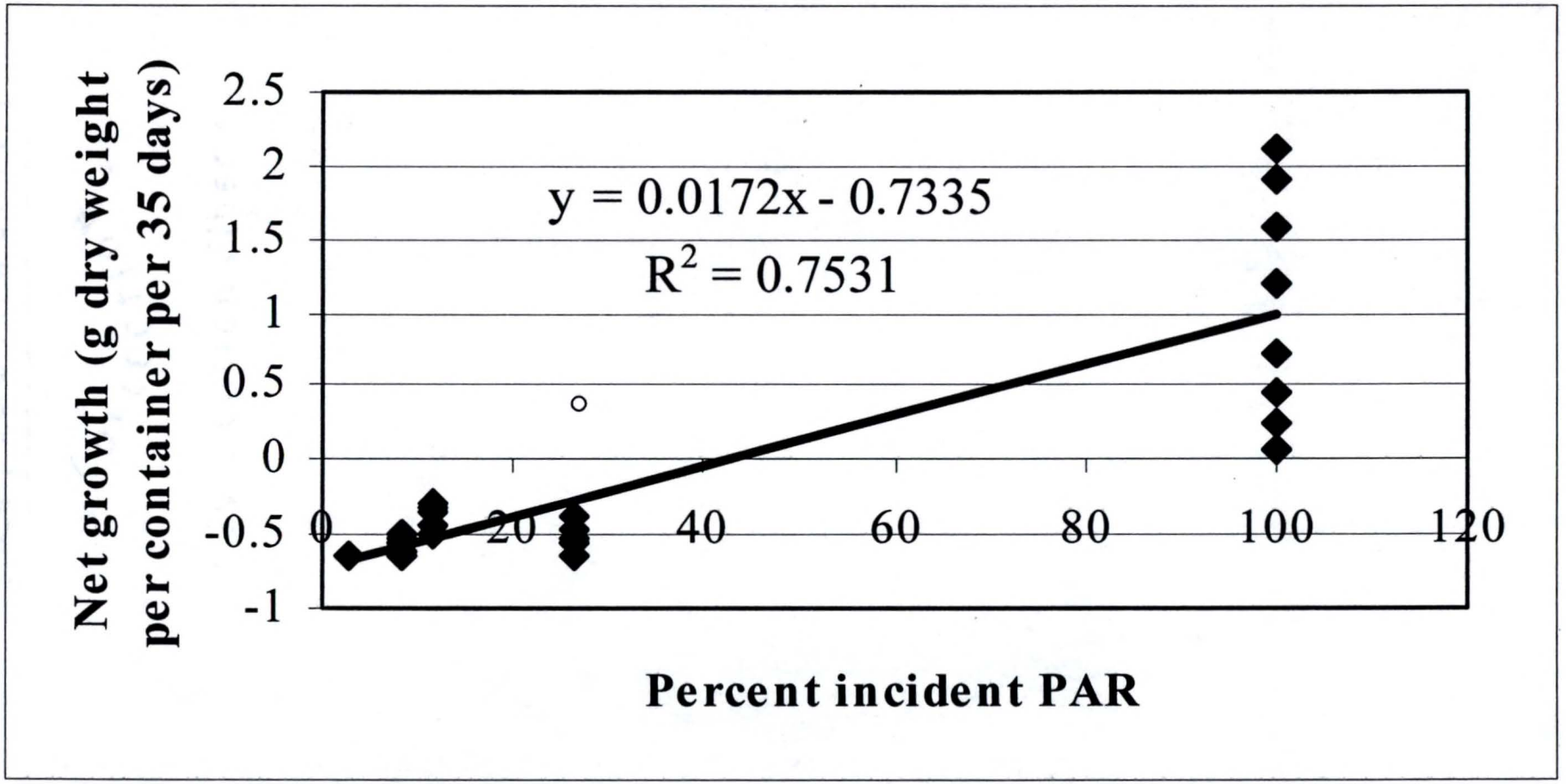


Figure 5-17: Propagule light requirements Experiment 3: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 35 days) of *P. illinoensis*. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

