THE CONCEPTUAL ECOLOGY AND MANAGEMENT OF PARROTFEATHER

[*Myriophyllum aquaticum* (Vell.) Verdc.]

By

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Parrotfeather [*Myriophyllum aquaticum* (Vellozo) Verdecourt] is a non-native aquatic plant from South America that was introduced into the United States in the 1890's. Research was conducted to elucidate seasonal life history, starch allocation patterns, and key environmental factors that may affect plant growth. Environmental factors identified in field studies were used to develop a conceptual model to display relationships between growth and environmental factors. The conceptual model served as a broad-based hypothesis to parameterize growth limiting factors as it related to *M. aquaticum* growth. Mesocosm experiments were then conducted to test relationships depicted in the model and define the growth requirements of this species.

Emergent shoot biomass, submersed shoot biomass, and sediment root biomass were related to light transmittance. Submersed shoot biomass was also related to water temperature. Stolons accounted for 40-95% of total biomass. Starch allocation was also greatest in stolons (78.1 g m^{-2}); where up to 16.3% of total starch was stored. Low points in biomass and starch occurred from October to March.

Biomass was greater when plants were grown in 30% shade, whereas plant length was greatest when plants were grown in 50% shade, with reductions observed in full sunlight. Biomass increased by 53% when nitrogen and phosphorus were added to the water column at 1.80 and 0.01 mg L^{-1}, respectively. *Myriophyllum aquaticum* yield response was positively related ($r^2 = 0.82$) to increasing nitrogen content and a critical concentration of 1.80% nitrogen and 0.20% phosphorus was identified for *M. aquaticum* growth. Plants grown at 0 cm water depth had 96% greater biomass than plants grown at water depths of 137 cm. Total length was 25% greater when plants were grown at water levels from 0-77 cm.

Winter drawdowns reduced biomass by 99% at 4 weeks when compared to pre drawdown biomass. Summer drawdown efficacy was more rapid where biomass was reduced by 98% at 2 weeks when compared to pre drawdown biomass. Subsurface herbicide applications were not more efficacious than herbicides applied to the foliage. The foliar application of 2,4-D was the only herbicide and application method that resulted in ≥ 90% biomass reduction of *M. aquaticum.*

DEDICATION

I dedicate this dissertation to God as nothing is possible without His grace. Also, to my family; my wife Melissa, and children Madeline, Aiden, Xavier, and Anne Catherine who provided the love and support I needed to continue down the long road of graduate school. Without their selfless sacrifices and unwavering patience with a husband and father trying to achieve his goal; this research, dissertation, and degree would not have been possible. I pray that I can now fill the void that I have left over the past five years as we start the next chapter in our lives. To each of them I owe a debt of gratitude, and I Love You.

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INTRODUCTION: A CONCEPTUAL FRAMEWORK TO GUIDE RESEARCH FOR THE DEVELOPMENT OF MANAGEMENT TECHNIQUES ON PARROTFEATHER *Myriophyllum aquaticum*

Biology and Ecology of *Myriophyllum aquaticum*

Parrotfeather [*Myriophyllum aquaticum* (Vellozo) Verdecourt] is a non-native invasive aquatic plant from South America that has been introduced into Southeast Asia, Australia, New Zealand, Japan, South Africa, and North America (Jacot-Guillarmod 1979; Cooke 1985). The earliest specimen recorded in the United States was collected April 20, 1890, from Haddonfield, New Jersey (Nelson and Couch 1985). *Myriophyllum aquaticum* is a common component of aquaria landscaping because of its aesthetic appearance and its ease of cultivation (Sutton 1985). Aquarium plant providers in the San Francisco Bay Area would plant *M. aquaticum* into local waterways to have convenient sources of saleable plant material (Aiken 1981). The ease of cultivation and attractiveness as a pond plant has led to the escape and subsequent colonization of natural areas by *M. aquaticum*. The stems of this species are brittle and easily fragmented; these small fragments root easily in mud to establish new colonies (Orchard 1981). In the United States, *M. aquaticum* has spread to 26 states, including Hawaii, and its current distribution is as far north as New York on the East Coast, the state of Washington on the West Coast, and in nearly every southern state.

Although *M. aquaticum* is not considered a major noxious aquatic weed throughout most of its range, it can cause severe localized problems in shallow ditches, streams, ponds, and shallow lakes (Sutton 1985). Large populations of *M. aquaticum* can impede water movement in streams and ditches, resulting in increased flood duration and intensity (Timmons and Klingman 1958). In South Africa, *M. aquaticum* infests all of the major river systems, where it poses a direct threat to the country's water supply (Jacot-Guillarmod 1977a). In areas such as the western United States where water resources are becoming depleted, dense populations of *M. aquaticum* may result in significant water loss through plant transpiration (Sytsma and Anderson 1993a). Furthermore, *Anopheles* mosquito larvae preferred dense *M. aquaticum* growth (1000 stems m^2) where it served as a refuge from predation (Orr and Resh 1989; Orr and Resh 1992). Female mosquitoes also had increased ovipostion rates when *M. aquaticum* shoots reached similar densities (Orr and Resh 1989; Orr and Resh 1992). The relationships between *M. aquaticum* and mosquitoes from a human health perspective have prompted some states to develop research and control measures for *M. aquaticum* (Sytsma and Anderson 1993b).

Godfrey and Wooten (1981) describe *M. aquaticum* as "stout, stems moderately elongate, partially submersed but with portions of leafy branches emergent. Leaves [are] whorled, stiff, usually with 20 or more linear filiform divisions, appearing feather-like and grayish green. Flowers are all pistillate, borne in the axils of unreduced leaves." *Myriophyllum aquaticum* is a dioecious species; however, only pistillate plants are found outside of its native range. In fact, staminate plants are rare even in native populations of South America (Orchard 1981). For this reason, seed production is not known to occur

(Aiken 1981), and reproduction is exclusively vegetative (Orchard 1981). Vegetative reproduction occurs solely by fragmentation of emergent and submersed shoots.

Myriophyllum aquaticum is heterophyllous, meaning it has both a submersed and emergent leaf form. Submersed shoots are comprised of whorls of four to six filamentous, pectinate leaves arising from each node (Mason 1957). Submersed leaves lack stomata but have perforations on each leaflet (Sutton and Bingham 1973). Conversely, emergent leaves have sunken anomocytic stomata (Sutton and Bingham 1973). The emergent plants of *M. aquaticum* have numerous air canals and aerenchyma (Sutton and Bingham 1973). In the leaves and the roots, this aerenchyma is continuous from one end of the organ to the other; however, the canals in the shoot are interrupted at the nodes (Sutton and Bingham 1973). When the submersed shoot emerges, the stem morphology changes so that emergent shoots become denser and contain more structural tissues than submersed shoots (Sytsma 1992). After plants reach the water surface, plant growth changes from vertical to horizontal to facilitate the rapid covering of the water surface, followed by extensive lateral branching and growth of new emergent shoots (Moreira et al. 1999).

Emergent shoots have a higher light saturation point than that of the submersed leaves (Salvucci and Bowes 1982). The saturation point is almost eight-fold higher in emergent leaves, approaching that of full sunlight (Salvucci and Bowes 1982). The light saturation point of the submersed leaves is between 250-300 μ E m^{-2} s^{-1} and indicates that photosynthesis of submersed plants is adapted to a shade environment. The anatomical and morphological differences in the submersed and emergent forms of *M. aquaticum*

may result from physiological adaptations to conditions in their respective environments (Sculthorpe 1967).

The uptake of nitrogen and phosphorus from sediment and their allocation have been documented in both natural and laboratory populations (Sytsma and Anderson 1993a,b,c,d). However, *M. aquaticum* develops adventitious roots that may be an important site for nutrient uptake in low nutrient environments. *Myriophyllum aquaticum* has shown to be resilient to changing environmental factors, as it inhabits areas over much of the United States.

Myriophyllum aquaticum is not seriously affected by frost, and the only cycling in growth rate appeared to be a result of environmental temperature and light availability (Moreira et al. 1999). Growth of *M. aquaticum* initiates when water temperatures reach 8 C and displays a direct relationship with temperature but can be limited by other factors such as light availability (Moreira et al. 1999). *Myriophyllum aquaticum* grows from the sediment so that environments where light can penetrate to the bottom generally favor *M. aquaticum* colonization (Moreira et al. 1999). In general, depths of less than 100 cm are optimum (Moreira et al. 1999); however, *M. aquaticum* has been observed growing in waters up to 2 m deep (Sutton 1985). *Myriophyllum aquaticum* can survive in coastal waters where frequent inundation of salt water occurs (Sutton 1985). The exposure to salt water can promote root growth and establishment (Haller et al. 1974). Regardless of environmental conditions, once *M. aquaticum* is established, it usually persists in spite of variations in the environment (Moreira et al. 1999).

Management of *Myriophyllum aquaticum*

Chemical Control

Herbicides currently used for *M. aquaticum* control include those herbicides that are applied to foliage, including 2,4-D, triclopyr, glyphosate, diquat, carfentrazone-ethyl, imazapyr, and imazamox. The use of 2,4-D and triclopyr as foliar applications have resulted in consistent control of *M. aquaticum* (Moreira et al. 1999, Hofstra et al. 2006). Glyphosate is generally not recommended, as this herbicide only kills emergent shoots and plants often regrow in greater densities (Moreira et al. 1999). Diquat is a contact herbicide that will kill the vegetation it comes in contact with, but significant regrowth is common (Westerdahl and Getsinger 1988; Moreira et al. 1999). Carfentrazone-ethyl will not control *M. aquaticum* as a foliar application (Richardson et al. 2008). The use of imazapyr and imazamox have been evaluated on small infestations with excellent and fair results, respectively (Wersal and Madsen 2007).

Subsurface herbicide applications for *M. aquaticum* control have received much less attention. To date, only carfentrazone-ethyl, diquat, and 2,4-D have been evaluated as subsurface applications (Glomski et al. 2006; Gray et al. 2007; Wersal et al. 2010). Carfentrazone-ethyl will not control *M. aquaticum* and is not recommended as a stand-alone treatment (Glomski et al. 2006; Gray et al. 2007). However, when carfentrazone-ethyl was combined with 2,4-D, it resulted in excellent control of small *M. aquaticum* populations (Gray et al. 2007). Diquat applied to the water column resulted in the fragmentation of *M. aquaticum* and may not be the best option for *M. aquaticum* control (Wersal et al. 2010). Multiple applications are likely necessary to completely control *M.*

aquaticum. The effectiveness of herbicide applications will be site-specific and depend upon the environmental conditions at the time of application.

Physical and Mechanical Control

Hand pulling and harvesting may offer temporary control; however, this approach is very labor intensive, as dense mats are heavy and difficult to haul out of the water (Jacot-Guillarmod 1977b; Shibayama 1988). Furthermore, the long term effectiveness of harvesting *M. aquaticum* has not been established. Raking and chaining (long chains of sharp blades pulled by tractors) may not be feasible due to the rapid biomass production of *M. aquaticum*, as dense mats are heavy and may damage equipment. Sabbatini and others (1998) reported that *M. aquaticum* was tolerant to mechanical disturbance (raking and chaining), and the repeated application of mechanical techniques favored *M. aquaticum* dominance in canals. Care must be taken to remove all plant parts (emergent shoots, submersed shoots, and roots) as well as fragments created or re-growth will occur.

Water drawdown may be a viable option for *M. aquaticum* control; however, the effectiveness of this approach has yet to be determined. To be successful, a drawdown would have to be sustained long enough to completely dry the soil, as *M. aquaticum* can and will survive in moist soil. Conversely, increasing the water depth may deplete energy reserves by forcing plants to continually grow towards the water surface that may, in turn, reduce total biomass. However, there are no empirical data regarding *M. aquaticum* response to increased water depths. Observations of reduced macrophyte growth have been documented in Florida where years of above average lake stages (> 2.7

m) in Lake Okeechobee almost eliminated submerged vegetation (Harwell and Havens 2003; Havens et al. 2004). Robel (1962) demonstrated that sago pondweed (*Stuckenia pectinata* (L.) Börner) biomass declined at water depths >46 cm with complete exclusion of the plant at depths greater than 100 to120 cm.

Biological Control

Biological agents that have been evaluated on *M. aquaticum* include the following: grass carp, several species of beetles, tortricids, and Lepidoptera (Habeck 1974; Habeck and Wilkerson 1980; Cordo and Deloach 1982a,b), and the fungi *Pithium carolinianum* (Bernhardt and Duniway 1984). Grass carp are not recommended for *M. aquaticum* control, as fish generally avoid eating this plant (Pine and Anderson 1991; Catarino et al. 1997). The leaf-feeding beetle (*Lysathia* spp.) showed some promise in South Africa by significantly reducing emergent shoot biomass (Cilliers 1999); however, this agent is not approved for use in the United States. Any successful biological control agent would have to effectively target both the emergent and the submersed portions of *M. aquaticum*, or regrowth will occur.

Phenology and Carbohydrate Allocation

Phenology is the study of the seasonal timing of critical stages in the life of plants and animals (Madsen and Owens 1998). One such event is the seasonal allocation of carbohydrates to various structures within a plant. *Myriophyllum aquaticum* has no real specialized structures for carbohydrate storage, yet once it is established, it persists even after the deployment of management techniques. This persistence suggests that energy is being stored in some structure of the plant in large enough quantities for regrowth when

favorable conditions return. However, carbohydrate allocation patterns have not been identified for *M. aquaticum*. Previous research has successfully documented carbohydrate allocation patterns on other aquatic plants such as Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Madsen 1997), hydrilla (*Hydrilla verticillata* L.f. Royle) (Madsen and Owens 1998), curlyleaf pondweed (*Potatmogeton crispus* L.) (Woolf and Madsen 2003), and Brazilian egeria (*Egeria densa* Planch.) (Pennington and Sytsma 2009). By determining and understanding carbohydrate allocation patterns, management can then target and exploit times of low energy within the plant and improve control of the target species (Madsen 1993).

Conceptual Approach to Managing *Myriophyllum aquaticum*

Conceptual models are descriptions of the general functional relationships among components in an ecosystem (Fischenich 2008). These models are an abstract view of reality to express an understanding of more complex systems and can serve as the basis for scientific debate (Fischenich 2008). Conceptual models can identify where there is agreement about stressors on a natural system and provide qualitative explanations of how these natural systems have been altered by environmental or anthropogenic stressors (Ogden et al. 2005a). Modeling had become a useful tool in guiding and plan formation in a number of management programs (Fischenich 2008).

Models have little utility during the implementation phases of restoration or management programs due to their abstract nature, but are imperative for monitoring and adaptive management programs (Fischenich 2008). For example, conceptual ecological models are an integral part of South Florida's restoration and planning process because

both scientists and managers depend on the models to build consensus regarding ecosystem linkages and responses. More importantly, they depend upon them to guide assessment of management operations and to identify new research needs (Ogden et al. 2005a). Conceptual models appeal to managers because of the ease of organizing and applying existing science to decision making and to the implementation of management programs (Ogden et al. 2005a). Scientists value the intellectual and integrative processes of developing hypotheses and links in the conceptual model and then using models to identify gaps in knowledge (Ogden et al. 2005a).

Currently in South Florida, several conceptual models exist to guide management, restoration, and research for the Comprehensive Everglades Restoration Plan. These models include entire system models to specific aquatic, marine, and terrestrial habitats (Barnes 2005; Browder et al. 2005; Crigger et al. 2005; Davis et al. 2005a; Duever 2005; Havens and Gawlik 2005; Ogden 2005; Ogden et al. 2005b; Rudnick et al. 2005; Sime 2005; VanArman et al. 2005). Each of these models were developed using a similar pathway: drivers, stressors, ecological effects, and attributes (Figure 1.1). In general, external drivers (environmental or anthropogenic) create internal stressors that have effects on the ecosystem, and these effects are observed as some change in the system (attributes) (Ogden et al. 2005a).

- Drivers – major forces operating outside the natural system that have large scale influences on the natural system. These include natural forces such as weather or anthropogenic forces such as surrounding land use.
- Stressors – physical or chemical changes that occur in the system that are brought about by the drivers.

- Ecological Effects – a physical, chemical, or biological response caused by the stressor.
- Attributes – these are real or hypothetical results or outcomes of the effects of the stressors.

Conceptual models have typically focused on depicting and guiding large scale research and restoration projects; species-specific ecological and management models are uncommon. Therefore, building upon conceptual frameworks for ecosystem management, a single-species conceptual model was developed to guide controlled mesocosm experiments on *M. aquaticum* growth (Figure 1.2). Linkages in the model were developed using existing *M. aquaticum* information collected under both laboratory and field conditions (Sytsma and Anderson 1993a,b,c,d), and new data collected from natural populations (see Chapter II). The main objectives were to identify important environmental and anthropogenic factors that can affect *M. aquaticum* growth; to describe *M. aquaticum* response to manipulation of its growing environment; to try to predict possible outcomes or environments where *M. aquaticum* can become a nuisance; and to develop new management recommendations for *M. aquaticum* based upon the outcome of the controlled experiments.

Previous research on *M. aquaticum* has largely been small scale laboratory and greenhouse studies (Sutton 1985; Maberly and Spence 1989; Kane et al. 1991). Few studies have examined *M. aquaticum* growth in natural populations as it relates to environmental factors (Moreira et al. 1999), and only one study has documented seasonal biomass and nutrient allocation patterns under field conditions (Sytsma and Anderson 1993b). Therefore, additional data are needed to elucidate the relationships between *M.*

aquaticum growth and environmental factors, to determine biomass and starch allocation patterns in *M. aquaticum*, and to determine how altering important environmental factors will affect growth characteristics of *M. aquaticum*.

The overall objective of this dissertation was to develop a species-specific conceptual model to parameterize factors that may limit the growth of *M. aquaticum*, which would lead to more effective management strategies for this species. Once the model was created, controlled mesocosm experiments were conducted to test important relationships or linkages in the model. The goal is to understand important relationships influencing the growth *M. aquaticum*, thereby allowing for the creation of predictive spatial models of habitat suitability. Habitat suitability models could then be used to identify likely areas of infestation across a landscape so that resources and monitoring intensity are not wasted surveying in unfavorable habitats.

The following research focuses on seasonal life history and carbohydrate allocation patterns, plant response to variations in environmental factors, and the evaluation of various management strategies. Chapter II is a two-year life history analysis to determine biomass and starch allocation patterns and to determine important relationships between seasonal plant growth and key environmental factors. Field data were then used to construct the conceptual model to determine plant response to key environmental factors.

Environmental Factors

Chapter III examines changes in growth characteristics of *M. aquaticum* in response to changes in light intensity and tests the environmental linkage in the conceptual model.

Surrounding Land Use

Chapter IV describes *M. aquaticum* growth when water column nutrient concentrations are altered, a possible result of land use changes and nutrient runoff in the surround landscape as depicted in the conceptual model.

Management

Chapter V is a study evaluating the effect of water depth on *M. aquaticum* biomass and will elucidate whether increasing water depth can be used as a physical control technique as part of an integrated management approach. Chapter VI describes the efficacy of a winter and summer drawdown that will further evaluate the use of physical control techniques for *M. aquaticum* management. Chapter VII examines the efficacy of chemical control options and application methods on *M. aquaticum*. In chapter VIII I offer management recommendations based upon the current studies and building upon information previously reported.

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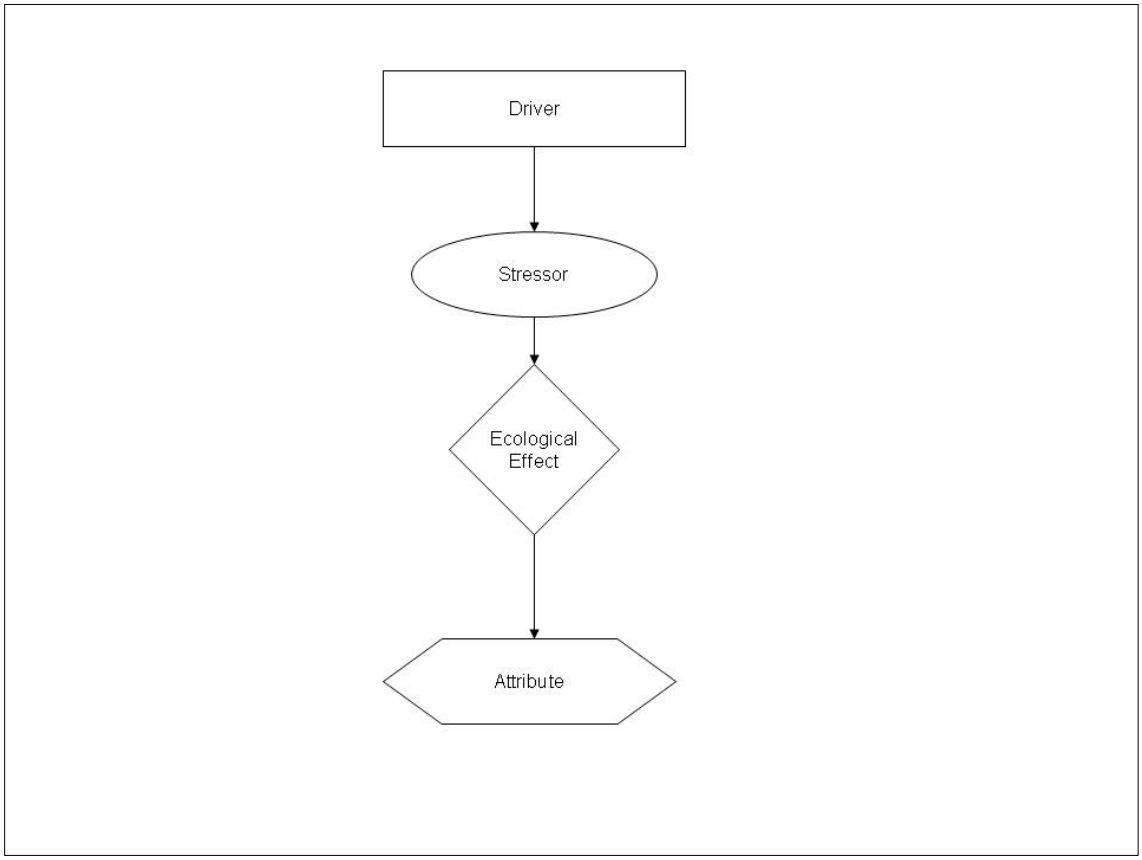

Figure 1.1 A simplified diagram outlining the important parts of a conceptual model as described by Ogden et al. (2005a).

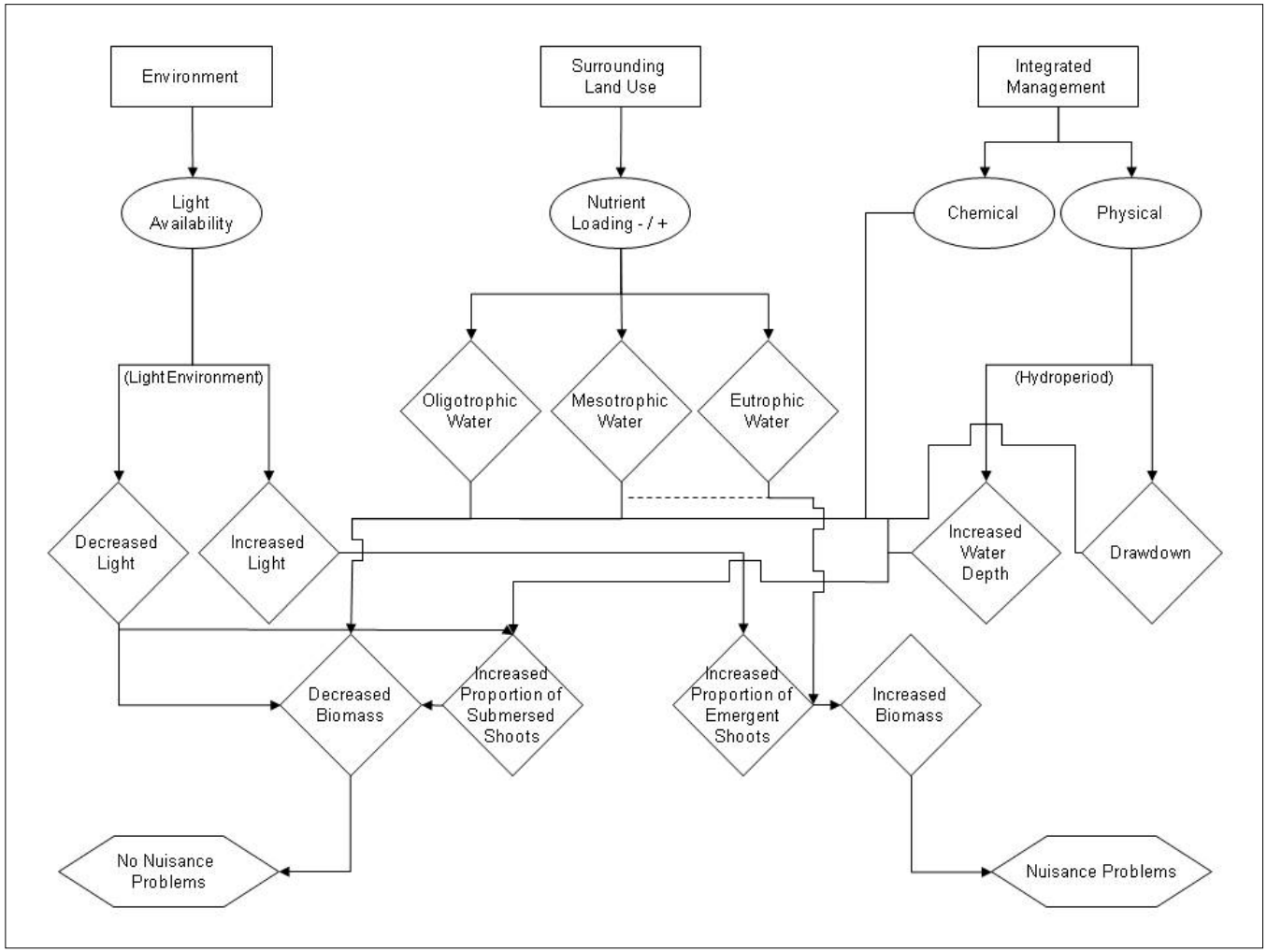

Figure 1.2 A conceptual model depicting relationships between *Myriophyllum aquaticum* biomass and environmental factors.

CHAPTER II

SEASONAL PHENOLOGY, STARCH ALLOCATION PATTERNS, AND THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE GROWTH OF *Myriophyllum aquaticum*

Abstract

Seasonal biomass and starch allocation patterns were determined from natural populations of *Myriophyllum aquaticum* in Mississippi, USA to identify potential low points in the seasonal phenological cycle for improved management of this species. *Myriophyllum aquaticum* was sampled monthly from four populations from January 2006 to December 2007. Water temperature, water depth, light intensity, light transmittance, pH, and conductivity were also recorded during each sampling event. Emergent shoot biomass ($p=0.02$), submersed shoot biomass ($p=0.03$), and sediment root biomass ($p<0.01$) were related to light transmittance. Submersed shoot biomass was also related ($p=0.01$) to water temperature. The r^2 of the mixed models ranged from only 0.06-0.20, indicating that other factors were influencing *M. aquaticum* growth. Biomass was greater in 2006 than in 2007, where peak biomass was 510.7 g m^{-2} and 39.6 g m^{-2} respectively for those years. Stolons accounted for 40-95% (mean 65.9 ± 2.7%) of total biomass followed by emergent shoot, submersed shoot, and sediment root biomass. Starch allocation was greatest in stolons (78.1 g m^{-2}), where up to 16.3% of total starch was stored, indicating that stolons are likely the primary storage location for carbohydrates.

Submersed shoots stored 0.6-11.0% of total starch from *M. aquaticum* followed by emergent shoots (0.4-7%). Sediment roots of *M. aquaticum* stored less than 3.8% of total starch, and much of the time starch content was below 2.0%. Sediment roots are not considered to be the primary site for energy acquisition and storage. Low points in both biomass and starch allocation occurred from October to March, where total biomass was less than 30.2 g m^{-2} and starch content was less than 2.0 g m^{-2}. An integrated approach should be implemented to either exploit the times of low energy reserves (fall and winter), or remove emergent shoots to gain access to the stolons and other submersed tissues. Management activities that target only the emergent shoots will not be effective at controlling this species as the majority of energy reserves are stored in stolons and submersed tissues.

Introduction

Phenology is the study of the seasonal timing of critical life stages in plants and animals (Madsen and Owens 1998). The allocation of biomass and other resources such as carbohydrates are fundamental aspects in the life history of plants. Plants in temperate regions typically allocate and store carbohydrates as starch in roots, rhizomes, and specialized structures for winter survival (Cyr et al. 1990). Plants allocate and store carbohydrates to support growth, photosynthesis, and maintenance throughout the growing season (Chapin et al. 1990; Spencer et al. 1997). Aquatic plants utilize many structures for storing starch, including roots (Madsen 1997; Madsen and Owens 1998), rhizomes (Gallagher et al. 1984), stems (Madsen 1997; Madsen and Owens 1998; Pennington and Sytsma 2009), stembases (Tucker and DeBusk 1981), tubers (Owens and

Madsen 1998), winter buds (Titus and Adams 1979), and turions (Woolf and Madsen 2003).

In most cases, aquatic plants will display distinct seasonal patterns in biomass (Wersal et al. 2006) and carbohydrate allocation (Woolf and Madsen 2003); where storage peaks in summer or fall and is depleted in spring when plant growth resumes (Madsen 1991). Understanding these annual growth cycles will allow for the determination of seasonal low points in energy reserves. Timing management to coincide with seasonal low points can exploit reduced energy reserves within the plant and possibly enhance efficacy of the management techniques; thereby reducing the ability of the target plant to re-grow, or survive an overwinter period (Madsen 1997). Herbicide treatments on alligatorweed (*Alternanthera philoxeroides* Mart. Griseb.) were found to be more effective when applied during times of low carbohydrate storage (Weldon and Blackburn 1968). The use of harvesting on Eurasian watermilfoil (*Myriophyllum spicatum* L.) had documented reductions in carbohydrate concentrations in plants (Perkins and Sytsma 1987), and the inability to overwinter (Kimbel and Capenter 1981). Harvesting has also been shown to reduce carbohydrates in hydrilla (*Hydrilla verticillata* L.f. Royle); and, if harvesting was maintained, tuber production was significantly reduced (Fox et al. 2002). However, the practical application of this strategy is dependent upon the location, knowing the phenological cycle of the target plant, and ultimately timing management to that cycle, though management decisions are often dictated by anthropogenic reasons and not for maximizing treatment efficacy (Pennington and Sytsma 2009).

Parrotfeather [*Myriophyllum aquaticum* (Vellozo) Verdecourt] is a non-native invasive aquatic plant from South America that was introduced into the United States in 1890, likely near Haddonfield, New Jersey (Nelson and Couch 1985). *Myriophyllum aquaticum* is a common component of aquaria landscaping, which had undoubtedly served as the primary vector of spread for this species (Sutton 1985). Although it is not considered a major noxious aquatic weed throughout most of its range, it can cause severe localized problems in shallow ditches, streams, ponds, and shallow lakes. Dense populations can impede water movement in streams and ditches, resulting in increased flood duration and intensity (Timmons and Klingman 1958). *Myriophyllum aquaticum* poses a direct threat to drinking water supplies in South Africa (Jacot-Guillarmod 1977). In the western United States where water resources are becoming depleted, dense populations may result in significant water loss from irrigation ditches through plant transpiration (Sytsma and Anderson 1993a). Furthermore, female *Anopheles* mosquitoes have increased ovipostion rates when shoot densities reached approximately (1000 stems m^2), as *M. aquaticum* serves as a refuge from predation (Orr and Resh 1989; Orr and Resh 1992).

Myriophyllum aquaticum can colonize a diverse range of habitats and tolerate disturbances in its growing environment. Plants are not seriously affected by frost, and the only cycling in growth rate appeared to be a result of temperature and light availability (Sytsma and Anderson 1993b; Moreira et al. 1999). Growth initiates when water temperatures reach 8 C and displays a direct relationship with temperature but can be limited by other factors such as light availability (Moreira et al. 1999). In general, depths of less than 100 cm are optimum (Moreira et al. 1999); however, *M. aquaticum*

has been observed growing in waters up to 2 meters deep (Sutton 1985). *Myriophyllum aquaticum* can survive in coastal waters where frequent inundation of salt water occurs, promoting sediment root growth and establishment (Haller et al. 1974). Survival and spread of *M. aquaticum* depends solely on vegetative reproduction via fragmentation, as this species does not produce any specialized reproductive or storage structures such as seeds, tubers, or turions, and likely relies on shoots and stolons to meet these needs although data are needed to verify this.

The objectives of this study were to: (1) document seasonal phenology (biomass allocation) over multiple years as it relates to environmental factors and plant tissues; and (2) quantify seasonal starch allocation patterns within the different plant tissues of *M. aquaticum*. To my knowledge, this is a first account of *M. aquaticum* seasonal phenology as it relates to environmental factors in the United States; and a first account of seasonal starch allocation patterns for this species.

Materials and Methods

Seasonal Biomass Collection

Myriophyllum aquaticum biomass was harvested monthly from four locations (32°20’45.859”N 89°20’43.1”W; 32°40’34.715”N 89°38’56.758”W; 33°26’30.332”N 88°54’13.453”W; 33°16’0.238”N 88°47’33.994”W) in Mississippi from January 2006 to December 2007. Harvest locations were generally small ponds or backwater areas of rivers that are typical habitats for *M. aquaticum* in the southeastern United States (Godfrey and Wooten 1981). Waterbody size ranged from approximately 0.1 to 15 ha; however, samples were harvested from only 0.1 to 0.2 ha of each waterbody that

contained *M. aquaticum*, to ensure consistency with sample area between the four locations. Water depths of sample locations ranged from moist soil to approximately 80 cm and varied throughout the year.

At each sample location, during every month, 30 biomass samples were harvested using a 0.018 m^2 PVC coring device (Madsen et al. 2007) for a total of 1880 samples; although in some months samples were not taken at some sites because the site was dry. The coring device was placed at least 20 cm into the sediment, and subsequent cores rinsed through a 19-L pail with a 0.25-cm^2 wire mesh bottom to separate plant material from sediment. Biomass samples obtained from the pail were then placed into appropriately labeled 3.79-L Ziploc® bags, stored in a cooler, and transported to Mississippi State University for processing. Plant biomass was rinsed to remove sediment and debris, and then divided into emergent shoots, submersed shoots, stolons, and sediment roots. Emergent shoots were separated by cutting the shoots at approximately the third node below the last whorl of emergent leaves. Adventitious roots were left on stolons and were incorporated into stolon biomass. Plant parts were dried for at least 72 hours at 70 C in a constant temperature oven and then weighed to ± 0.0001 g using a Mettler Toledo AB104-S balance (Greifensee, Switzerland). *Myriophyllum aquaticum* total biomass as well as its constituent parts is expressed as g m^{-2} for each month.

Environmental Monitoring

During all harvest times water depth was recorded for each sample at all locations prior to collecting a core. Additionally pH, conductivity, and turbidity were recorded

once at each site every month with a Eureka Environmental Multi-Probe (Eureka Environmental, Austin, Texas). A HOBO temperature probe (Onset Computer Corporation, Pocasset, Maine) was deployed at each of the four harvest locations to record water temperature in 1 h intervals for the two years of sampling. Light profiles in 25 cm increments from the water surface to the bottom sediment were determined monthly at each harvest location using a LI-1400 data logger with a LI-190 photometric sensor (incident light) and a LI-192 submersible sensor (LI-COR Biosciences, Lincoln, Nebraska). Incident and submersed light readings were used to calculate percent light transmittance through the water column.

Starch Analysis Procedure

Myriophyllum aquaticum biomass harvested during the life history evaluation was used to assess the seasonal allocation patterns of starch in emergent shoots, submersed shoots, stolons, and sediment roots. Starch was chosen because it is generally the long-term storage carbohydrate that can be readily reconverted to sugars. Dried biomass was composited into three groups of 10 samples (i.e. life history biomass samples 1 through 10 were composited into tissue sample 1, biomass samples 11 through 20 were composited into tissue sample 2, and so on) to obtain three tissue samples for each plant constituent at each sample location. Compositing biomass samples ensured that adequate tissue mass was available for analytical techniques, and to reduce the number of tissue analyses required (Woolf and Madsen 2003).

After compositing biomass samples, samples were ground using a Cyclone Sample Mill (UDY Corporation, Fort Collins, Colorado) to pass through #40 mesh screen

(1 mm). Approximately 50 mg of the ground sample was transferred into plastic centrifuge tubes for storage and preparation for starch analysis. Starch extraction and determination was conducted using the amylase/amyloglucosidase method through commercially purchased STA20 starch assay kits from Sigma Aldrich (Sigma Aldrich, St. Louis, Missouri). The complete method can be found in the STA20 Technical Bulletin (Sigma Aldrich 2010). In addition to *M. aquaticum* samples, wheat starch standards that were included with the kits as 84% pure starch, and two sets of duplicate *M. aquaticum* samples were assayed to determine the reliability of starch data. A total 1178 samples were assayed for starch content. Standard curves (n=43) were also developed to ensure that starch data were within the range of what the kits could detect, and to assess data accuracy. Assay precision, as determined by the percent difference of the duplicate samples, was 10.6% ± 0.8 SE. Accuracy as determined by standard curves was 2% ($r^2 = 0.98$). Starch recovery was 98.3% ± 1.9 SE which was determined using a known mass and purity of the wheat standard provided with the kits.

Data Analysis

Biomass data were analyzed using a Mixed Procedures models in SAS (SAS Institute, Inc., Cary, North Carolina) to determine relationships between environmental factors and *M. aquaticum* biomass (Littell et al. 1996; Wersal et al. 2006). The models accounted for repeated measures in the sampling design. Emergent shoot, submersed shoot, stolon, sediment root, and total biomass were included as dependent variables. Water temperature, water depth, incident light, light transmittance, and year were included as the independent variables in all models. Turbidity and pH were not included

in the models because light transmittance was used instead to test water clarity, and pH remained fairly constant between 7 and 8 and therefore no relationships were expected. Analyses were conducted at a p=0.05 significance level.

Starch data are presented as percent starch for all tissue types and g starch m^{-2} as determined by monthly biomass samples. Grams starch m^{-2} was calculated by multiplying monthly biomass data by the concentration of starch for a given tissue. There were no additional analyses conducted on starch data because results should be similar to those found for biomass data. Starch data are presented with biomass to show trends over time.

Results

Seasonal Biomass Allocation and Environmental Factors

Myriophyllum aquaticum biomass was greater in 2006 than in 2007 where peak biomass was 510.7 g m^{-2} and 39.6 g m^{-2} respectively for those years. There was a drought in the summer of 2006 which caused two sample sites to completely dry and therefore reduced biomass yield in the fall of 2006 and all of 2007. Stolon biomass accounted for 40 to 95% (mean of 65.9 ± 2.7% 1 SE) of total *M. aquaticum* biomass, with peak accumulation occurring from August to September in both years (Figure 2.1). Emergent shoot biomass accounted for 6 to 43% (mean of 19.8 ± 2.1%) of total *M. aquaticum* biomass with peak accumulation beginning in March. Submersed shoot biomass ranged from 0.2 to 23.1% (mean 8.1 ± 1.5%) of total biomass and peaked in February of both years. Sediment root biomass accounted for 0.6 to 15.6% (mean 6.1 ± 0.7%) of total biomass and remained fairly constant over time.

Seasonal changes in environmental factors are depicted in Figure 2.2. Overall there were few significant relationships observed between the environmental factors tested and *M. aquaticum* seasonal biomass as determined by repeated measures mixed procedures models (Table 2.1). There were no significant relationships observed between total biomass or stolon biomass and any of the environmental variables included in the model. A significant year effect was always observed regardless of plant tissue type, this was due to the greater biomass observed in 2006 than in 2007. There was a relationship between light transmittance and emergent shoot, submersed shoot, and sediment root biomass. Submersed shoot biomass was also related to temperature. However, the r^2 of these models ranged from only 0.06-0.20 indicating that other factors were influencing *M. aquaticum* growth.

Seasonal Biomass and Starch Allocation

Overall, plant tissues varied in proportion and allocation patterns over time. Starch allocation was greatest in stolons, where up to 16.3% of total starch was stored, indicating that stolons are likely the primary storage location for carbohydrates (Figure 2.3). Submersed shoots stored up to 10.8% of total starch from *M. aquaticum* followed by emergent shoots (up to 7.7%) and sediment roots. Sediment roots of *M. aquaticum* stored up to 3.8% of total starch, and for much of both years, starch content was $\leq$ 2%.

Due to the significant year effect ($p \leq 0.01$), data are displayed separately for 2006 and 2007 to more clearly show trends in biomass and starch over time. In general, total starch allocation followed biomass production in both years (Figure 2.4 A and B), with more starch being present in 2006 than 2007 (Figure 2.4 C and D). The greatest starch

content was 58.8 to 78.1 g m^{-2} and was observed between May and July 2006. Low points in starch content were observed between November and March for both years where there was 2.3 and 0.5 g m^{-2} of starch present in *M. aquaticum* tissues in 2006 and 2007 respectively. The low points in total starch content also corresponded to low points in total biomass.