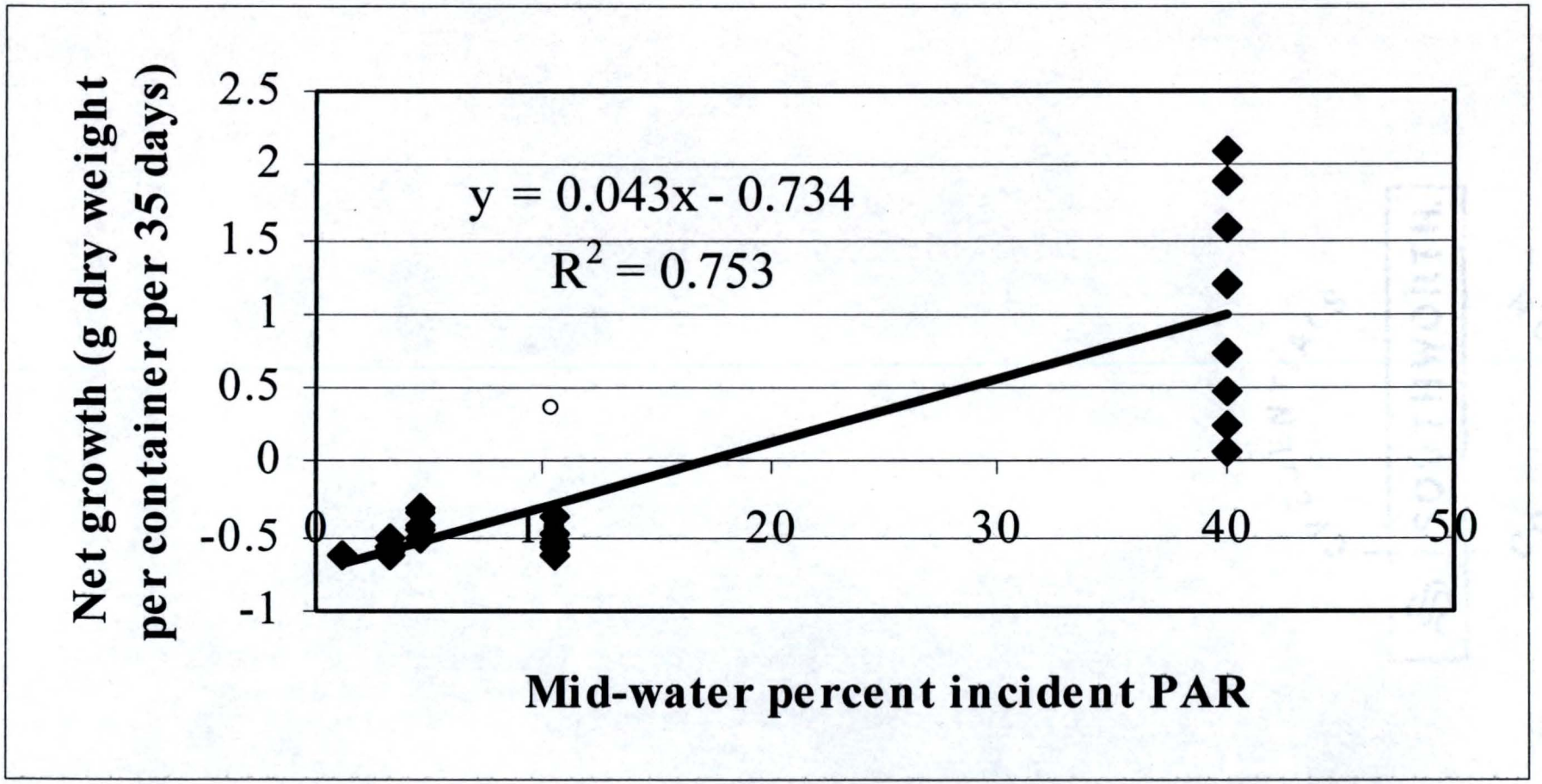


Figure 5-18: Propagule light requirements Experiment 3: Linear regression of mid-water PAR, measured at the sediment-water interface, versus the net growth (g dry weight per container per 35 days) of *P. illinoensis*. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

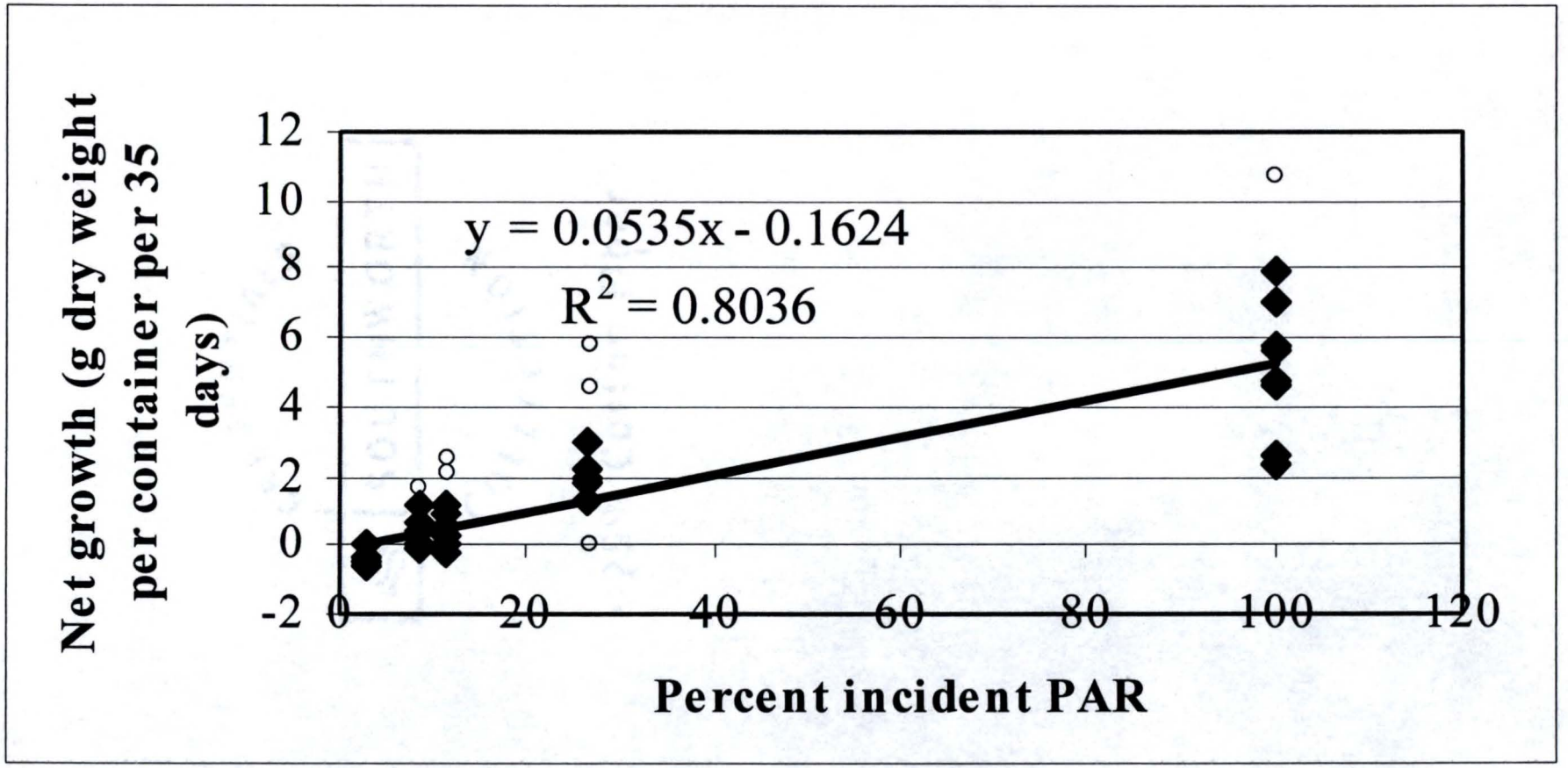


Figure 5-19: Propagule light requirements Experiment 3: Linear regression of PAR at the air-water interface versus the net growth (g dry weight per container per 35 days) of *Chara* sp. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