Seasonal low points in emergent shoot biomass and starch content were between October and March for 2006, where starch content was 0.03 g m^{-2} and followed that of biomass (Figure 2.5 A and C). After March 2006, there was a rapid reallocation of starch to emergent tissues with peak starch content (9.3 g m^{-2}) occurring in April and May, and a decline in starch content beginning in June. Both biomass and starch content were highly variable in 2007 and therefore, the only discernable trend was that of a seasonal low point in starch content which occurred from September to December (Figure 2.5 B and D).

Biomass and starch content in submersed shoots was generally low throughout most of 2006 and 2007 (Fig 2.6 A-D). Starch content in submersed shoots was greatest in 2006 where starch content was between 0.0 and 1.1 g m^{-2} (Figure 2.6 C). Peak biomass and starch content occurred in February of both years followed by a rapid decline. Following the peak in February, biomass and starch fluctuated very little throughout the remainder 2006 and 2007.

Stolon biomass and starch content peaked in July 2006 at approximately 78.1 g m^{-2}, whereas starch content peaked in May in 2007 at approximately 2.5 g m^{-2} (Figure 2.7 A-D). The peaks in starch were generally more discernable than that of biomass. Seasonal low points in both biomass and starch content of stolons occurred

between October and February where starch content remained below 10.0 g m^{-2} in 2006 and 1.0 g m^{-2} in 2007. Overall, stolons accounted for the greatest proportion of biomass and starch content of *M. aquaticum*.

Sediment roots always comprised the smallest proportion of biomass and starch content, generally < 50.0 g m^{-2} and 0.8 g m^{-2} respectively (Figure 2.8 A-D). There was little change in sediment root biomass and starch content between years with the exception of an unexplained peak in April of 2006 (Figure 2.8 A). There was less than 1.0 g m^{-2} of starch stored in sediment roots throughout 2006 and 2007, with the exception of April 2006, indicating that roots are not the primary storage tissue for carbohydrates.

Discussion

Seasonal Biomass Allocation and Environmental Factors

Peak biomass observed in 2006 was within the range reported for *M. aquaticum* populations in California, where biomass ranged from 234±74 g m^{-2} to 1001±84 g m^{-2} depending upon the water depth in which plants were sampled (Sytsma and Anderson 1993b). Biomass in 2007 was much lower than in previously reported populations. In Japan, *M. aquaticum* fresh weight was reported to be 13.3 kg m^{-2} (Shibayama 1988), and in Portugal, fresh weight ranged from 22 to 26 kg m^{-2} (Monteiro and Moreira 1990). Sytsma and Anderson (1993b) reported a dry weight:fresh weight ratio of 0.21; therefore, fresh weight biomass in this study would have been approximately 2.4 kg m^{-2} in 2006 and 0.1 kg m^{-2} in 2007, much lower than previously reported. The reduced biomass is attributed to a drought over the summers of 2006 and 2007 where by June of both years, two of the sample sites contained moist soil or were completely dry. *Myriophyllum*

aquaticum survived in the remaining moist soil as small emergent shoots or was killed after the sediment dried. Maltchik and others (2007) suggested that *M. aquaticum* may be tolerant of drawdown events (complete removal of surface water) lasting 9 months if the sediment remains saturated. In 2006, water did not return to these sites until November when the rainy season began and therefore subsequent biomass in the fall of 2006 and all of 2007 was reduced.

Although biomass was lower than in other populations, biomass allocation to specific tissues was comparable to previously reported populations. In California, stolon biomass accounted for 72 to 95% of the total biomass, followed by emergent shoots (≤ 24%), sediment roots (> 12%) and submersed shoots (1 to 3%) (Sytsma and Anderson 1993b). The Mississippi populations allocated more biomass to submersed shoots than sediment roots. The allocation of biomass to submersed shoots was likely triggered by environmental cues such as light availability (transmittance) and temperature. In fact, there was a significant relationship between submersed shoot biomass and these environmental factors.

Light availability is often the primary environmental factor influencing submersed plant biomass (Barko et al. 1986). For *M. aquaticum*, reductions in light transmittance during winter months stimulated the growth of submersed shoots. This response is typical in milfoil species, as reduced water clarity causes the submersed Eurasian watermilfoil (*Myriophyllum spicatum* L.) to grow rapidly to the water surface and produce a canopy (Smith and Barko 1990). In Mississippi, reductions in light availability occurred during winter months and thus colder water temperatures were also present.

Water temperature influences plant performance, especially photosynthetic rates, and can ultimately have a regulatory effect on phenology and resource allocation (Madsen 1991).

Biomass allocation to submersed shoots was over a short period of time beginning in January of both years; a peak in February, and declining in March when water temperatures and light intensities began to increase. The peak in submersed shoot biomass indicates that this growth form is adapted to shade environments and is capable of reduced photosynthetic rates to survive in these environments (Salvucci and Bowes 1982). In contrast, the photosynthetic light saturation point is almost eight-fold higher in emergent leaves, approaching that of full sunlight (Salvucci and Bowes 1982); and as light intensities increased beginning in March the allocation to emergent shoots also increased. Therefore, submersed shoot growth is transient and only utilized for short overwintering periods, times of reduced light and temperature, or to survive disturbances in the growing environment. *Myriophyllum aquaticum* will rapidly reallocate biomass to emergent shoots when conditions are favorable and maintain emergent growth as long as energy stores are available. Prolonged exposure to adverse growing conditions will result in reductions in biomass or plant mortality as observed after the summer droughts in this study. Drawdown may be an effective method of removing biomass and carbohydrate reserves and thus managing this species if the sediment can be sufficiently dried.

Seasonal Biomass and Starch Allocation

Seasonal starch allocation patterns followed that of seasonal biomass, where peaks in biomass coincided with peaks in starch content. *Myriophyllum aquaticum* does not produce specialized structures for perrenation or overwintering and therefore would

have to store carbohydrates in other plant tissues. The congeneric *M. spicatum,* which also does not have specialized storage structures, stores up to 15% of total starch in lower stem tissues and up to 20% starch in root crowns during overwintering periods (Madsen 1997). This is in contrast to *M. aquaticum*, which stores the majority of its starch in stolons, and therefore stolons are considered the primary storage site for starch.

Myriophyllum aquaticum is often described as a creeping perennial species with active growing points sometimes meters away from its rooted position in the sediment. Furthermore, the stolons and shoots are brittle and fragment easily, so having a centralized energy store throughout the year would benefit *M. aquaticum* more so than concentrating its starch stores at or near the sediment. Insoluble starch is the long-term storage carbohydrate in the plant, but cannot be translocated through the plant because of the molecule size (Madsen et al. 1993). However, starch can be readily reconverted to sugars, which can be translocated, to support plant growth (Madsen et al. 1993). Having a centralized store would allow for a more rapid conversion of starch to sugars near areas of new shoot growth along the stolons. Plant fragments should also have a greater chance of survival during dispersal, and increased colonization success if the fragment finds suitable habitat for growth, by utilizing stored starch in stolon tissues.

The starch concentration in sediment roots was low and fluctuated little over time. The low concentrations of stored energy suggest that new growth is likely not initiated from sediment roots and they serve only to anchor the plant in the sediment. This is further supported by considering the uptake and allocation of nutrients in *M. aquaticum* as it has received much more attention than carbohydrates. Sediment roots are highly cuticularized which may limit nutrient uptake from the sediment (Sutton and Bingham

1973) and subsequent carbohydrate production. *Myriophyllum aquaticum* has a low sediment root:shoot ratio, further reducing the ability of sediment roots to contribute to the total nutrient supply for plants (Sytsma and Anderson 1993a). Plant growth did not reduce sediment nutrient concentrations over the course of a laboratory study, suggesting that plant growth was being sustained from other plant structures (Sytsma and Anderson 1993a). Nitrogen allocation to sediment roots is generally below 10% (Sytsma and Anderson 1993b). Emergent shoots comprised greater than 80% of the total phosphorus pool in these natural populations with no annual accumulation of phosphorus in other tissues (Sytsma and Anderson 1993b). Therefore, nutrient uptake and storage are low in sediment roots, and carbohydrate production and storage are occurring elsewhere within the plant. Future research needs to identify the role that adventitious roots have on nutrient uptake and subsequent carbohydrate production.

The allocation of resources is a common strategy in plants that undergo periods of stress (Mooney 1972). In the case of aquatic plants, species can produce specialized structures such as tubers, turions, and winter buds to store carbohydrates (Madsen and Owens 1998; Woolf and Madsen 2003); or store carbohydrates in several locations throughout the plant. *Myriophyllum spicatum* has starch stores in root crowns and lower stems (Madsen 1997), as does *Egeria densa* Planch. (Pennington and Sytsma 2009). However, *M. aquaticum* relies on only one structure to store the bulk of its energy resources and management can be directed towards exploiting this trait.

Knowing where starch is being stored and when low points exist may offer insights into the efficacy of management options, and the potential regrowth capability of plants after management techniques have been implemented. Primary low points for

aquatic plants in temperate regions typically occur during spring regrowth when plants are relying on stored energy to initiate growth of plant tissues until photosynthesis can begin (Madsen 1997). Low points in total biomass and starch concentrations of *M. aquaticum* in Mississippi occurred from October to March in both years. These low points coincided with reduced water temperatures and light transmittance which subsequently caused the senescence of emergent shoots and the reliance on stolons and submersed shoots for winter survival. Timing management during October to March may result in increased efficacy due to reductions in emergent shoot biomass and starch stores in stolon tissues.

Myriophyllum aquaticum management is typically conducted during summer months when biomass is at its peak and emergent shoots cover the water surface. Previous attempts have focused on the use of foliar-applied herbicides resulting in poor efficacy. In Portugal, foliar treatments of glyphosate and diquat were not effective for controlling *M. aquaticum* and often permitted rapid re-infestation (Moreira et al. 1999). In New Zealand, applications of clopyralid, fluridone, triclopyr, glyphosate, endothall, and dichlobenil were evaluated; resulting in no control with fluridone and clopyralid, and significant regrowth following glyphosate applications (Hofstra et al. 2006). Applications of triclopyr were effective at reducing *M. aquaticum* cover in field situations; though regrowth of emergent shoots was observed several weeks following the applications (Hofstra et al. 2006). Targeting the emergent shoots will often result in poor control and significant regrowth because *M. aquaticum* does not allocate and store large concentrations of resources in emergent shoots. Once these shoots have been killed or removed, new shoots will re-grow from nodes on the stolons within a day or two. If

management is to be successful, efforts need to focus on the stolons, as this is the primary location for regrowth and energy storage. Future research needs to evaluate whether the observed low points in biomass and starch (October to March) can be exploited to improve management efficacy and determine effective techniques to target stolons, such as submersed herbicide applications and drawdown.

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Table 2.1 Solutions for fixed effects of the mixed procedures model analyzing *Myriophyllum aquaticum* biomass and environmental factors from four populations in Mississippi in 2006 and 2007.

Tissue	Effect	t Value	P Value
Total Biomass	Temperature	0.76	0.96
	Depth	-0.54	0.58
	Incident Light	1.59	0.11
	Transmittance	-1.57	0.12
	Year	4.30	<0.01
Emergent Shoot Biomass	Temperature	0.80	0.42
	Depth	0.52	0.60
	Incident Light	1.57	0.12
	Transmittance	-2.35	0.02
	Year	2.73	0.01
Submersed Shoot Biomass	Temperature	-2.77	0.01
	Depth	-1.19	0.24
	Incident Light	-0.62	0.54
	Transmittance	-2.16	0.03
	Year	2.54	0.01
Stolon Biomass	Temperature	0.87	0.38
	Depth	-0.80	0.42
	Incident Light	1.62	0.10
	Transmittance	-0.70	0.48
	Year	4.31	<0.01
Sediment Root Biomass	Temperature	0.60	0.55
	Depth	0.25	0.80
	Incident Light	1.09	0.27
	Transmittance	-3.12	<0.01
	Year	2.61	0.01

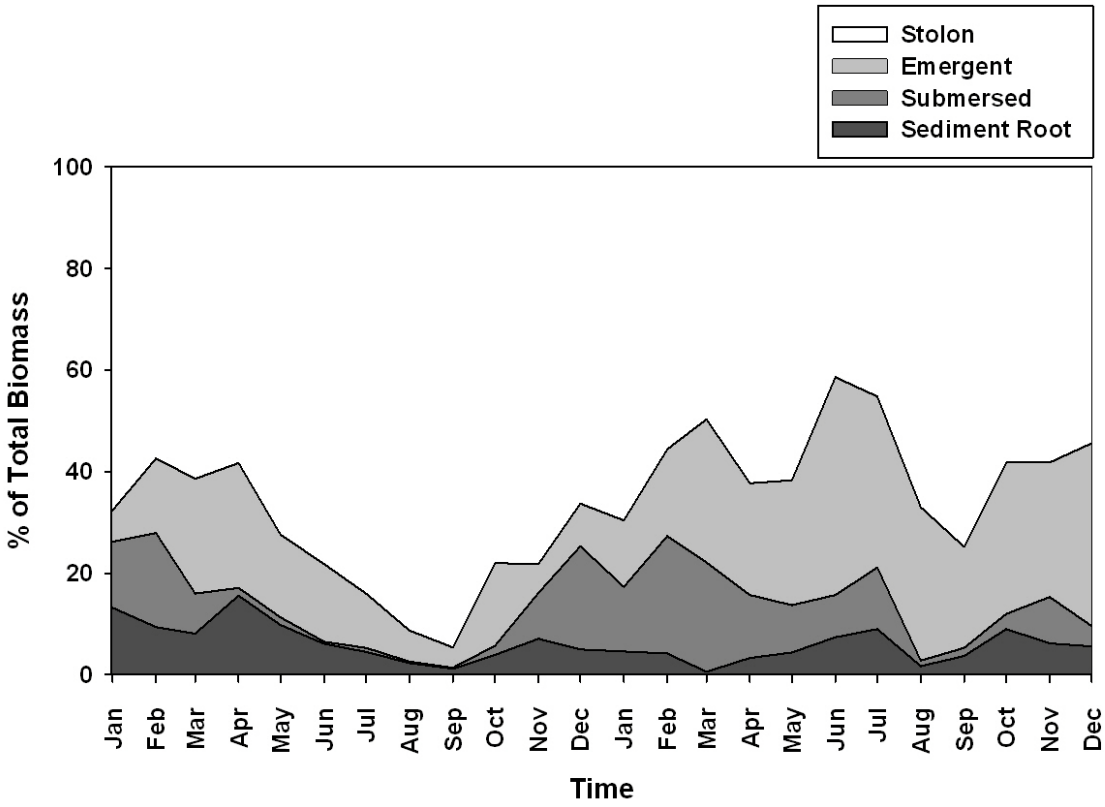

Figure 2.1 *Myriophyllum aquaticum* seasonal biomass allocation (as % of total biomass) patterns for individual plant tissues from four populations in Mississippi in 2006 and 2007.

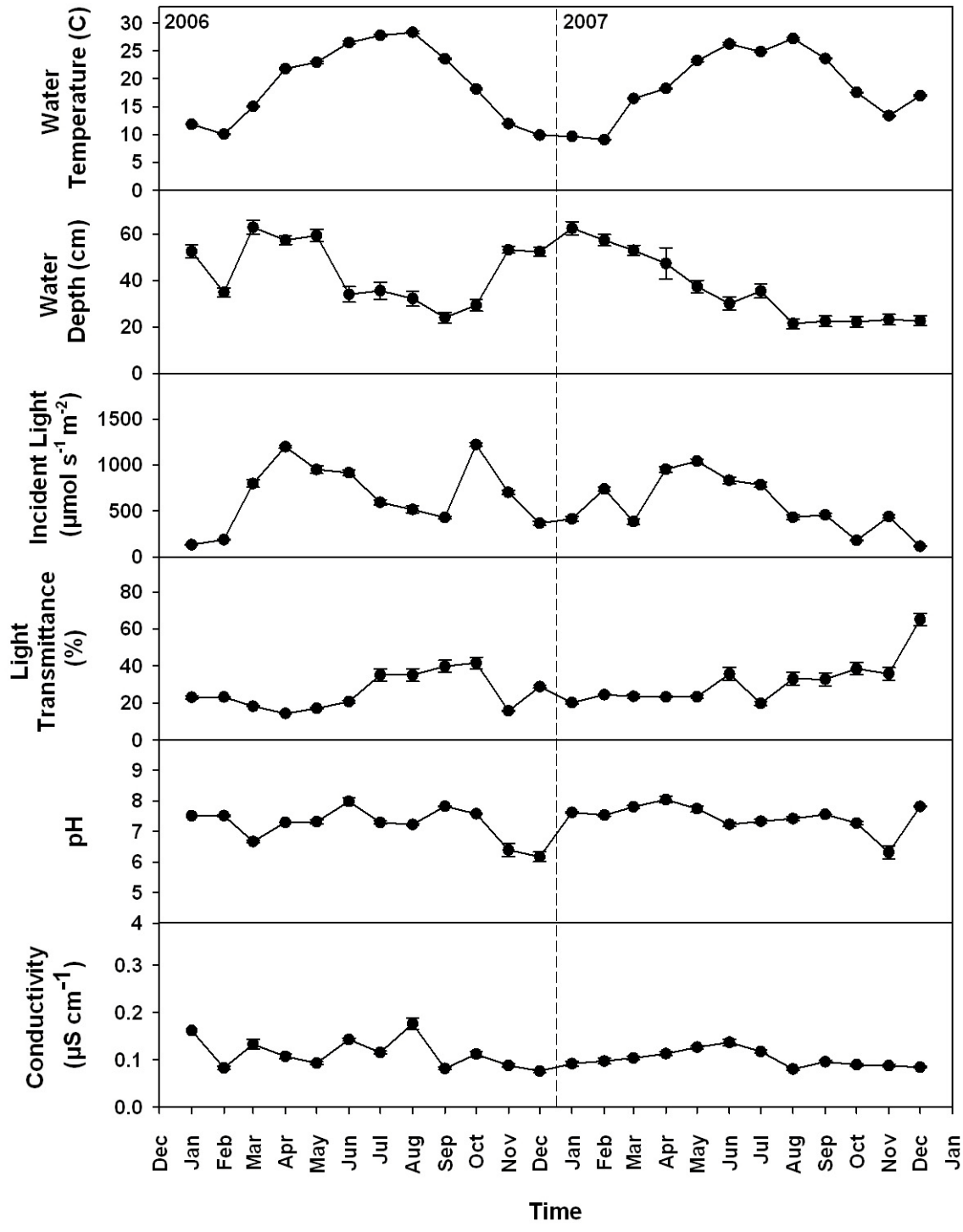

Figure 2.2 Seasonal fluctuations in mean (± 1 SE) environmental factors measured at four locations in Mississippi in 2006 and 2007.

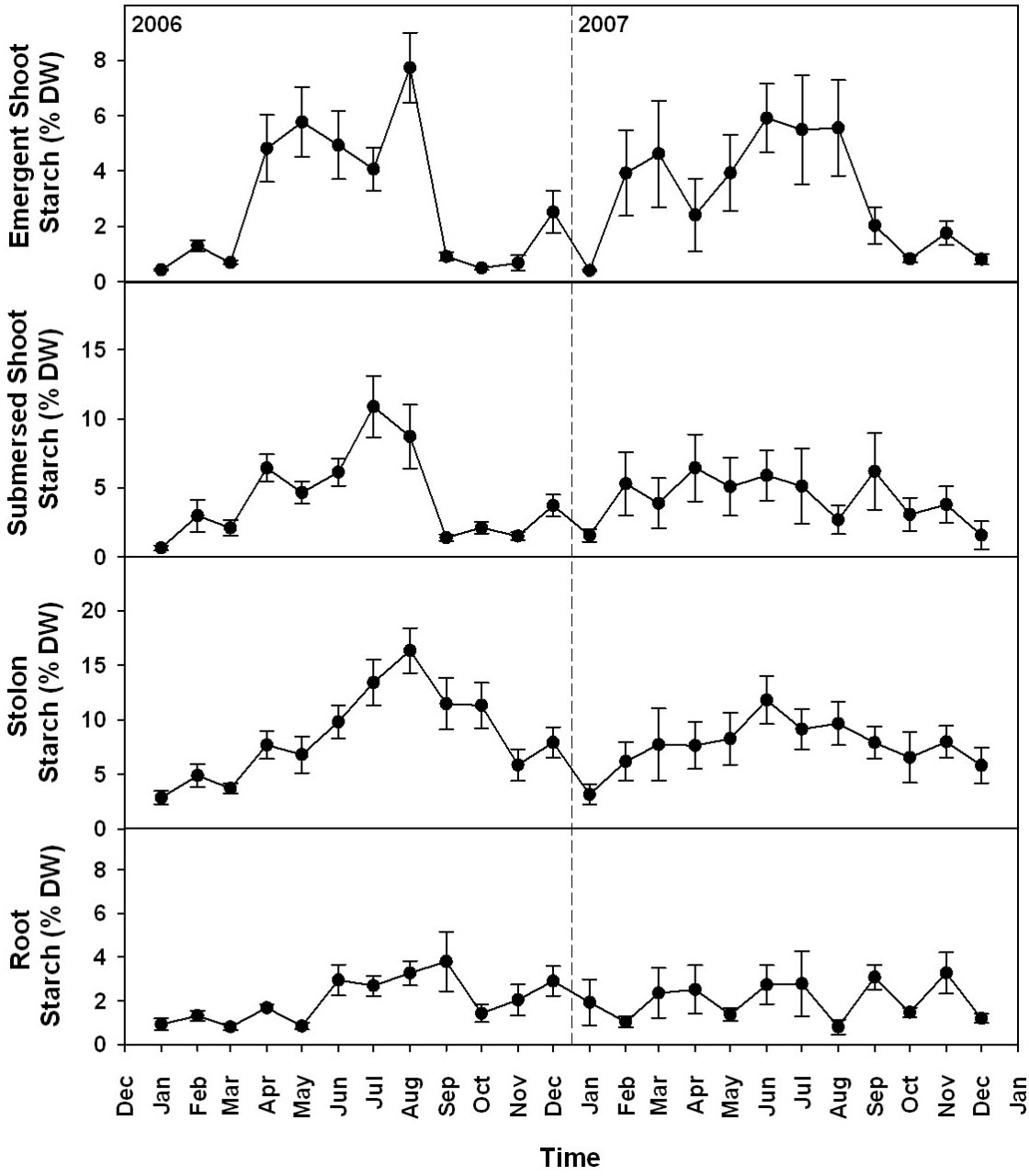

Figure 2.3 Mean (± 1 SE) seasonal percent starch (as % of dry weight) in individual tissues of *Myriophyllum aquaticum* from four populations in Mississippi in 2006 and 2007.

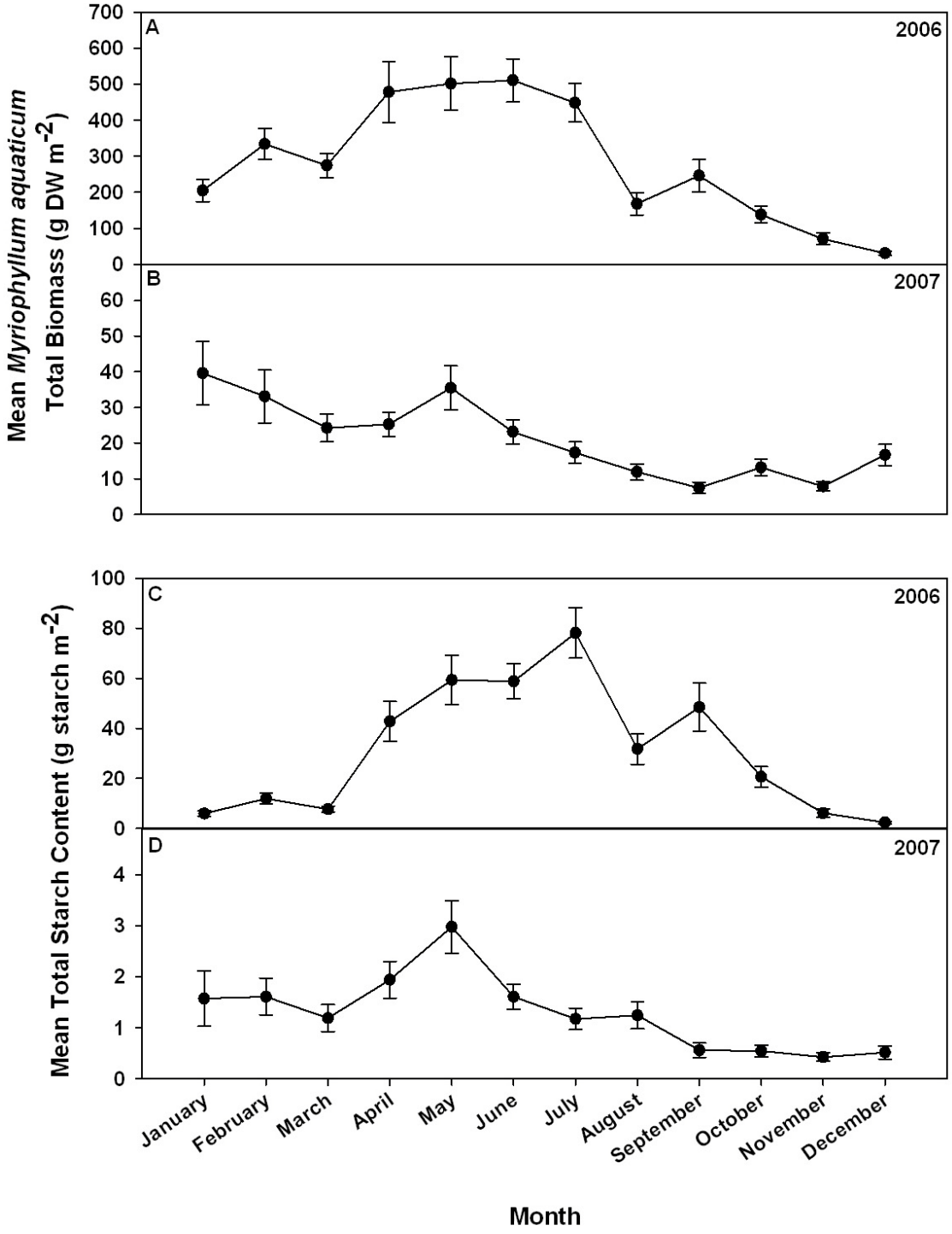

Figure 2.4 Mean (± 1 SE) *Myriophyllum aquaticum* total seasonal biomass (A, B g m^{-2}) and starch content (C, D g starch m^{-2}) from four populations in Mississippi in 2006 and 2007.

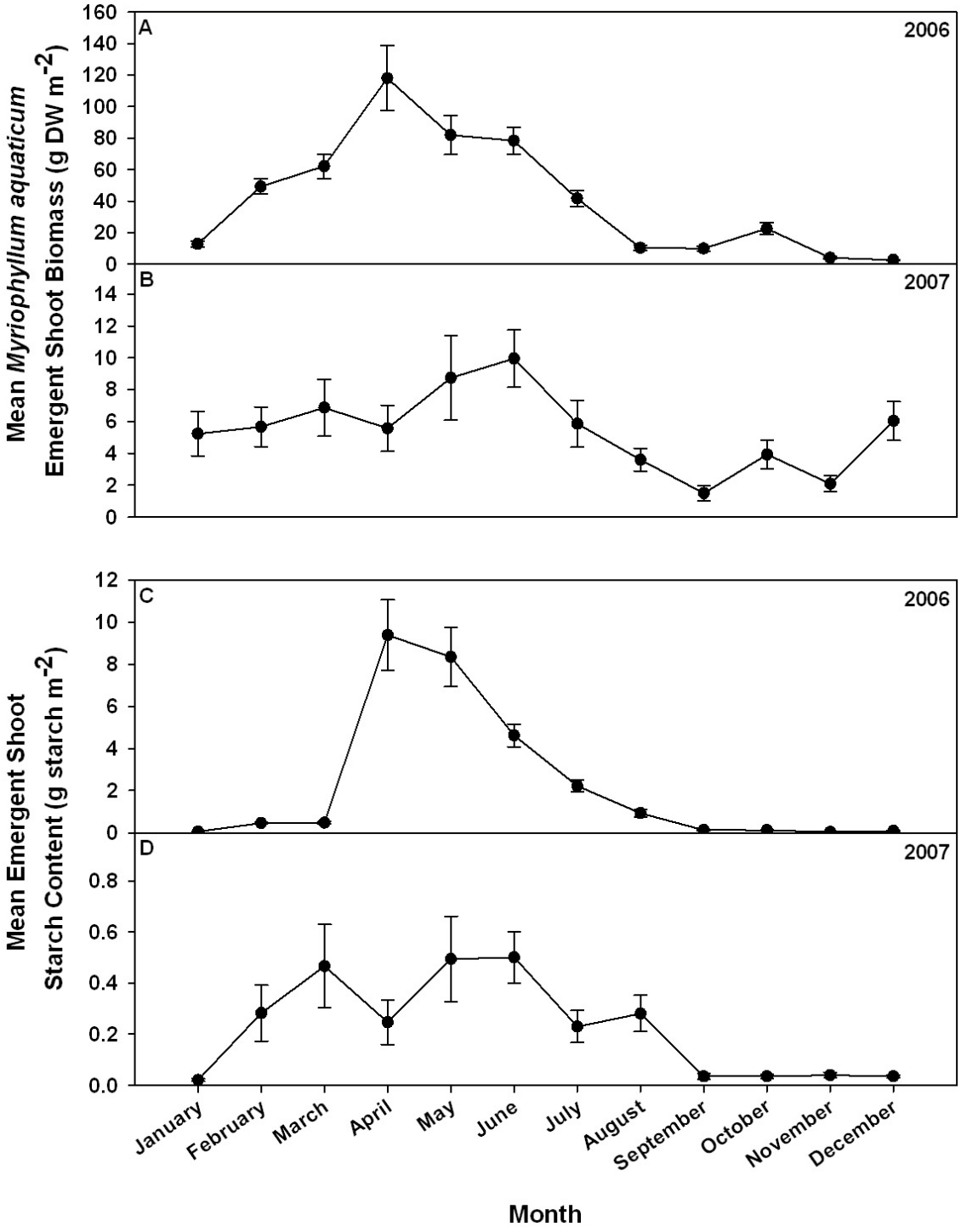

Figure 2.5 Mean (± 1 SE) *Myriophyllum aquaticum* seasonal emergent shoot biomass (A, B g m^{-2}) and starch content (C, D g starch m^{-2}) from four populations in Mississippi in 2006 and 2007.

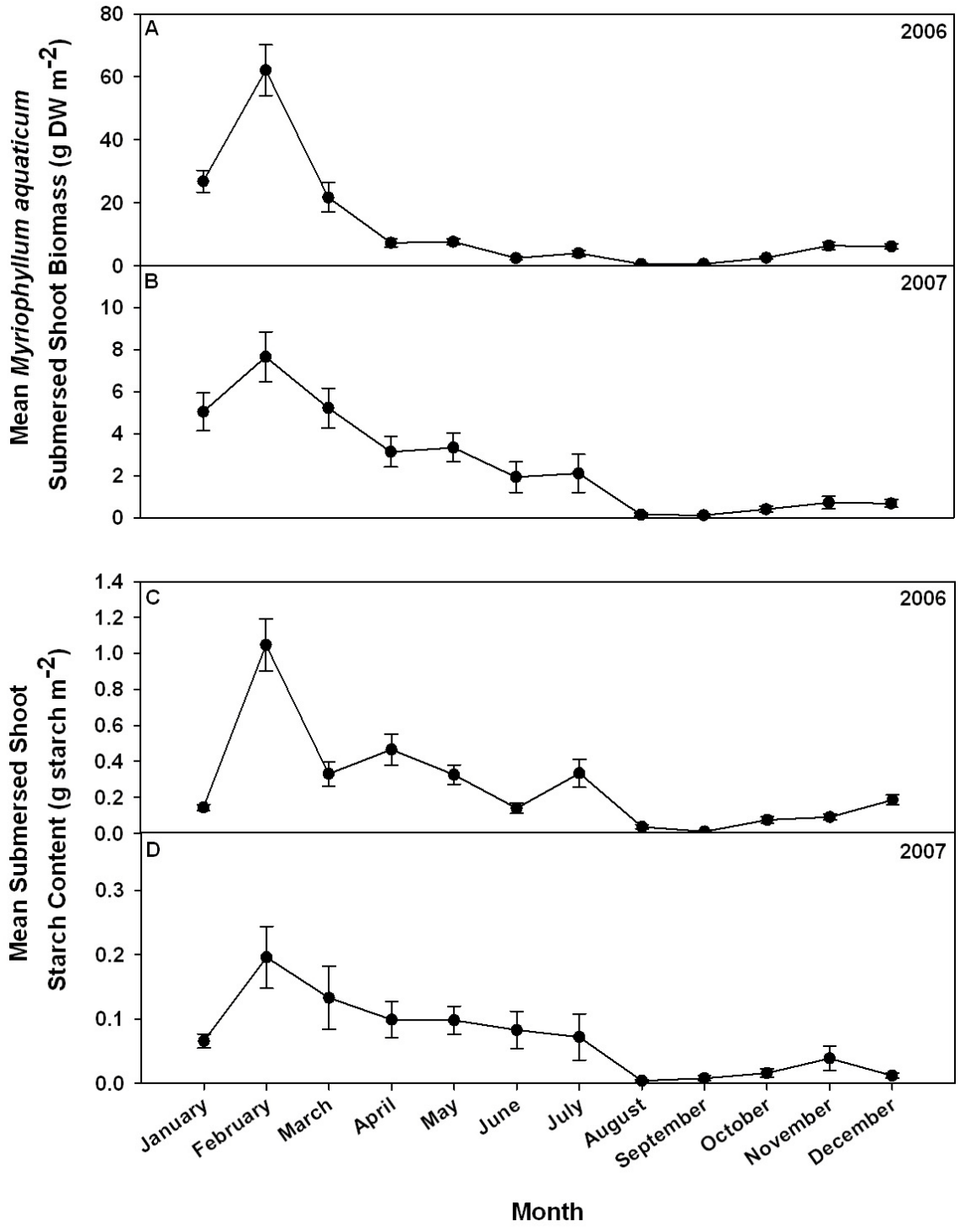

Figure 2.6 Mean (± 1 SE) *Myriophyllum aquaticum* seasonal submersed shoot biomass (A, B g m^{-2}) and starch content (C, D g starch m^{-2}) from four populations in Mississippi in 2006 and 2007.

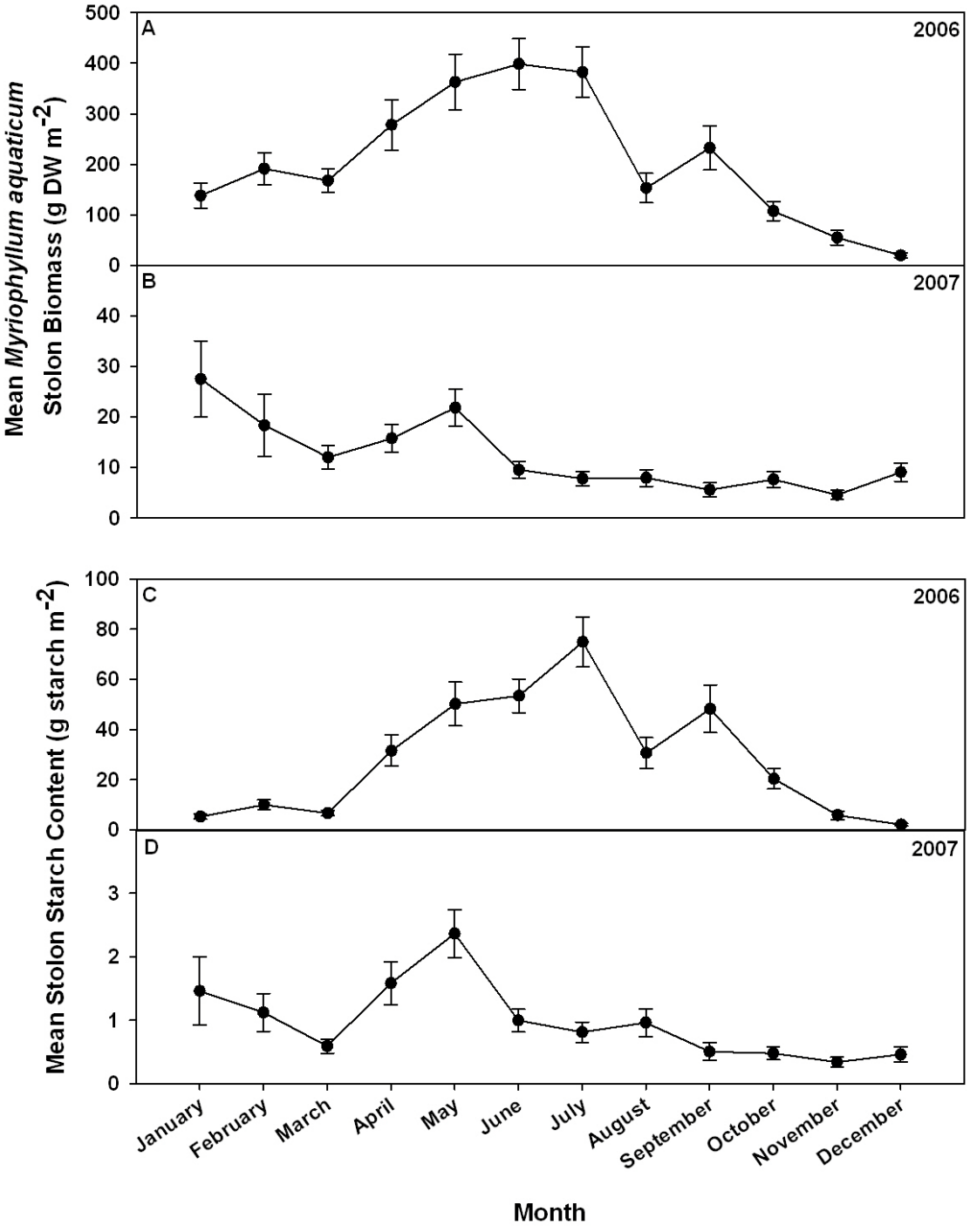

Figure 2.7 Mean (± 1 SE) *Myriophyllum aquaticum* seasonal stolon biomass (A, B g m^{-2}) and starch content (C, D g starch m^{-2}) from four populations in Mississippi in 2006 and 2007.

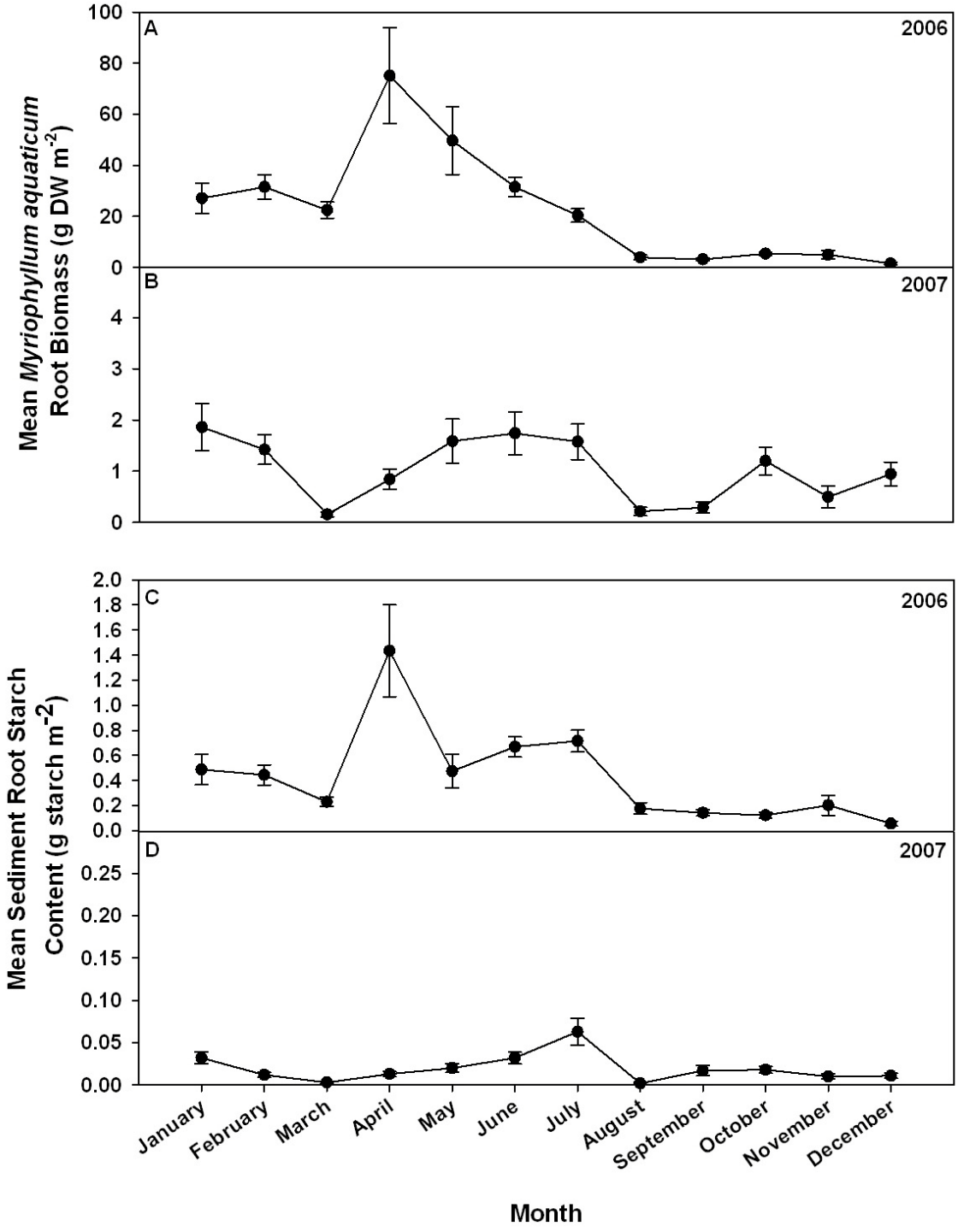

Figure 2.8 Mean (± 1 SE) *Myriophyllum aquaticum* seasonal sediment root biomass (A, B g m^{-2}) and starch content (C, D g starch m^{-2}) from four populations in Mississippi in 2006 and 2007.