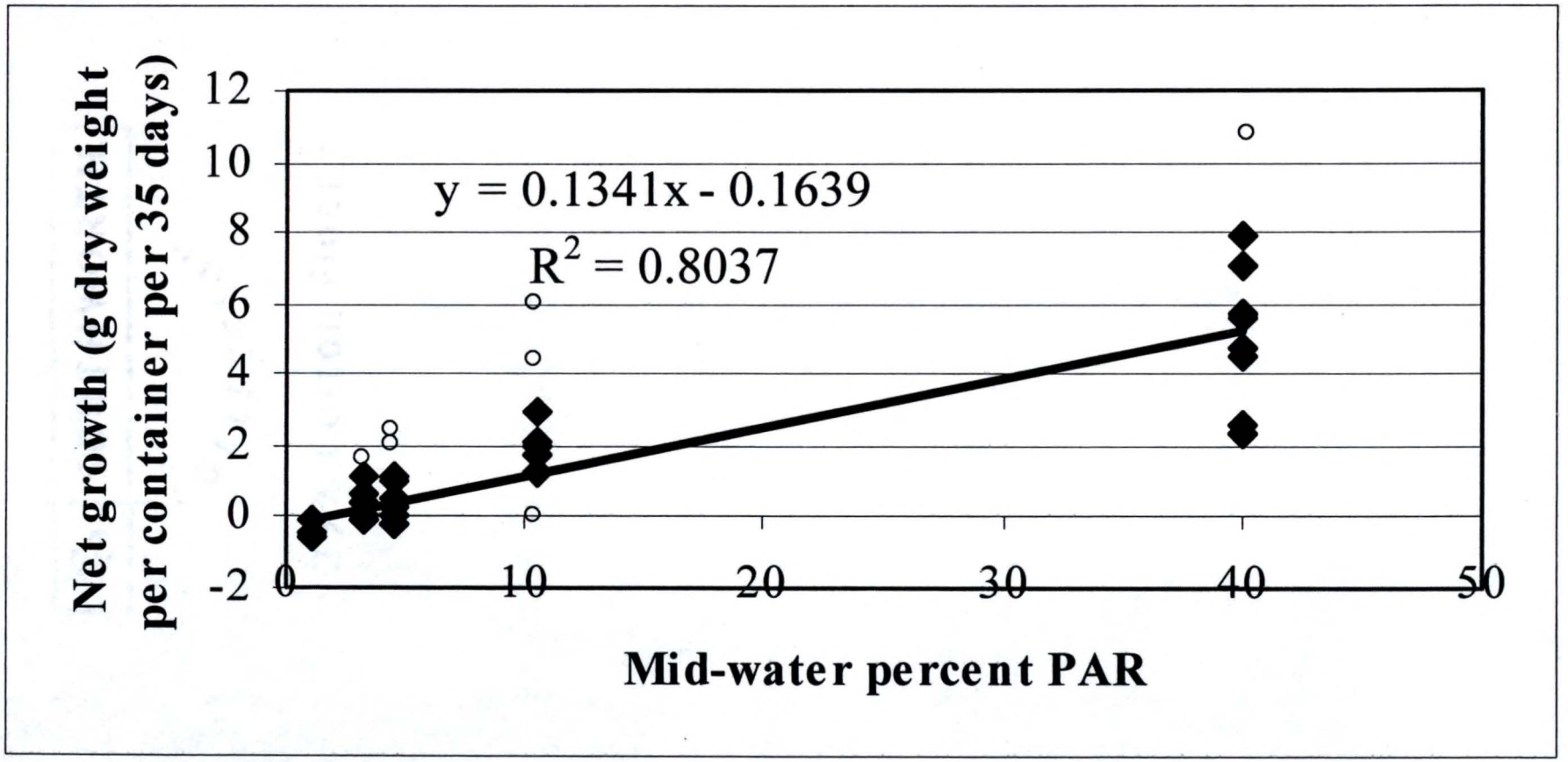


Figure 5-20: Propagule light requirements Experiment 3: Linear regression of mid-water PAR, measured at the sediment-water interface, versus the net growth (g dry weight per container per 35 days) of *Chara* sp. Outlying data points were excluded from analysis and are indicated in the figure as open circles.