CHAPTER III

INFLUENCES OF LIGHT INTENSITY VARIATIONS ON GROWTH CHARACTERISTICS OF THE INVASIVE AQUATIC MACROPHYTE *Myriophyllum aquaticum*

Abstract

Parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc] is a nonnative aquatic heterophyllous plant. Having both emergent and submersed leaves may allow *M. aquaticum* to invade and colonize highly disturbed or less than optimal environments through changes in growth habit. The reallocation of resources to emergent or submersed growth likely allows *M. aquaticum* to overcome changes in light availability. The objective of this study was to determine the effects of light availability on growth characteristics such as plant length, biomass, and relative growth rate of *M. aquaticum* through replicated mesocosm experiments. Experiments were conducted in May through August of 2006 and 2007 to determine the response of *M. aquaticum* grown in full sunlight, 30%, 50%, and 70% shade. Measurements were taken of total plant length, emergent shoot length, submersed shoot length, and the total of number of emergent and submersed shoots were recorded. Plants were sorted to emergent shoots, submersed shoots, sediment roots, and stolons, dried then weighed. After 12 weeks, *M. aquaticum* biomass mass was different ($p < 0.01$) between light treatments. Differences in plant mass were a result of greater plant growth in the 30% light treatment. Total plant length

was greatest ($p < 0.01$) in the 50% light treatment, with a reduction in plant length observed in full sunlight. Emergent shoot length was reduced ($p < 0.01$) in full sunlight, while an increase in submersed shoot length occurred in 70% shade. These data suggest that intermediate light availability is optimal for *M. aquaticum* growth and that the growth of two leaf forms is a physiological response to changes in light availability.

Introduction

The presence and spread of invasive species are often associated with the activities of humans and habitat degradation as a result of these activities (Mills et al. 1994). Wetlands and shallow lakes are often prone to invasion due to the increased frequency at which disturbances occur. Disturbances that can alter the light environment, such as changes in the water regime, can cause a shift in species dominance and species composition within a waterbody (van der Valk 2005). If native species are removed, this may facilitate invasions by opening niche space resulting in more access to resources for invading species (Davies et al. 2005; Lockwood et al. 2005; Capers et al. 2007). In light of the negative impacts often associated with species invasions, it is important to gain an understanding of the factors that may limit a species' ability to invade a particular habitat (Chadwell and Engelhardt 2008).

In aquatic habitats, light can often be the most important factor limiting the growth of aquatic macrophytes (Barko et al. 1986), and can determine community composition as well as zonation within a waterbody (Spence 1967; Seabloom et al. 1998). Those species that have morphological adaptations to optimize the capture of light will most often be successful in colonizing and establishing populations in low-light

environments (Barko et al. 1986). Such adaptations include changes in whole plant morphology, specific leaf morphology, stem elongation, and canopy production (Barko et al. 1982). Submersed aquatic plants such as Eurasian watermilfoil (*Myriophyllum spicatum* L.) will produce fewer longer shoots with longer leaves that have increased surface areas in response to low light conditions. However, some plant species have adapted alternate growth forms to survive frequent disturbances in the environment.

Parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.] is a heterophyllous herbaceous perennial plant that is not native to the United States. *Myriophyllum aquaticum* has two distinct leaf forms that can grow together on the same plant or more commonly the growth form will be dictated by growing conditions. Emergent leaves are feather-like and grayish green, stiff, and grow in whorls around the emergent shoot (Godfrey and Wooten 1981). These leaves have stomata, a thick waxy cuticle, and short cylindrical leaflets (Sutton and Bingham 1973). Submersed leaves are typically orange to red, lack both stomata and a leaf cuticle, and grow in whorls around submersed shoots (Mason 1957). The anatomical and morphological differences in the submersed and emergent form of *M. aquaticum* may result from physiological adaptations to conditions in their respective environments (Sculthorpe 1967).

Having two distinct growth forms may give *M. aquaticum* the ability to overcome extreme disturbances in the water regime and convey a competitive advantage over macrophytes that are more sensitive to changes in their growing environment. In the Sinos River Basin, Brazil, *M. aquaticum* growth occurred during both a flooded period and a drawdown period (Maltchik et al. 2007). These changes in water regime caused the rapid shift in *M. aquaticum* leaf forms to allow survival in flooded or drawdown

situations. The reallocation of resources to emergent or submersed leaves likely allows *M. aquaticum* to overcome changes in light availability and to optimize the use of light in their respective environments. Therefore, the objective was to determine the direct effects of light intensity on growth characteristics of *M. aquaticum* and to determine growth-limiting levels. Understanding of the environmental constraints posed by light intensities will indicate what environments *M. aquaticum* can colonize and exploit to establish new infestations. These areas can be targeted for more aggressive monitoring to identify infestations at their onset before plants become firmly established.

Materials and Methods

A mesocosm study was conducted at the R.R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS (33°28'29.76" N, 88°46'24.70" W) for 12 weeks from June 5 to August 30, 2006 and repeated from June 6, to August 27, 2007. Both studies were conducted in 24, 1100-L mesocosms (L 161 cm, W 175 cm, H 64 cm) with six repetitions per light treatment: full sun, 30% shade, 50% shade, and 70% shade. Shade cloth of desired percentage was suspended above and on all four sides of a grouping of six tanks with the exception of the full sun treatment. Water was supplied to each mesocosm from an irrigation reservoir adjacent to the mesocosm facility. All mesocosms were filled to a water depth of approximately 50 cm. Air was supplied to all mesocosms from a regenerative air blower using 2.5 cm stone diffusers and a PVC lift pipe. Daily incident light intensity measurements were recorded in each light treatment between the hours of 12:00 and 2:00 using a LI-1400 data logger with a LI-190 photometric sensor (LI-COR Biosciences, Lincoln, NE). A HOBO temperature probe

(Onset Computer Corporation, Pocasset, Maine) was deployed in each mesocosm to record temperature in 1 h intervals for the duration of the study.

Planting

Planting of *M. aquaticum* consisted of placing two apical emergent shoots, approximately 20 cm in length, into each of 336, 3.78 L pots containing a top soil, loam, and sand mixture (3:2:1). Sediment was amended at a rate of 2 g L^{-1} in each pot using Osmocote 19-6-12 fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH). After planting, 14 pots of *M. aquaticum* were placed into each mesocosm. *Myriophyllum aquaticum* biomass was assessed at 0, 2, 4, 6, 8, 10, and 12 weeks after start (WAS) by removing two pots from each tank. Plants were removed from the pots and rinsed to remove sediment, debris, and algae growing on the plants. After rinsing, total plant length (cm) was recorded for each plant by measuring from the sediment roots (sediment line) to the longest emergent tip. Plants were then separated into emergent shoots, submersed shoots, stolons, and sediment roots. Total number of emergent and submersed shoots was recorded, and then the length of each shoot measured and recorded (cm). Plant tissues were then placed into a forced air oven and dried at 70 C for 72 hours. *Myriophyllum aquaticum* biomass is expressed as g DW pot^{-1} for total biomass and each plant tissue.

Data Analysis

Statistical analyses were conducted using SAS software (SAS Institute, Inc., Cary, NC, USA). A mixed procedures model was utilized to examine main effects of light treatments on biomass, plant length, and shoot number of *M. aquaticum*; year and

subsequent interactions with year were considered random effects in the model (Littell et al. 1996). Data were analyzed within WAS to account for a treatment by WAS interaction. If a significant main effect was observed, treatment means were separated using least squares means and grouped using the Least Significant Difference method. Relative growth rates (RGR) (ln g DW pot^{-1} day^{-1}) were calculated for each WAS and light treatment for total, emergent shoot, submersed shoot, stolon and sediment root biomass using the following equation outlined by Hunt (1982):

$$r = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1} \quad (3\text{-}1)$$

where W_1 and W_2 are plant dry weights at times t_1 and t_2. A mixed procedures model was also utilized to determine differences in RGR within WAS for each biomass tissue type.

Results

Light intensity measurements and water temperature are displayed in Figure 3.1. On average incident light was reduced by 35.8 ± 9.1%, 59.4 ± 7.2%, and 78.8 ± 4.1% of full sunlight for the 30, 50, and 70% light treatments respectively. These data indicate that the shade cloth offered the desired levels of light attenuation for the study. The variation in light levels did not result in a difference in the total number of emergent shoots ($p = 0.48$) or submersed shoots (0.96) produced by *M. aquaticum* over 12 weeks of plant growth (data not shown). Additionally, daily water temperatures were on average 29.6 ± 0.03 C, 28.2 ± 0.01 C, 27.2 ± 0.01 C, and 26.3 ± 0.04 C for the full sun, 30%, 50%, and 70% light treatments respectively; and were different between treatments.

Total plant length, however, was affected by light levels as early as 4 WAS where plants grown in 50% shade were on average 15% longer than plants in the other treatments (Figure 3.2). By 12 WAS, *M. aquaticum* length was still greater when plants were grown in 50% shade. Plant length was 159.7 ± 3.7 cm pot^{-1} when grown in 50% shade whereas plant lengths were 126.3 ± 3.9, 145.5 ± 4.1, and 149.6 ± 3.4 cm pot^{-1} for the full sunlight, 30% and 70% light treatments, respectively 12 WAS. Differences in emergent shoot length were not as well defined as with total plant length by 12 WAS (Figure 3.3). However, from 6 WAS to the conclusion of the study, emergent shoot length was always greater when plants were grown in 30-70% shade as opposed to full sunlight. By 12 WAS, *M. aquaticum* grown under shaded conditions had emergent shoots that were on average 24% longer than plants grown in full sunlight. Submersed shoot length of *M. aquaticum* was greatest when plants were grown in 70% shade as early as 2 WAS (Figure 3.4). However at 6, 8, and 10 WAS, submersed shoot length was similar to plants grown at 30 and 50% shade. By 12 WAS submersed shoot length was significantly greater (18%) when plants were grown in 70% shade versus plants grown in the other light treatments.

Pretreatment biomass was 1.5 ± 0.9 g DW pot^{-1}. At the conclusion of the study, biomass was > 40.0 g DW pot^{-1} which indicates that plants were actively growing in all light treatments throughout the study. Total biomass was greater when plants were grown in 30 and 50% shade at 8 and 10 WAS; however, by 12 WAS total biomass was greatest when grown in 30% shade (Figure 3.5). At the conclusion of the study, total biomass was reduced when plants were grown in 70% shade as compared to all other light treatments. Total biomass after 12 weeks in the 30% treatment was 109.1 ± 7.4 g

DW pot^{-1}, whereas biomass in the 70% treatment was 49.6 ± 3.6 g DW pot^{-1}, a 55% decrease in biomass. Total biomass of *M. aquaticum* grown in full sunlight was 80.5 ± 6.0 g DW pot^{-1} at 12 WAS.

Emergent shoot biomass followed a similar pattern as total biomass where *M. aquaticum* responded more favorably to the 30 and 50% light treatments at 8 and 10 WAS (Figure 3.6). Biomass was 27.0 ± 1.4 g DW pot^{-1} 12 WAS when plants were grown in 30% shade, whereas emergent shoot biomass was 16.2 ± 1.1 and 19.7 ± 1.1 g DW pot^{-1} for plants in the 70% and full sunlight treatments respectively. Emergent shoot biomass at 12 WAS was greater when plants were grown in 30% shade when compared to other light treatments. Emergent shoot biomass comprised 12 to 45% of total biomass across light treatments and WAS. Submersed shoot biomass comprised the smallest proportion of total biomass throughout the study, where it never exceeded 2% of total biomass. At the conclusion of the study submersed biomass only accounted for 1.8, 1.1, 1.3, and 1.6% of total biomass for full sunlight, 30, 50, and 70% light treatments, respectively. Submersed shoot biomass was not different ($p = 0.05$) between light treatments at 12 WAS (Figure 3.7).

Stolon biomass consistently comprised the greatest proportion of total biomass where it ranged from 34 to 81% across light treatments and WAS. Biomass was lower ($p < 0.01$) for plants grown in 70% shade from 4 to 12 WAS (Figure 3.8). Stolon biomass was similar between the full sunlight, 30%, and 50% treatments from 6 to 12 WAS, where on average biomass was > 50% than stolon biomass in the 70% light treatment.

Sediment root biomass was greatest in the 30% light treatment at 8 WAS (Figure 3.9). However, at 12 WAS, biomass was similar between plants grown in 30 and 50%

shade, and sediment root biomass was similar between plants grown in 50 and 70% shade. Sediment root biomass of plants grown in 30% shade was always greater than plants grown in full sunlight which reflects the pattern observed for total biomass and emergent shoot biomass. Sediment root biomass comprised 6 to 20% of total biomass across light treatments and WAS.

The relative growth rates of *M. aquaticum* tissues varied greatly throughout the study and most often light intensity did not affect growth rates (Table 3.1). However, a general pattern is visible with respect to RGR, tissue type, and when significance was observed. Significant effects were observed for total biomass, emergent shoot biomass, and stolon biomass between 2 and 6 WAS times of increased growth and canopy production. Submersed shoot RGR was only affected by light intensity after 6 weeks when plants had reached the water surface and new shoot production began or an emergent canopy had formed causing self shading. Similarly root RGR effects were observed at 8 and 10 WAS, which would correspond to times after plants had emerged from the water column and formed a canopy.

Discussion

Increasing light availability did not result in increased growth of *M. aquaticum*. Results indicated that optimal growth occurs in intermediate light intensities, particularly 30% shade. *Myriophyllum aquaticum* did grow in full sunlight and survived in 70% shade through adaptations to optimize its capture and use of light; however, biomass was reduced when *M. aquaticum* was grown in 70% shade. Within a few days of planting, the apical tips changed from the emergent leaf form to the submersed leaf form in all

mesocosms. The change in leaf form is likely a result of reduced light availability and an inability of emergent leaves to process inorganic carbon. In general, when plants are submersed the availability of inorganic carbon for photosynthesis is reduced due to slow diffusion rates in water and the buildup of boundary layers (Madsen and Sand-Jensen 1994). The leaves of emergent shoots have sunken stomata, a thick waxy cuticle, and short cylindrical leaflets, whereas submersed leaves lack stomata and a cuticle (Sutton and Bingham 1973). These morphological changes in leaf structure likely promote gas exchange within the water column. The light saturation point of submersed leaves is between 250-300 μmol m^{-2} s^{-1}, eight times lower than that of emergent leaves (Salvucci and Bowes 1982). The lower photosynthetic rate of submersed leaves suggests that this growth form is adapted to a shade environment (Salvucci and Bowes 1982).

In the shaded treatments, submersed shoots elongated to reach the water surface and maximize photosynthesis, which is evident by the increased shoot length in the 70% treatment. Eurasian watermilfoil (*Myriophyllum spicatum* L.), hydrilla (*Hydrilla verticillata* Royle), and egeria (*Egeria densa* Planch.) increased shoot length with increasing levels of shade (Barko and Smart 1981). In low light environments, these submersed species reallocated energy to the development of a canopy through shoot elongation and an increase in upper branches and leaf whorls (Barko and Smart 1981). However, once the submersed shoots of *M. aquaticum* reached the water surface, growth reverted back to the emergent leaf form. Optimal photosynthesis of *M. aquaticum* occurs as the emergent form. Therefore, *M. aquaticum* will not remain as a submersed plant for long periods of time as the photosynthetic rate of submersed leaves may not be sufficient to support plant growth (Salvucci and Bowes 1982). The submersed leaf form is an

intermediate growth state and is only utilized for short overwintering periods, times of reduced light and temperature (Sytsma and Anderson 1993a), or to survive disturbances in the growing environment. Prolonged exposure to adverse growing conditions, such as reduced light intensity, will result in reductions in growth or plant mortality. *Myriophyllum aquaticum* grown in 70% shade had reduced total biomass, emergent shoot biomass, and stolon biomass when compared to the other treatments.

Water temperatures were different between light treatments in this study, where a 4 C difference was noted between the full sunlight and 70% light treatment. However, it was not possible to determine specific effects or relationships between water temperature and plant growth because the shade was causing the changes in water temperature. Though, other milfoil species such as Eurasian watermilfoil (*Myriophyllum spicatum* L.), can photosynthesize over a broad range of temperatures (Smith and Barko 1990); and growth increases with increasing water temperatures up to 32 C (Barko and Smart 1981). *Myriophyllum spicatum* is also capable of appreciable photosynthesis at water temperature as low as 10 C (Stanley and Naylor 1972). *Myriophyllum aquaticum* being a milfoil species should have exhibited increased growth in the full sunlight treatment due to increases in light availability and temperature.

Overall, *M. aquaticum* has a light saturation point that approaches full sunlight and therefore it would be expected that plants exposed to full sunlight would have increased growth (Salvucci and Bowes 1982). However, current data of reduced biomass and shoot length in full sunlight as compared to 30% shade, full sunlight light may not be optimal for this species even with the emergent leaf form. Increased light availability is often correlated to increases in water temperature, which may have resulted in water

stress of *M. aquaticum*, where transpiration from emergent shoots exceeded water uptake. In laboratory studies, however, Sytsma and Anderson (1993b) concluded that water loss due to transpiration was only 15 ml d^{-1} and biomass was produced with an economy of water use similar to C_4 terrestrial plants. *Myriophyllum aquaticum*, however, is a C_3 plant (Salvucci and Bowes 1982); therefore, photorespiration may have decreased as temperatures increased resulting in greater energy use in full sunlight and an overall reduction in plant growth as photorespiration can range from high to very low depending upon the environment in which it is growing (Salvucci and Bowes 1982). Aquatic habitats that subject plants to reduced CO_2 availability, high O_2, light, and temperature may enhance CO_2 loss via photorespiration and adversely impact plant growth (Van et al. 1976); though no research has been conducted studying the direct effects of temperature on *M. aquaticum* growth and photosynthesis. However, one way to offset costs associated with harsh growing conditions is to have alternative growth forms that are better adapted to current growing conditions.

Myriophyllum aquaticum is described as an amphibious responder, or a species that grows in a variety of habitats and conditions, and displays a high level of morphological plasticity (heterophylly) in response to changes in its growing environment (Casanova and Brock 2000). There have been many factors cited for having a role in inducing heterophylly in aquatic plants including temperature (Deschamp and Cooke 1984; Goliber and Feldman 1990; Kane and Albert 1982), photoperiod (Cook 1969), and light intensity (Goliber 1989). Light quality has also been attributed to the induction of heterophylly (Lin and Yang 1999). We observed a general increase in submersed shoot biomass in the 30% and 50% light treatments 6 WAS and an increase 10

WAS, followed by a switch to emergent shoots when plants reached the water surface. *Myriophyllum aquaticum* was likely maximizing growth under reduced light conditions by growing submersed shoots. When plants reached the water surface and light availability increased, the growth form changed to emergent shoots in order to maximize photosynthesis.

In its native habitat of South America, *M. aquaticum* is often found growing in palustrine habitats, or areas that are prone to frequent water level variations (Rolon and Maltchik 2006). Variations in water depth effects light quality and quantity and the overall ability of plants to reach the water surface (Casanova and Brock 2000). Under stable water regimes, different species will exhibit different depth tolerances as a result of light availability, resulting in plant zonation within the community with submersed plants becoming dominant at deeper depths (Seabloom et al. 1998; Seabloom et al. 2001; van der Valk 2005). *Myriophyllum aquaticum* is adapted to habitats that have frequent short periods of inundation where plants survive by growing submersed shoots. It was observed that the duration of flooding was an important factor controlling the growth and establishment of amphibious plant species (Casanova and Brock 2000).

Flood duration determines whether there is sufficient time for plants to respond to flood conditions by changing morphology or elongation of stems (Casanova and Brock 2000). Under sustained flooding of 12 weeks, *M. aquaticum* biomass was reduced when water depths were > 30 cm (Wersal, unpublished data). It was concluded that the reduced light availability in deeper water depths and the previously reported low photosynthetic rate of the submersed shoots limited shoot elongation to the water surface and the subsequent growth of an emergent canopy, ultimately resulting in reduced

biomass. The light treatments utilized in the current study may have had similar light intensities to what *M. aquaticum* would experience under prolonged flooded conditions. *Myriophyllum aquaticum* had greater shoot elongation under shaded conditions, which would be similar to shoot elongation to the water surface in deeper water. However, prolonged exposure to low light conditions reduced biomass.

Unlike plant length and biomass, RGR was much less sensitive to light effects, although a few interesting patterns were observed that may highlight life history traits and allocation patterns of *M. aquaticum*. Changes in RGRs were only observed for total biomass and stolon biomass prior to 8 WAS. Changes in RGR were observed for emergent shoot biomass at 4 WAS. The time period from planting to 8 WAS represented rapid shoot production, elongation to the water surface, and the initiation of an emergent canopy to sustain plant growth. The rapid growth of stolons and emergent shoots contributed to the higher RGR for total plant biomass between 2 and 4 WAS. In contrast, submersed shoot RGR and sediment root RGR was only significant after 8 WAS. This suggests that *M. aquaticum* had sufficiently established an emergent canopy and was reallocating energy to the formation of a root crown and the growth of new submersed shoots that would in turn grow to the water surface to fill gaps in the emergent shoot canopy. By 12 WAS the emergent canopy covered the water surface resulting in self shading of new submersed shoots in the water column. *Myriophyllum spicatum* will undergo self shading when a surface canopy is produced. Leaves below 1 m of the surface canopy begin to senesce and slough due to the light attenuation of the surface canopy (Madsen et al. 1991). The morphology of submersed *M. aquaticum* leaves is

similar to that of *M. spicatum*, and therefore self shading could be a plausible explanation for the observed negative RGR of submersed shoots during this time period.

Aquatic plants are generally very plastic in their response to environmental factors. Most often in reduced light environments plant and leaf morphology will change, in general producing fewer, longer shoots and leaves (Barko and Smart 1981; Barko et al. 1982). The anatomical and morphological differences in the emergent and submersed forms of *M. aquaticum* likely result from physiological adaptations to conditions in their respective environments (Sculthorpe 1967; Salvucci and Bowes 1982). The ability to adapt to changing environmental conditions are important determinants for success in plant growth, especially in low light environments (Barko et al. 1986). Species such as *M. aquaticum* that are capable of elongating to the water surface and forming a canopy may have a competitive advantage over other species (Haller and Sutton 1975; Barko and Smart 1981). *Myriophyllum aquaticum* can not only produce a surface canopy, it can survive as a submersed plant at reduced light intensities for short durations, and survive drawdown conditions for up to 9 months (Maltchik et al. 2007).

Myriophyllum aquaticum could possibly invade a wide range of habitats through shifts in its growth form and annual life history characteristics. However, to fully understand the invasion potential of *M. aquaticum*, more experiments are needed to determine direct effects of environmental variability, resource availability, resource use, and resource allocation, on specific attributes of plant growth (Trémolières 2004). The ability to predict potential habitats suitable for invasion would be invaluable for monitoring and management programs of invasive species. In order to gain predictability

more information is needed on plant response to environmental factors and resource availability across a landscape.

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Table 3.1 Mean relative growth rates (ln g DW d^{-1}) for *Myriophyllum aquaticum* biomass. Standard error is ≤ 0.01for all RGR estimates. Analyses were conducted within tissue type and WAS, values sharing the same letter are not statistically different at a $p < 0.05$ significance level.

	Weeks After Start					
Light Treatment	**2**	**4**	**6**	**8**	**10**	**12**
Total Biomass						
Full Sun	0.01ab	0.02ab	0.04	0.06	0.07	0.07
30% Shade	-0.02b	0.04a	0.04	0.09	0.05	0.07
50% Shade	0.01a	0.05a	0.06	0.09	0.05	0.05
70% Shade	0.02a	0.00b	0.04	0.08	0.06	0.06
	p = 0.02	p = 0.01	p = 0.34	p = 0.17	p = 0.49	p = 0.10
Emergent Shoot						
Full Sun	-0.10	0.05bc	0.07	0.09	0.05	0.05a
30% Shade	-0.13	0.12a	0.05	0.11	0.05	0.04a
50% Shade	-0.12	0.10ab	0.10	0.09	0.05	0.01b
70% Shade	-0.10	0.01c	0.08	0.13	0.05	0.03ab
	p = 0.18	p < 0.01	p = 0.31	p = 0.16	p = 0.88	p = 0.02
Submersed Shoot						
Full Sun	-0.17	0.06	0.05	0.00b	0.07	0.02a
30% Shade	-0.15	0.05	0.01	0.08a	0.03	-0.03b
50% Shade	-0.17	0.09	0.00	0.09a	0.02	-0.03b
70% Shade	-0.13	0.04	0.00	0.07a	0.03	-0.03b
	p = 0.23	p = 0.44	p = 0.16	p < 0.01	p = 0.24	p = 0.02
Stolon Biomass						
Full Sun	0.02	0.02a	0.03b	0.05	0.08	0.07
30% Shade	0.00	0.04a	0.02b	0.07	0.06	0.08
50% Shade	0.00	0.02ab	0.07a	0.07	0.06	0.06
70% Shade	0.01	-0.02b	0.04ab	0.05	0.06	0.08
	p = 0.15	p = 0.02	p = 0.01	p = 0.29	p = 0.45	p = 0.39
Root Biomass						
Full Sun	-0.16	0.03	0.01	0.06b	0.09a	0.10
30% Shade	-0.17	0.02	0.05	0.15a	0.02b	0.12
50% Shade	-0.15	0.04	0.02	0.09ab	0.08a	0.09
70% Shade	-0.17	0.01	0.03	0.09b	0.07a	0.12
	p = 0.66	p = 0.76	p = 0.29	p = 0.02	p < 0.01	p = 0.64

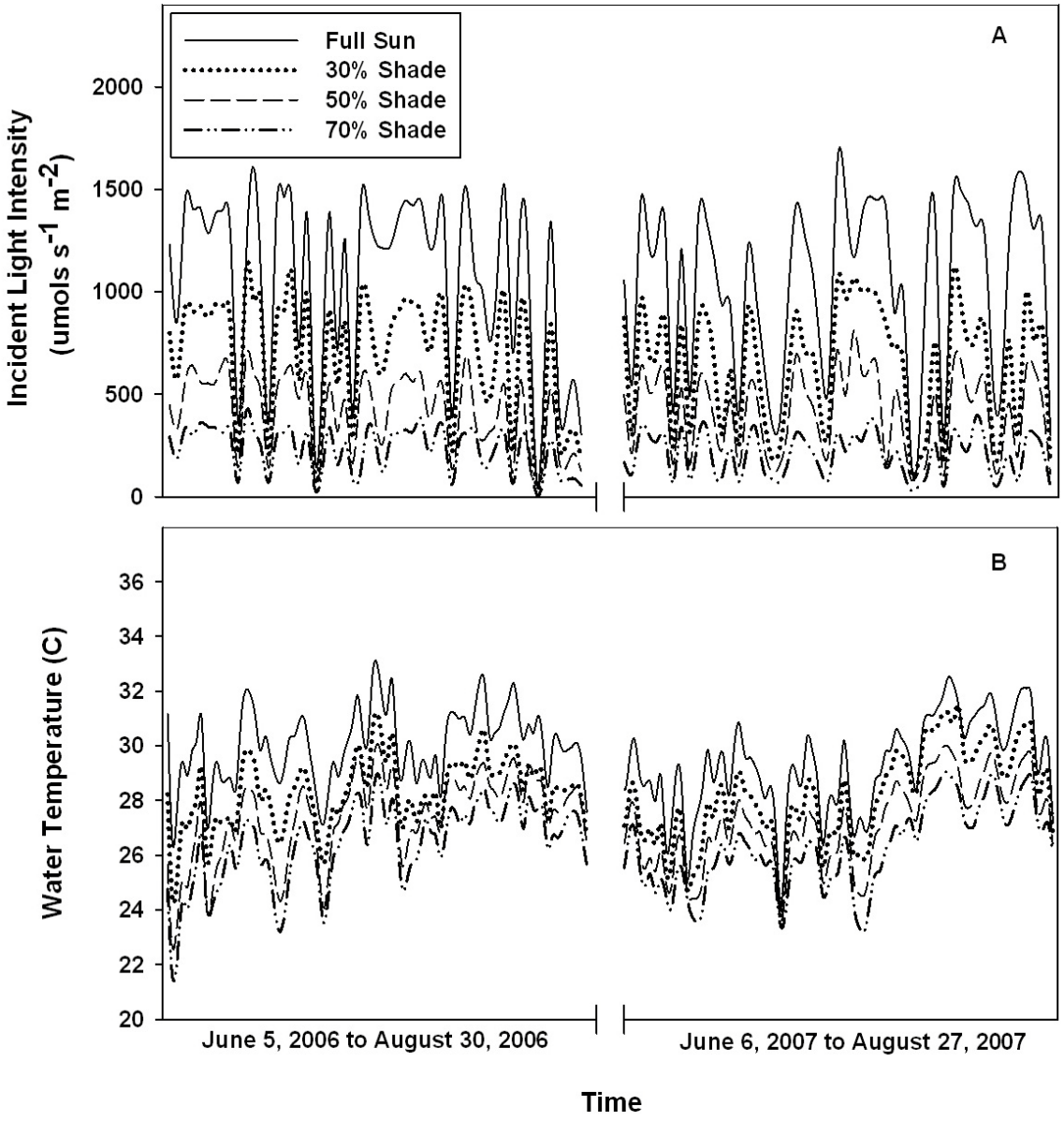

Figure 3.1 Light intensity measurements (A) and water temperature (B) collected throughout the studies conducted in 2006 and 2007.

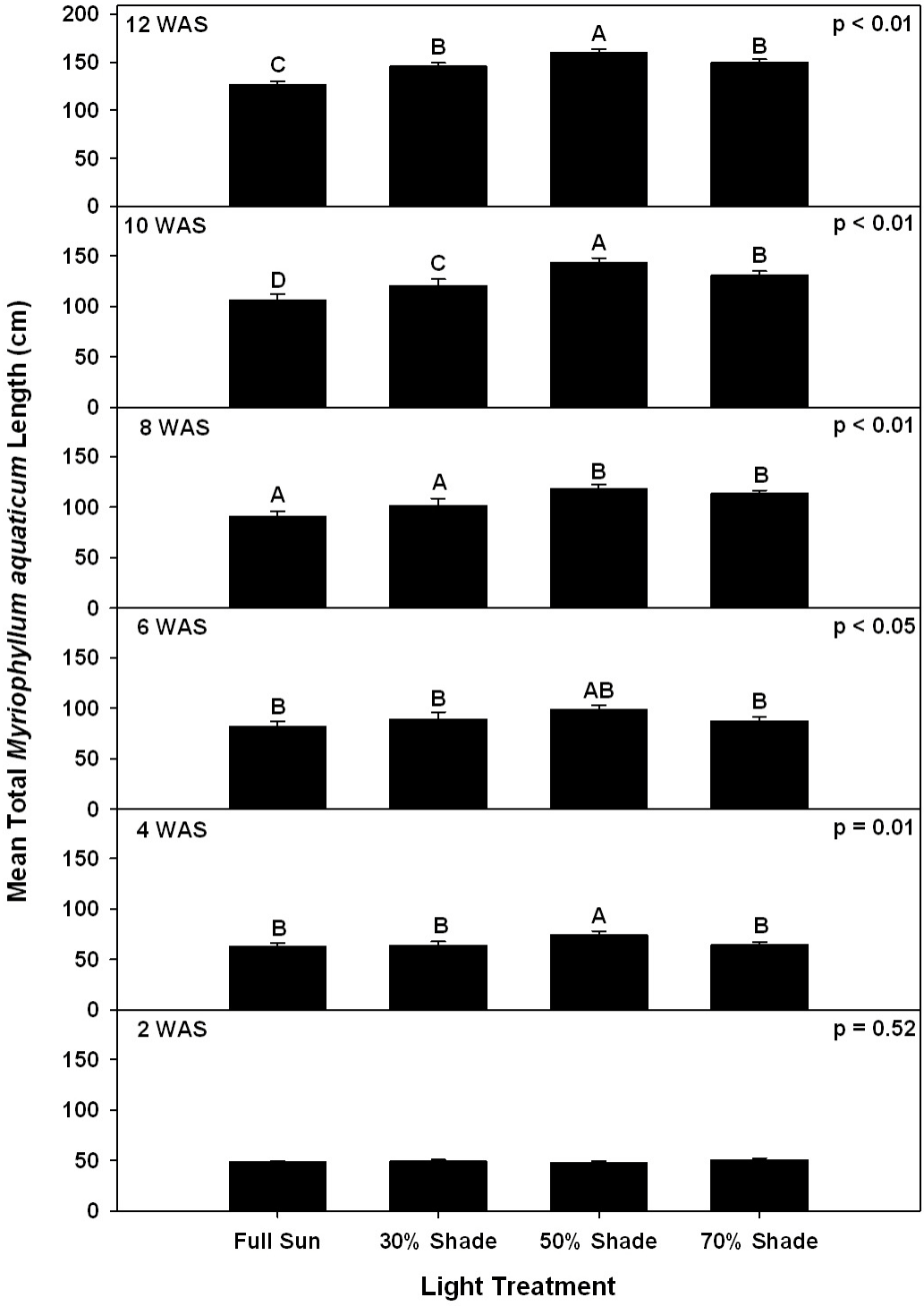

Figure 3.2 Mean (± 1 SE) total plant length of *Myriophyllum aquaticum* at each harvest interval. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

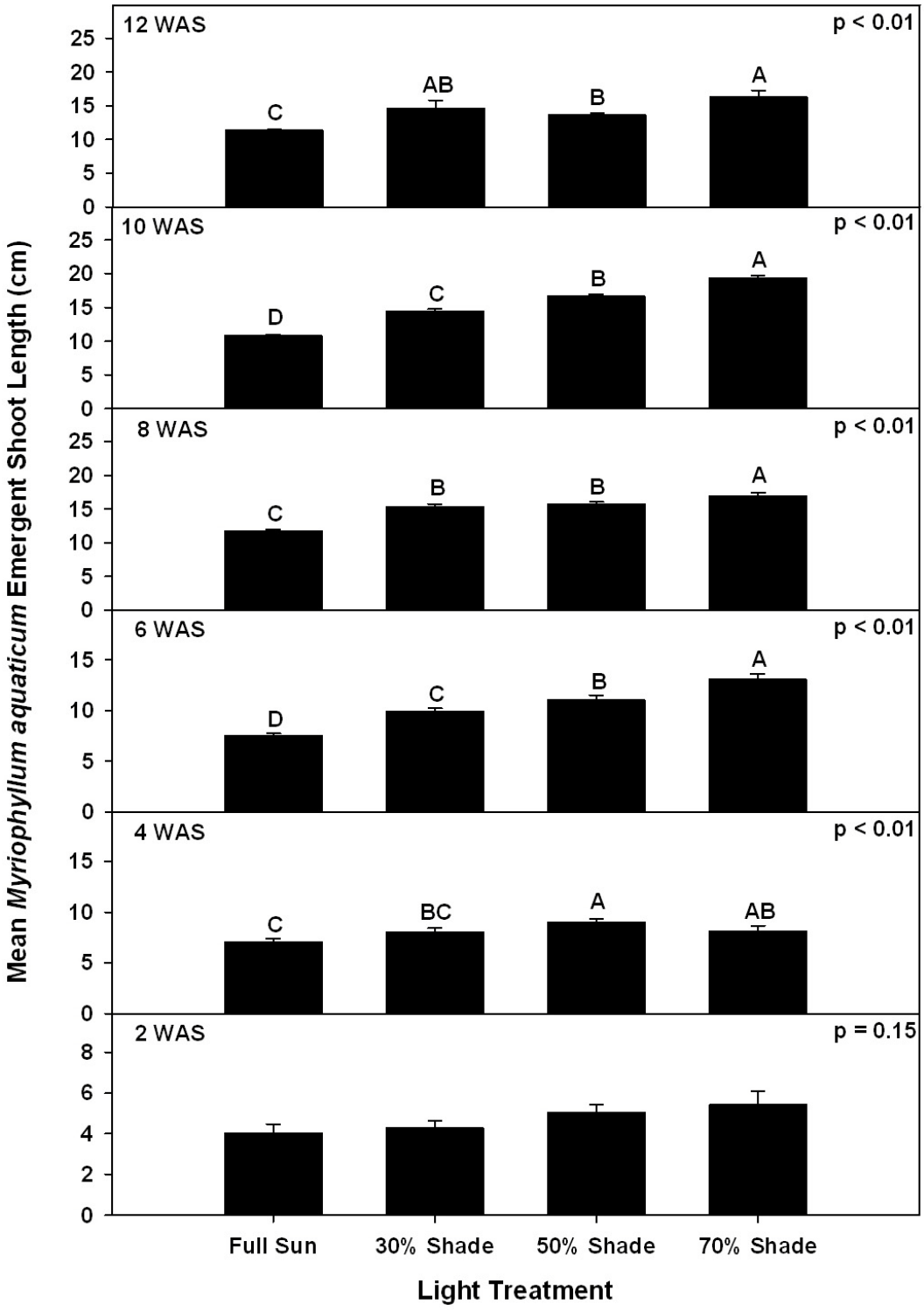

Figure 3.3 Mean (± 1 SE) emergent shoot length of *Myriophyllum aquaticum* at each harvest interval. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

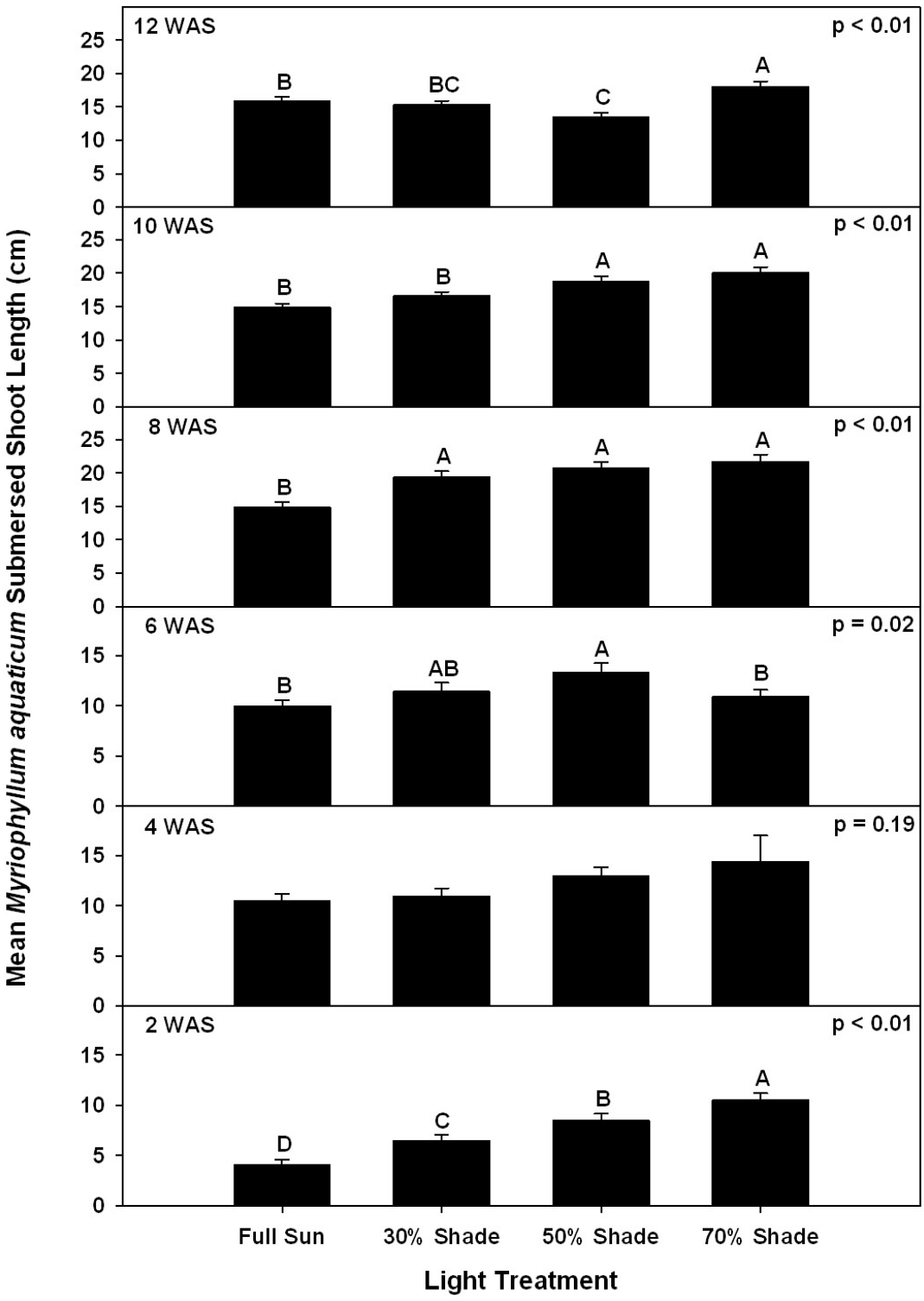

Figure 3.4 Mean (± 1 SE) submersed shoot length of *Myriophyllum aquaticum* at each harvest interval. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

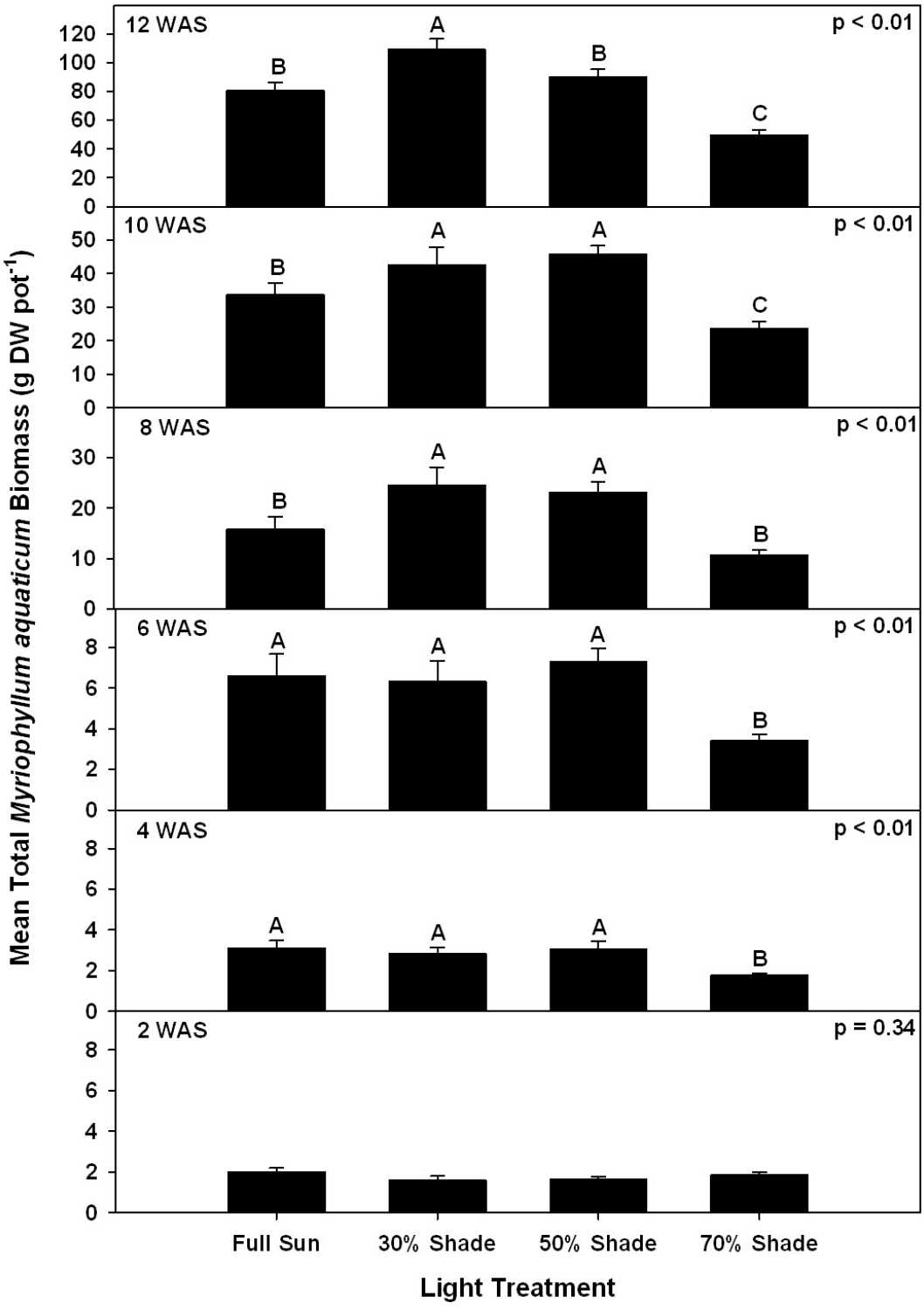

Figure 3.5 Mean (± 1 SE) total plant biomass of *Myriophyllum aquaticum* at each harvest interval. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

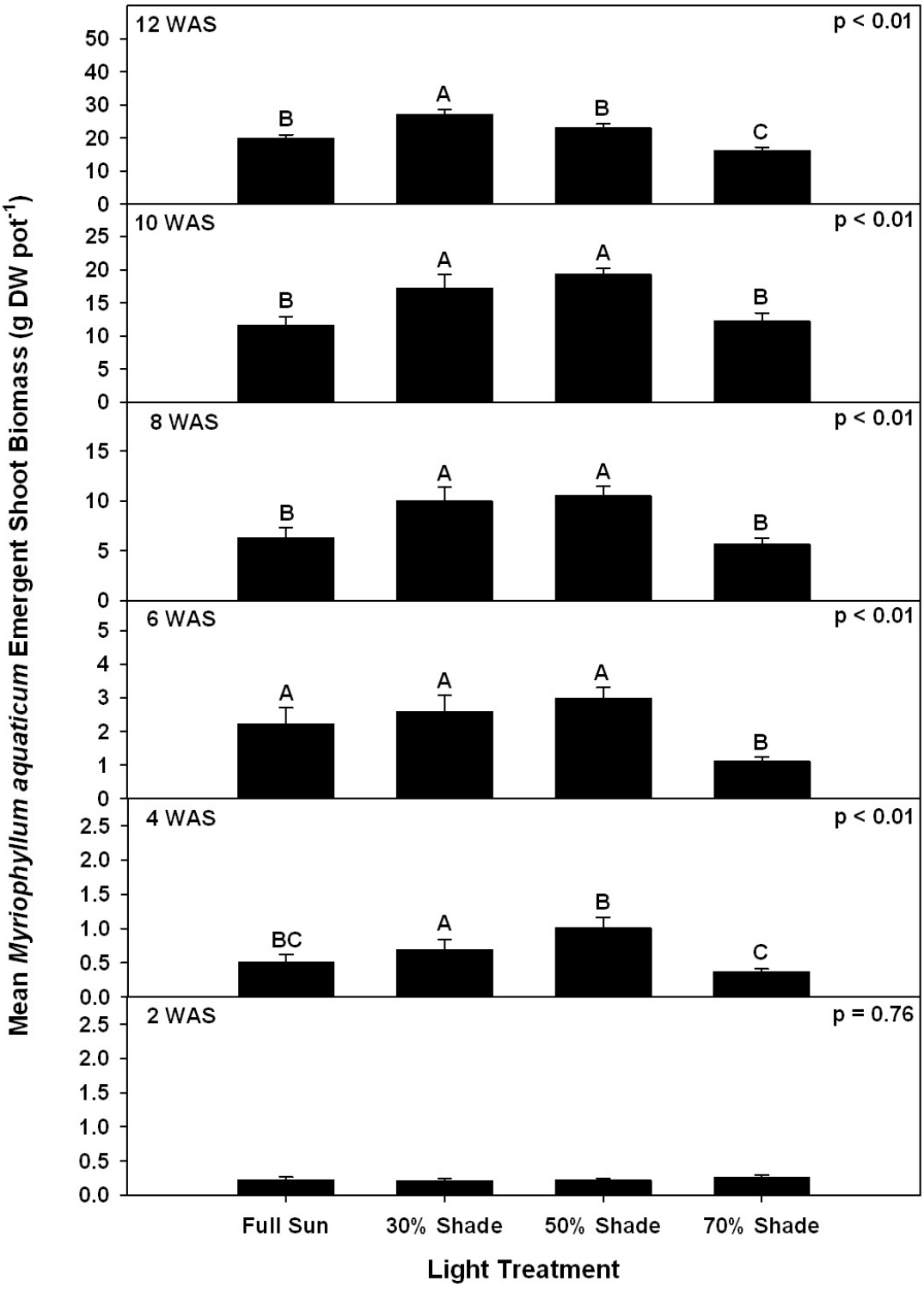

Figure 3.6 Mean (± 1 SE) emergent shoot biomass of *Myriophyllum aquaticum* at each harvest interval. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

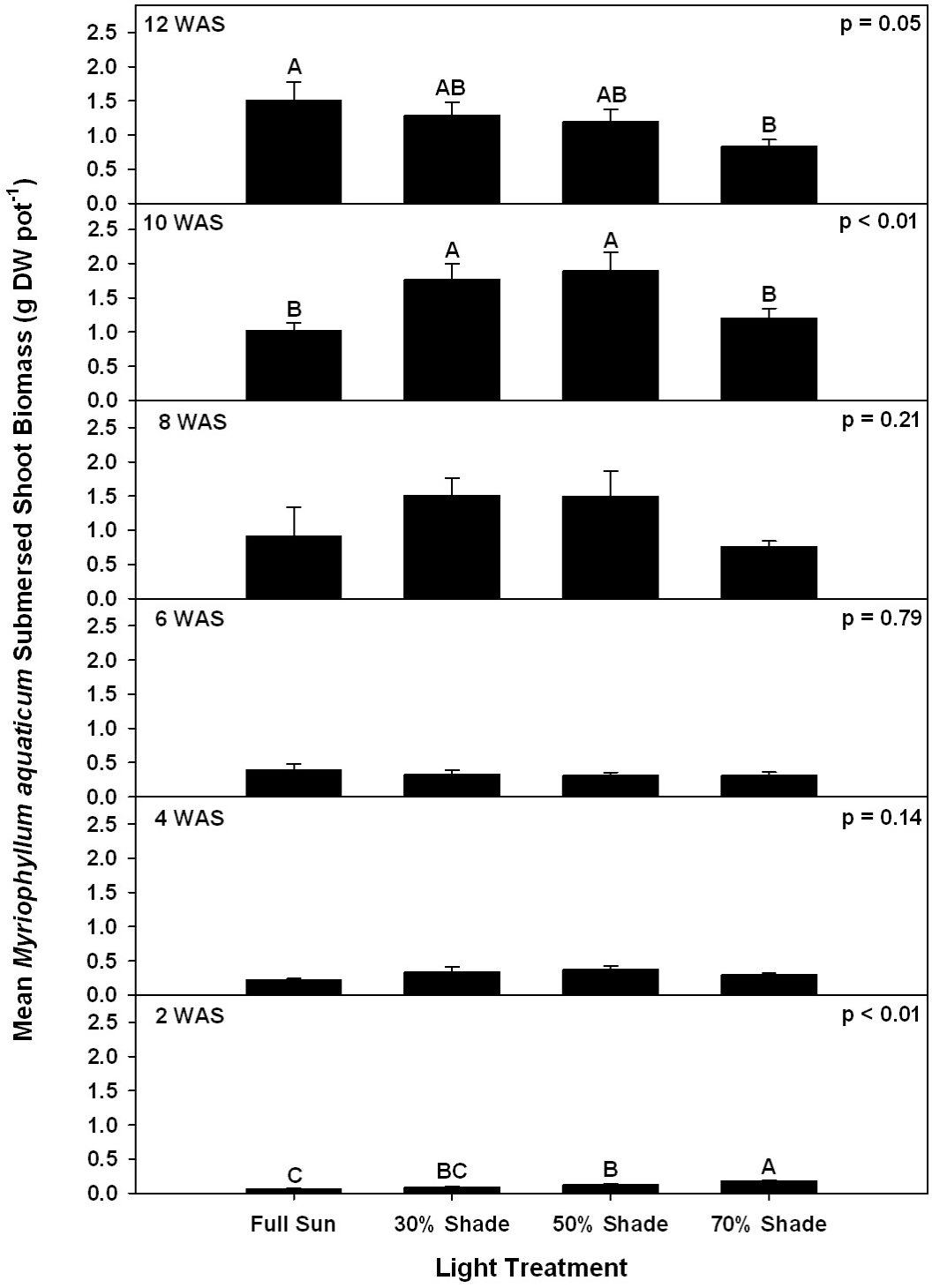

Figure 3.7 Mean (± 1 SE) submersed shoot biomass of *Myriophyllum aquaticum* at each harvest interval. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

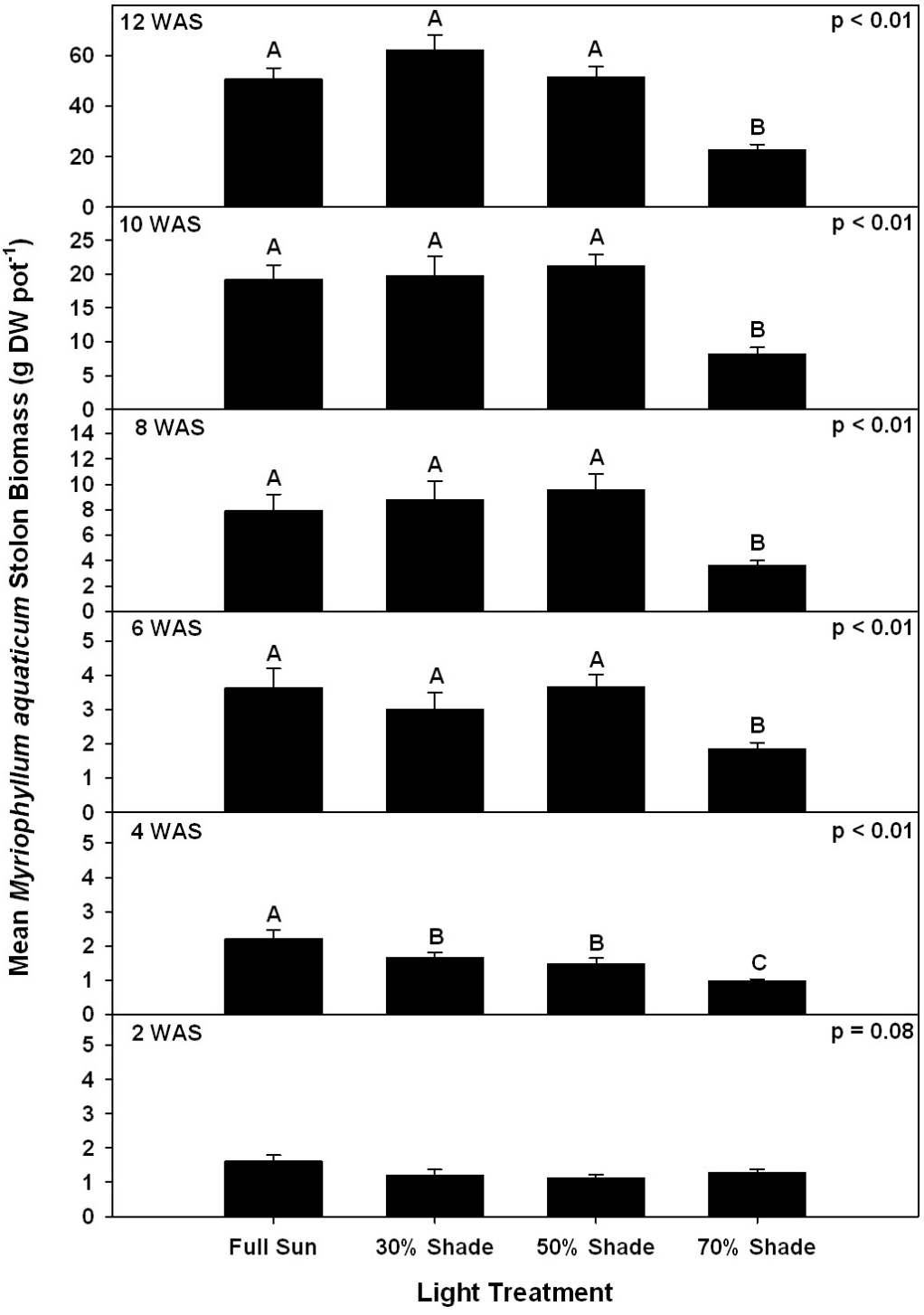

Figure 3.8 Mean (± 1 SE) stolon biomass of *Myriophyllum aquaticum* at each harvest interval. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

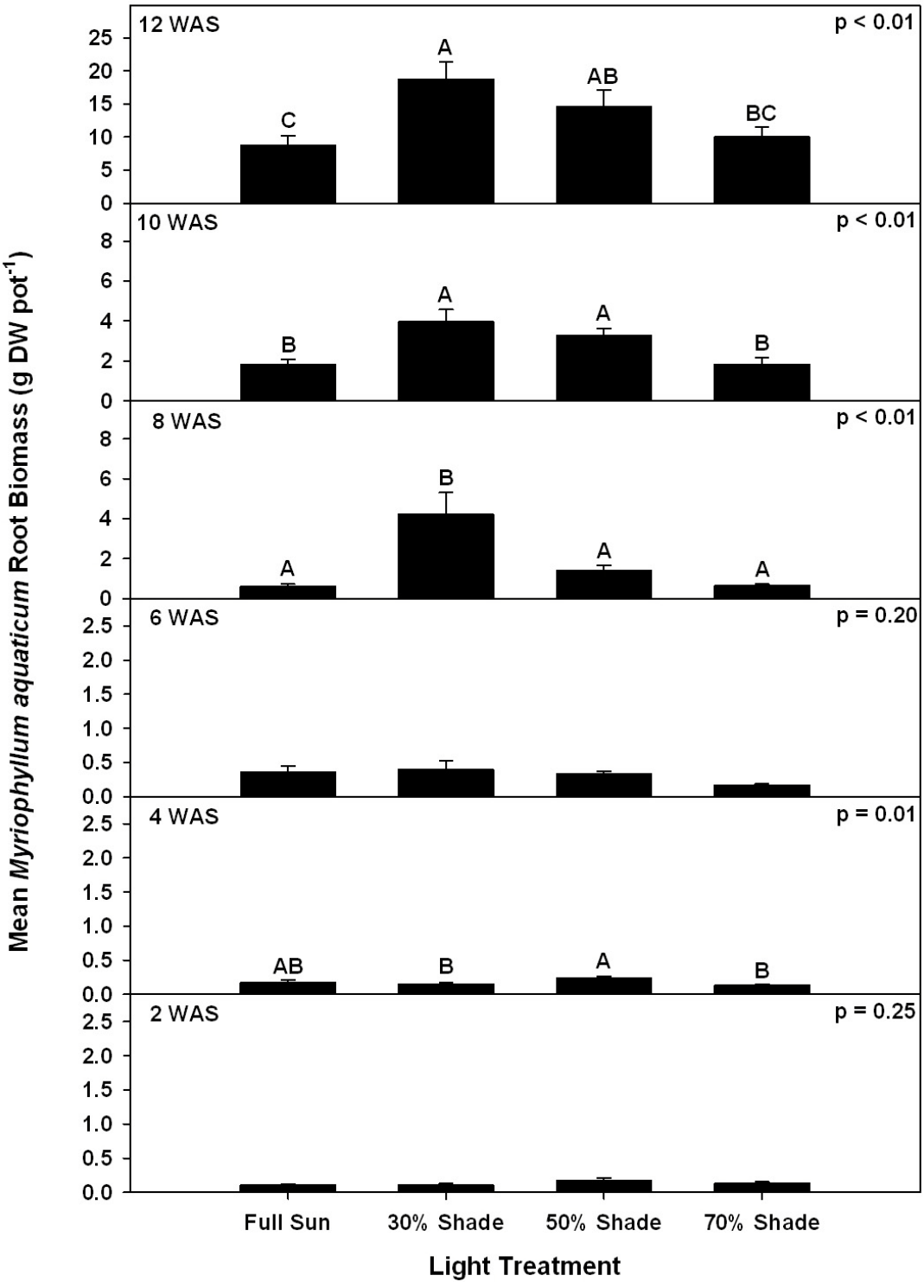

Figure 3.9 Mean (± 1 SE) sediment root biomass of *Myriophyllum aquaticum* at each harvest interval. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

CHAPTER IV

INFLUENCES OF WATER COLUMN NUTRIENT LOADING ON GROWTH CHARACTERISTICS OF THE INVASIVE AQUATIC MACROPHYTE *Myriophyllum aquaticum*

Abstract

Nuisance growth of *Myriophyllum aquaticum* has often been attributed to high amounts of nutrients. The uptake of nitrogen and phosphorus from sediments and their allocation have been documented in both natural and laboratory populations. However, nutrient loading to surface water is increasingly becoming an important issue for water quality standards. Aquatic macrophytes that develop adventitious roots may be able to survive through the uptake of water column nutrients. The objectives were to determine water column nutrient effects on whole plant biomass as well as each tissue type of *M. aquaticum*; and to determine the biomass yield response as nutrient content increased. Mesocosm experiments were conducted where nitrogen (1.80, 0.80, and 0.40 mg L^{-1}; high, medium, and low) and phosphorus (0.09, 0.03, 0.01 mg L^{-1}; high, medium, and low) concentrations were paired and added to the water column. After 12 weeks, the combination of high:low N:P resulted in greater ($p < 0.01$) total biomass and greater biomass for all plant tissues. Total biomass at the high:low N:P combination was 53% greater than biomass at all other combinations. The yield response of *M. aquaticum* was a quadratic function of tissue nutrient content. Yield was positively ($r^2 = 0.82$) related to

increasing nitrogen content, whereas a negative ($r^2 = 0.89$) relationship was determined for increasing phosphorus content. The negative relationship is likely due to increased nutrient competition and shading by algae resulting in reduced *M. aquaticum* growth. Tissue nutrient content indicated that critical concentrations (1.80% nitrogen and 0.20% phosphorus) for growth were not attained except for nitrogen in plants grown in the high:low N:P combination. These data provide further evidence that *M. aquaticum* requires high levels of nitrogen to achieve nuisance growth. Uptake of water column nutrients may be a mechanism for survival during adverse conditions, a means of long distance dispersal of fragments, or may offer a competitive advantage over species that rely on sediment nutrients.

Introduction

Habitats around the world are experiencing an increasing number of invasions of non-indigenous species (Vitousek et al. 1997). Most species fail to successfully establish, but some species will colonize and grow to nuisance levels, often with negative consequences on the local plant community composition, ecosystem functions, and human uses and economic resources (Vitousek et al. 1996; Chapin et al. 2000; Pimental et al. 2000). Environmental changes as a result of species invasions highlight the importance of understanding the factors that may limit a species ability to invade a particular habitat (Chadwell and Engelhardt 2008). The theory of fluctuating resource availability implies that a plant community becomes more susceptible to invasion whenever there is an increase in unused resources (Davis et al. 2000). An increase in unused resources can occur from a decline in resource use from native species, or

resource supply can increase at a faster rate than native species can sequester it (Davis et al. 2000). Whenever resource supply goes up, there are more resources available to invading species which makes a particular habitat more vulnerable to invasion (Davis et al. 2000).

A key resource that is often limiting in aquatic systems is the availability of nutrients for macrophyte growth. In recent years, the amount of nutrients of anthropogenic origin are increasingly finding their way into waterbodies worldwide, which has resulted in declines of macrophyte diversity and changes in community structure (Phillips et al. 1978; Vitousek et al. 1997; Bedford et al. 1999; Montante et al. 2003). Increased nutrient availability may be a key component in the plant invasion process (Elton 1958). Some invasive species are able to increase their growth rates in response to increases in nutrient availability and out-compete native species that cannot respond in a similar fashion (Burke and Grime 1996; Vitousek et al. 1997; Kennedy et al. 2009). These new competitors that are capable of higher growth rates may have long term negative impacts to native community composition (Kennedy et al. 2009).

Nutrient amendment studies for aquatic plants have typically focused on enrichment of sediment nutrients, as the sediment is often considered the most important source of nutrients for aquatic plants (Barko and Smart 1981; Barko and Smart 1986; Spencer and Ksander 1995). However, as the amount of nutrients finding their way into waterbodies increases, understanding the effects of water column enrichment on macrophyte communities, invasive species, and the invasion process will become more important (Kennedy et al. 2009). One invasive aquatic macrophyte that is becoming

problematic in shallow lakes, ponds, streams, rivers, and irrigation and drainage canals is *Myriophyllum aquaticum*.

Previous studies of nutrient uptake by *M. aquaticum* indicate that the majority of nitrogen and phosphorus required for growth could be obtained from the sediment (Bristow and Whitcombe 1971; Barko and Smart 1981). However, Sytsma and Anderson (1993a) reported that only about 2% of water transpired by *M. aquaticum* originated in the sediment, and they concluded that mass flow did not enhance nutrient supply to or from sediment roots. The relative growth rate of sediments roots was similar to zero, further indicating a general lack of importance of sediment roots with respect to nutrient transport within the plant (Sytsma and Anderson 1993a). Therefore, mesocosm experiments were conducted to determine how *M. aquaticum* would respond to the loading of different combinations of nitrogen and phosphorus to the water column. The objectives were to determine water column nutrient effects on whole plant biomass as well as each tissue type of *M. aquaticum*; and to determine the biomass yield response as nutrient content increased. These data should offer insights into habitat types in which *M. aquaticum* could be a successful invader and the potential importance of water column nutrients to invasive aquatic macrophyte growth.

Materials and Methods

A mesocosm study was conducted at the R.R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS (33°28’29.76” N, 88°46’24.70” W) for 12 weeks from September 20 to December 8, 2006 and repeated from September 7 to November 30, 2007. Both studies were conducted in 36, 1100-L mesocosms (L 161 cm,

W 175 cm, H 64 cm) with a 3 by 3 factorial arrangement of treatments arranged in a randomized complete block design with 4 repetitions per nutrient combination. Nutrient combinations consisted of all possible pairings of nitrogen (1.8, 0.8, and 0.4 mg $L^{-}1$; high, medium, low, as ammonium nitrate) and phosphorus (0.09, 0.03, 0.01 mg L^{-1}; high, medium, and low as potassium phosphate) to determine growth limitations of water column nutrients. Nitrogen and phosphorus concentrations were determined based upon concentrations found in eutrophic, mesotrophic, and oligotrophic waters (Wetzel 2001).

Planting of *M. aquaticum* consisted of placing two apical shoots, approximately 20 cm in length, into each of 288, 3.78-L pots containing a washed pea gravel substrate. Pea gravel was used as a substrate to ensure there were no additional nutrients present that would otherwise occur in a soil substrate. Eight pots of planted *M. aquaticum* were placed into each mesocosm that were filled with 757 L of water. Water was supplied to each mesocosm from an irrigation reservoir adjacent to the mesocosm facility. Air was supplied to all mesocosms from a regenerative air blower using 2.5 cm stone diffusers and a PVC lift pipe.

After planting, pretreatment plant and water samples were collected to assess biomass and nutrient concentrations in the water column of each mesocosm prior to nitrogen and phosphorus amendments. Water samples were collected and transported to the Mississippi State University Forestry, Soils, and Hydrology Lab, where; total nitrate and phosphate were determined using the APHA method 4110: determination of anions by ion chromatology (Eaton et al. 2005). Following pretreatment sampling, the total amount of nitrogen and phosphorus (mg) for use in treatment combinations was determined based on a water volume of 757 L. Appropriate amounts of nitrogen and

phosphorus were measured using an analytical balance and added to appropriately labeled mesocosms at 0, 3, 6, 9, and 12 weeks after start (WAS). Prior to any nutrient amendments at all treatment times, water samples were collected in a similar fashion to pretreatment samples and the water volume returned to 757 L in all mesocosms. In 2007, *in vivo* chlorophyll *a* was recorded in each mesocosm at 3, 6, 9, and 12 WAS using a handheld fluorometer (Turner Designs, Sunnyvale, CA) to estimate algal density for each nutrient combination.

Myriophyllum aquaticum biomass was assessed at 3, 6, 9, and 12 WAS by harvesting the plants from two pots in every mesocosm. Plants were washed and sorted to emergent shoots (2 to 3 nodes below the last green leaf), submersed shoots, stolon, and sediment roots. Plant tissues were dried at 70 C for 72 hours then weighed; subsequent biomass is expressed as g DW pot^{-1} for each WAS and plant tissue. At 12 WAS, the dried emergent shoots were sent to the Mississippi State Chemical, Industrial and Agricultural Services Laboratory, Mississippi State University, where the percent nitrogen and phosphorus was determined using the AOAC Official Method 990.03, combustion method (AOAC International, 2000).

Data Analysis

Statistical analyses were conducted using SAS software (SAS Institute, Inc., Cary, NC, USA). A Mixed Procedures model was utilized to examine nutrient combination effects on total biomass, emergent shoot, submersed shoot, stolon, and sediment root biomass of *M. aquaticum*; year, block, and their subsequent interactions were considered random effects in the model (Littel et al. 1996). Data were analyzed within WAS to

account for a treatment by WAS interaction. If a significant main effect was observed, treatment means were separated using least squares means and grouped using the Least Significant Difference method. Relative growth rates (RGR) (ln log g DW pot^{-1} day^{-1}) were also calculated for each WAS and nutrient combination for total, emergent shoot, submersed shoot, stolon and sediment root biomass using the following equation outlined by Hunt (1982):

$$r = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1} \quad (4\text{-}1)$$

where W_1 and W_2 are plant dry weights at times t_1 and t_2. A mixed procedures model was also utilized to determine differences in RGR, tissue nutrient content, and chlorophyll *a* across nutrient combinations.

Polynomial regression analysis was used to determine the relationship between total *M. aquaticum* biomass yield and nutrient content in tissues. Regression models were sequentially fit beginning with a linear model. Polynomial terms were then added one at a time and lack of fit determined using partial t-tests. Regression analysis allowed for the estimation of a critical nutrient content for plant tissues. All analyses were conducted at a $p < 0.05$ level of significance.

Results

Mean (± 1SE) water column nutrient data are summarized in Table 4.1. Pretreatment (0 WAS) nutrient concentrations were 0.02 ± 0.01 mg L^{-1} for nitrate and 0.00 mg L^{-1} for phosphate, indicating there were very little nutrients present in the water column prior to amendments, and all nutrients available for plant growth would come

from the treatment combinations. Overall, there was very little nitrate or phosphate detected in the water across nutrient combinations. The exceptions being the medium:low and high:low N:P combinations which had an accumulation of nitrate by 12 WAS. Relative growth rates for each tissue type and nutrient combination across WAS ranged from 0.0 to 0.02 for total biomass, -0.10 to 0.03 for emergent shoot biomass, -0.15 to 0.05 for submersed shoot biomass, -0.01 to 0.02 for stolon biomass, and -0.19 to 0.07 for sediment root biomass. The large negative values are indicative of the planting technique used in the study. The emergent fragments used for planting did not have submersed shoots, stolons, or roots attached and therefore, the calculation of RGR for the time interval from planting to 3 WAS would always be negative when using a natural logarithm approach because the weight at time two is subtracted (most often a negative number) from the weight at time one which is 0 for the above mentioned tissues. Analyses of RGR resulted in no significant differences ($p > 0.05$) between nutrient combinations and all *M. aquaticum* biomass (total and plant parts) across WAS which is likely a result of nutrient deficiency, therefore, there will be no further discussion of these data.

Total *M. aquaticum* biomass was significantly greater at the high:low N:P combination by 6 WAS than biomass at all other nutrient combinations (Figure 4.1). Biomass was on average 42% greater at the high:low N:P combination during this time. At 12 WAS, biomass was 53% greater at the high:low N:P combination, significantly higher than all other nutrient combinations. When nitrogen was held constant at 1.80 mg L^{-1} and phosphorus increased from 0.01 to 0.09 mg L^{-1} there was a significant decrease in *M. aquaticum* biomass beginning at 6 WAS and continued to 12 WAS.

Myriophyllum aquaticum stolon biomass was slower to respond to water column nutrient amendments as differences were not observed until 9 WAS (Figure 4.2). Stolon biomass in the high:high N:P combination at 9 WAS was significantly lower (43 and 36% respectively) than the high:low and high:medium N:P combinations. At 12 WAS, the only difference in stolon biomass occurred at the high:low N:P combination where biomass was on average 43% greater than biomass in other nutrient combinations.

Sediment root biomass was greatest at the high:low N:P combination at 6 WAS (Figure 4.3). However, at 9 and 12 WAS root biomass was similar between the high:low and high:medium N:P combinations. When phosphorus was supplied at 0.09 mg L^{-1}, sediment root biomass was reduced when compared to the high:low N:P combination.

Submersed shoots constituted the smallest proportion of total biomass and was generally similar across nutrient combinations and WAS with the exception of the high:low N:P combination. Submersed shoot biomass was greatest at the high:low N:P combination by 9 WAS (Figure 4.4). When phosphorus was increased to 0.03 and 0.09 mg L^{-1} it resulted in reductions in submersed shoot biomass when nitrogen was fixed at 1.80 mg L^{-1}, although there was no difference in biomass between the medium and high phosphorus combinations.

Emergent shoot biomass of *M. aquaticum* was also significantly higher at the high:low N:P combination (Figure 4.5). Emergent shoot biomass was on average 53, 68, and 76% greater at the high:low N:P combination than biomass at all other nutrient combinations at 6, 9, and 12 WAS, respectively. Similar to total biomass, emergent shoot biomass decreased as phosphorus concentration increased when nitrogen was fixed at 1.80 mg L^{-1}.

Yield response of *M. aquaticum* was a quadratic function of both nitrogen (r^2 = 0.82) and phosphorus (r^2 = 0.78) content in plant tissues. The relationship was positive for nitrogen and negative for phosphorus (Figure 4.6). As nitrogen increased, total yield increased, only after nitrogen concentrations rose above 1.80% of plant tissue, indicating a growth limiting nitrogen level. Conversely, as phosphorus content increased, biomass decreased. The decrease in biomass is evident after phosphorus content exceeded 0.20% (Figure 4.6). Overall, *M. aquaticum* was nutrient-limited as all combinations were at or near critical nutrient levels with the exception of the high:low N:P combination where nitrogen content was above the critical threshold (Figure 4.7).

The addition of nutrients to the water column resulted in the growth of algae as determined by chlorophyll *a* measurements in all nutrient combination treatments (Figure 4.8). However, only the high:high N:P combination resulted in significantly more algae by 12 WAS. Visually there was an increase in filamentous algae as the phosphorus concentration increased, suggesting that algae were responding to phosphorus additions to the water column.

Discussion

Myriophyllum aquaticum growth was limited by nitrogen when concentrations in the water were supplied below 1.80 mg L^{-1}, and by phosphorus concentrations in the water column throughout this study. *Myriophyllum aquaticum* yield increased with increasing nitrogen content after 1.8%, which suggests that nutrient uptake was in fact occurring from the water column. The critical limiting nutrient threshold was estimated to be 1.80% nitrogen and 0.20% phosphorus in plant tissues. These values support

previously published data for emergent shoots where critical values were estimated at 1.54% and 0.19% for nitrogen and phosphorus respectively for shoots grown in nutrient solutions (Sytsma and Anderson 1993b). Tissue concentrations of nitrogen and phosphorus were close to or below the critical thresholds established in this and previous studies. Nutrient limitation is further supported in that biomass did not differ across nutrient combinations until nitrogen increased to 1.80 mg L^{-1}. This suggests that plants grown at the lower nutrient combinations were not acquiring sufficient amounts of nutrients from the water column to initiate or sustain high biomass production. The combination of high:low N:P had tissue nitrogen above the critical threshold, but phosphorus was below the critical threshold which may suggest that nitrogen has a larger role in *M. aquaticum* growth than phosphorus when supplied to the water column at concentrations at or above 1.80 mg L^{-1}.

Uptake of both nitrogen and phosphorus from the water column is facilitated via adventitious roots. These roots grow from each node of the stolon where growth begins as soon as old emergent shoots are submersed in the water column. Adventitious roots and can grow to lengths of approximately 30 to 50 cm giving greater access to water column nutrients than other macrophyte species. Adventitious roots generally have a higher RGR than even total RGR (Sytsma and Anderson 1993a). A dense population of *M. aquaticum* with adventitious roots along each stolon of every plant would have increased access to water column nutrients. However more research is needed to determine the real function of adventitious in the growth of *M. aquaticum*, although these data and previous studies suggest they may be the primary site of nutrient uptake, especially for plants growing in deeper water (Sytsma and Anderson 1993c).

Myriophyllum aquaticum does have sediment roots; however, they are highly cuticularized which may limit nutrient uptake from the sediment (Sutton and Bingham 1973). *Myriophyllum aquaticum* has a low sediment root:shoot ratio further reducing the ability of sediment roots to contribute to the total nutrient supply for plants. Plant growth did not reduce sediment nutrient concentrations over the course of a laboratory study due to a shift in allocation patterns from sediment roots to adventitious roots after the development of emergent shoots (Sytsma and Anderson 1993a). In natural populations, stolons and emergent shoots were the sink for nitrogen. Allocation was >80% throughout the year with the majority of nitrogen stored in stolon tissues (Sytsma and Anderson 1993d). Nitrogen allocation to sediment roots never exceeded 18% and was below 10% the majority of the time (Sytsma and Anderson 1993d). Emergent shoots comprised >80% of the total phosphorus pool in these same natural populations with no annual accumulation of phosphorus in other tissues, suggesting that *M. aquaticum* relies on phosphorus uptake from the water column (Sytsma and Anderson 1993d).

An inverse relationship was observed between *M. aquaticum* yield and increasing phosphorus content. In general, increasing or decreasing phosphorus availability typically affects root growth as is indicated in agricultural plants (Cassman et al. 1980; Linkhor et al. 2002); though in this study root biomass did not respond to changes in phosphorus concentration. Therefore, the negative relationship in yield response and phosphorus availability is attributed to competition for light and nutrients with algae. Algae assimilate phosphorus at rates more rapid than what is actually used for growth; and if other conditions are adequate, enrichments of phosphorus in the water often result in immediate increases in algal photosynthesis and growth rates (Wetzel 2001).

This would also be a plausible explanation for the reduction in total and emergent shoot biomass at the high:medium and high:high N:P combinations. There was a significant increase in chlorophyll *a* in the high:high N:P combination after 12 weeks. By 9 WAS, mats of filamentous algae were floating on the water surface and growing on *M. aquaticum* plants the highest phosphorus concentration with little to no filamentous algae growing in the lowest phosphorus combinations. The filamentous algae coated the surface of emergent stems, stolons, and adventitious roots when phosphorus was increased.

Aquatic macrophytes are often attaching points for filamentous algae and other epiphytic organisms or serve to cycle nutrients within a waterbody. Phosphorus uptake from the water column by rooted macrophytes is often much less than by attached algae (Wetzel 2001). Epiphytes may reduce macrophyte growth by intercepting light and nutrients that would have otherwise been absorbed through leaf surfaces (Phillips et al. 1978; Ruesink 1998). Epiphyte production was found to be higher on *Myriophyllum spicatum* L. than native or plastic plants in a controlled study (Cattaneo and Kalff 1979). The authors attributed the greater epiphyte production to the highly dissected leaves that are characteristic of *Myriophyllum* spp., which may have allowed epiphytes to better utilize light and dissolved nutrients in the water. In natural macrophyte communities 3.4 to 8.9% of phosphorus present in epiphytes was contributed by macrophytes (Carignan and Kalff 1982). Furthermore, *M. spicatum* alone was estimated to increase total phosphorus load to the water column by 2.2%, of which, more than half of this amount is readily available to epiphytes and algae (Carignan and Kalff 1982).