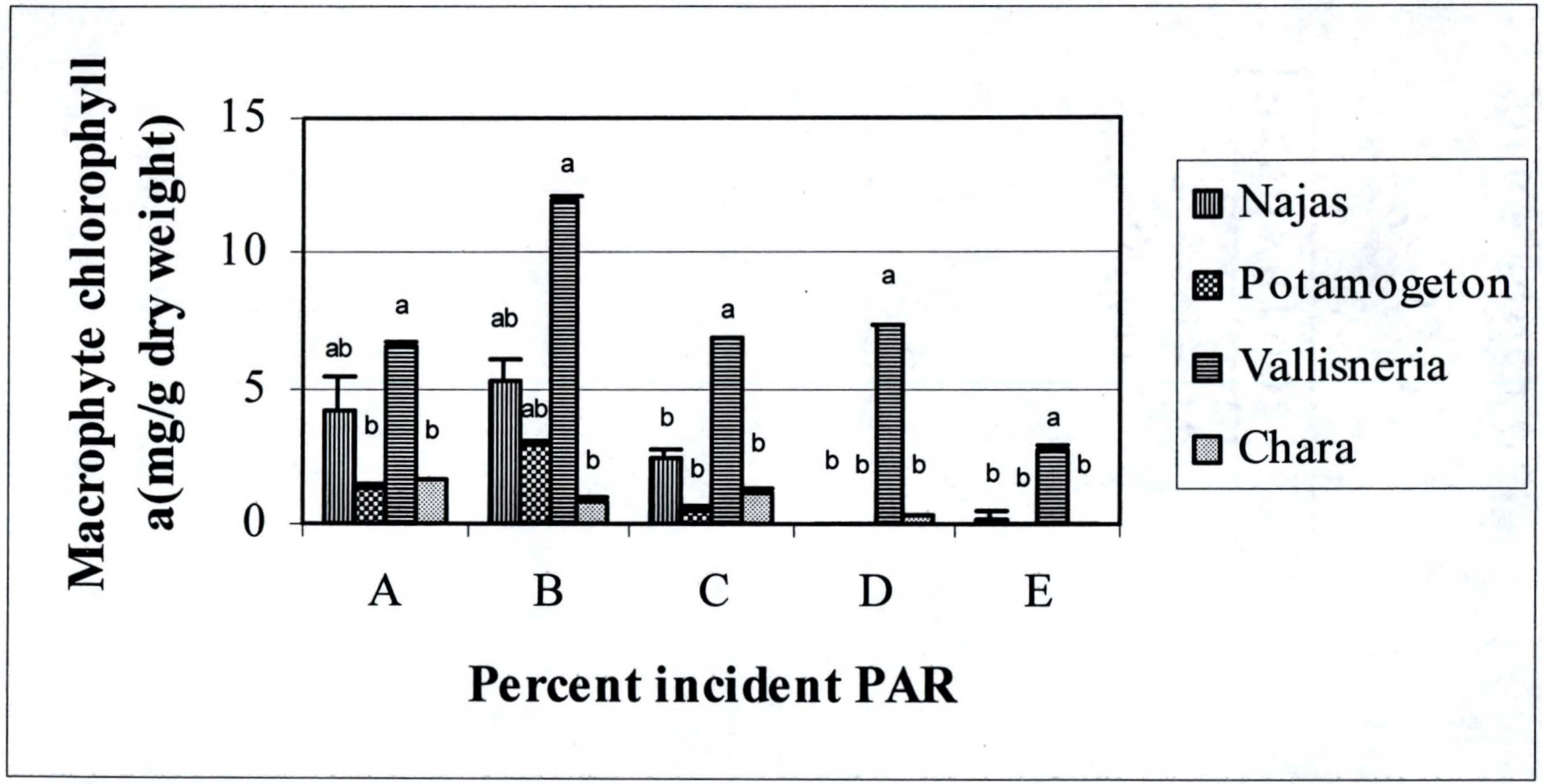


Figure 5-21: Among species comparisons of macrophyte chlorophyll *a* produced during propagule plant light requirement Experiment 1 (4/27 to 7/14/02). Bars represent the means of three samples (n=3) per macrophyte species. Error bars represent the standard error. Means with the same letter are not significantly different at the 5% level according to Tukey's HSD Method.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Submerged vascular macrophytes are an important ecological component of aquatic systems. These primary producers provide habitat for invertebrates, epiphytes, fish and a variety of other organisms. The distribution and growth of submersed macrphytes are influenced by a variety of environmental factors including sediment and light.

The results of Objective 1 of this study indicated that the sediments occurring at stations B, D, F and H in Lake Hollingsworth were suitable substrates for the growth of *Najas guadalupensis*, *Potamogeton illinoensis*, *Vallisneria americana* and *Chara* sp. when grown in experimental growth tanks. These results, in combination with the observation that root to shoot ratios were always less than 1 for all plant types, indicate that the sediment nutrient concentrations investigated in this study were adequate to support the growth of all four species. Field-testing of these results is necessary in order to determine the effect of such factors as sediment quantity on the applicability of these findings to the natural environment. Late spring appeared to be the optimum time in which to introduce propagules of *Najas guadalupensis*, *Potamogeton illinoensis*, *Vallisneria americana* and *Chara* sp. into systems to be restored. *N. guadalupensis*, *V. americana* and *Chara* sp. all exhibited the most luxurious growth during the summer months. *P. illinoensis* also produced vigorous summertime growth. Accordingly, planting in late spring should provide sufficient

time for the plants to acclimate to their new environment and in order to be able to maximize their growth potential during the summer. This period of strong growth should allow the plants to firmly establish. Plant growth appeared to be influenced by a combination of factors including light, water temperature and sediment nutrients.

The results of Objective 2 indicated that there was a decrease in total biomass produced by *Najas guadalupensis*, *Potamogeton illinoensis*, *Vallisneria americana* and *Chara* sp. in response to decreasing PAR. Above to below-ground biomass ratios for all species also decreased as the light decreased. The perennial species, *P. illinoensis* and *V. americana*, produced significantly greater below-ground biomass as compared to the annual, *N. guadalupensis*. Comparison of the growth response of mature and propagule representatives of the same species indicted that propagules of *N. guadalupensis, P. illinoensis* and *V. americana* had higher light requirements for net growth as compared with mature plants of the same species. This observation is probably best explained by the large quantity of energy required to power all of the metabolic activities involved in the initial extension of shoot material up through the water column. This trend was reversed for *Chara sp.* The reason for this discrepancy is presently unknown. Temporal varaiation in macrophyte light requirements was also observed. Generally, macrophytes exhibited higher light requirements for net growth during the late winter to early spring culture period. Establishment of the study species during the growing season will require PAR levels that will provide sufficient energy for the growth of each of the individual species. The findings of this research indicated that the light required for no net loss of mature plants ranged from 2 to 50% incident irradiance while propagule light requirements ranged from 3 to 22.5%. This

variation in light requirements among the species and between mature and propagule plants suggests that greater quantities of light would be required to establish a diverse habitat than would be required to grow any individual species.

V. americana, appeared to be the most well-adapted study species for survival in low light environments. Light requirements for mature plants ranged from 2 to 6% incident light and 8.3 to 18.3 % for propagule plants. The combination of low light capability, high concentration of chlorophyll *a* and extensive root structure possibly confer an advantage to *V. americana* in shallow turbid systems.

The results of this study indicate the need for further investigation of the effects of sediment and light on native submerged macrophytes. The limited range of sediment organic matter contents and nutrient levels investigated probably obscured macrophyte growth responses to some sediment factors in this study. High variances observed in the data collected in both Objectives also probably confounded the identification of statistically significant differences in many cases.

Many questions remain unanswered for lake managers seeking guidelines for the use in selecting and establishing desirable native plants in restored systems. In order to provide these answers, additional investigation of the relationship between plant nutrition and sediment physical and chemical composition is needed. Further study is also needed in order to quantify the amount of light required by submersed macrophytes in all stages of their life cycles. A better understanding of the role of light in inter-specific competition is also needed.

APPENDIX
METHODS

Grid Method

Lake Hollingsworth is a shallow, highly productive lake with the potential for highly variable organic sediment distribution. In their 1996 study of the variability of sediment distribution in shallow Florida lakes, Whitmore et al. (1996) indicate the importance of using systematic mapping to locate optimal coring sites. In order to ensure representative assessment of littoral sediments, a sampling scheme was developed by superimposing a grid on the bathymetric map of Lake Hollingsworth drawn on April 13, 1992 (Figure A.1). Use of the grid method facilitated identification of sampling stations distributed evenly throughout the lake littoral zone to ensure equal area coverage of the littoral region (Hakanson 1981). The convention, when using the grid method, is that stations can either be located within the space between the lines or at the intersection of lines. In this study, stations were located within the spaces between the lines. The sampling grid has twelve littoral stations of which eight were sampled. Four of the grid stations did not include the 0.8 m contour line and were disregarded. Grid stations included in the survey were lettered from A to H proceeding in a counterclockwise direction. Stations on the grid were an average of 0.45 kilometers apart. Navigation to sampling stations was achieved using a Trimble CDS1 GPS unit.