Myriophyllum aquaticum has both an emergent and submersed leaf form as well as adventitious roots, offering more attachment points for algae. As reported earlier, >80% of total phosphorus is located in the emergent shoots of *M. aquaticum* (Sytsma and Anderson 1993d); meaning that a large source of phosphorus for algal growth is concentrated at or near the water surface and readily accessible by free floating or epiphytic algae. Therefore, as phosphorus was added to the mesocosms over the course of the current study, algae densities increased and could directly uptake nutrients more quickly than *M. aquaticum*. Filamentous algae could have also directly inhibited nutrient uptake from the water column by growing on *M. aquaticum* plants and possibly limited photosynthesis through shading of the water column. Although phosphorus content of algae was not directly measured, nutrient concentrations in the water column were documented during regular water sampling events where very little NO_3 and PO_4 were recorded. Furthermore, tissue nutrient concentrations of *M. aquaticum* indicated that nutrients were often below critical limits. The low water column nutrient concentrations and low tissue nutrient concentrations offer further support for algal interference with nutrient uptake by *M. aquaticum*; although a mass balance of total nitrogen and phosphorus would be needed to confirm this.

Myriophyllum aquaticum is not considered a major noxious aquatic weed throughout most of its range; however, it can cause severe localized problems (Sutton 1985). Its reliance on high nutrient environments (Sutton 1985; Sytsma and Anderson 1993b,d) may be an important predictor as to where this species can colonize and the severity of the invasion. *Myriophyllum aquaticum* typically invades shallow wetlands, slow moving streams, irrigation reservoirs or canals, edges of lakes, ponds, sloughs, or

backwaters (Sutton 1985; Timmons and Klingman 1958). These areas typically have frequent nutrient pulses and can support luxurious plant growth. In Florida, USA, nitrate concentrations are rising in freshwater waterbodies due to non-point anthropogenic sources (Bacchus and Barile 2005). Non-native species that can exploit these nutrient inputs have the ability to have severe negative impacts on native plant community composition (Kennedy et al. 2009). *Hydrilla verticillata* (L. f.) Royle produced more biomass than both native species *Sagittaria kurziana* Glück and *Vallisneria americana* Michx. in a controlled study when nitrate was held constant (Kennedy et al. 2009). When nitrate concentrations were elevated, *H. verticillata* more than doubled its biomass (Kennedy et al. 2009).

Understanding the relationships between nutrient loading and invasive plant growth is becoming more important as anthropogenic nutrient sources increase. Current data provides further evidence to support previous claims that *M. aquaticum* growth and distribution are controlled in large part by environmental nutrient supply (Sutton 1985 Sytsma and Anderson 1993b,d); and in habitats where eutrophication is occurring, *M. aquaticum* may become very problematic through increased nutrient uptake from the water column. These data provide basic ecological information and, when combined with other growth limiting data, predictive models can be developed to identify which habitats are most prone to invasion by *M. aquaticum*. These data indicate that *M. aquaticum* could invade a range of habitats including oligotrophic lakes. Colonization success and nuisance growth would likely be limited to eutrophic habitats or areas where nutrient competition with other algae or other macrophytes is low.

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Table 4.1 Summary of mean (± 1 SE) nitrate and phosphate concentrations for each water column nutrient combination. Pretreatment (0 WAS) nutrient concentrations were 0.02 ± 0.01 mg L^{-1} for nitrate and 0.00 mg L^{-1} for phosphate.

Target Concentration N:P (mg L^{-1})	Weeks After Start							
	3		6		9		12	
	NO_3	PO_4	NO_3	PO_4	NO_3	PO_4	NO_3	PO_4
0.40:0.01	0.17±0.06	0.02±0.01	0.03±0.02	0.00±0.00	0.02±0.01	0.03±0.03	0.04±0.01	0.00±0.00
0.40:0.03	0.17±0.07	0.05±0.02	0.01±0.00	0.01±0.01	0.01±0.00	0.00±0.00	0.01±0.00	0.01±0.00
0.40:0.09	0.17±0.06	0.18±0.05	0.01±0.01	0.02±0.01	0.00±0.00	0.04±0.02	0.03±0.01	0.19±0.06
0.80:0.01	0.30±0.11	0.02±0.01	0.02±0.01	0.00±0.00	0.18±0.07	0.05±0.02	0.25±0.06	0.00±0.00
0.80:0.03	0.30±0.11	0.05±0.02	0.01±0.00	0.01±0.01	0.01±0.00	0.01±0.01	0.06±0.02	0.00±0.00
0.80:0.09	0.31±0.11	0.14±0.05	0.02±0.01	0.02±0.01	0.01±0.00	0.05±0.01	0.06±0.03	0.11±0.04
1.80:0.01	0.92±0.14	0.02±0.01	0.59±0.23	0.00±0.00	3.39±0.32	0.01±0.01	5.95±0.33	0.00±0.00
1.80:0.03	0.53±0.22	0.04±0.02	0.03±0.01	0.01±0.01	0.12±0.06	0.04±0.03	0.47±0.17	0.00±0.00
1.80:0.09	0.63±0.24	0.14±0.05	0.01±0.01	0.03±0.01	0.04±0.03	0.05±0.02	0.27±0.11	0.03±0.02

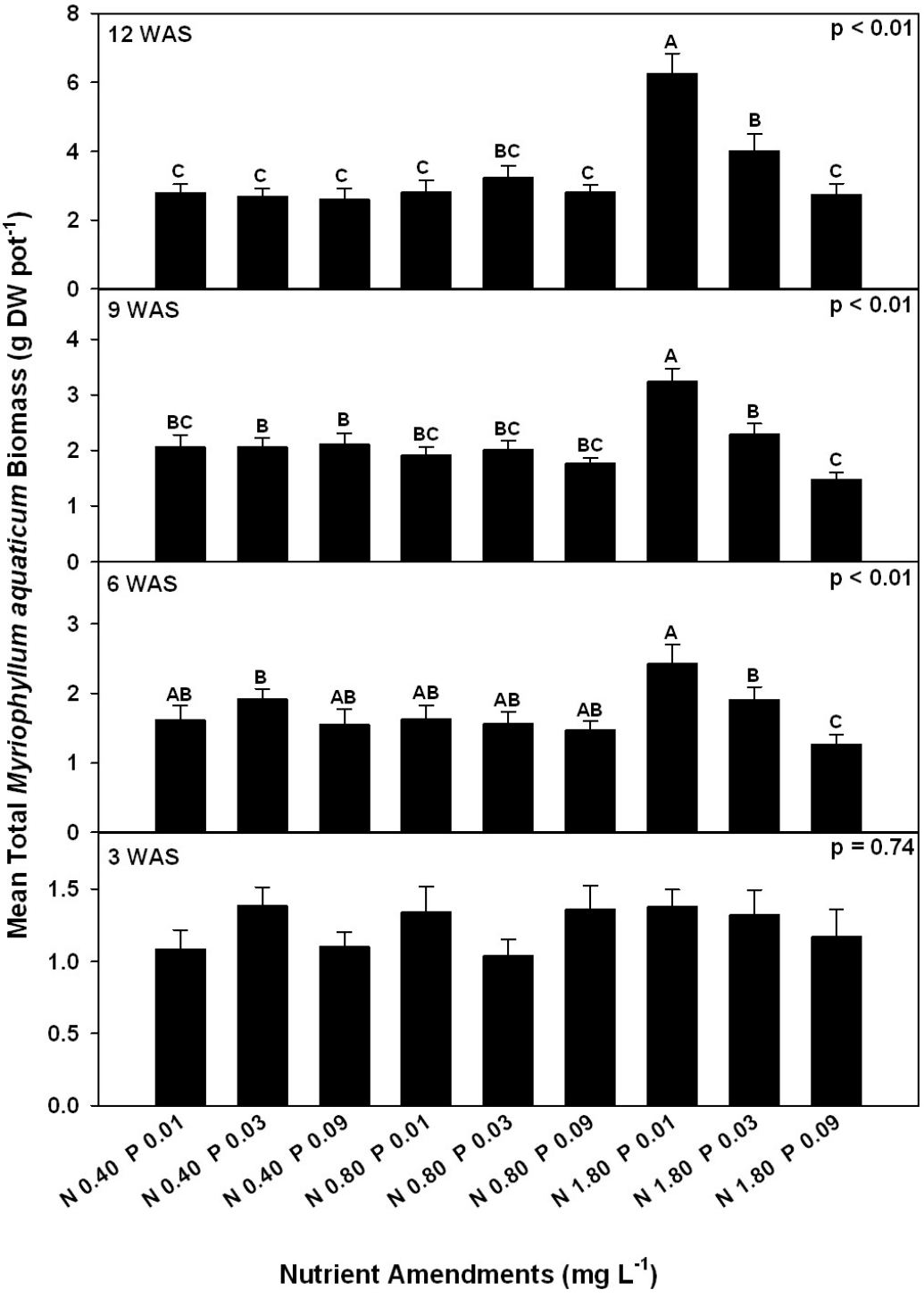

Figure 4.1 Mean (± 1 SE) total biomass of *Myriophyllum aquaticum* grown in varying nitrogen and phosphorus combinations added to the water column. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

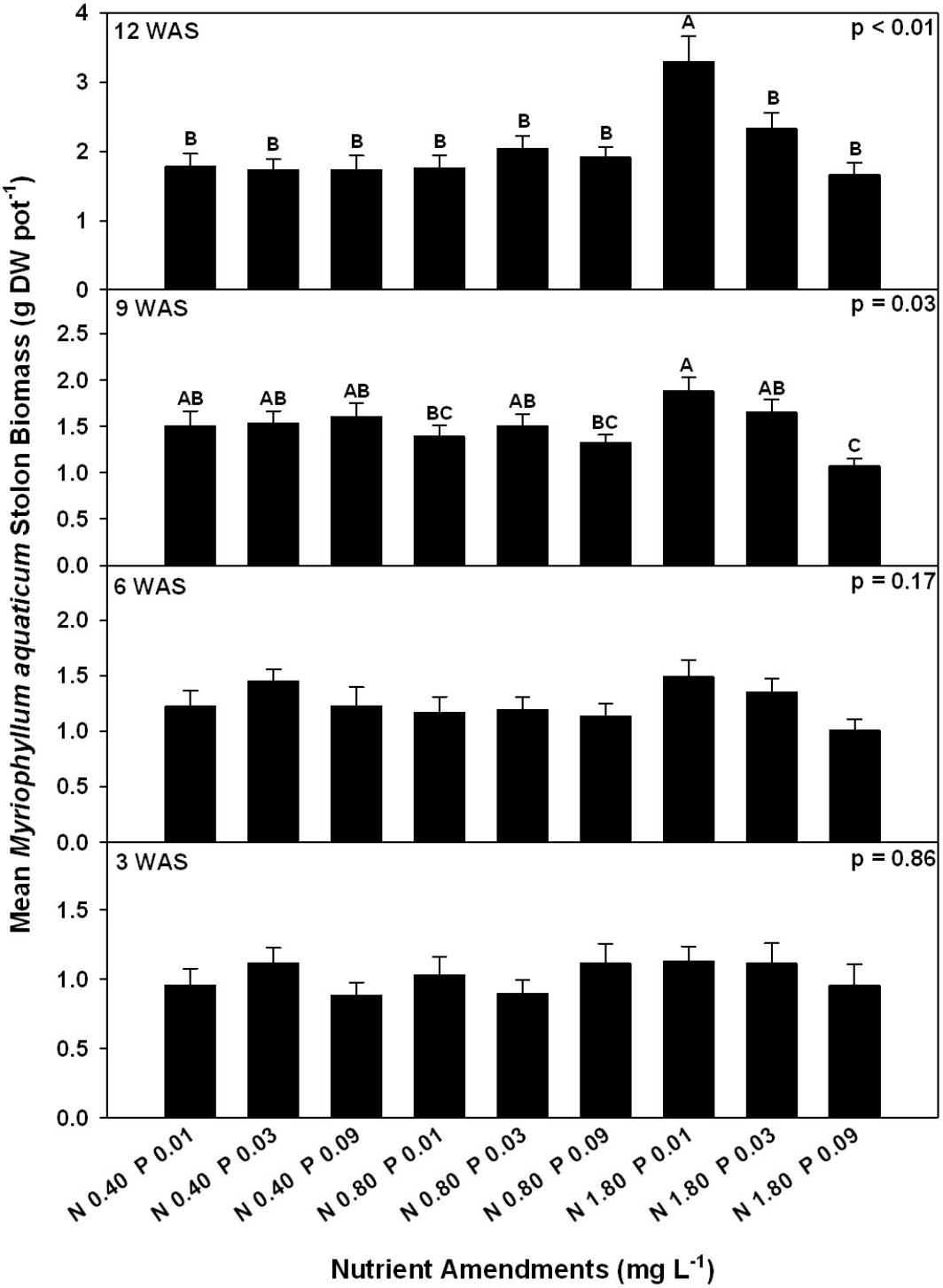

Figure 4.2 Mean (± 1 SE) stolon biomass of *Myriophyllum aquaticum* grown in varying nitrogen and phosphorus combinations added to the water column. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

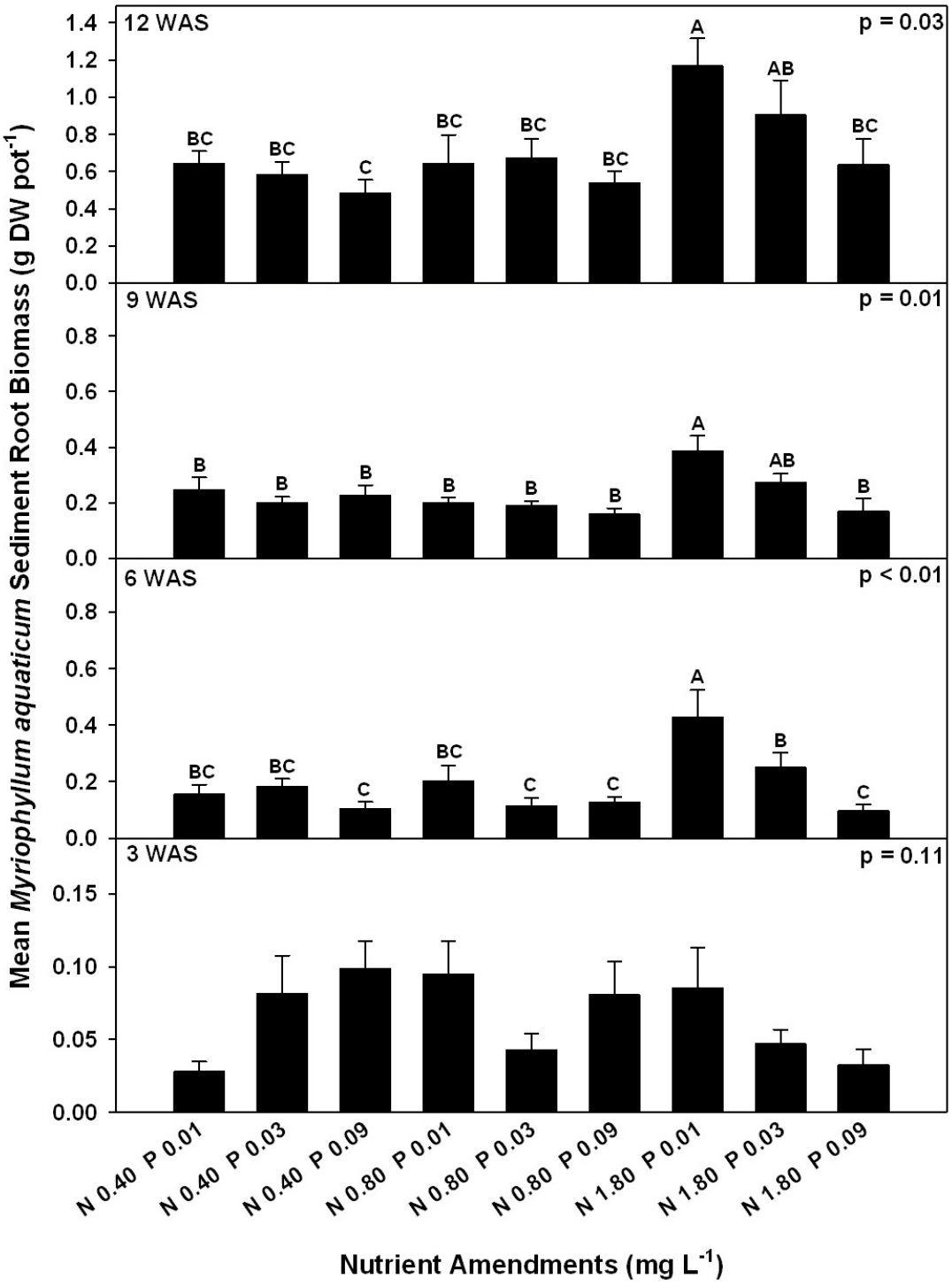

Figure 4.3 Mean (± 1 SE) sediment root biomass of *Myriophyllum aquaticum* grown in varying nitrogen and phosphorus combinations added to the water column. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

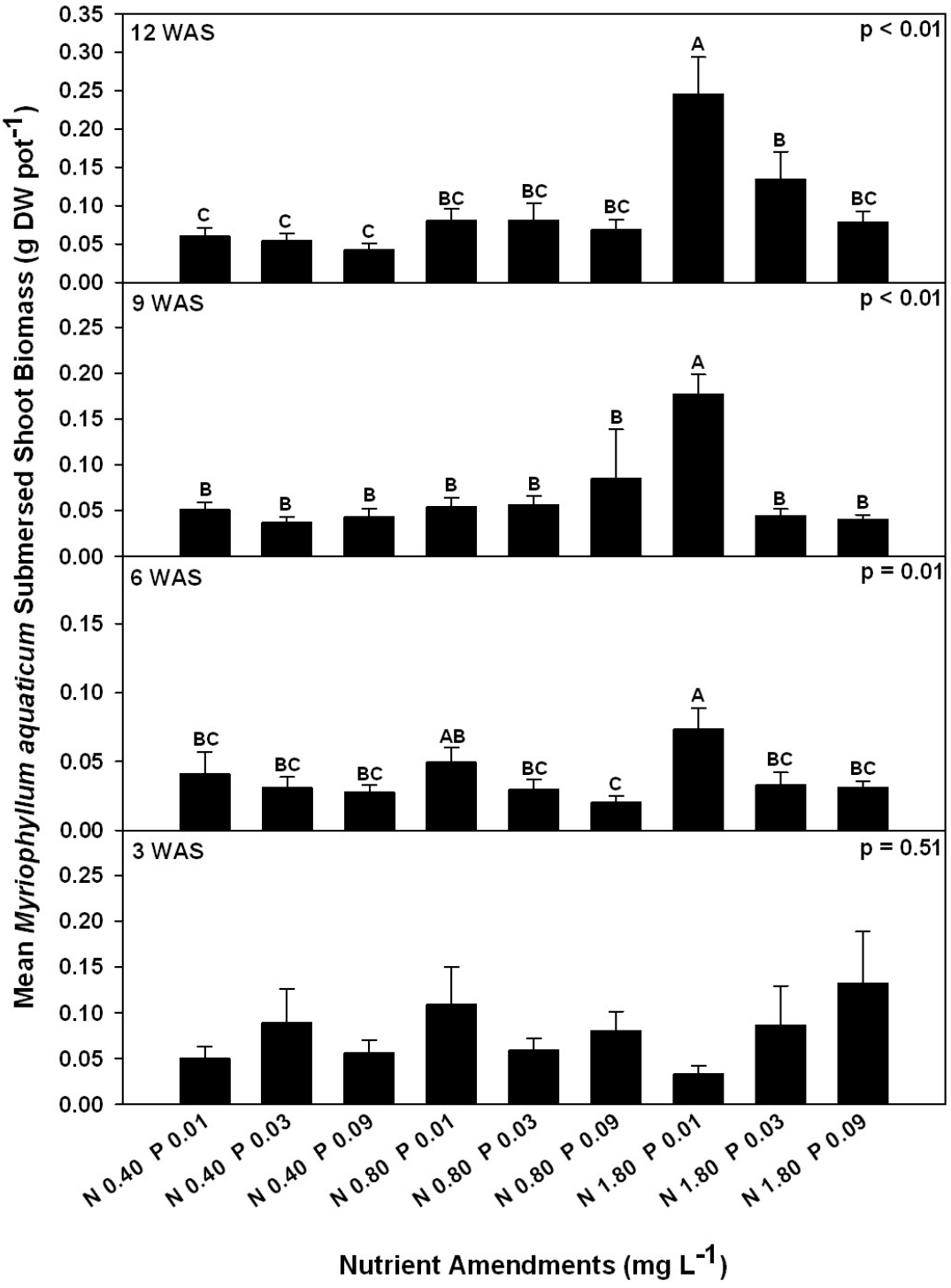

Figure 4.4 Mean (± 1 SE) submersed shoot biomass of *Myriophyllum aquaticum* grown in varying nitrogen and phosphorus combinations added to the water column. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

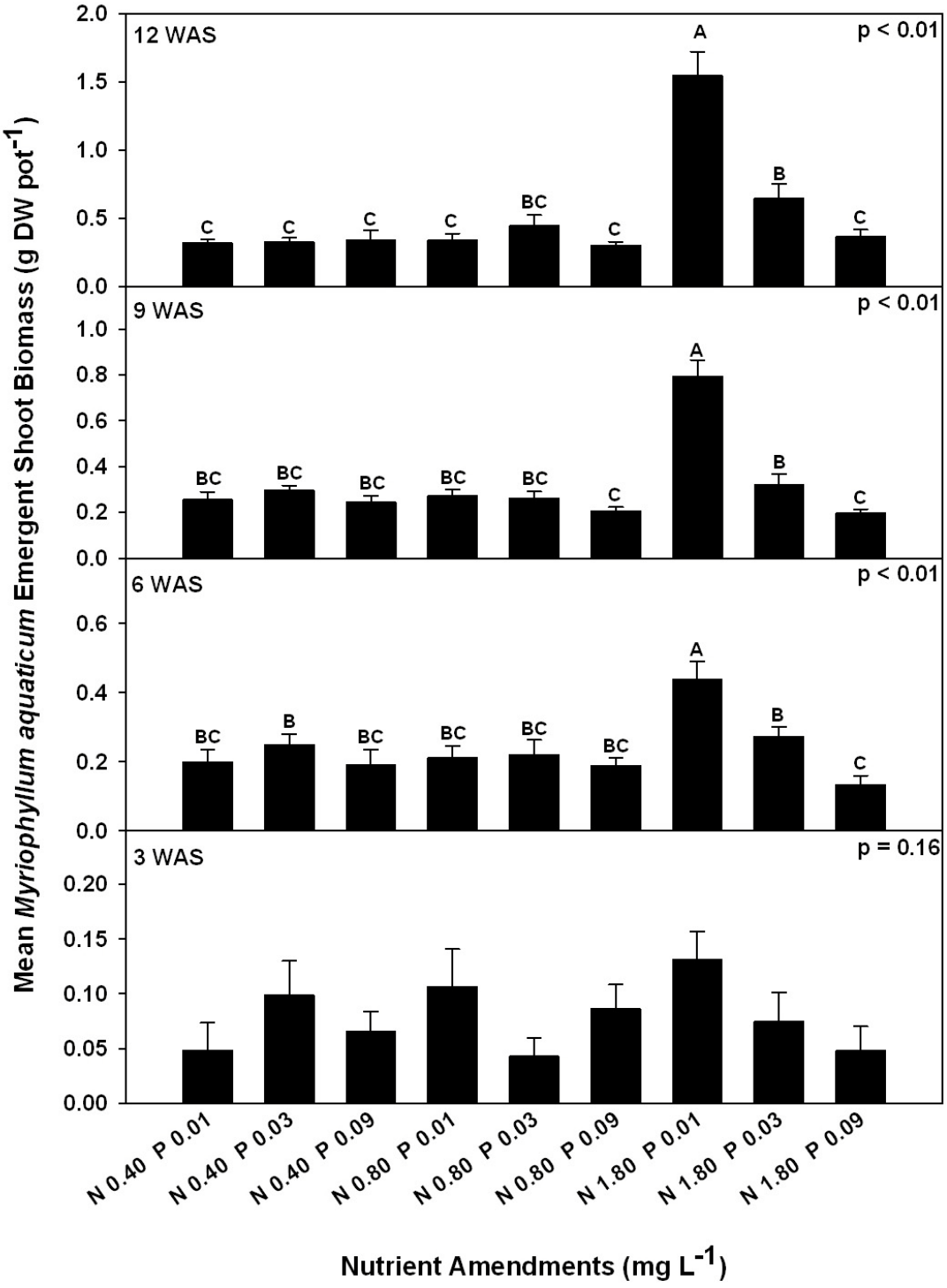

Figure 4.5 Mean (± 1 SE) emergent shoot biomass of *Myriophyllum aquaticum* grown in varying nitrogen and phosphorus combinations added to the water column. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

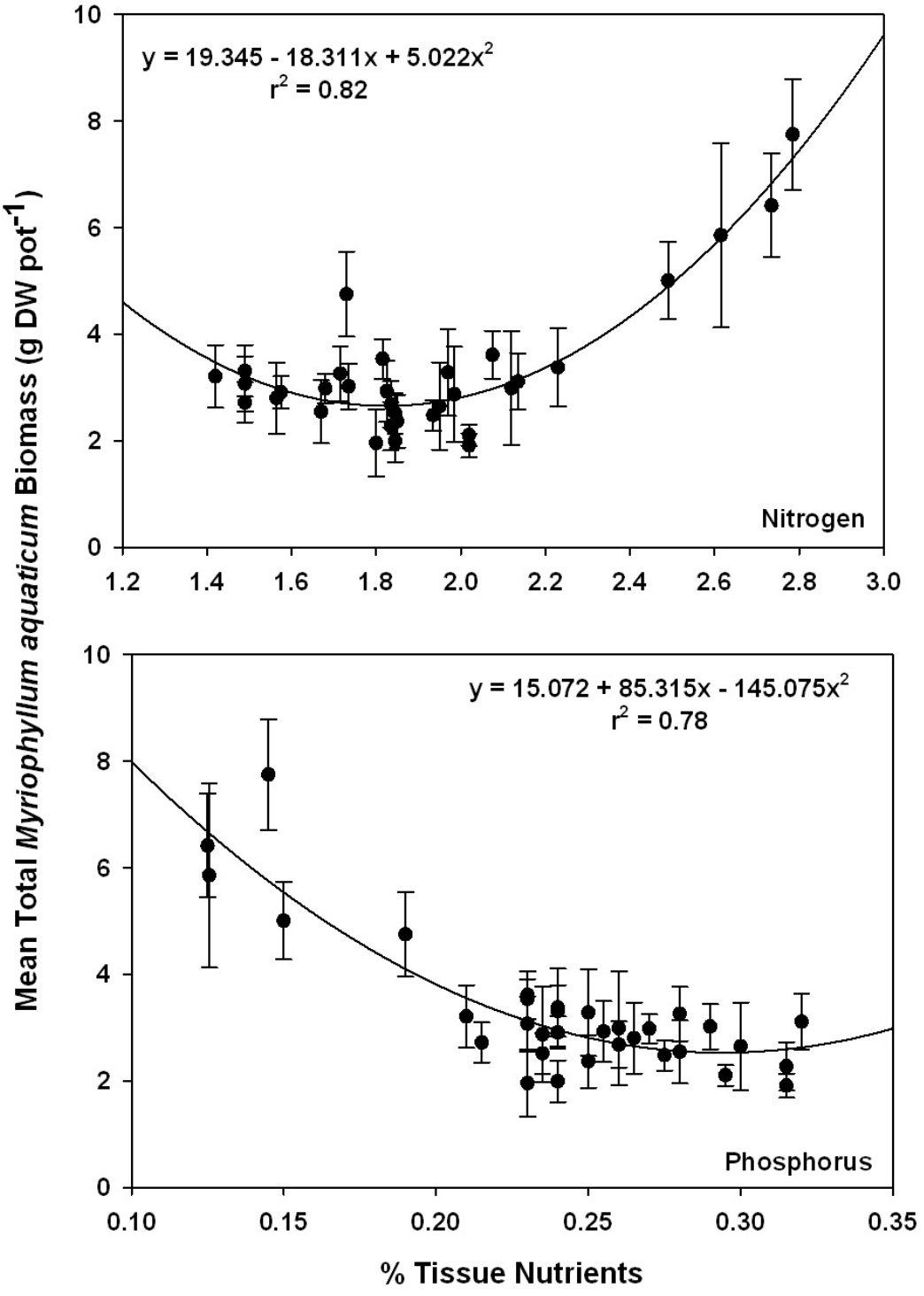

Figure 4.6 Mean (± 1 SE) total yield response of *Myriophyllum aquaticum* to nitrogen (top) and phosphorus (bottom) concentrations in plant tissues. The regression line represents the best fit of a polynomial regression analysis. Total yield response is a quadratic function of tissue nutrient concentration.

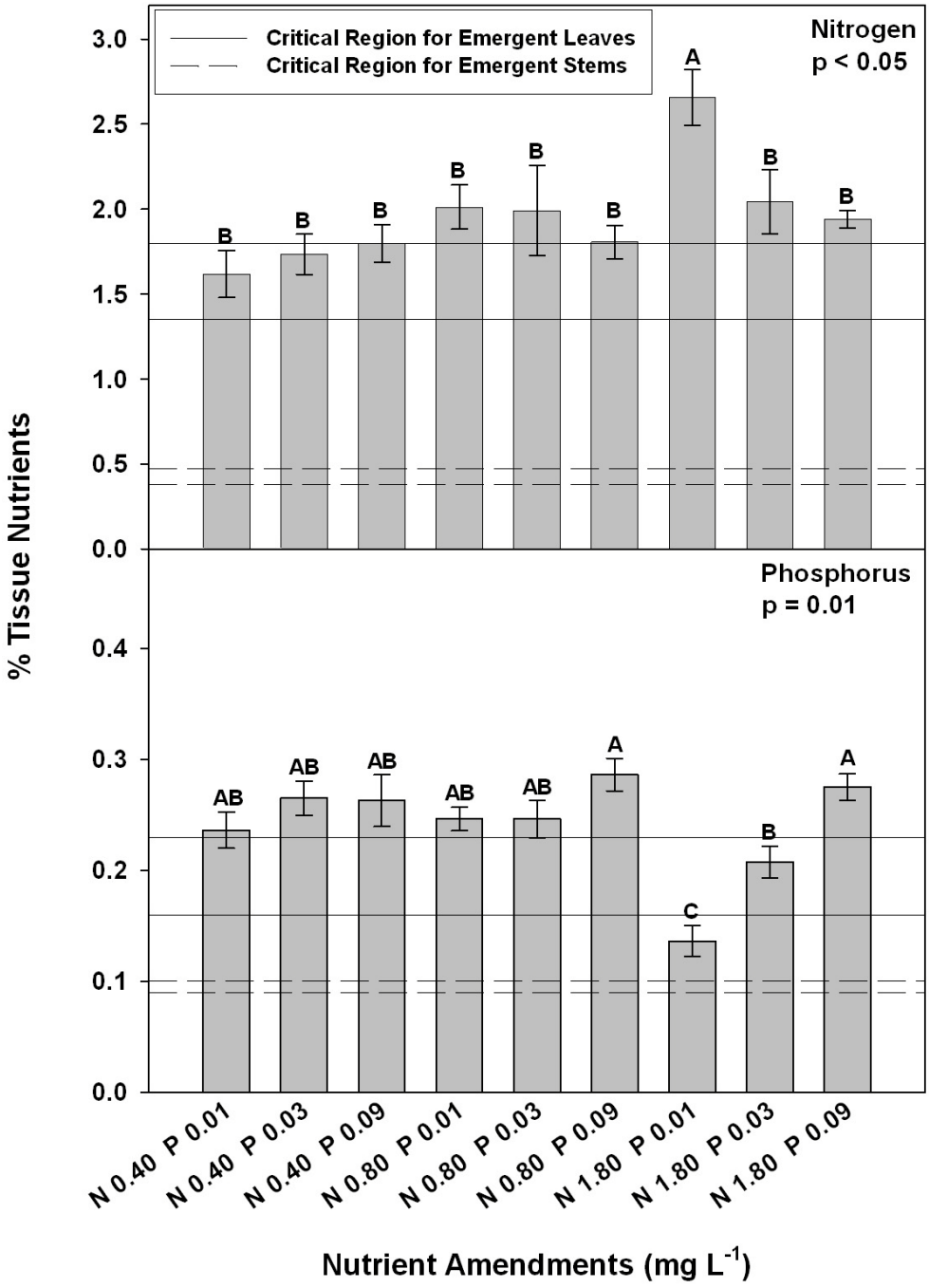

Figure 4.7 Mean (± 1 SE) nitrogen (top) and phosphorus (bottom) content in emergent shoots of *Myriophyllum aquaticum* grown in varying nitrogen and phosphorus combinations added to the water column. Critical concentration lines were established from values reported by Sytsma and Anderson (1993c). Bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

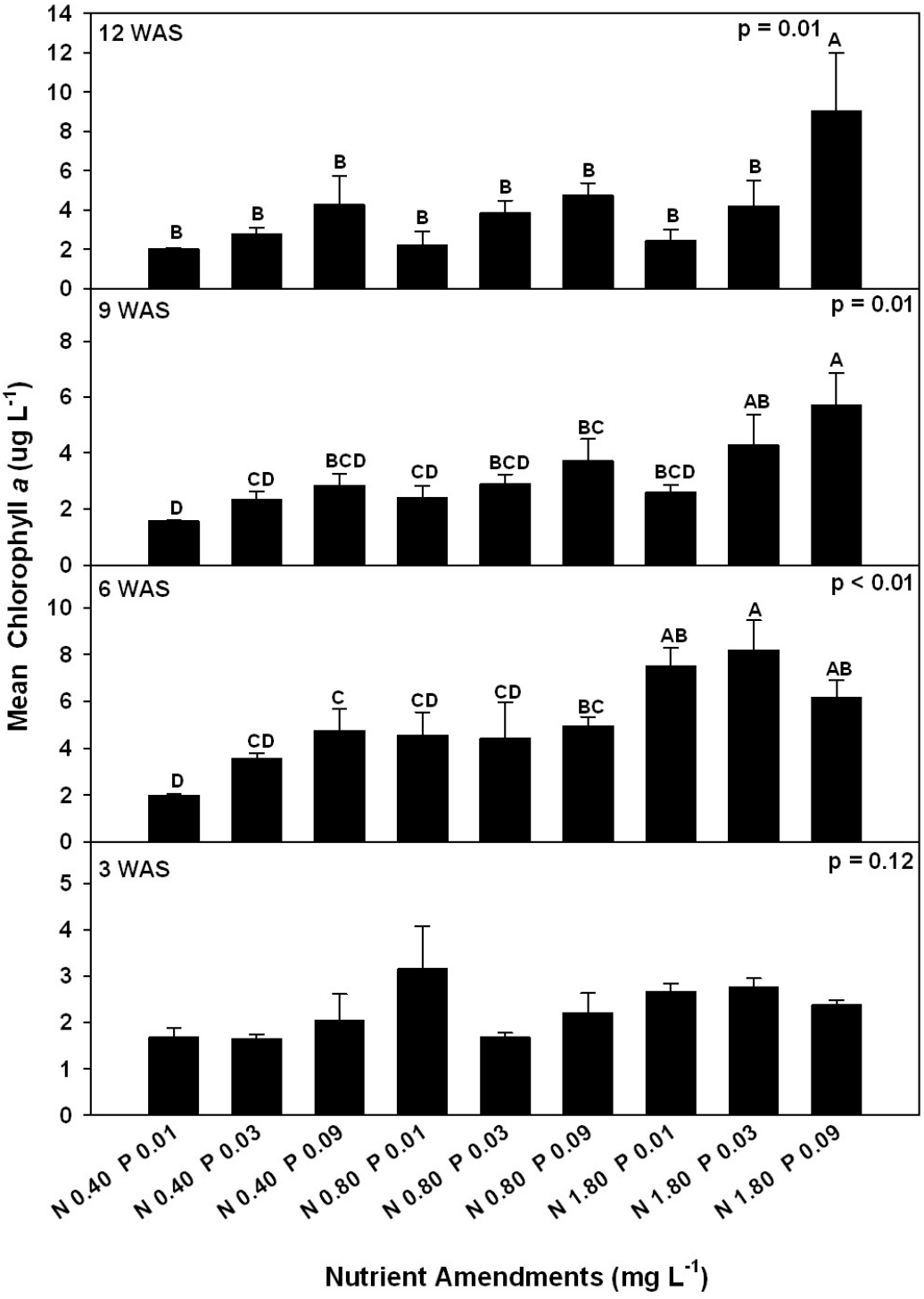

Figure 4.8 Mean (± 1 SE) chlorophyll *a* concentration for each nutrient combination. Chlorophyll *a* was only measured in 2007. Analyses were conducted within WAS and bars sharing the same letter are not significantly different at a $p < 0.05$ level of significance.

CHAPTER V

COMPARATIVE EFFECTS OF WATER LEVEL VARIATIONS ON GROWTH CHARACTERISTICS OF THE INVASIVE AMPHIBIOUS PLANT *Myriophyllum aquaticum*

Abstract

Myriophyllum aquaticum (Vell.) Verdc. (parrotfeather) is a non-native heterophyllous aquatic plant that has invaded a range of habitats in the United States, including irrigation and drainage ditches, wetlands, lakes, and streams. *Myriophyllum aquaticum* reduces native species richness, impacts water quality, reduces habitat quality for fish and wildlife, and impacts human uses. Despite having a submersed leaf form, *M. aquaticum* is not typically a problem as water level increases; however, the colonization potential of this species based upon water level is not well defined. In fact, little data exist describing the biological and ecological mechanisms affecting *M. aquaticum* growth. The objectives of this study were to evaluate *M. aquaticum* response to increasing water levels under controlled mesocosm conditions. Light transmittance through the water column was negatively ($R^2 = 0.99$) related as a quadratic function of water depth. *Myriophyllum aquaticum* biomass at 0 cm was 96% greater than plants grown at 137 cm. Biomass of emergent shoots, stolons, and sediment roots was also greater when *M. aquaticum* was grown at the 0 cm water level. Submersed shoot biomass was on average 99% greater at 37, 57, and 77 cm. However, submersed shoots

comprised only a small fraction 0.1-12% of total biomass, depending on the water level. Total *M. aquaticum* length was 25% greater when plants were grown at water levels from 0-77 cm over plants grown at 97, 117, 137 cm. Overall, *M. aquaticum* growth was greatest when water levels were shallow. Shallow water is often easier to invade and is subject to greater disturbance which benefits *M. aquaticum*, as this species is dependent upon fragmentation for reproduction and spread. Nuisance *M. aquaticum* growth is likely dependent upon plants emerging from the water column. As water levels increase, emergence becomes increasingly more difficult as a result of the reduced photosynthetic ability of submersed leaves to support plant growth to the water surface. These results can be used to identify suitable areas for *M. aquaticum* invasion and spread and for the development of early detection and rapid response programs.

Introduction

Hydrologic variations within wetlands and shallow lakes often determine patterns of plant zonation and community structure (Casanova and Brock 2000; van Geest et al. 2005). The water regime of a given habitat is often characterized by the depth, duration, and frequency of flood and drawdown events (Casanova and Brock 2000). Sustained or frequent flooding can lead to a more stable environment and a shift in species dominance and ultimately species composition (van der Valk 2005). More stable environments created by flooding often inhibit emergent macrophyte growth (Casanova and Brock 2000), and favor submersed aquatic macrophytes such as evergreen perennial species. Water level fluctuations can be viewed as disturbance to the plant community, and disturbance is often the primary mechanism that facilitates invasions through removing

native species and opening niche space for colonizing species (Davies et al. 2005; Lockwood et al. 2005; Capers et al. 2007).

Habitats around the world are experiencing an increasing number of invasions of non-indigenous species (Vitousek et al. 1997). Most species fail to successfully establish, but some species will colonize and grow to nuisance levels, often with negative consequences on the local plant community composition, ecosystem functions, and human uses (Chapin et al. 2000). Environmental changes as a result of species invasions highlight the importance of understanding the factors that may limit a species ability to invade a particular habitat (Chadwell and Engelhardt 2008).

Parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.] is a herbaceous perennial aquatic plant that is not native to the United States and is increasingly becoming problematic in shallow streams, irrigation ditches, ponds, and shallow lakes. *Myriophyllum aquaticum* typically invades shallow waterbodies that are prone to disturbances such as repeated and frequent water level fluctuations. Once established, disturbances that can fragment plants, such as harvesting, mowing, chaining, or the rapid rise and fall of water level, will favor the growth and spread of this species. Sabbatini and others (1998) reported that *M. aquaticum* was tolerant to mechanical disturbances and the repeated occurrence of these events favored *M. aquaticum* dominance in canals. Survival and spread of *M. aquaticum* depends solely on vegetative reproduction via fragmentation, as this species does not produce any specialized reproductive structures such as seeds, tubers, or turions (Sutton 1985).

Myriophyllum aquaticum is heterophyllous, meaning it has a distinct submersed and emergent leaves, and can change leaf forms in response to environmental changes

(Trémolières 2004; Winn 1999). An inundation gradient can be a major factor which induces plasticity in plants (Trémolières 2004). Having two distinct growth forms may give *M. aquaticum* the ability to overcome extreme disturbances in the water regime and convey a competitive advantage over macrophytes that are more sensitive to changes in their growing environment.

The impact of water level and duration of flooding on wetland macrophyte communities, particularly emergent and submersed species, is well documented at the field scale (Casanova and Brock 2000; Maltchik et al. 2007; Richardson et al. 2002; van der Valk et al. 1994; van der Valk 2005), with some effects reported for amphibious species (Casanova and Brock 2000; Maltchik et al. 2007). Casanova and Brock (2000) reported on the influence of water depth on macrophyte establishment; however, the deepest depth in their study was 60 cm. Hussner et al. (2009) reported differences in *M. aquaticum* total shoot length, shoot biomass, root biomass and total biomass, though water level was either 10 cm above the sediment surface, 20 cm below the sediment surface, or completely drained. *Myriophyllum aquaticum* is capable of growing in deeper water depths; however the direct effects of water level on its growth characteristics are unknown. Therefore, the objectives of this study were to examine changes in the growth form of *M. aquaticum* as water levels increase and offer insights into the colonization potential of this species based on water level.

Materials and Methods

Studies were conducted in a mesocosm facility located at the R.R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS (33°28’29.76” N,

88°46’24.70” W) from June 8 to September 4, 2008 and repeated from June 2 to August 28, 2009. Both studies were conducted in 28, 1900-L mesocosms (137 cm diameter by 157 cm deep) arranged in a randomized complete block design with 4 repetitions per water level treatment for 12 weeks. Water level treatments were 0 (pots just below the water surface which represents shoreline habitat), 37, 57, 77, 97, 117, and 137 cm.

Water Level Manipulation and Planting

Platforms were constructed from sheets of galvanized metal to reduce rust formation when submersed and to maximize platform strength when potted plants were placed on them. The platforms were 130 cm long by 30.5 cm wide with grooves to hold pots from falling off the platforms. Platforms were suspended at the appropriate water level using vinyl coated chain. Water levels were determined based upon the total height of the planting containers (pots were 16.5 cm diameter by 20 cm deep), and the depth from the top of the pot to the water surface was considered the treatment depth. Therefore, when pots were placed on the bottom of the mesocosms the treatment level was 137 cm. Platforms were then suspended at appropriate depths within designated mesocosms to achieve the treatment water levels from the top of the pot to the water surface. The 0 cm water level was achieved by immersing the pots just below the water surface to maintain moist soil. Water was supplied to each mesocosm from an irrigation reservoir adjacent to the mesocosm facility.

Myriophyllum aquaticum was harvested from a local waterbody and transported to Mississippi State University for planting. Planting consisted of placing two apical shoots of *M. aquaticum*, approximately 20 cm in length, into each of 168, 3.78-L pots

containing a top soil, loam, and sand mixture (3:2:1). Sediment was amended at a rate of 2 g L pot^{-1} using Osmocote 19-6-12 fertilizer (The Scotts Company, Marysville, OH). Six pots of planted *M. aquaticum* were placed onto the platforms of each tank with the exception of the 137 cm water level in which pots were placed directly on the bottom of the mesocosm. A 30% shade cloth was installed over the top of all mesocosms to mediate heat effects, as *M. aquaticum* biomass is not affected by shade cloth up to 50% when compared to plants grown in full sunlight (Wersal, unpublished data).

Light intensity, both incident and submersed, was recorded at each water level in each mesocosm using a LI-1400 data logger with a LI-190 photometric sensor (incident light) and a LI-192 submersible sensor (LI-COR Biosiences, Lincoln, NE). Light data were recorded approximately twice per week for 12 weeks during both studies. After 12 weeks, all pots were removed from the tanks, total plant length was determined from the sediment to the longest apical tip for each plant, and all biomass was harvested including sediment roots. Plants were washed and sorted to emergent shoots (2 to 3 nodes below the last green leaf), submersed shoots, stolon, and sediment roots. Plant tissues were dried at 70 C for 72 hours then weighed. Biomass is expressed as g DW pot^{-1}.

Data Analysis

Statistical analyses were conducted using SAS software (SAS Institute, Inc., Cary, NC, USA). A mixed procedures model using year as a random effect was utilized to examine water level effects on total biomass, emergent shoot, submersed shoot, stolon, and sediment root biomass of *M. aquaticum* as well as total plant length (Littell et al. 1996). If a significant main effect was observed, treatment means were separated using

least squares means and grouped using the Least Significant Difference method. Light transmittance was calculated by dividing the submersed values by incident values for each mesocosm and is presented as a percent. Polynomial regression analysis was used to determine the relationship between water depth and percent light transmittance. Regression models were sequentially fit beginning with a linear model. Polynomial terms were then added one at a time and lack of fit determined using partial t-tests. There was no block effect ($p = 0.85$) for biomass or plant length ($p = 0.07$). All analyses were conducted at a $p < 0.05$ level of significance.

Results

Total biomass of *M. aquaticum* decreased by 96% when plants were grown at 137 cm (5.4 ± 0.9 g DW pot^{-1}) compared to plants at grown at 0 cm (140.2 ± 7.1 g DW pot^{-1}) (Figure 5.1). Biomass at 37 cm was 58% less than plants grown at 0 cm. In fact, *M. aquaticum* biomass at the 0 cm water level was significantly ($p < 0.01$) greater than biomass at all water levels. Although total biomass is generally a good metric to evaluate plant response under controlled conditions, other plant tissues such as emergent shoots, submersed shoots, stolons, and sediment roots may respond differently to water level.

Emergent shoot biomass was 35.9 ± 1.9 g DW pot^{-1} at the 0 cm water level which was 96% greater than emergent shoot biomass of plants grown at the 137 cm water level (1.6 ± 0.4 g DW pot^{-1}) (Figure 5.2). Stolon biomass of *M. aquaticum* was also greater at the 0 cm water level (91.6 ± 5.6 g DW pot^{-1}) and overall, stolon biomass accounted for approximately 45-70% of total biomass across all water levels. Sediment root biomass was 6.5 ± 0.5 g DW pot^{-1} at the 0 cm water level, and was also greater than root biomass

at any other water level. Sediment root biomass comprised 4.5 to 9% of total biomass across water levels with a larger proportion of root biomass relative to total biomass as water levels increased.

Submersed shoot biomass was greatest when *M. aquaticum* was grown at the 37, 57, and 77 cm water levels (Figure 5.2). Average submersed shoot biomass across these levels (37, 57, 77 cm) was 3.1 ± 0.4 g DW pot^{-1}, which was 90% greater than all other water levels combined where biomass was only 0.8 ± 0.2 g DW pot^{-1}. Submersed shoot biomass never accounted for more than 12% of total biomass for a given water depth.

Myriophyllum aquaticum plant length was similar across the 0, 37, 57, and 77 cm water levels and was greater ($p < 0.01$) than plants grown at the 97, 117, and 137 cm levels (Figure 5.3). Plant lengths were 111.7 ± 2.8 cm, 112.1 ± 4.1 cm, 118.8 ± 5.9 cm, 118.0 ± 8.8cm for the 0, 37, 57, and 77 cm water levels respectively, and 85.8 ± 6.8 cm, 93.9 ± 7.2 cm, and 75.7 ± 6.9 cm for the 97, 117, and 137 cm levels respectively. *Myriophyllum aquaticum* had difficulty reaching the water surface in the deeper water levels and therefore plant lengths are lower than the treatment level.

Incident light was similar ($p = 0.52$) across all water level treatments indicating the same amount of light was reaching the surface of each mesocosm. Light transmittance through the water column however, was negatively ($R^2 = 0.99$) related as a quadratic function to increasing water depth, meaning light attenuation was rapidly occurring as water levels increased even though the bottom of all mesocosms could be observed (Figure 5.4). The trend in light availability corresponds to the observed decreases in *M. aquaticum* biomass as there is a similar trend in biomass data.

Discussion

Biomass of *Myriophyllum aquaticum* was negatively affected as water levels increased with the exception of submersed shoot biomass which increased at intermediate water levels. In natural populations, total *M. aquaticum* biomass was 1001 g m^{-2} when plants were harvested from water depths < 0.5 m, which represented a 77% increase in biomass from plants collected at sites (234 g m^{-2}) that were 0.5–1.5 m in depth (Sytsma and Anderson 1993a). Biomass allocation to emergent shoots is also greater when plants grow in water depths < 0.5 m (Sytsma and Anderson 1993a). These results are attributed to the heterophyllous growth of *M. aquaticum* and the response to light intensity in its growing environment. *Myriophyllum aquaticum* grown at the 0 cm water level did not have to switch growth forms and could allocate energy to horizontal growth over the water surface, growth of stolons, and growth of adventitious roots. The presence of adventitious roots has been suggested as an important site for water and nutrient uptake and reduced reliance upon sediment roots. *Myriophyllum aquaticum* growth did not reduce sediment nutrient concentrations over the course of a controlled study when adventitious roots were present, and the water column provided 98% of water utilized by plants (Sytsma and Anderson 1993b). In the current study, the proportion of sediment root biomass to total plant biomass increased as water levels increased, suggesting a reliance on sediment roots in the absence of adventitious roots. Sediment roots are typically heavily cuticularized, thick, stiff, and lack root hairs (Sutton and Bingham 1973; Sytsma and Anderson 1993b). The formation of a cuticle on roots may inhibit the uptake of water and nutrients and may have limited *M. aquaticum* growth as water levels increased and plants remained submersed.

When *M. aquaticum* becomes submersed, the leaf form rapidly changes from emergent tissues to submersed tissues; in the current study this switch occurred in a matter of days. Plants in the intermediate water levels were able to reach the water surface and begin emergent shoot growth as well as promote new growth from root crowns, which accounted for the increase in submersed shoot biomass in these water level treatments. The submersed shoots in the deepest water levels were responsible for maintaining plant growth and for plant elongation to the water surface. Light transmittance was ≥ 25% in all treatments, which is sufficient to promote submersed plant growth (Chambers and Kalff 1985). However, the observed significant declines in biomass and plant length as water levels increased, suggest that submersed leaves alone cannot sustain *M. aquaticum* growth for long periods of time.

The optimal photosynthetic rate of *M. aquaticum* occurs as the emergent form and therefore, *M. aquaticum* will not remain as a submersed plant for long periods of time as the photosynthetic rate of submersed leaves may not be sufficient to support plant growth (Salvucci and Bowes 1982). The photosynthetic light saturation point is almost eight-fold higher in emergent leaves, approaching that of full sunlight, whereas the light saturation point of the submersed leaves is between 250-300 μ E m^{-2} s^{-1} (Salvucci and Bowes 1982). The lower photosynthetic rate of submersed leaves suggests that this growth form is adapted to a shade environment (Salvucci and Bowes 1982). However, the congeneric Eurasian watermilfoil (*Myriophyllum spicatum* L.), which grows completely submersed, will undergo self-shading when a surface canopy is produced; leaves below 1 m of the surface canopy begin to senesce and slough due to the light attenuation of the surface canopy (Madsen et al. 1991). Leaf morphology of submersed

M. aquaticum is similar to that of *M. spicatum*, and it therefore may not be as shade tolerant as once believed. The light levels recorded in this study may have been enough to mimic the self-shading effect of a surface canopy at the deeper water depths; or when plants reached the surface and began emergent growth this would have created a self shading environment for plants still in the water column. This may explain the reduced submersed shoot biomass at the 0 cm water depth and the overall reduction in biomass at the deeper water depths. Submersed growth is transient and only utilized for short overwintering periods, times of reduced light and temperature (Sytsma and Anderson 1993a), or to survive disturbances in the growing environment; prolonged exposure to adverse growing conditions will result in reductions in growth or plant mortality.

Myriophyllum aquaticum is described as an amphibious fluctuation responder, or a species that grows in a variety of habitats and conditions such as flooded, damp, or drawdown conditions; and has morphological plasticity (heterophylly) in response to water level variations (Casanova and Brock 2000). In a study conducted in a 2 ha palustrine wetland in the Sinos River Basin, Brazil, *M. aquaticum* was collected during both a flooded period and a drawdown period, but was more associated with wet growing conditions (Maltchik et al. 2007). These authors also suggest that *M. aquaticum* may be tolerant of drawdown events (complete removal of surface water) lasting 9 months if the sediment remains saturated. It was also reported that near-permanent wetland and flooded wetland conditions were dominated by amphibious fluctuation-responder plant species under mesocosm conditions (Casanova and Brock 2000). These studies show that *M. aquaticum* is well adapted to survive both drawdown and flooding events of various durations. Shorter flooding durations allow for amphibious species to recover

between flooding events, and survival at intermediate durations required plants to tolerate both immersion and emersion (Casanova and Brock 2000). Casanova and Brock (2000) concluded that flood duration would determine if there is sufficient time for amphibious plants to respond by changing leaf morphology or elongation of stems.

In this study, *M. aquaticum* was subjected to different water levels and one flood duration of 12 weeks. The plants responded quickly to immersion by changing leaf morphology; however, plants in deeper water levels were unable to sufficiently grow to the water surface and begin emergent growth. *Myriophyllum aquaticum* may have responded differently if the duration of flooding was reduced. These data suggest that this species does not grow well under sustained deep flood conditions. The reduced biomass and plant length observed in this study, along with evidence of reduce photosynthetic rates of the submersed leaves from previous studies, may offer some evidence that there is an energetic cost associated with heterophylly. Aquatic plant populations that experience frequent changes in the water regime also exhibit the greatest degree of heterophylly (Cook and Johnson 1968), and a reduction in heterophylly in populations from more stable environments suggests that there may be costs associated with heterophylly (DeWitt et al. 1998). Heterophylly is a trait that must have some adaptive value, otherwise it would not be found in nature (Trémolières 2004). In its native habitat of South America, *M. aquaticum* is often found growing in palustrine habitats, or areas that are prone to frequent water level variations (Rolon and Maltchik 2006). Therefore, heterophylly allows *M. aquaticum* to survive in its native palustrine habitats and to invade highly disturbed habitats.

The objective then would be to predict the invasion potential of habits not only by *M. aquaticum* but other non-native species as well based upon life history traits (Trémolières 2004). Often however, basic biological and ecological data are overlooked and the focus is placed solely on management of the problematic species. Based on results from the current study, the establishment and growth of *M. aquaticum* is going to be limited to shallow, less than 80 cm, areas where fragments can root and plants can grow rapidly to the water surface and establish an emergent canopy and adventitious roots. In deeper water, invasion and growth is going to be limited or inhibited by light availability, fragment establishment, and the ability of submersed plants to grow to the water surface, unless flood duration is reduced to allow plant growth to the water surface. Other studies are needed to address flood duration effects on *M. aquaticum*. To fully understand invasion processes, more experiments are needed to determine effects of environmental variability and resources availability on specific attributes of non-native plant growth in aquatic systems (Trémolières 2004), thereby allowing the identification of optimal areas for invasion and the development of early detection and rapid response programs.

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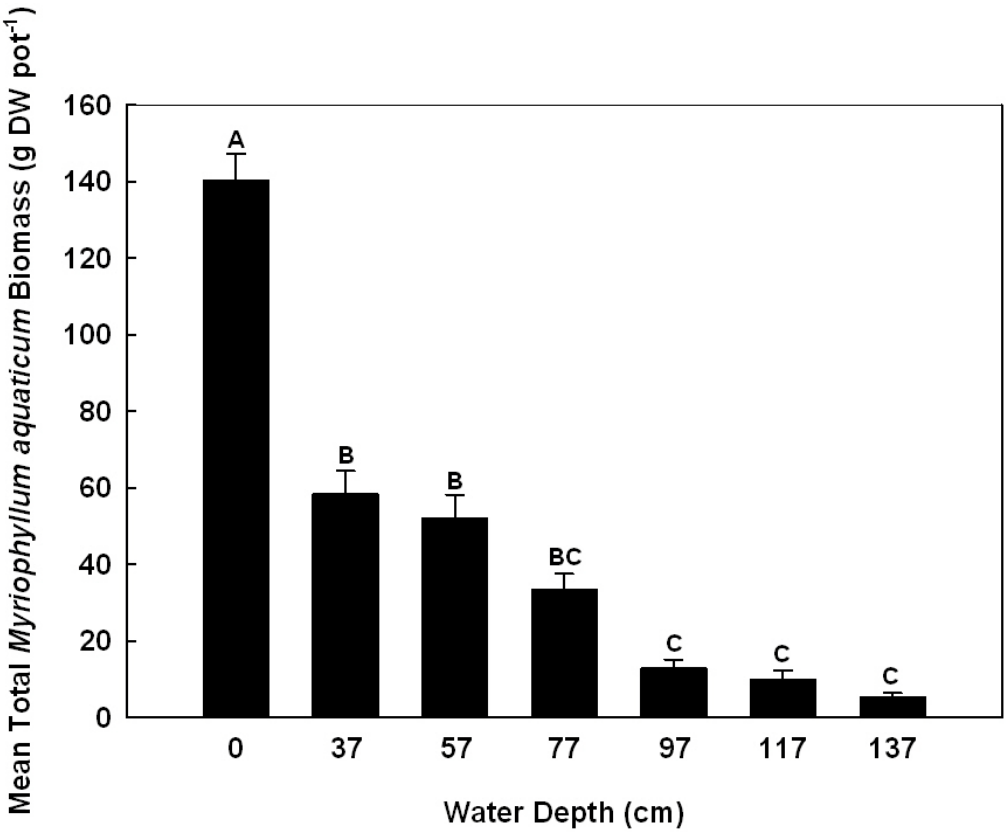

Figure. 5.1 Mean (± 1 SE) *Myriophyllum aquaticum* biomass at increasing water levels. Bars sharing the same letters are not significantly different according to the LSD procedure using a Mixed Procedures Model at a $p < 0.05$ significance level.

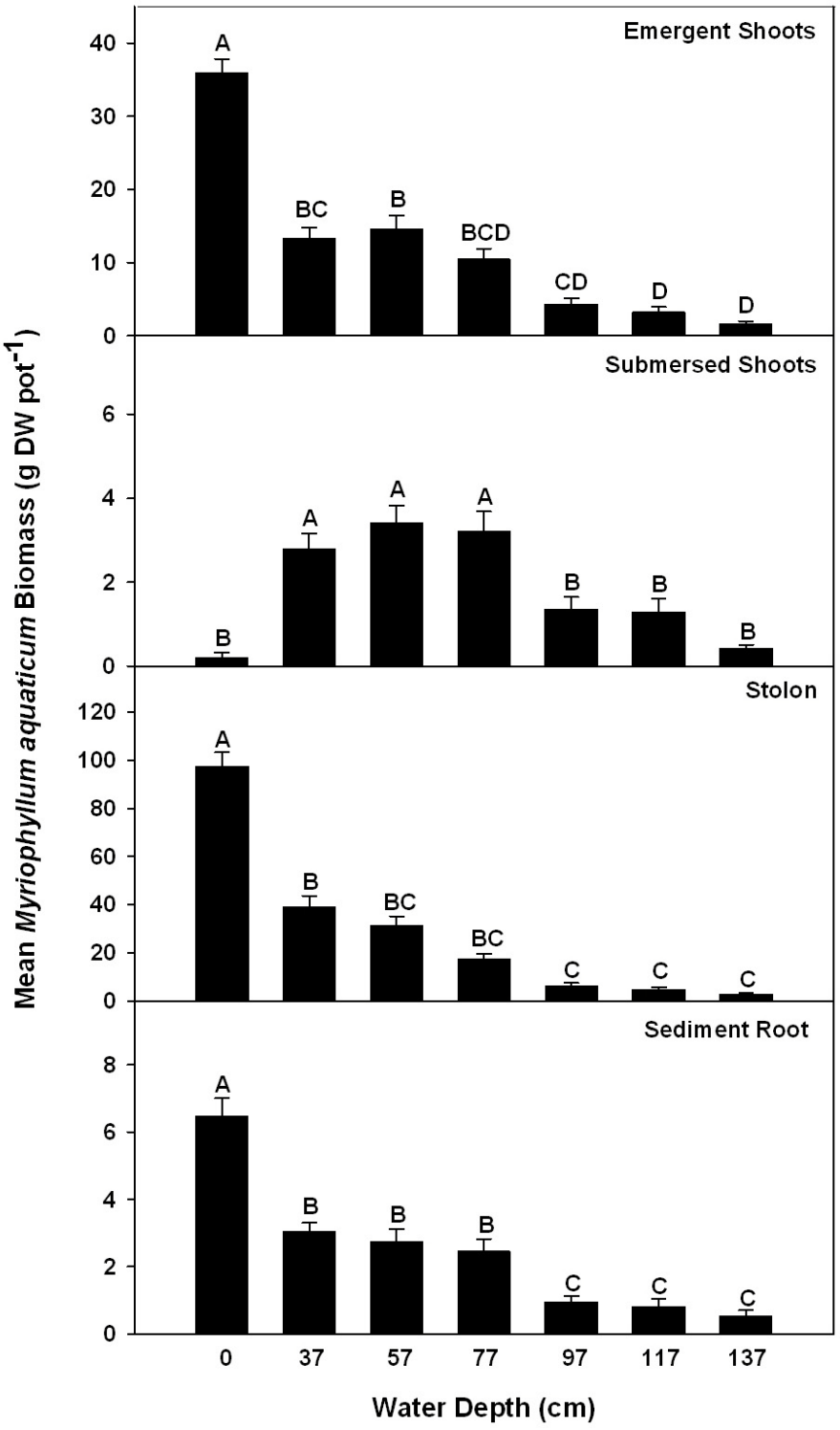

Figure 5.2 Mean (± 1 SE) emergent shoot, submersed shoot, stolon, and sediment root biomass of *Myriophyllum aquaticum* at increasing water levels. Bars sharing the same letters are not significantly different according to the LSD procedure using a Mixed Procedures Model at a $p < 0.05$ significance level.

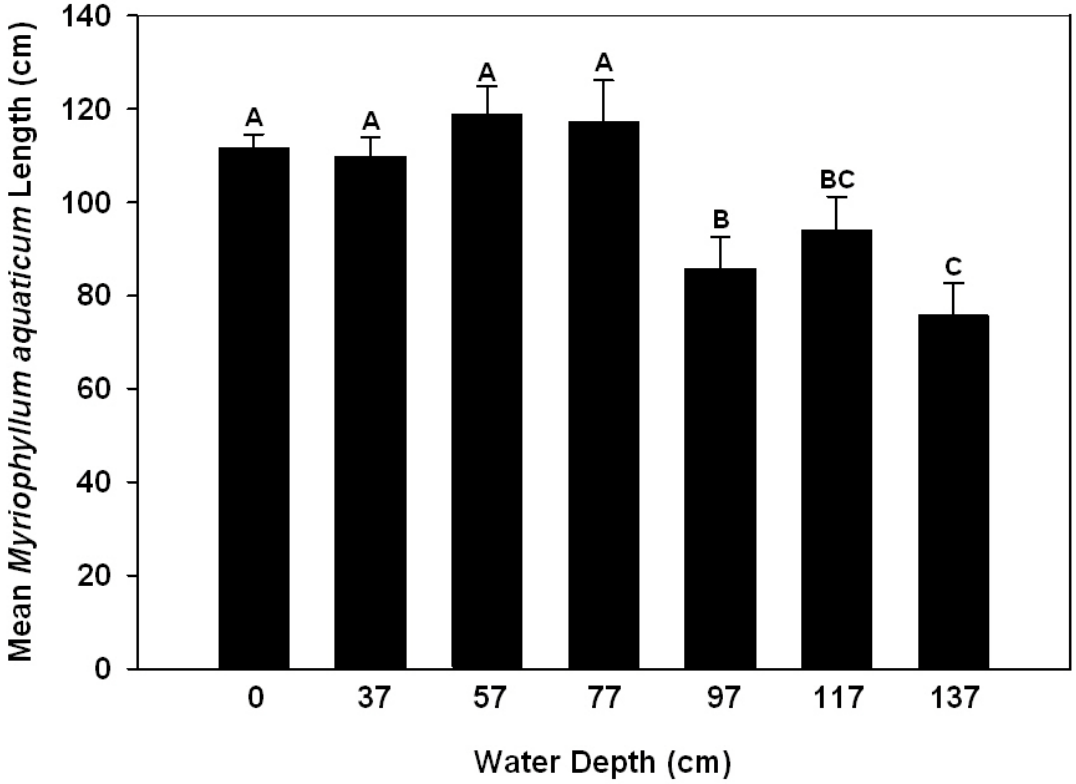

Figure 5.3 Mean (± 1 SE) total *Myriophyllum aquaticum* length at increasing water levels. Bars sharing the same letters are not significantly different according to the LSD procedure using a Mixed Procedures Model at a $p < 0.05$ significance level.

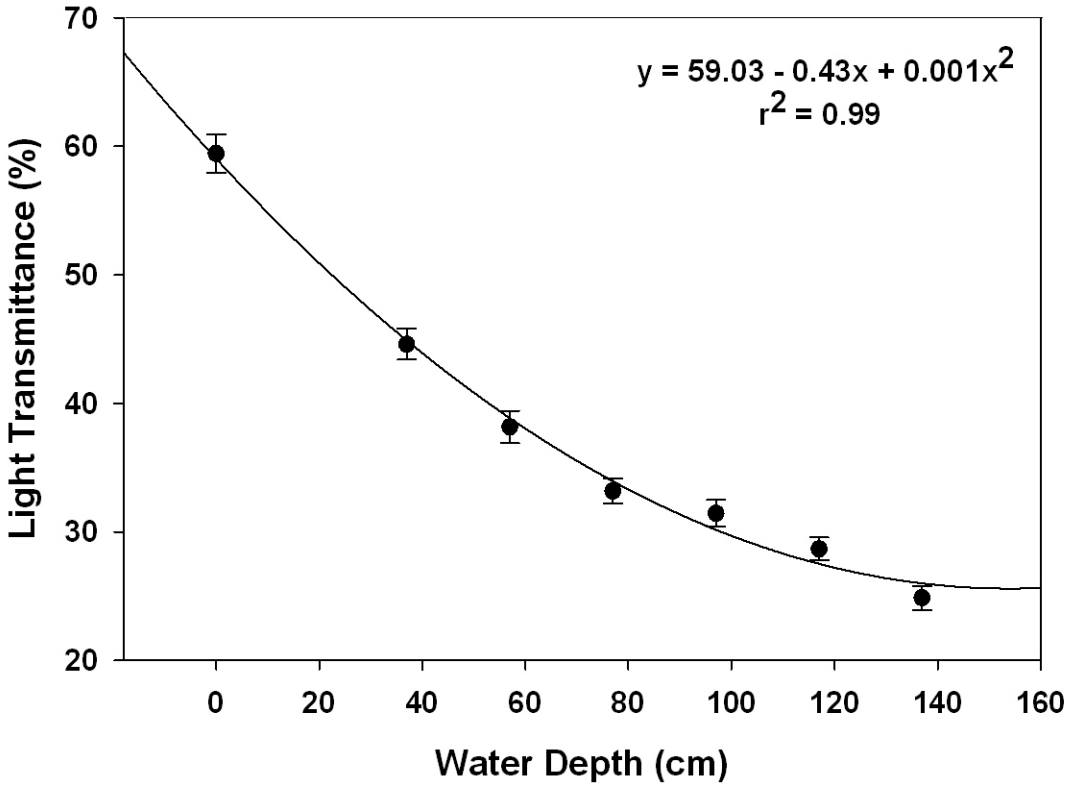

Figure 5.4 Polynomial regression analysis of mean (± 1 SE) light transmittance calculated for each water level over the course of 12 weeks.

CHAPTER VI

EVALUATION OF WINTER AND SUMMER DRAWDOWNS FOR CONTROL OF THE NON-NATIVE AQUATIC PLANT *Myriophyllum aquaticum*

Abstract

Non-native aquatic plants can often invade and rapidly outgrow native species in shallow waterbodies resulting in the establishment of monotypic populations of the invading plant. Parrotfeather [*Myriophyllum aquaticum* Vell. Verdc.] is a non-native species that is becoming an increasing nuisance in shallow waterbodies across the southeastern United States, with few effective management options. Therefore, a 0, 2, 4, 8, and 12 week winter and summer drawdown was conducted under controlled mesocosm conditions to evaluate *M. aquaticum* response to seasonal effects of drawdown events. Overall, both the winter and summer drawdowns were effective at reducing *M. aquaticum* biomass. The winter drawdown reduced ($p=0.003$) biomass by 99% at 4 weeks when compared to pre drawdown levels. The summer drawdown reduced ($p<0.01$) biomass by 98% at 2 weeks when compared to pre drawdown levels. Regrowth of *M. aquaticum* was evident in all drawdown treatments upon reflooding, indicating that this species can survive drawdowns of 12 weeks; and longer drawdown durations may be required for complete control.

Introduction

Disturbance within a waterbody is often the primary mechanism that facilitates invasions through removing native species and opening niche space for colonizing species (Davies et al. 2005; Lockwood et al. 2005; Capers et al. 2007). Wetlands and shallow lakes are often prone to invasion by non-native species due to the frequency at which disturbances occur. Variations in hydrology such as extreme flooding or drawdown events often determine the structure of macrophyte communities in a given habitat (van Geest et al. 2005). Sustained or frequent flooding can lead to a more stable environment and a shift in species dominance and ultimately species composition (van der Valk 2005). More stable environments created by flooding often inhibit emergent macrophyte growth (Casanova and Brock 2000), and favor submersed aquatic macrophytes such as the evergreen perennial species.

Drawdowns are also very important in determining the composition of wetland and aquatic macrophyte communities (van der Valk 1981). Drawdown events that expose sediments will favor annual macrophyte species or those species that have long lived propagules in the sediment (van der Valk 1981). The drying out of sediments have extreme effects on aquatic vegetation and often results in the removal of all or most aboveground biomass (Richardson et al. 2002). In lakes that were repeatedly disturbed by drawdowns, the macrophyte community had shifted to those species that were tolerant to desiccation (van Geest et al. 2005). Therefore, the use of drawdown events, whether intentional or following the natural hydrologic cycle of the habitat, may be efficacious in managing non-native aquatic plants while restoring a diverse wetland or aquatic macrophyte community.

Parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.] is a herbaceous perennial aquatic plant that is not native to the United States. This species readily invades shallow waterbodies that are prone to disturbance. Sabbatini et al. (1998) reported that *M. aquaticum* was tolerant to mechanical disturbances and the repeated application of these techniques favored *M. aquaticum* dominance in canals. Dense beds of *M. aquaticum* have resulted in reductions in dissolved oxygen in the water column, which may be detrimental to fish (Fonseca 1984; Moreira et al. 1999). *Myriophyllum aquaticum* can inhibit the growth of more desirable plant species such as pondweeds and coontail (Ferreira and Moreira 1994), which are readily utilized by waterfowl as food items (Wersal et al. 2005). A strong correlation was also determined between the density of *M. aquaticum* growth and the presence of mosquito eggs and larvae (Orr and Resh 1989; Orr and Resh 1992), which may lead to increases in mosquito-borne diseases that could infect wildlife and humans.

Unlike the congeneric *M. spicatum*, *M. aquaticum* is heterophyllous meaning it has a distinct submersed and emergent leaf form. Having two distinct growth forms may give *M. aquaticum* the ability to overcome extreme disturbances in the hydrologic regime of a waterbody, or convey a competitive advantage over macrophytes that are more sensitive to changes in their growing environment. In a study conducted in a 2 ha palustrine wetland in the Sinos River Basin, Brazil, *M. aquaticum* was collected during both a flooded period and a drawdown period, but was more associated with wet growing conditions (Maltchik et al. 2007). Maltchik and others (2007) suggested that *M. aquaticum* may be tolerant of drawdown events (complete removal of surface water) lasting 9 months if the sediment remains saturated. Survival and spread of *M. aquaticum*

depends solely on vegetative reproduction via fragmentation, as this species does not produce any specialized reproductive structures such as seeds, tubers, or turions (Sytsma and Anderson 1993a). *Myriophyllum aquaticum* is a dioecious species however, pistillate flowers are most common in all naturalized populations including its native range, with staminate flowers rarely observed (Orchard 1979). During a comprehensive study of *Myriophyllum* species, Orchard (1981) found only a few staminate flowers, and two plants with immature fruits, on specimens collected from South America. Therefore, little is known regarding the appearance of staminate flowers, fruit, or seed; and no information is available on factors affecting pollination, fruit development, and seed germination since staminate flowers are rare (Sutton 1985). The paucity of staminate flowers indicates that seed production likely does not occur and therefore this species would rely on vegetatiive means for reproduction and survival.

The lack of specialized reproductive structures may allow drawdown events to be efficacious against *M. aquaticum* if the sediment can be dried sufficiently and over a long enough duration to cause desiccation of root crowns. Currently there is little data regarding the effects of drawdown events on *M. aquaticum* and no data from controlled drawdown experiments, or the seasonal effects of drawdown events on *M. aquaticum*. Therefore, the objectives of this study were to examine the efficacy of winter and summer drawdown events lasting 2 to 12 weeks under controlled mesocosm conditions. Summer drawdown events should be more effective for controlling *M. aquaticum* because of warmer temperatures and reduced soil moisture.

Materials and Methods

Experiments were conducted at the R.R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS from June 2008 through September 2009. Both the winter and summer drawdown experiments were conducted in 20, 1100 L mesocosms arranged in a completely randomized experimental design. Drawdown durations were 0, 2, 4, 8, and 12 weeks. All drawdown durations were repeated in four mesocosms during both experiments.

Planting

Myriophyllum aquaticum was harvested from a local pond and transported to Mississippi State University for planting. Planting consisted of placing two apical shoots of *M. aquaticum*, approximately 20 cm in length, into each of 272, 3.78 L pots containing a top soil, loam, and sand mixture (3:2:1). Sediment was amended at a rate of 2 g L pot^{-1} with Osmocote® 19-6-12 fertilizer. Fourteen pots of planted *M. aquaticum* were placed into each of the 2, 4, 8, and 12 week mesocosms and 12 pots placed into the 0 week mesocosms. All mesocosms were filled with water so that the water level was approximately 12 cm above the plants. Water was supplied to each mesocosm from an irrigation reservoir adjacent to the mesocosm facility. Air was continuously supplied to all mesocosms during the growth phase of each experiment by a regenerative air blower using 2.5 cm stone diffusers and a PVC lift pipe placed in each mesocosm. Once the drawdowns were initiated air was removed with the exception of the reference tanks which had continuous air. Air was re-supplied to all mesocosms during the refill (recovery) stage of both experiments to circulate water in the mesocosms.

Winter and Summer Drawdown Experiments

Planting for the winter drawdown occurred on September 8, 2008 followed by a 4 month growth period. The growth period was used to establish a mature population of *M. aquaticum* in each mesocosm. The winter drawdown was initiated on January 16, 2009 with the final biomass harvest on May 8, 2009. Planting for the summer drawdown occurred on February 2, 2009 followed by a 4 month growth period. The summer drawdown was initiated on June 15, 2009 and final biomass harvest on September 28, 2009.

At the conclusion of the 4 month growth periods plants had completely covered the water surface in all mesocosms. Prior to drawdown initiation, 2 pots were removed from each mesocosm and plants were harvested at the sediment surface, dried at 70 C, and weighed to assess pre drawdown biomass. Following the pre drawdown harvest, water was removed from all mesocosms with the exception of the 0 week drawdown as this would serve as the reference to assess plant recovery. After the specified drawdown duration (for example 2 weeks) had been reached, 2 pots were removed from these mesocosms, all living plant material harvested, dried at 70 C, and weighed to assess post drawdown biomass. These mesocosms were then refilled with water and a recovery period of 4 weeks was used to assess plant re-growth after the drawdown. Following the recovery period the remaining 10 pots were removed from these mesocosms, living plants harvested at the sediment surface, dried at 70 C, and weighed to assess recovery biomass. This sequence was followed for the 4, 8, and 12 week durations for both the winter and summer drawdown studies. After the 12 week drawdown sequence all remaining mesocosms including references were harvested, dried at 70 C, and weighed.

Environmental Monitoring

Weather data were recorded in 1 hour intervals over the duration of both experiments by a HOBO Weather Station (Onset Computer Corporation, Pocasset, MA). The weather station was located on site within 15 meters of the mesocoms. Soil moisture probes (EC-5, Decagon Devices, Pullman, WA) were placed into one pot for each mesocosm to monitor soil moisture. The EC-5 model probes were chosen because they perform better at high soil moisture contents and are field ready for most soils with no calibration while maintaining a ± 3% accuracy (Decagon Devices 2006). Kizito and others (2008) also found that sensor calibrations were robust over a limited range of soil types, bulk densities, and electrical conductivities. Percent soil moisture was recorded from each mesocosm once a week throughout both experiments. Soil moisture was also measured in an air dried sample to serve as a reference.

Data Analysis

A paired t-test was used to compare pre drawdown biomass to post drawdown biomass for the 2, 4, 8, and 12 week drawdown durations. A mixed procedures model was developed using SAS® (Cary, NC) to analyze seasonal and interaction effects for *M. aquaticum* recovery biomass (Littell et al. 1996). There was a significant ($p<0.01$) season and season*treatment effect; therefore, the winter and summer drawdown experiments were analyzed separately. A mixed procedures model was used to determine differences in biomass between the 0, 2, 4, 8, and 12 week drawdown durations with means separated using least squares means and grouped using the Least Significant Difference method. All analyses were conducted at a $p < 0.05$ level of significance. Soil moisture data were

averaged within drawdown duration and reported as the mean (± 1SE) percent for each duration across both experiments. Similarly, weather data were averaged across months and the means (± 1SE) are reported.

Results

Pre drawdown *M. aquaticum* biomass was different ($p<0.01$) between the winter and summer drawdowns. Initial biomass for the winter drawdown experiment was 28.1 ± 5.3 g DW pot^{-1} and 52.9 ± 9.1 g DW pot^{-1} for the summer experiment, a 47% increase in biomass between seasons. The significant seasonal effects observed in the models are attributed to the difference in total *M. aquaticum* biomass between the winter and summer experiments which is a result of the seasonal life history of the plant.

The 2 week winter drawdown treatment resulted in no reduction ($p=0.88$) in *M. aquaticum* biomass (Figure 6.1). After 4 weeks however, there was a significant ($p=0.003$) reduction in *M. aquaticum* biomass following drawdown. Biomass reductions were 99, 99, and 97% respectively for the 4, 8, and 12 durations when compared to pre drawdown biomass samples collected during corresponding sampling events. *Myriophyllum aquaticum* regrowth was observed following all drawdown durations after the recovery period for the winter experiment; however biomass was still lower ($p < 0.0001$) than reference samples (Figure 6.2). *Myriophyllum aquaticum* biomass was 0.99 g DW pot^{-1} following a 12 week winter drawdown, and subsequent biomass increased 50% to 1.92 g DW pot^{-1} after the recovery period.

The summer drawdown resulted in significant biomass reductions across all drawdown durations when compared to pre drawdown samples (Figure 6.1). Biomass

reductions during summer for all drawdown durations were > 98%. Regrowth of *M. aquaticum* was observed after the recovery period for all drawdown durations, but similar to the winter experiment, biomass values were significantly ($p < 0.0001$) lower than reference samples (Figure 6.2). *Myriophyllum aquaticum* biomass prior to refill in the 12 week mesocosms was 0.01 g DW pot^{-1}; at the conclusion of the recovery period biomass had increased 92% to 0.13 g DW pot^{-1}.

Environmental Monitoring

Soil moisture during the winter drawdowns never fell below the complete soil saturation line and therefore did not approach dry soil (Figure 6.3). In contrast, soil moisture during the summer drawdowns immediately fell below complete soil saturation upon draining the mesocosms with the exception of the reference mesocosms in which the soil remained completely submersed and thus saturated. Complete saturation for the ECH_2O probes are typically 40-50% soil moisture (Decagon Devices 2006), but some of the completely submersed pots gave readings as low as 30% during summer months; therefore 30% soil moisture was considered complete saturation for this study. Kizito and others (2008) indicated that a 10 degree shift in temperature causes changes in the volumetric water content readings. Temperature, humidity, and photosynthetically active radiation (PAR) are summarized for both experiments in Table 6.1.

Discussion

In the current study, a winter and summer drawdown resulted in > 95% biomass reduction across all drawdown durations, with the exception of the winter 2 week drawdown which did not result in a reduction of *M. aquaticum*. During winter drawdown

events soil moisture never fell below complete saturation. However, when drawdown events were initiated in summer, soil moisture rapidly fell to levels near that of dry soil. Soil moisture during this time closely tracked that of the dry soil until the refill occurred at 2, 4, and 8 weeks after initial drawdown. After the refill, soil moisture rose quickly to complete saturation. The soil moisture in the 12 week mesocosms increased abruptly after only 8 weeks drawdown exposure. The increase in soil moisture corresponds to increased amounts of rain received during late summer.

Total rainfall from July through September 2009 was 14.5 cm greater than the same time period in 2008. It rained 35 out of 61 days from August through September 2009 which kept soil saturated and allowed *M. aquaticum* to survive where mortality was expected. These results corroborate those reported from a field trial where *M. aquaticum* was found to be more associated with the wet phase of the hydrologic cycle in Brazil, (Maltchik et al. 2007). Although biomass values reported for the 12 week drawdown events in this study are minimal it does indicate the capacity of this species to survive adverse environmental conditions and regrow when conditions become favorable. This was particularly unexpected for a macrophyte species that does not produce any sort of seed, tuber, or turion.