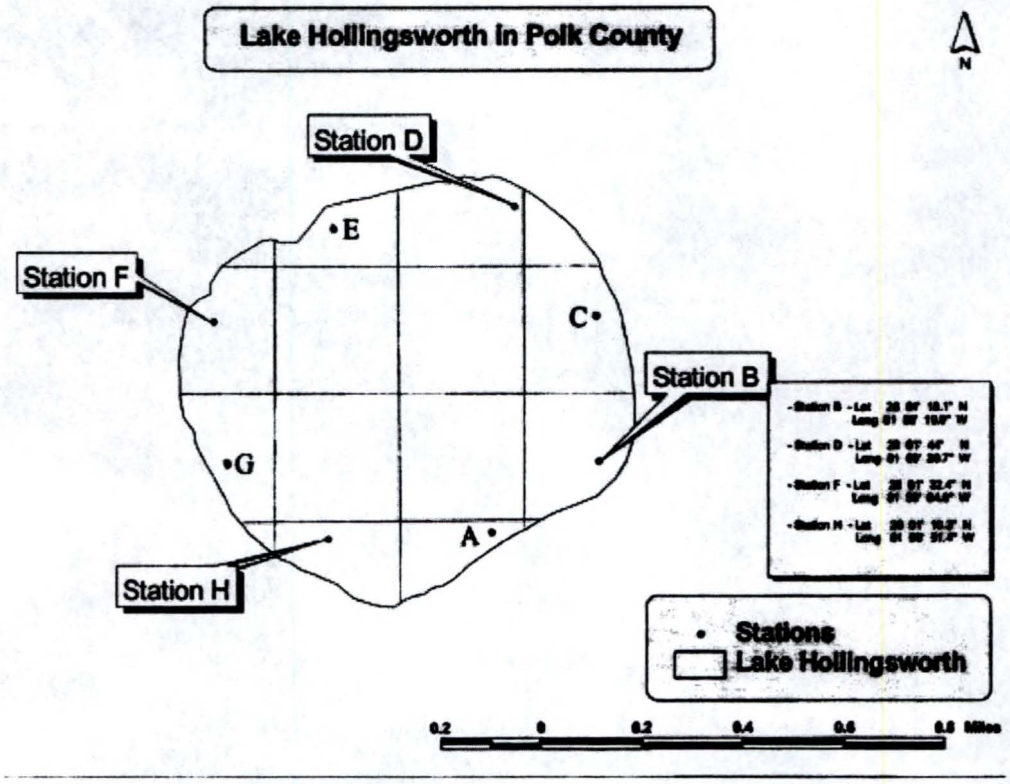


Figure A1. Sampling grid for identifying sediment survey study station locations. Spaces within lines mark sampling sites and were established by superimposing a grid on the bathymetric map of Lake Hollingsworth drawn on April 13, 1992.

Sediment Nutrient Analyses

Sediment nutrient analyses were performed using the Mehlich-1 Extraction Procedure (Southern Region Information and Exchange Group on Soil Testing and Plant Analyses 1983) by the Analytical Research Laboratory of the Soil and Water Science Department, University of Florida, Gainesville. As described in the UF/IFAS Extension Soil Testing Laboratory (ESTL) Analytical Procedures and Training

Manual written by Rao S. Mylavarapu and Elizabeth D. Kennelley, 4-cm3 of dry soil (approximately 5 g mineral soil) are scooped into an extracting bottle. Twenty mL of Mehlich-1 Extracting Solution is added to the bottle. The extracting solution consists of a combination of 0.0125M H_2SO_4 and 0.05M HCl. Samples are shaken on a reciprocal shaker for 5 min. Samples are then filtered and sediment nutrient concentrations are measured using inductively coupled argon plasma (ICAP) spectroscopy. Instrument readings, reported in mgL^{-1} are then converted to $mgKg^{-1}$ DWT using the following equation:

$$\frac{mg}{L} * \frac{1L}{1000mL} * \frac{mLsoln}{gsoil} * \frac{1000g}{1kg} = \frac{mg}{kg}$$