Water use may be an explanation for the rapid efficacy of both a winter and summer drawdown and also why plants survived in moist soil until mesocosms were refilled. When *M. aquaticum* emerges from the water surface it begins to grow adventitious roots in the water column, after which the reliance on sediment roots is reduced. *Myriophyllum aquaticum* growth did not reduce sediment nutrient concentrations over the course of a controlled study when adventitious roots were present

(Sytsma and Anderson 1993b). This indicates that *M. aquaticum* relies almost exclusively on water column nutrients for growth. In fact, the water column provided 98% of the water transpired by *M. aquaticum* which suggests that the majority of nutrients used for growth would also come from the water column (Sytsma and Anderson 1993b). By removing the water, the *M. aquaticum* mat collapsed, thereby exposing the stolons and the adventitious roots resulting in the rapid desiccation of these tissues. Adventitious roots are likely important sites for water and nutrient uptake, and stolons store the majority of starch that would be needed to support plant growth. The desiccation of these tissues resulted in plant death.

The *M. aquaticum* that survived drawdown events in these studies were short (approximately 4-6 cm) emergent shoots growing in the moist soil of the pot. These shoots re-grew during the recovery period when mesocosms were refilled with water. These shoots may have been able to survive, albeit at a reduced growth rate, on the interstitial water in the soil. Sediment interstitial water accounts for approximately 2% of the water transpired by *M. aquaticum* (Sytsma and Anderson 1993b), and plants would have had to survive on what was available in the sediment until favorable conditions in the mesocosms returned. The emergent form of *M. aquaticum* has a transpiration coefficient of 260 ml H_2O mg DW^{-1} which is similar to C-4 terrestrial plants (Sytsma and Anderson 1993b). Furthermore, the leaves of emergent shoots have sunken anomocytic stomata (Sutton and Bingham 1973), a thick waxy cuticle, and short cylindrical leaflets. These traits are typical for reducing transpiration and are common in plants growing in more xerophytic environments. Sytsma and Anderson (1993b) concluded that low water use may be advantageous only during some critical phase in the life cycle of *M.*

aquaticum, or in ephemeral environments with fluctuating water levels where drawdown would result in water stress. Therefore, if only small shoots of emergent *M. aquaticum* are present, plants may be able to survive extended periods of time at reduced growth rates without standing water. Maltchik and others (2007) reported that *M. aquaticum* was present and composed a significant portion of macrophyte biomass during flood events, but also constituted 88.3% of macrophyte biomass during a drawdown event in a Brazilian wetland as long as the sediment remained moist.

Myriophyllum aquaticum has proven to be resilient towards management techniques and once established it persists in spite of management or environmental conditions (Moreira et al. 1999). A drawdown conducted in winter or summer was very effective at reducing *M. aquaticum* biomass, thereby alleviating the problems associated with nuisance growth. A summer drawdown lasting 12 weeks or more may offer longer term efficacy as plants would have to survive the drawdown and then the winter season at a reduced rate of growth. Conversely, plants that survived a winter drawdown would begin growth in more hospitable conditions, such as having a longer photoperiod and warmer temperatures; and would likely have a better chance at re-establishing a population during spring and summer. Therefore, the effectiveness of a drawdown will depend upon the life history strategies of the target plants.

Myriophyllum aquaticum being a herbaceous perennial responded well to the use of a drawdown. However, submersed aquatic plants such as *Hydrilla verticillata* (L.f.) Royle that produce tubers and turions have a mechanism to survive several years of drawdown and often become the dominant plant when the environment becomes favorable again. *Hydrilla verticillata* was initially controlled by a winter drawdown,

however after two growing seasons it became one of the dominant species in Lake Ocklawaha, Florida (Hestand and Carter 1975). Biomass of *Myriophyllum spicatum* L. was reduced 99% from a maximum biomass of 2000 g DW m^{-2} in NNR Břehyňskỳ Fishpond, Czech Republic (Adamec and Husák 2002). It was also reported that after the removal of *M. spicatum*, the desirable aquatic plants *Nymphaea candida* (Presl.) and *Myriophyllum verticillatum* L. recovered. The removal of a non-native plant canopy and the exposure of the soil as a result of a drawdown generally favor native seed producing annual species (van der Valk 1981; Smith and Kadlec 1983).

However, tradeoffs exist when deciding upon proper management techniques to control non-native aquatic plants. Tradeoffs can include economic, social, and environmental issues that need to be addressed when developing a management plan. The use of drawdown in a lake or reservoir is typically inexpensive, does not have the negative outlook that is often associated with herbicide use, and is effective on large scales. However, a drawdown is non-selective and therefore there will be a loss of all or most submersed aquatic macrophytes. A drawdown will result in the removal of aquatic invertebrates and fish, and result in the loss of use of the waterbody for the duration of the drawdown. Therefore, management techniques should be site-specific based on environmental factors, and chosen to maximize control of the target species. Decisions should be based upon the desired use and desired outcomes of the habitat being managed.

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Table 6.1 Summary of mean (± 1 SE) monthly environmental data collected for the duration of both the winter and summer drawdown experiments. Rain fall data are totals for each month. Minimum PAR was 1.2 μmol m^{-2} sec^{-1} for all dates.

Date	Rain (cm)	Temperature (C)	Min	Max	Relative Humidity (%)	Min	Max	PAR (μmol m^{-2} sec^{-1})	Max
2008									
Jul	5.9	27.1±0.4	23.3±0.4	32.4±1.0	86.3±1.7	62.4±3.2	99.9±0.1	463.3±61.5	1615.7±187.9
Aug	24.6	25.4±0.3	21.5±0.3	30.3±0.6	85.8±1.6	63.5±3.2	99.9±0.1	627.1±182	1510.5±87.9
Sep	13.2	22.8±0.5	18.8±0.7	28.1±0.5	86.3±1.6	64.7±2.8	99.9±0.0	404.4±24.5	1390.1±80.5
Oct	5.9	16.4±0.8	10.9±1.0	22.8±0.7	81.1±2.0	54.8±2.8	98.1±1.1	371.1±18.1	1421.8±64.9
Nov	7.5	10.1±0.7	4.4±0.8	16.3±0.9	77.1±2.5	53.5±4.0	95.1±1.5	257.0±22.0	1023.4±76.4
Dec	31.8	8.2±1.1	3.6±1.1	13.5±1.1	81.7±2.7	64.3±4.1	94.7±1.8	180.4±17.6	806.8±64.3
2009									
Jan	17.9	6.5±1.0	1.4±1.1	12.2±1.1	75.5±2.9	56.2±3.9	90.5±2.3	232.1±91.0	972.7±68.2
Feb	4.9	9.3±1.1	2.9±1.1	15.6±1.0	68.4±2.9	42.9±3.7	90.5±2.2	314.6±23.1	1261.0±74.8
Mar	19.9	13.4±1.0	8.3±1.0	18.8±1.0	76.7±2.8	54.1±3.9	95.7±1.5	365.3±26.4	1410.3±78.6
Apr	9.7	16.9±0.9	10.7±0.9	29.6±6.9	73.0±2.0	47.9±2.5	94.9±1.6	485.4±27.8	1703.4±65.0
May	27.4	21.1±0.6	18.0±0.6	24.9±0.9	94.3±1.6	80.8±4.4	100.0±0.0	355.1±41.8	1378.3±129.3
Jun	10.4	27.1±0.6	20.2±1.2	32.6±1.4	64.8±3.2	36.3±4.0	96.2±3.9	870.0±145.9	2026.2±2.5
Jul	13	26.9±0.5	21.3±0.4	32.5±0.4	83.4±1.7	60.1±2.8	99.3±0.4	532.1±27.8	1783.8±52.0
Aug	17.2	26.4±0.5	21.2±0.4	31.7±0.5	88.3±1.1	64.0±2.2	100.0±0.0	501.4±23.0	1697.9±54.0
Sep	28.0	28.2±0.3	20.9±0.4	31.0±0.7	94.4±1.2	78.5±3.1	99.9±0.1	334.8±27.8	1415.5±76.4

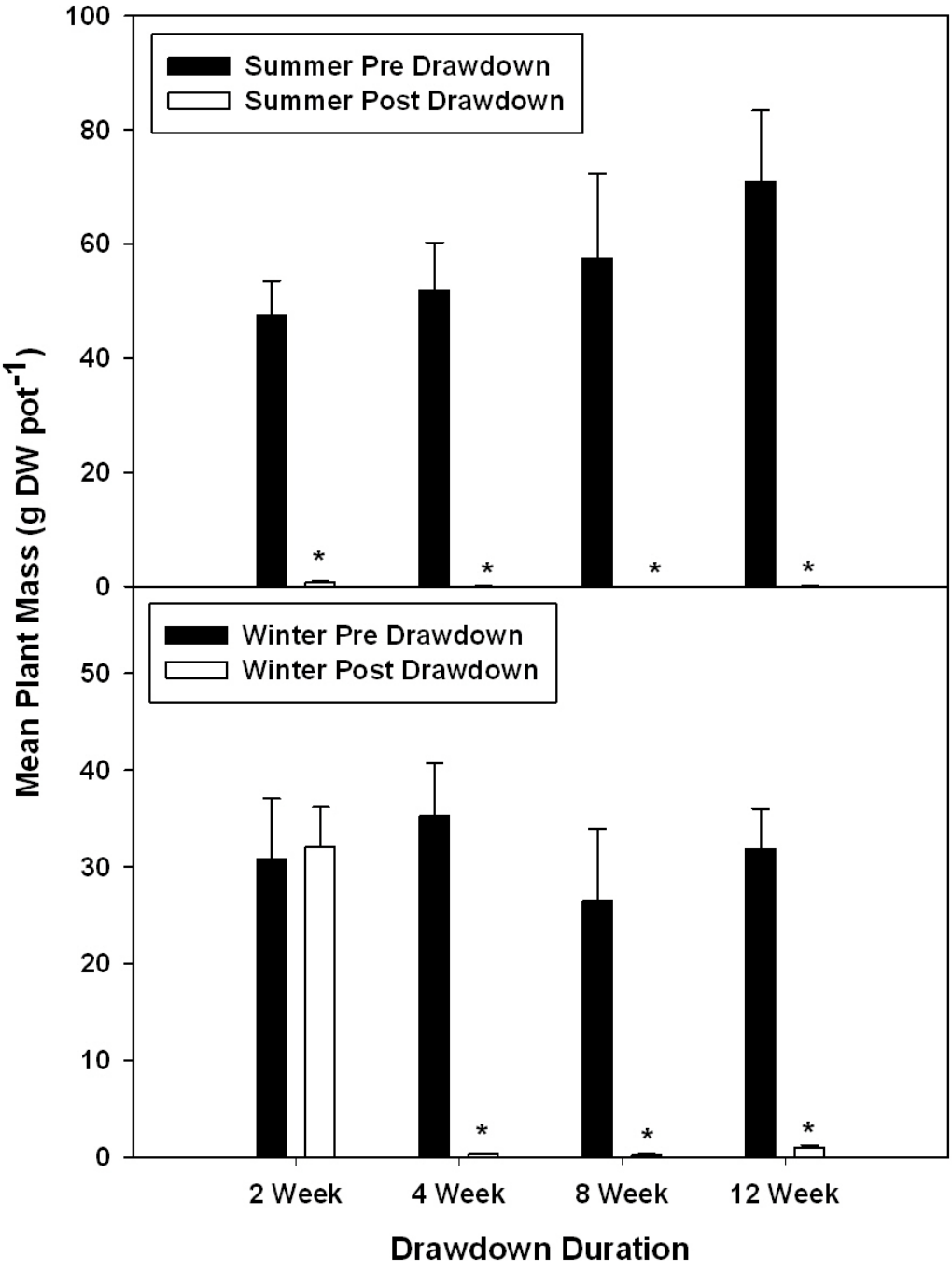

Figure 6.1 Mean (± 1 SE) *Myriophyllum aquaticum* biomass from pre and post drawdown (prior to refilling) sampling times for both the winter (bottom) and summer (top) drawdown events. An asterisk indicates a significant difference as determined by a paired t-test at a $p < 0.05$ level of significance.

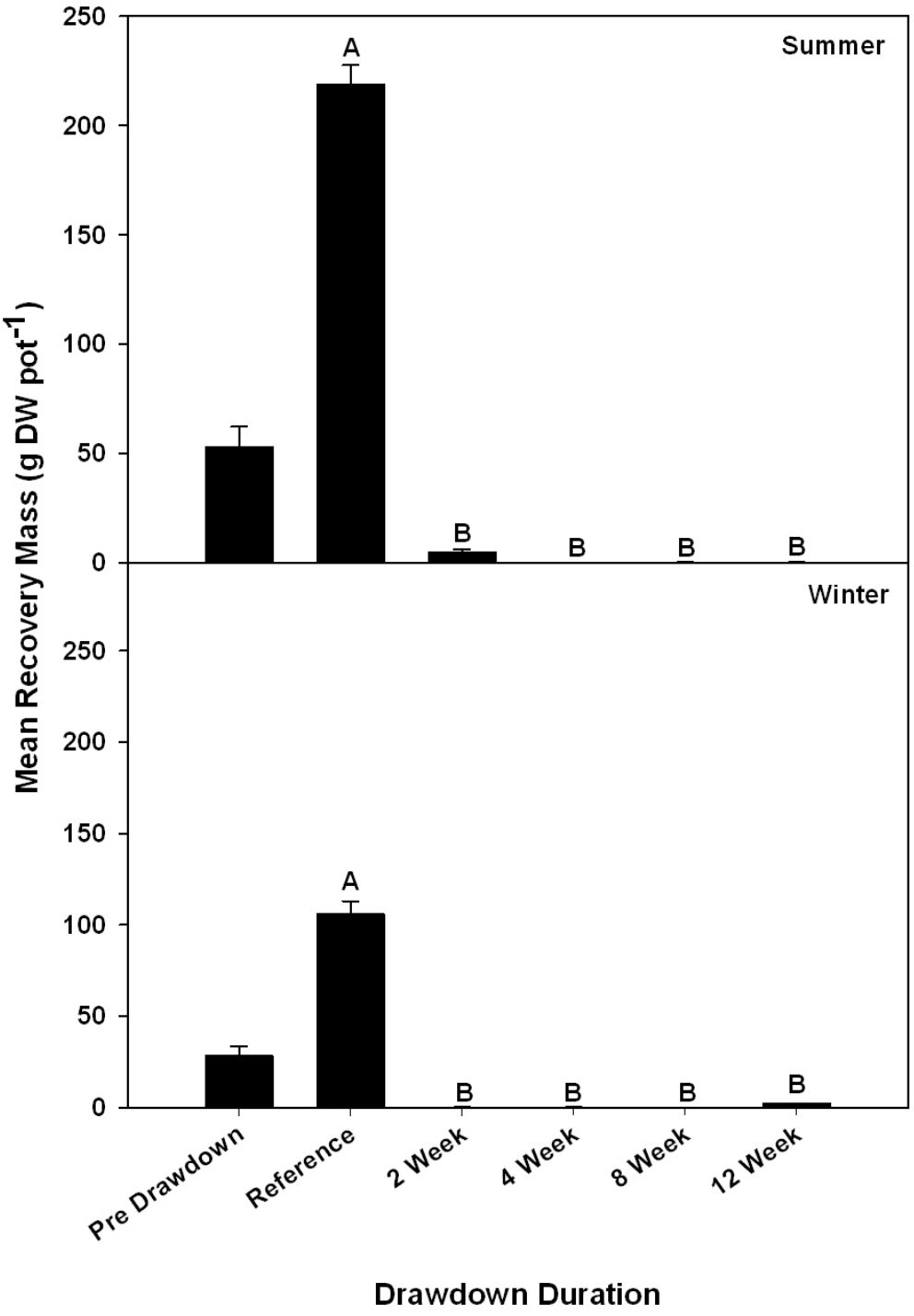

Figure 6.2 Mean (± 1 SE) *Myriophyllum aquaticum* biomass harvested after the four week recovery period. Bars sharing the same letter are not significantly different according to the LSD procedure at a $p < 0.05$ level of significance.

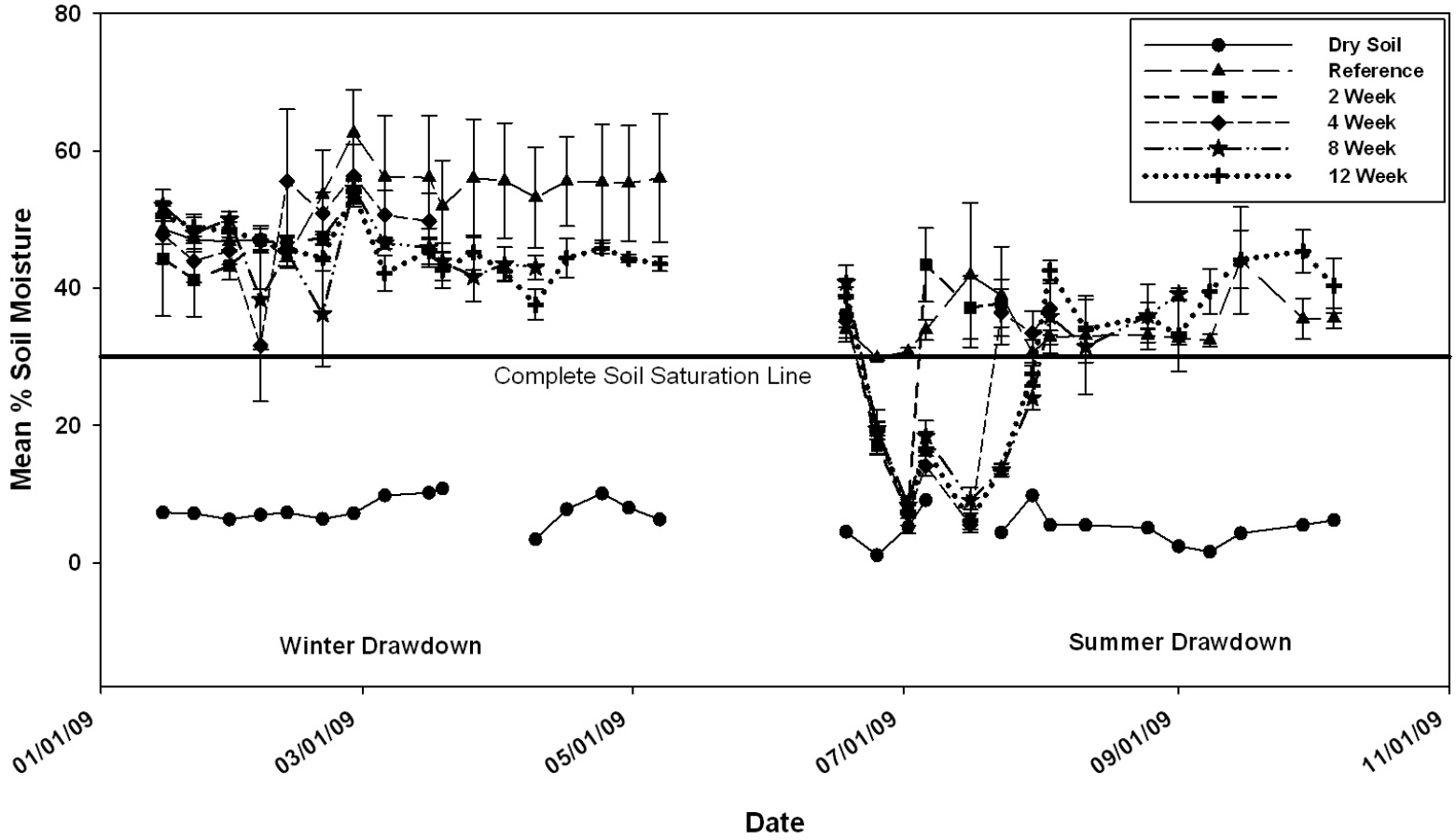

Figure 6.3 Mean (± 1 SE) percent soil moisture for both the winter and summer drawdown durations.

CHAPTER VII

COMPARISON OF SUBSURFACE AND FOLIAR HERBICIDE APPLICATIONS FOR CONTROL OF *Myriophyllum aquaticum*

Abstract

Myriophyllum aquaticum is an invasive aquatic plant in the United States that is native to South America. *Myriophyllum aquaticum* has impaired the use of waterbodies throughout the United States and is difficult to control, despite using a variety of management techniques. The objectives of this study were to examine the efficacy of subsurface applications of seven herbicides labeled for aquatic use and to compare those applications to herbicides that can also be applied to emergent foliage. A replicated mesocosm study was conducted in 378 L tanks beginning in August 2007 and repeated during the same period in 2008. The maximum and half-maximum labeled rates of copper chelate, diquat (6,7-dihydrodipyrido [1,2-a:2',1'-c] pyrazinediium), endothall (7-oxabicyclo [2.2.1]heptane-2,3-dicarboxylic acid), fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone), triclopyr [(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid), 2, 4-D ((2,4-dichlorophenoxy)acetic acid), and carfentrazone-ethyl (ethyl α,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate) were applied to the water column for a 48 h time period in designated mesocosms. The maximum labeled rate for foliar applications of diquat, triclopyr, and 2,4-D were used to compare treatment methods. Six weeks after

treatment (WAT), copper, endothall, fluridone, and carfentrazone-ethyl were not efficacious for controlling *M. aquaticum*. Diquat at all rates and application methods resulted in 70-90% biomass reduction. Triclopyr at both the highest aqueous concentration and foliar application resulted in an 84 and 86% reduction in biomass at 6 WAT. The foliar application of 2,4-D was the only herbicide and application method that resulted in ≥ 90% biomass reduction of *M. aquaticum*. In these studies, regrowth occurred in all tanks regardless of herbicide or treatment method, indicating multiple applications would be necessary to provide longer-term plant control. Future work should identify possible herbicide combinations and/or timing of applications to maximize treatment efficacy.

Introduction

Parrotfeather [*Myriophyllum aquaticum* (Vell. Verdc.] is a non-native invasive aquatic plant that was introduced to the United States from South America in the 1890's. *Myriophyllum aquaticum* has caused major problems in water-bodies throughout the United States, where infestations have reduced access, use, and runoff in ditches, streams, ponds, and shallow lakes (Sutton 1985). Large populations of *M. aquaticum* can impede runoff to such an extent that flooding of adjacent lands occurs (Timmons and Klingman 1958). In South Africa, *M. aquaticum* infests all of the major river systems, posing a direct threat to the country's water supply (Jacot-Guillarmod 1977). *Myriophyllum aquaticum* also provides mosquito larvae a refuge from predation and can indirectly aid in the spread of insect born diseases (Orr and Resh 1989; Orr and Resh 1992). The problems posed by *M. aquaticum* are often perpetuated as this species is widely

cultivated and sold in the United States via the water garden industry (Aiken 1981). Once established, it is capable of thriving in a variety of environmental conditions and is difficult to control using a variety of management techniques (Moreira et al. 1999).

Previous research has often focused on foliar herbicide applications to control *M. aquaticum*. Contact herbicides such as diquat and endothall have been evaluated, but these herbicides offer short term control and repeat applications are often necessary (Moreira et al. 1999; Westerdahl and Getsinger 1988). When triclopyr (Garlon®3A) was applied at rates greater than 2.0 kg acid equivalent (ae)/ha it resulted in complete control of parrotfeather for up to 30 weeks after treatment (WAT) (Hofstra et al. 2006). Wersal and Madsen (2007) reported 50-100% control of *M. aquaticum* with imazamox (2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid) and imazapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)5-oxo-1H-imazol-2-yl]-3-pyridinecarboxylic acid), respectively.

The resiliency of *M. aquaticum* may in part be attributed to its submersed growth form. Submersed tissues of *M. aquaticum* become light saturated at a much lower level than emergent tissues. The light saturation point of the submersed leaves is between 250-300 μ E/m/s and indicates that photosynthesis of submersed plants is adapted to reduced light environments (Salvucci and Bowes 1982). The growth of submersed tissues was also found to have an inverse relationship with both light transmittance and water temperature, whereas, when both environmental variables increased, biomass of submersed tissues decreased (Wersal, unpublished data). This would suggest that a higher percentage of submersed biomass would occur in fall and winter. In California, submersed biomass was an important component in *M. aquaticum* growth only in winter,

but submersed biomass never exceeded 3% of the total annual biomass of the plant (Sytsma and Anderson 1993). Therefore, subsurface herbicide applications may offer increased control of *M. aquaticum* by targeting those times in the plant's life cycle when biomass is reduced, such as the formation of submersed tissues.

Currently, of the herbicides labeled for aquatic use, only 2,4-D, diquat, and carfentrazone-ethyl have been evaluated as subsurface applications against *M. aquaticum* (Glomski et al. 2006; Gray et al. 2007; Wersal et al. 2010). Therefore, a thorough evaluation of subsurface herbicide applications would offer insight into whether this application method is efficacious on *M. aquaticum* and which herbicides would result in control. The objectives of this study were to examine the efficacy of subsurface applications of seven herbicides labeled for aquatic use and to compare those applications to herbicides that can also be applied to emergent foliage.

Materials and Methods

Planting

A mesocosm study was conducted at the R.R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS, from August to October 2007 and was repeated in 2008. The study was conducted during late summer and early fall in order to promote submersed shoot growth and to follow the natural phenology of *M. aquaticum* in Mississippi. The study was conducted in 72, 378 L mesocosms. Planting consisted of placing two apical shoots of *M. aquaticum*, approximately 20 cm in length, from greenhouse stock into each of 432, 3.78 L pots containing a top soil, loam, and sand mixture (3:2:1). Sediment was amended at a rate of 2 g L/pot using Osmocote 19-6-12

fertilizer. Six pots of planted *M. aquaticum* were placed into each of the 72 mesocosms that were filled with 246 L water. Water was supplied to each mesocosm from an irrigation reservoir adjacent to the mesocosm facility. Air was supplied to all mesocosms using 2.5 cm stone diffusers and a PVC lift pipe. *Myriophyllum aquaticum* was allowed to grow until the shoots began to reach the water surface to achieve a mixture of submersed and emergent tissues for herbicide applications. Prior to herbicide application, one pot from each tank was harvested by cutting the plants at the sediment surface. Plants were dried for at least 48 h at 70 C and weighed for pre-treatment biomass.

Treatment Methods

Herbicide applications consisted of the maximum and half-maximum labeled rates of copper, diquat, endothall, triclopyr, 2,4-D, and carfentrazone-ethyl with a 48 h exposure time (Table 7.1). A concentrated aqueous solution of each herbicide was applied to each mesocosm such that, when diluted in 246 L, it provided the desired herbicide concentration. To achieve the 48 h exposure, designated mesocosms were drained and refilled with fresh water to remove remaining residues. Fluridone was applied under static exposure conditions. Since *M. aquaticum* was listed as being partially controlled on the fluridone label with no recommended herbicide rate of application, we choose to use concentrations that are considered lethal to Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Netherland et al. 1993; Crowell et al. 2006).

The maximum labeled rate for foliar applications of diquat, triclopyr, and 2,4-D were used to compare treatment methods (Table 7.1). Foliar herbicide applications were

made evenly over the water surface using a spray volume of 934 L/ha with a CO_2 pressurized single nozzle (8002 flat fan) spray system. A non-ionic surfactant was added to the spray solution of the foliar applications at a rate of 0.5% v:v. Water in foliar applied mesocosm tanks was drained and replaced with fresh untreated water after application to remove herbicide residues that may have entered the water column during application. Draining the water in these mesocosms ensured that any effects from foliar applications were due to the herbicide uptake from the emergent portion of the plant and not from submersed plant tissues in the water column. All herbicide treatments were repeated in four mesocosms.

Data Analysis

Myriophyllum aquaticum was rated weekly for percent control (0 = no control, 100 = complete control) for six weeks. Six weeks after treatment (WAT), noticeably live plant material was harvested at the sediment surface, dried for at least 48 h at 70 C, and weighed to determine plant mass. A general linear model was used to determine differences between control ratings within weeks, and a Fisher's Protected LSD was used to separate any differences. A similar analysis was conducted on biomass 6 WAT. All analyses were conducted at a $p < 0.05$ level of significance. There was no difference ($p = 0.10$) between years, therefore, data were pooled.

Results and Discussion

Visual Ratings

Copper, endothall, fluridone, carfentrazone-ethyl, and the subsurface 2,4-D applications were not efficacious on *M. aquaticum* at 6 WAT (Table 7.2). The foliar rate of diquat (4.5 kg ai/ha) resulted in 90% control 1 WAT, however, by 6 WAT, control was only 60%. Diquat at 0.37 mg ai/L provided 70% control 6 WAT. This level of control with diquat was surprising for a fast acting contact herbicide. The maximum carfentrazone-ethyl concentration did show some activity, although not to the extent of diquat, on *M. aquaticum* 1 WAT as visual ratings were different ($p < 0.01$) than untreated reference plants. Carfentrazone-ethyl may have been more efficacious if water pH was more acidic. The water used in this study was taken from an irrigation reservoir where the pH fluctuates between 7.8 and 9. A pH approaching 9 would result in a half life of approximately 3 to 4 hours, reducing the contact of the plants to a lethal dose of the herbicide (Ngim and Crosby 2001). However, the initial activity of this herbicide may offer increased control when combined with a systemic herbicide such as 2,4-D or triclopyr. During a similar mesocosm trial, 100% control of *M. aquaticum* was achieved 3 WAT when carfentrazone-ethyl was combined with several concentrations of 2,4-D as a subsurface application (Gray et al. 2007).

Combinations of a contact and a systemic herbicide may be of benefit to exploit the rapid effects of the contact herbicide and to maintain the long term control typically offered by the systemic herbicide. However, this will depend upon herbicide selection as significant antagonism has been found with combinations of diquat and penoxsulam (2-

(2,2-difluoroethoxy)-*N*-(5,8 dimethoxy [1,2,4] triazolo [1,5-*c*] pyrimidin-2-yl)-6 (trifluoromethyl) benzenesulfonamide) applied to the foliage of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) (Wersal and Madsen 2010). In the current study, 2,4-D as a foliar application and triclopyr as both the maximum subsurface and foliar application resulted in significant control of *M. aquaticum* as compared to reference plants when applied alone; however, there was no rate or application method that achieved $\geq$ 90% control. The foliar application of 2,4-D (2.1 kg ae/ha) resulted in 85% control of *M. aquaticum* 6 WAT which was the best control out of all herbicides and application methods.

Biomass

Copper did not reduce *M. aquaticum* biomass at any herbicide concentration. Endothall at 5.0 mg ae/L and fluridone at 0.02 mg ai/L did reduce *M. aquaticum* biomass however reductions were only 30 and 26% of untreated reference plants, respectively, at 6 WAT (Figure 7.1). The lack of efficacy with fluridone was somewhat surprising in that the label states that *M. aquaticum* is partially controlled by this chemical. There was some shoot reddening and bleaching of leaves observed by 4 WAT at the highest concentration, but these symptoms were transient. The concentrations of fluridone were within the range typically used in controlling *M. spicatum*, as specific recommendations *M. aquaticum* were not available (Pedlow et al. 2006; Crowell et al. 2006). However, the exposure time in this study was only 45 days, and this likely limited maximum efficacy. Netherland et al. (1993) reported that an exposure time of approximately 60 days is needed for fluridone concentrations of 12 µg ai/L to control Eurasian watermilfoil. The

symptoms observed on *M. aquaticum* in the current study indicate that fluridone has activity, but higher concentrations and/or longer exposure times are needed for *M. aquaticum* control.

The systemic herbicides 2,4-D as a foliar application and triclopyr as both the maximum subsurface and foliar application resulted in > 80% biomass reduction. It was interesting that the subsurface 2,4-D applications resulted in poor control with the lowest concentration not being different from untreated reference plants (Figure 7.1). In a previous study, a 1.0 mg ae/L 2,4-D concentration resulted in complete *M. aquaticum* control 3 WAT (Gray et al. 2007). The difference between that study and the current study is the exposure of *M. aquaticum* to the herbicide. The study conducted by Gray et al. (2007) utilized a static exposure where this study had a 48 h exposure time. Therefore, in order for a subsurface 2,4-D application to be effective, exposure times need to be longer than 48 h.

Similar results were observed in this study for triclopyr with the exception of the 1.25 mg ae/L concentration. In New Zealand, triclopyr offered significant *M. aquaticum* control in both mesocosm and field trials where they reported significant reductions in percent cover of *M. aquaticum* under controlled conditions and > 90% control for field applications out to 12 WAT (Hofstra et al. 2006). However, similar to results from this study, triclopyr did not result in complete control of *M. aquaticum* as regrowth was evident by 5 WAT. Plant recovery was from root crowns as new submersed shoots grew to the water surface and produced a new emergent apical tip. The regrowth from the sediment indicates that triclopyr may not have been fully translocated to the root crown or roots, and sufficient energy reserves remained to initiate new growth. The maximum

labeled rates of triclopyr as both a foliar and subsurface application were evaluated in this study. The higher rates may have limited herbicide translocation through rapid tissue destruction, and, therefore, a lethal dose of triclopyr was not present in tissues below the sediment surface, thereby allowing plant regrowth (Gardner and Grue 1996).

The use of diquat at all rates and application methods resulted in significant reductions in *M. aquaticum* biomass 6 WAT. This was particularly surprising as diquat typically offers rapid plant control with subsequent regrowth (Moreira et al. 1999). Plant recovery from diquat exposure was from the sediment similar to that described for triclopyr. More interesting was the fact that subsurface applications of diquat resulted in fragmentation of *M. aquaticum* plants. A necrotic region formed on the stolons of treated plants at the water/air interface, causing the emergent shoots to separate from the stolons. These free floating emergent shoots rapidly grew adventitious roots and survived throughout the remainder of the study and were included in biomass determinations. It is unclear if these fragments would have been viable, but given the fact that tissues were still intact and all fragments were growing adventitious roots, it is likely that under field conditions these fragments could have re-populated the treated area or spread to new habitats. The mechanism causing the fragmentation is unknown and further investigation is needed, but it has been reported under similar controlled circumstances (Wersal et al. 2010). It appears that diquat did not move once in the plants. In a laboratory study, ^{14}C diquat did not move from the roots of treated *M. aquaticum* plants and did not enter the xylem of treated plants to facilitate translocation (Sutton and Bingham 1970).

There was no difference in applying herbicides as a foliar spray or to the water column based on the results of this study, with the exception of 2,4-D. In this study, the

most effective herbicides for *M. aquaticum* control were diquat, 2,4-D, and triclopyr; however, the use of diquat as a subsurface treatment caused plant fragmentation that may result in new infestations in field situations. Copper chelate, carfentrazone-ethyl, endothall, and fluridone did not control *M. aquaticum* in this study. Although significant *M. aquaticum* control was achieved, there was no herbicide or application method that resulted in complete control of *M. aquaticum*.

In general, foliar applications are easier to make and typically less expensive than subsurface herbicide applications therefore, the use of diquat, 2,4-D, or triclopyr as a foliar spray are recommended based on the results of this study. However, when considering the industry standards and labeled rates for these herbicides, 2,4-D would be the most economical choice when there are no restrictions of its use. Diquat and triclopyr are generally three times the cost per liter of herbicide as 2,4-D and maximum labeled rates per hectare for these herbicides are four times greater than that of 2,4-D, resulting in a 12 fold increase in application costs to control *M. aquaticum* using foliar applications. Future work should evaluate herbicides, herbicide combinations, and application timings that could maximize treatment efficacy as well reduce the cost of herbicide application.

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Table 7.1 Herbicide selection, rates, and application methods for control of *Myriophyllum aquaticum*.

Herbicide	Rate	Subsurface	Foliar
Copper Chelate	1.0 mg ai/L	X	
	0.5 mg ai/L	X	
Diquat	0.37 mg ai/L	X	
	0.19 mg ai/L	X	
	4.5 kg ai/ha		X
Endothall	5.0 mg ae/L	X	
	2.5 mg ae/L	X	
Fluridone	0.02 mg ai/L	X	
	0.01 mg ai/L	X	
Triclopyr	2.5 mg ae/L	X	
	1.25 mg ae/L	X	
	6.7 kg ae/ha		X
2,4-D	4.0 mg ae/L	X	
2,4-D	2.0 mg ae/L	X	
2,4-D	2.1 kg ae/ha		X
Carfentrazone-ethyl	0.20 mg ai/L	X	
Carfentrazone-ethyl	0.10 mg ai/L	X	

Table 7.2 Visual percent control ratings of *Myriophyllum aquaticum* following subsurface and foliar aquatic herbicide applications.

Herbicide Treatment[c]	Method[d]	Weeks After Treatment[ab]					
		1	2	3	4	5	6
Carfentrazone-ethyl 0.10	S	10f	10f	5f	5f	5fg	0f
Carfentrazone-ethyl 0.20	S	40d	35e	20e	20e	20ef	10e
Copper Chelate 0.50	S	0g	0g	0f	0f	0g	0f
Copper Chelate 1.0	S	0g	0g	0f	0f	0g	0f
Diquat 0.19	S	60c	60c	55c	55c	50bc	50d
Diquat 0.37	S	80b	80b	80b	75b	70a	70b
Diquat 4.5	F	90a	85ab	80b	70b	70ab	60c
Endothall 2.5	S	0g	0g	0f	0f	0g	0f
Endothall 5.0	S	0g	0g	0f	0f	0g	0f
Fluridone 0.01	S	0g	0g	0f	0f	0g	0f
Fluridone 0.02	S	0g	0g	0f	0f	0g	0f
Triclopyr 1.25	S	45d	45d	35d	30d	25de	15e
Triclopyr 2.5	S	85a	85ab	80ab	75b	70a	70b
Triclopyr 6.7	F	90a	90a	80ab	80b	70a	70b
2,4-D 2.0	S	5g	10f	10f	5f	5fg	0f
2,4-D 4.0	S	30e	30e	20e	20de	20ef	15e
2,4-D 2.1	F	90a	90a	90a	90a	85a	85a
Untreated Reference		0g	0g	0f	0f	0g	0f
LSD		6	7	10	10	20	9

[a]Means in a column followed by the same letter are not statistically different according to a Fisher's Protected LSD test at a $p<0.05$ level of significance.
[b]Analyses were conducted within weeks
[c]Subsurface applications are given as mg ai or ae/L; Foliar applications are given as kg ai or ae/L depending upon the herbicide used
[d]S=subsurface; F=foliar

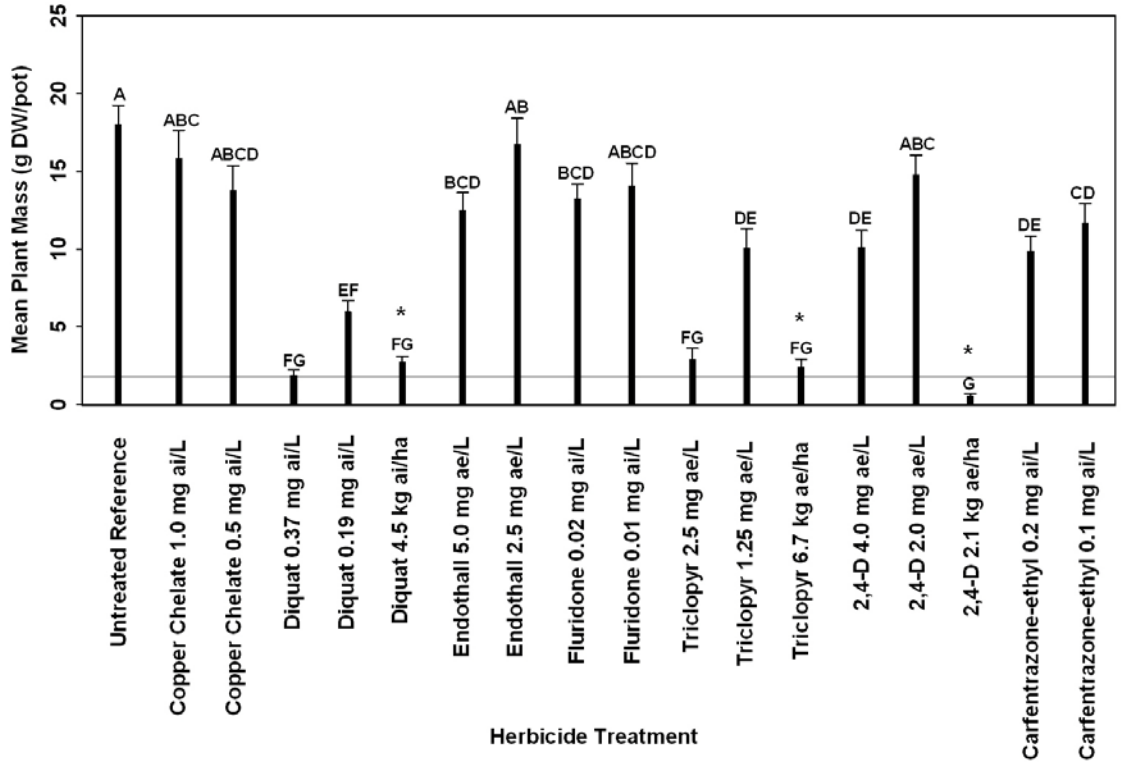

Figure 7.1 Mean (± 1 SE) dry weight biomass of *Myriophyllum aquaticum* harvested 6 weeks after treatment with selected aquatic labeled herbicides. Pre-treatment biomass was 1.51 g DW/pot and by 6 WAT reference plant biomass increased 92% to 18.01 g DW/pot indicating plants were actively growing throughout the study. Bars sharing the same letter do not differ according to a Fisher's Protected LSD test at a $p < 0.05$ level of significance. The solid horizontal line represents a 90% reduction in biomass from untreated reference plants. Asterisks denote foliar applied herbicides.