Epiphyte Biomass Determination Method

The mechanical removal method described by Zimba and Hopson (1997) was used to separate the epiphytic algae from the individual macrophytes. Three randomly selected individual macrophytes were placed in separate 1-L plastic bottles containing 100 mL of distilled water. Each bottle was then agitated by hand at approximately 180 revolutions per minute. A subsample of the resultant epiphyte suspension was filtered through 1 mm screening to remove macrophyte fragments.

The epiphyte slurry was subsampled to facilitate the investigation of two parameters. A subsample of the suspension was concentrated onto glass fiber filters (0.7μm porosity). Epiphyte chlorophyll samples were processed and chlorophyll a and phaeophytin a and chlorophyll a, b and c were determined in accordance with Standard Methods (SM 10200 H) (A.P.H.A. 1995) guidelines and equations. Epiphyte chlorophyll concentrations were normalized to dry weight of host plant.

Mean epiphyte biomass per gram dry weight calculated for each macrophyte species was used to estimate the total epiphytic component of the final biomass (g dry weight) measured for each macrophyte sample. Chlorophyll *a* corrected for phaeophytin *a* and relative percentages of chlorophyll *a*, *b* and *c* were used to investigate epiphyte response to the treatment groups. Chlorophyll data was also used to determine if the epiphytic community exhibited any host specificity. In addition, macrophyte biomass was corrected by subtracting epiphyte weight from the final dry weight measured for each macrophyte species in response to each treatment group.

Macrophyte Chlorophyll Method

Triplicate samples of macrophyte tissue approximately 5 cm in length were collected for each macrophyte species for each treatment group. Apical tips were collected for *N. guadalupensis*, *P. illinoensis* and *Chara sp.*. Leaf tips were collected for *V. americana*. Epiphyte biomass was removed using the mechanical removal method described by (Zimba and Hopson 1997) (see above). Wet weight (g) was measured. Macrophyte samples were then stored in the dark in the freezer at -20°C for no more than 90 days until processed and analyzed for chlorophyll *a* and phaeophytin *a*. The chlorophyll was extracted by freezing the leaf segments with approximately 2 mL of liquid nitrogen, pulverizing them with a mortar and pestle, and extracting them in 90% acetone for 24 hours in the freezer at -20°C. When necessary, pigments were diluted 1:5 with 90% acetone. The optical densities of the extracts were measured using a Beckman DU520 General Purpose UV/VIS Spectrophotometer. Macrophyte chlorophyll was determined using the equations outlined in Standard Methods (SM 10200 H.) (A.P.H.A. 1995).

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BIOGRAPHICAL SKETCH

Margaret S. Hopson-Fernandes was born in Jacksonville, Florida on March 6, 1967. Margaret grew up in Atlantic Beach, Florida. She graduated third in a class of 485 students from Duncan U. Fletcher High School in 1985.

Margaret began her university studies at Jacksonville University in Jacksonville, Florida where she majored in biology and minored in chemistry. She received her Bachelor of Arts degree in 1989, graduating *magna cum laude*. Margaret entered the University of Florida for the first time as a postbaccalaureate student in 1989. She was admitted to Graduate School at the University of Florida in the Department of Botany in January of 1990. Margaret worked as a graduate teaching assistant for the Department of Botany throughout her tenure as a Masters student. Margaret received her Master of Science degree in 1995. After completing her thesis, Margaret moved to Brasilia, Brazil, where she taught various grades and subjects at the American School of Brasilia. She married fellow ecologist and Gator, Carlos Fernandes, in January of 1997. Their first child, Eddie was born in June of 1998. The Fernandes family returned to Gainesville in January of 1990 when Margaret began her doctoral program in the Department of Environmental Engineering Sciences. Margaret was awarded a Graduate Assistance in Areas of National Need (GAANN) Fellowship in August of 1990. Margaret's daughter, Maria, was born on September 25, 2001, the day after Margaret returned from harvesting the second replication of Experiment 1. During her tenure as a doctoral student, Margaret was a teaching

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ben Koopman, Chair
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