CHAPTER VIII

CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

In this chapter, I will summarize the major findings of each of the previous chapters and give specific management recommendations for *Myriophyllum aquaticum* based upon current data and those published in previous studies, and the feasibility of control using these options.

Chapter Summaries

Chapter I: A Conceptual Approach to Biomass Management

Developing a conceptual model allowed for a simplified visual representation of data needed to gain a greater understanding of the growth requirements of *M. aquaticum*. Once these data needs were determined from previously published literature and field studies conducted in Mississippi, appropriate controlled mesocosm experiments were developed to determine plant response to changes in important environmental factors such as light availability, nutrient loading, and water regime.

Chapter II: Life History and Starch Allocation Patterns

Seasonal biomass and starch allocation patterns were determined from four natural populations of *M. aquaticum* in Mississippi. Biomass was greater in 2006 than in 2007 where peak biomass was 510.7 g m^{-2} and 39.6 g m^{-2} respectively for those years.

The reduction in biomass in 2007 was largely the result of a drought in 2006. Two of the four sampling locations were completely dry from June to November 2006.

Overall, stolons accounted for 40-95% (mean 65.9 ± 2.7%) of total biomass followed by emergent shoot, submersed shoot, and sediment root biomass. Starch allocation was greatest in stolons (78.1 g m^{-2}); where up to 16.3% of total starch was stored, indicating that stolons are likely the primary storage location for carbohydrates. Submersed shoots stored 0.6-11.0% of total starch followed by emergent shoots (0.4-7%). Sediment roots of *M. aquaticum* stored less than 3.8% of total starch, and therefore are not considered to be the primary site for energy acquisition and storage. Low points in both biomass and starch allocation occurred from October to March, where total biomass was less than 30.2 g m^{-2} and 7.4 g m^{-2} in 2006 and 2007 respectively. Starch concentrations at their lowest point were less than 2.0 g m^{-2} and 0.4 g m^{-2} for 2006 and 2007 respectively.

Emergent shoot biomass ($p=0.02$), submersed shoot biomass ($p=0.03$), and sediment root biomass ($p<0.01$) were related to light transmittance. Submersed shoot biomass was also related to ($p=0.01$) to water temperature. Biomass and starch allocation to submersed shoots peaked in February, followed by a rapid decline in March when water temperatures and light intensities began to increase. The peak in submersed shoot biomass indicates that this growth form is adapted to shade environments and is capable of reduced photosynthetic rates to survive in these environments (Salvucci and Bowes, 1982). Therefore, submersed shoot growth is transient and only utilized for short overwintering periods, times of reduced light and temperature, or to survive disturbances in the growing environment.

Chapter III: Effects of Varying Light Intensity

Plants such as *M. aquaticum* can change leaf morphology (heterophylly) in response to changes in the environment. In this study, as light intensity was reduced from full sunlight, total plant length, emergent shoot length, and submersed shoot length increased; with greater total plant biomass in the 30% light treatment, and increased submersed shoot length in the 70% light treatment. These results are typical of plants in low light environments where shoot elongation occurs to reach adequate levels of sunlight. However, total biomass was reduced when plants were grown in 70% shade likely due to reduced photosynthetic rates of emergent shoots under these conditions (Salvucci and Bowes 1982).

Chapter IV: Water Column Nutrient Loading

Total biomass at the 1.80:0.01 N:P combination was 53% greater than biomass at other combinations. The biomass response of *M. aquaticum* was a quadratic function of tissue nutrient content. Biomass yield was positively ($r^2 = 0.82$) related to increasing nitrogen content, whereas a negative ($r^2 = 0.89$) relationship was determined for increasing phosphorus content, likely due to competition with algae for phosphorus and available light. Tissue nutrient content indicated that critical concentrations (1.8% nitrogen and 0.2% phosphorus) for growth were not attained in most treatments. These data provide further evidence that *M. aquaticum* requires high levels of nutrients to achieve nuisance growth. Survival through uptake of water column nutrients may be a mechanism for survival, a means of long distance dispersal of fragments, or may offer a competitive advantage over species that rely on sediment nutrients.

Chapter V: Effects of Varying Water Depths

Despite having a submersed leaf form, *M. aquaticum* is not typically problematic as water level increases; however, the colonization potential of this species based upon water level is not well defined. Biomass at 0 cm was 96% greater than plants grown at 137 cm. Biomass of emergent shoots, stolons, and sediment roots were also greater when *M. aquaticum* was grown at the 0 cm water level. Submersed shoot biomass was on average 99% greater at 37, 57, and 77 cm. However, submersed shoots comprised only a small fraction, 0.1-12% of total biomass depending on the water level. Total *M. aquaticum* length was 25% greater when plants were grown at water levels from 0-77 cm over plants grown at 97, 117, 137 cm. Shallow water is often easier to invade and subject to greater disturbance which benefits *M. aquaticum* as this species is dependent upon fragmentation for reproduction and spread. Survival depends upon the plants ability to emerge from the water column and prolong growth as the submersed leaf form will result in significant declines in the plant population.

Chapter VI: Drawdown as a Management Option

Myriophyllum aquaticum does not produce specialized structures for perrenation or carbohydrate storage such as seeds, tubers, turions, or winter buds. The lack of specialized reproductive structures may allow drawdown events to be efficacious if the sediment can be dried over sufficient duration to cause desiccation of stolons and root crowns. Both the winter and summer drawdowns were effective at reducing plant biomass. The winter drawdown reduced ($p=0.003$) biomass by 99% at 4 weeks when compared to pre drawdown biomass levels. The summer drawdown reduced biomass

($p<0.01$) more rapidly, as a 98% reduction was observed at 2 weeks when compared to pre drawdown levels. Regrowth of *M. aquaticum* was evident in all drawdown treatments upon reflooding, indicating that this species can survive drawdowns of 12 weeks. Longer drawdown durations may be required for complete control to sufficiently dry sediments and fully desiccate target plants.

Chapter VII: Subsurface Herbicide Evaluations

Subsurface herbicide applications were made to target submersed portions of *M. aquaticum*; and from Chapter 2, these submersed tissues also contain the majority of stored starch within the plant. Six weeks after treatment (WAT), copper, endothall, fluridone, and carfentrazone-ethyl were not efficacious for controlling *M. aquaticum*. Diquat at all rates and application methods had good efficacy as early as 1 WAT and also resulted in 70-90% biomass reduction at 6 WAT. Triclopyr, at the highest aqueous concentration and as the foliar application resulted in an 84 and 86%, respectively, reduction in biomass at 6 WAT. The foliar application of 2,4-D was the only herbicide and application method that resulted in greater than 90% biomass reduction. Regrowth did occur in all mesocosms regardless of herbicide or treatment method, indicating multiple applications would be necessary to provide longer term plant control.

Management Recommendations for Targeting Seasonal Phenology

Chemical Control

Myriophyllum aquaticum management is typically conducted during summer months when biomass is at its peak and emergent shoots cover the water surface.

Previous management attempts have focused on the use of foliar-applied herbicides resulting in poor efficacy. In Portugal, foliar treatments of glyphosate and diquat were not effective for controlling *M. aquaticum* and often permitted rapid re-infestation (Moreira et al. 1999). In New Zealand, applications of clopyralid, fluridone, triclopyr, glyphosate, endothall, and dichlobenil were evaluated resulting in no control with fluridone and clopyralid and significant regrowth following glyphosate applications (Hofstra et al. 2006). Applications of triclopyr were effective at reducing *M. aquaticum* cover in field situations (Hofstra et al. 2006). Targeting the emergent shoots will often result in poor control and significant regrowth, because *M. aquaticum* does not allocate and store large concentrations of resources in emergent shoots. Once these shoots have been killed or removed, new shoots will regrow from nodes on the stolons within a day or two.

If management is to be successful, efforts need to target stolons, as this is the primary location for growth and energy storage. Management should be implemented during times of low biomass and total starch concentrations (Figure 8.1). Triclopyr, 2,4-D and imazapyr (Wersal and Madsen 2007) are the most effective herbicides for controlling *M. aquaticum* (Table 8.1). Since *M. aquaticum* does not have reproductive or storage structures, an initial herbicide application would remove the majority of plant biomass and thus energy stores for regrowth. After removing the initial biomass, a follow-up application should be made in the same year to control new growth.

Biological, Mechanical, Physical, and Cultural Control

Biological, mechanical, physical, and cultural options are summarized in Table 8.2. Biological agents that have been evaluated on *M. aquaticum* include grass carp, several species of beetles, tortricids, and Lepidoptera (Habeck 1974; Habeck and Wilkerson 1980; Cordo and Deloach 1982a,b), and the fungi *Pithium carolinianum* (Bernhardt and Duniway 1984). Grass carp are not recommended, as fish generally avoid eating this plant (Pine and Anderson 1991; Catarino et al. 1997), grass carp are non-selective feeders and would consume non-target vegetation, and grass carp are mobile non-native additions to a waterbody; they will disperse to other waterbodies if given the opportunity. The leaf-feeding beetle (*Lysathia* spp.) showed some efficacy in South Africa by significantly reducing emergent shoot biomass (Cilliers 1999); however, this agent is not approved for use in the United States. Any successful biological control agent would have to effectively target both emergent and submersed tissues, or regrowth will occur.

Sytsma and Anderson (1993) recommended that a harvesting strategy which removes only emergent shoots could remove a significant portion of the total phosphorus pool in a waterbody, as greater than 80% of total phosphorus is stored in emergent tissues, and severely impact *M. aquaticum* growth. Harvesting would need to be frequent and sustained over time otherwise overwinter accumulation of carbohydrates will occur, resulting in significant regrowth the following spring (Perkins and Sytsma 1987). Harvesting aquatic plants on large scales is labor-intensive and very expensive, often times the cost is greater than \$2470 ha^{-1} (\$1000 $acre^{-1}$) (Langeland 1996). Harvesting may be feasible on small, new infestations when biomass accumulation is low. Larger,

denser infestations will require another management strategy to reduce biomass prior to harvesting.

The removal of phosphorus as discussed previously, by means of harvesting plant tissue, may not result in growth reductions depending upon surrounding land use patterns. Waterbodies near urban areas and agriculture are often prone to nutrient runoff and eutrophication. The amount of nutrients of anthropogenic origin are increasingly finding their way into waterbodies worldwide, which has resulted in declines of macrophyte diversity and changes in community structure (Vitousek et al., 1997; Montante et al., 2003). *Myriophyllum aquaticum* would be able to directly utilize the influx of nutrients to sustain growth or become a greater nuisance if nutrient concentrations in water are sufficient to cause plant tissues to exceed critical concentrations of 1.8% nitrogen and 0.2% phosphorus.

The use of drawdown can be very effective, with seasonality of *M. aquaticum* not an issue, as control was achieved using a 3 month winter or summer drawdown. Drawdown targets the whole plant causing the complete removal of aboveground biomass; and therefore stolons, and the majority of carbohydrate stores in the plant. A drawdown lasting more than 3 months, or consecutive drawdown events, may result in complete control, if sediment remains dry. *Myriophyllum aquaticum* tolerates drawdown events lasting 9 months if the sediment remains moist (Maltchik et al. 2007). Drawdown is typically inexpensive, does not have the negative outlook that is often times associated with the use of herbicides, and is effective on large scales. Though, a drawdown is non-selective and therefore there will be a loss of all submersed aquatic macrophytes that do not have specialized structures, such as tubers, or turions, in the sediment. Additionally,

drawdown will result in the removal of aquatic invertebrates and fish, and result in the loss of use of the waterbody for the duration of the drawdown. To mitigate potential losses of submersed plants, fish, and invertebrates, a partial drawdown may be used to expose *M. aquaticum* growing along the shoreline of a waterbody; as this is typically favorable habitat for *M. aquaticum*.

In general, management should be implemented to either exploit the times of low energy reserves (fall and winter) in *M. aquaticum*, or remove emergent shoots to gain access to the stolons and other submersed tissues. Management activities that target only the emergent shoots will not be effective at controlling this species; as the majority of energy reserves are stored in stolons and submersed tissues. Regardless of the target species, there are tradeoffs when deciding upon the proper management techniques to control non-native aquatic plants. These tradeoffs can include economic, social, and environmental issues that need to be addressed when developing a management plan. Therefore, management techniques should be site-specific, based on environmental factors, and chosen to maximize control of the target species. Management decisions should be based upon the desired use and desired outcomes of the habitat being managed.

Data Applicability and Future Research

The conceptual model, or individual parts of the model, created during this dissertation research can be transferred into ArcGIS® Model Builder to generate spatially referenced habitat suitability models for *M. aquaticum*. Spatial models will identify the most probable locations of *M. aquaticum* invasion and infestation across a landscape, and estimate the severity of an infestation based upon biomass yield response to

environmental factors. Parameters can be added to models as data become available and parameters can be weighted in importance to refine habitat suitability predictions. Additionally, this approach may be viable for other invasive species and could ultimately be incorporated into Early Detection Rapid Response programs to save on survey and monitoring costs. By determining suitable areas for plant growth *a priori*, directed surveys can be conducted in likely areas of infestation instead of conducting large scale surveys across the landscape; which is labor intensive, relatively slow, and expensive.

Future research needs to identify other environmental factors that may influence *M. aquaticum* growth such as sediment loading and temperature effects on plant growth. Timing of management techniques with low points in starch storage needs to be evaluated as well the use of integrated management techniques. For example the use of a short-term drawdown followed by a foliar herbicide application to control the small emergent shoots observed during the drawdown study. Additionally, research is needed to determine the role that adventitious roots have in nutrient uptake, water uptake, potential energy storage, and the implications of targeting adventitious roots for management purposes.

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Table 8.1 Aquatic labeled herbicides for use in controlling *Myriophyllum aquaticum*.

Herbicide	Type of Chemical	Method	Effectiveness[1]
2,4-D	Selective Systemic	Foliar	Excellent
Copper	Broad Spectrum Contact	Subsurface	Poor
Diquat	Broad Spectrum Contact	Foliar	Good
Endothall	Broad Spectrum Contact	Subsurface	Poor
Glyphosate	Broad Spectrum Contact	Foliar	Fair
Fluridone	Broad Spectrum Systemic	Subsurface	Fair
Imazapyr	Broad Spectrum Systemic	Foliar	Excellent
Imazamox	Broad Spectrum Systemic	Foliar	Fair
Triclopyr	Selective Systemic	Foliar or Subsurface	Good
Carfentrazone-ethyl	Broad Spectrum Contact	Foliar or Subsurface	Poor
Penoxsulam	Broad Spectrum Systemic	Foliar or Subsurface	Unknown

[1]Excellent = ≥ 90% control of treated plants
Good = 80% control of sprayed plants
Fair = < 80% control of sprayed plants; re-growth can be expected

Table 8.2 Management options for control of *Myriophyllum aquaticum*.

Category	Technique	Note	Rating
Biological	Grass Carp	Not a preferred food, use of grass carp has many drawbacks	Poor
	Leaf Feeding Beetle	Not approved for release in U.S.. Targets emergent shoots	Not Operational
Mechanical[1]	Harvesting	Small areas	Fair
	Raking or Chaining	Biomass production is too great for this method. Disturbance will create fragments and cause subsequent spread.	Poor
	Hand Pulling	Small areas	Fair
Physical[2]	Drawdown	Large-scale	Excellent
	Dredging	Large-scale, expensive	Excellent
Cultural	Nutrient Removal	Large-scale, dependent upon surrounding land use patterns	Unknown

[1]Care must be taken to remove all plant fragments.
[2]Plants can grow in moist soil. Drawdown should facilitate complete drying of sediment and needs to be maintained for longer than 3 months.

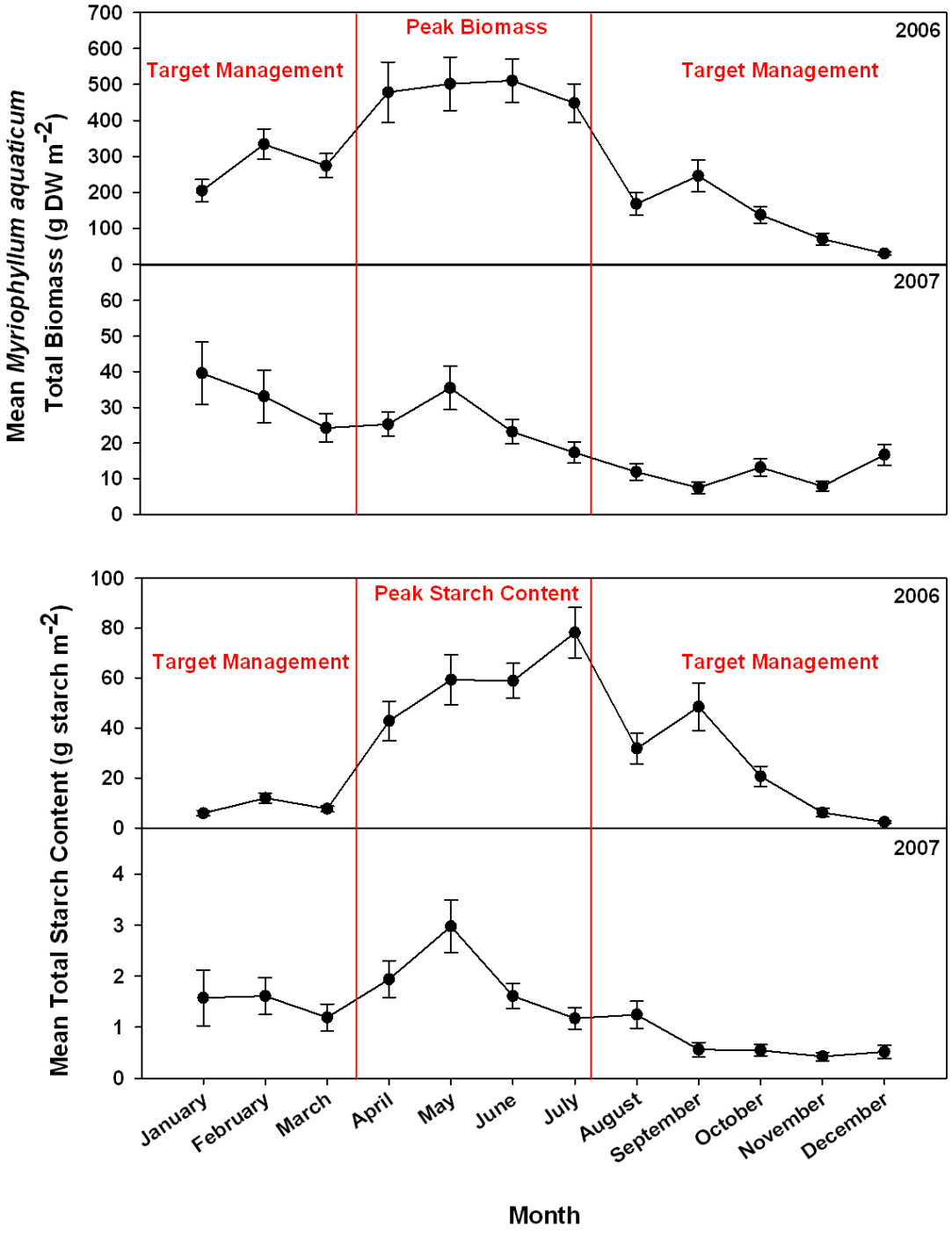

Figure 8.1 Times of peak and seasonal low points in *Myriophyllum aquaticum* total biomass and starch content, and proposed times to implement management strategies.

APPENDIX A

MAP OF *Myriophyllum aquaticum* BIOMASS SAMPLING LOCATIONS WITHIN MISSISSIPPI

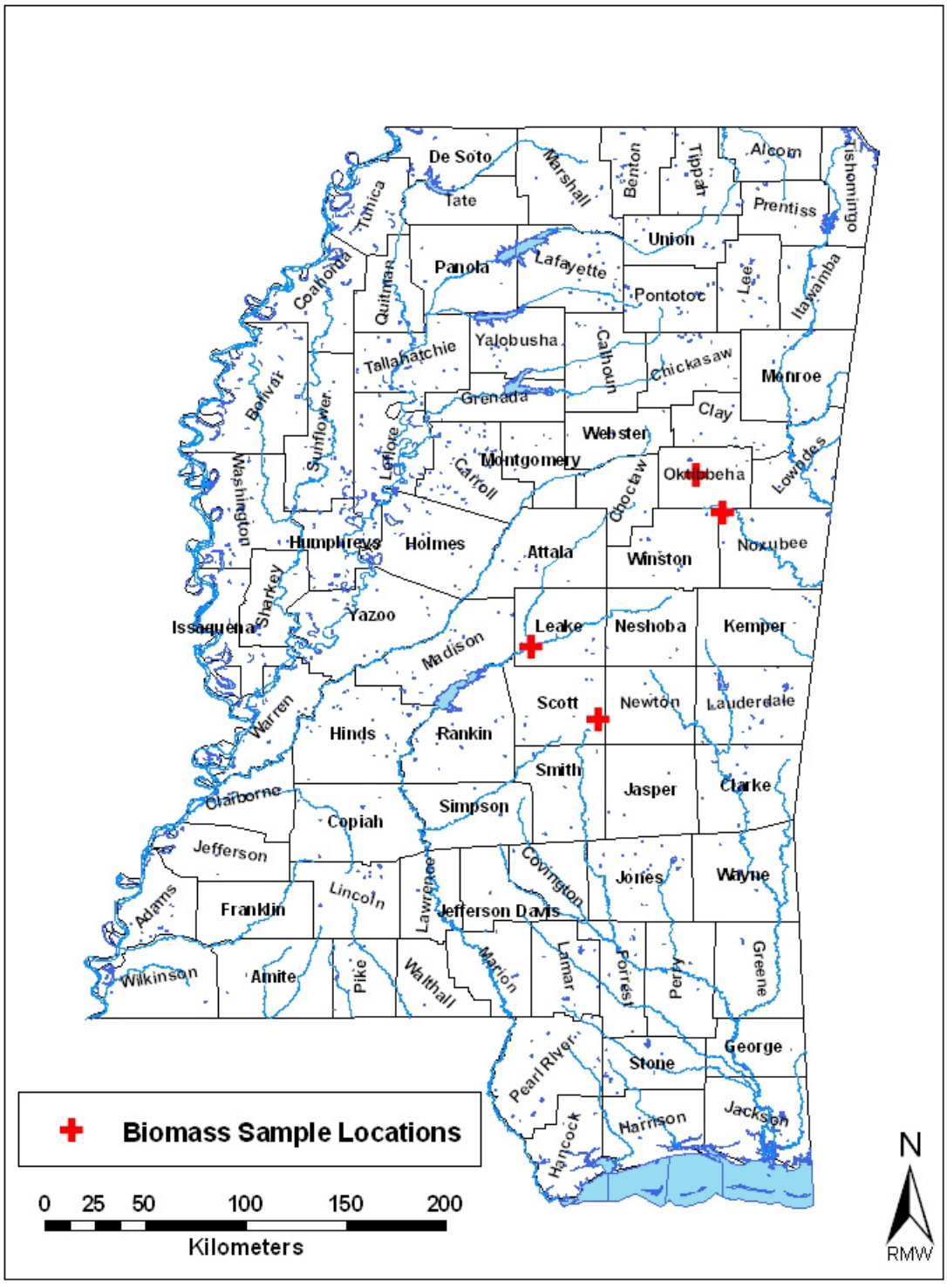

Figure A.1 Locations of *Myriophyllum aquaticum* sampling sites in Mississippi used for determining life history characteristics and starch allocation patterns in 2006 and 2007.

APPENDIX B

STARCH ASSAY METHOD

STA-20 Starch Assay Kit (Amylase/Amyloglucosidase Method)

Method outlined from: Sigma-Aldrich. 2010. STA20 Technical Bulletin. http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/Bulletin/sta20bul.Par.0001.File.tmp/sta20bul.pdf. Accessed March 23, 2010.

STA-20 Kit Description

The hydrolysis of starch to glucose is catalyzed by α-amylase and amyloglucosidase. Glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen peroxide reacts with *o*-dianisidine in the presence of peroxidase to form a colored product. Oxidized *o*-dianisidine reacts with sulfuric acid to form a more stable colored product. The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration.

Reagents

1. Heat stable α-Amylase was supplied as a solution in 25% propylene glycol and is ready to use.

2. Starch assay reagent was reconstituted with 20.0 ml of water. After addition of water. Each vial, when reconstituted with 20.0 ml of water, contains 50.0 units ml^{-1} of amyloglucosidase from *Aspergillus niger* and buffer salts.

3. Glucose oxidase/peroxidase reagent capsules contained 500 units of glucose oxidase from *Aspergillus niger*, 100 purpurgalin units of horseradish peroxidase, and buffer salts; and was reconstituted with 39.2 ml of water.

4. *o*-Dianisidine reagent contained 5.0 mg of *o*-dianisidine dihydrochloride. The reagent was reconstituted with 1.0 ml of water.

5. Glucose assay reagent was prepared by adding 0.8 ml of the reconstituted o-dianisidine reagent to the amber bottle containing 39.2 ml of the reconstituted glucose oxidase/peroxidase reagent.

6. Glucose standard solution was supplied as 1.0 mg ml^{-1} glucose in 0.1% benzoic acid.

7. Wheat starch was supplied ready to use at a purity of 84% to ensure assay reliability.

8. Corn starch was supplied ready to use at a purity of 93% to ensure assay reliability.

Reagents Necessary but not Provided with the STA-20 Kit

1. 12 N sulfuric acid solution prepared by a 3-fold dilution in water of concentrated ACS grade sulfuric acid (36 N).
2. 80% ethanol solution.
3. Dimethyl sulfoxide (DMSO) ACS grade.

Sample Preparation Instructions

Grind plant samples to < 0.5 mm (No. 40 mesh). Weigh 50.0 to 100.0 mg samples to 0.1 mg accuracy. Transfer the samples to appropriately marked test tubes. For wheat and corn starch controls, and samples with high starch content, reduce sample size to 1.0 to 10.0 mg. Samples that contain glucose or maltodextrins must be extracted with ethanol to remove these substances.

1. Add 5.0 ml of the 80% Ethanol Solution to each sample.
2. Incubate at 80 to 85 °C for 5 minutes.
3. Mix the contents of the tube and add another 5.0 ml of the 80% Ethanol Solution.
4. Centrifuge tube for 10 minutes at 1,000 g. Discard the supernatant.

5. Resuspend the pellet in 10.0 ml of the 80% Ethanol Solution and mix. Centrifuge for 10 minutes at 1,000 g. Pour off the supernatant and discard.

Using DMSO to Remove Polysaccharides such as Amyl pectin

1. Add 2.0 ml of DMSO to each sample.

2. Mix and incubate for 5 minutes in a boiling water bath.

3. Continue with starch digestion.

Starch Digestion

1. Add 0.2 ml of the 80% ethanol solution to each sample and to an empty test tube labeled "Starch Digestion Blank" and mix.

2. Pipette 3.0 ml of water and 0.02 ml of the α-Amylase (Reagent 1) into each sample and starch digestion blank.

3. Mix and incubate for 5 minutes in a boiling water bath.

4. Remove the tubes from the water bath and cool to room temperature.

5. Bring the volume in each tube up to 10.0 ml with water and mix.

6. To 1.0 ml of each test and blank solution from step 5, add 1.0 ml of the starch assay reagent (Reagent 2).

7. Mix and incubate for 15 minutes in a 60 °C shaking water bath.

8. Remove the tubes from the water bath and cool to room temperature.

9. Dilute 1.0 ml of each sample and blank to 10.0 ml with water.

10. Continue with glucose determination.

Glucose Determination

1. Pipette the following solutions into the appropriately marked test tubes:

Reagent (ml)	Standard Blank	Standard	Reagent Blank	Sample
Water	1.0	0.950	--	--
[a]Glucose Standard (Reagent 6)	--	0.05	--	--
Blank from Starch Digestion	--	--	1.0	--
Sample from Starch Digestion	--	--	--	1.0

[a]This can be included as a sample in a standard curve.

2. At time zero, start the reaction by adding 2.0 ml of the glucose assay reagent (Reagent 5) to the first tube and mix. Allow 30 to 60 second intervals between the addition of glucose assay reagent to each subsequent tube.

3. Incubate each tube exactly 30 minutes at 37 °C in a water bath. Stop each reaction at 30 to 60 second intervals by adding 2.0 ml of the 12 N sulfuric acid solution into each tube and mix thoroughly.

4. Measure the absorbance of each tube at 540 nm.

Calculations

$\Delta A_{STANDARD} = A_{STANDARD} - A_{STANDARD\ BLANK}$

$\Delta A_{TEST} = A_{TEST} - A_{REAGENT\ BLANK}$

$$\frac{(\Delta A_{TEST})\ (900)}{(\Delta A_{STANDARD})\ (\text{Sample Weight in mg})} = \%\ \text{Starch}$$

APPENDIX C

STANDARD CURVE FOR STARCH ASSAY METHOD

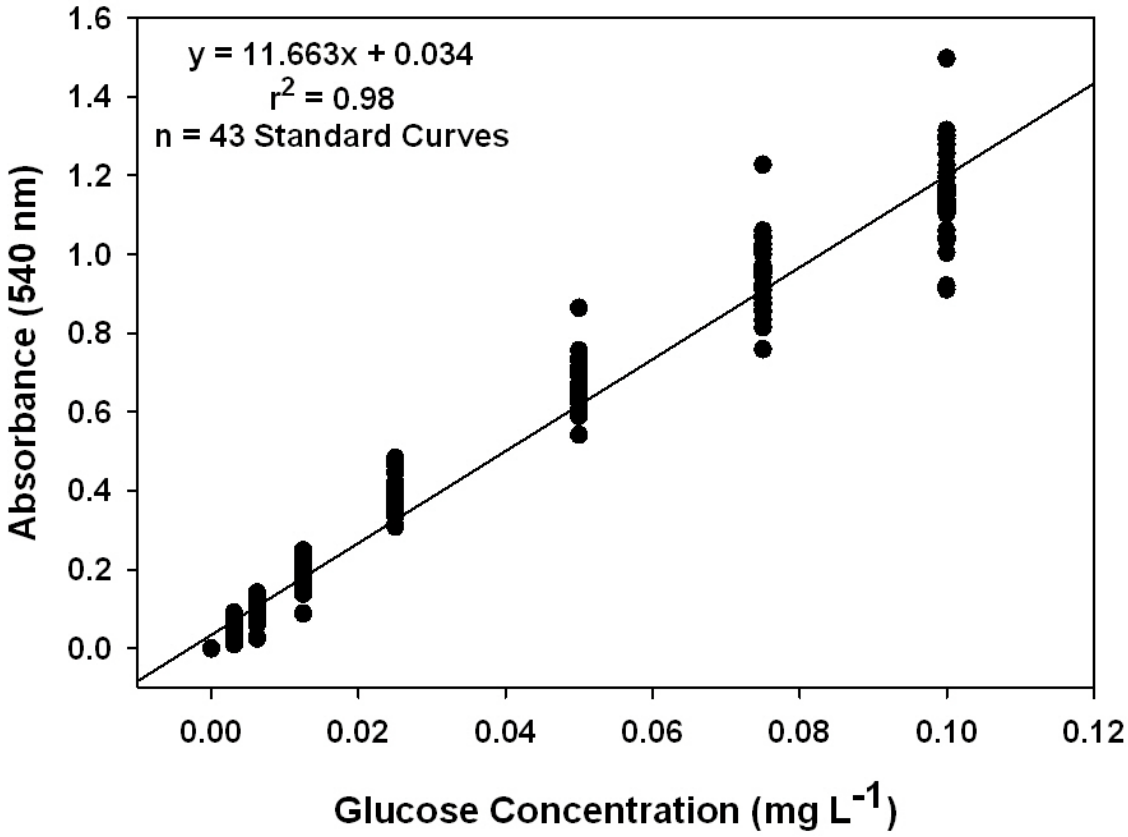

Figure C.1 Standard curve for starch assays using the STA-20 kit.

APPENDIX D

STARCH STANDARD ASSAY FOR THE STA-20 KIT

Table D.1 Percent starch recovery of corn standards provided in the STA-20 kit.

Sample	Type	Mass	% Starch	% Purity	% Recovery
1	corn	2.00	85.00	93	91.40
2	corn	2.10	50.34	93	54.13
3	corn	2.20	52.91	93	56.90
4	corn	4.00	73.85	93	79.40
5	corn	4.00	58.93	93	63.37
6	corn	4.00	68.86	93	74.05
7	corn	6.10	57.92	93	62.28
8	corn	6.10	50.15	93	53.93
9	corn	6.40	75.29	93	80.96
10	corn	8.10	62.78	93	67.51
11	corn	8.20	75.94	93	81.66
12	corn	7.90	70.47	93	75.77
Mean (± 1 SE)			65.20 ± 3.28		70.10 ± 3.52

Table D.2 Percent starch recovery of wheat standards provided in the STA-20 kit.

Sample	Type	Mass	% Starch	% Purity	% Recovery
1	wheat	2.30	72.76	84	86.62
2	wheat	2.30	86.77	84	103.29
3	wheat	2.30	108.69	84	129.39
4	wheat	4.00	72.03	84	85.75
5	wheat	3.90	72.08	84	85.81
6	wheat	4.00	73.81	84	87.87
7	wheat	6.30	77.39	84	92.13
8	wheat	6.20	60.07	84	71.52
9	wheat	6.00	65.05	84	77.44
10	wheat	8.00	61.34	84	73.02
11	wheat	8.30	69.86	84	83.16
12	wheat	8.30	72.58	84	86.40
Mean (± 1 SE)			74.36 ± 3.73		88.50 ± 4.40

APPENDIX E

WHEAT STANDARD ASSAY FOR STARCH RECOVERY DETERMINATION

Table E.1 Percent starch recovery of the wheat standard provided in the STA-20 kit. Wheat standards were included in the *Myriophyllum aquaticum* starch assays.

Sample	Month	Year	Standard	Mass	% Starch	% Purity	% Recovery
29	January	2006	Wheat	2.7	92.35	84	109.93
58	January	2006	Wheat	2.2	71.70	84	85.36
85	February	2006	Wheat	2.8	93.37	84	111.15
102	February	2006	Wheat	1.8	102.46	84	121.98
131	March	2006	Wheat	3.6	72.99	84	86.89
160	March	2006	Wheat	2.3	90.21	84	107.40
187	April	2006	Wheat	3.4	74.89	84	89.15
215	April	2006	Wheat	5.8	94.03	84	111.94
241	May	2006	Wheat	4.7	74.57	84	88.78
269	May	2006	Wheat	2.8	87.37	84	104.02
292	June	2006	Wheat	2.9	64.23	84	76.46
320	June	2006	Wheat	4.1	76.50	84	91.07
346	July	2006	Wheat	2.2	94.87	84	112.94
374	July	2006	Wheat	2.5	94.73	84	112.77
398	August	2006	Wheat	2.3	80.37	84	95.68
423	August	2006	Wheat	4.1	92.33	84	109.91
449	September	2006	Wheat	2.1	81.65	84	97.20
477	September	2006	Wheat	2.2	73.73	84	87.78
506	October	2006	Wheat	2.8	65.51	84	77.99
535	October	2006	Wheat	2.3	83.51	84	99.42
563	November	2006	Wheat	3.9	81.14	84	96.59
591	November	2006	Wheat	2.9	77.14	84	91.83
619	December	2006	Wheat	4.0	88.47	84	105.32
648	December	2006	Wheat	2.1	103.06	84	122.69
677	January	2007	Wheat	3.0	92.83	84	110.51
702	January	2007	Wheat	3.1	71.23	84	84.80
728	February	2007	Wheat	4.4	71.93	84	85.63
755	February	2007	Wheat	3.9	72.95	84	86.85
776	March	2007	Wheat	3.7	93.72	84	111.57
804	March	2007	Wheat	5.4	99.04	84	117.91
832	April	2007	Wheat	2.2	83.22	84	99.07
858	April	2007	Wheat	3.1	75.25	84	89.58
883	May	2007	Wheat	3.6	91.98	84	109.50
910	May	2007	Wheat	3.6	62.27	84	74.13
936	June	2007	Wheat	4.0	91.39	84	108.80

Table E.1 (continued)

Sample	Month	Year	Standard	Mass	% Starch	% Purity	% Recovery
960	June	2007	Wheat	4.2	83.91	84	99.89
985	July	2007	Wheat	4.4	87.80	84	104.53
1004	July	2007	Wheat	4.0	82.46	84	98.17
1023	August	2007	Wheat	3.7	89.25	84	106.25
1037	August	2007	Wheat	2.2	91.79	84	109.28
1054	September	2007	Wheat	3.6	59.83	84	71.23
1069	September	2007	Wheat	3.3	64.79	84	77.13
1083	October	2007	Wheat	2.5	75.27	84	89.60
1097	October	2007	Wheat	4.1	76.14	84	90.65
1120	November	2007	Wheat	5.2	80.35	84	95.65
1135	November	2007	Wheat	5.4	95.53	84	113.73
1162	December	2007	Wheat	4.0	68.35	84	81.36
1177	December	2007	Wheat	4.0	93.26	84	111.02
Mean (± 1 SE)					82.61 ± 1.60		98.30 ± 1.91
CV					13.47		13.47

APPENDIX F

PERCENT DIFFERENCE OF DUPLICATE *Myriophyllum aquaticum* STARCH SAMPLES

Table F.1 Percent difference between duplicate *Myriophyllum aquaticum* starch samples.

Month	Year	Site	Tissue	Mass	Starch	% Difference
January	2006	Pearl	Stol Dup	54.80	4.92	
January	2006	Pearl	Stol	54.40	5.56	11.47
January	2006	Doyle	Sub Dup	54.40	0.35	
January	2006	Doyle	Sub	54.70	0.29	21.20
January	2006	Maples	Stol Dup	54.00	1.53	
January	2006	Maples	Stol	54.60	1.23	24.02
January	2006	Lake	Sub Dup	54.00	0.45	
January	2006	Lake	Sub	54.40	0.64	29.88
February	2006	Pearl	Sub Dup	55.90	5.76	
February	2006	Pearl	Sub	56.40	7.25	20.58
February	2006	Maples	Stol Dup	58.80	0.82	
February	2006	Maples	Stol	58.20	0.67	21.54
February	2006	Lake	Emer Dup	55.80	1.43	
February	2006	Lake	Emer	55.00	1.44	0.24
February	2006	Lake	Sub Dup	55.40	1.64	
February	2006	Lake	Sub	56.00	1.75	5.83
March	2006	Pearl	Emer Dup	54.20	0.46	
March	2006	Pearl	Emer	55.30	0.45	3.08
March	2006	Doyle	Stol Dup	55.90	4.28	
March	2006	Doyle	Stol	54.50	4.08	4.92
April	2006	Doyle	Stol Dup	55.90	4.07	
April	2006	Doyle	Stol	55.20	3.16	28.85
April	2006	Lake	Root Dup	56.40	2.08	
April	2006	Lake	Root	54.00	2.05	1.43
April	2006	Pearl	Sub Dup	55.40	7.66	
April	2006	Pearl	Sub	56.40	7.44	2.92
April	2006	Maples	Root Dup	58.10	1.04	
April	2006	Maples	Root	54.60	1.06	1.43
May	2006	Maples	Stol Dup	55.10	5.36	
May	2006	Maples	Stol	54.70	5.06	6.01
May	2006	Doyle	Stol Dup	54.10	0.52	
May	2006	Doyle	Stol	55.20	0.57	9.72
May	2006	Pearl	Sub Dup	55.80	5.67	
May	2006	Pearl	Sub	58.90	4.74	19.65
June	2006	Maples	Root Dup	56.80	3.54	

Table F.1 (continued)

Month	Year	Site	Tissue	Mass	Starch	% Difference
June	2006	Maples	Root	57.40	3.44	2.79
June	2006	Lake	Stol Dup	55.80	13.69	
June	2006	Lake	Stol	58.80	14.56	5.98
June	2006	Pearl	Stol Dup	56.80	8.89	
June	2006	Pearl	Stol	56.40	7.69	15.58
July	2006	Doyle	Stol Dup	55.90	5.62	
July	2006	Doyle	Stol	59.20	5.03	11.85
July	2006	Lake	Root Dup	55.30	5.59	
July	2006	Lake	Root	59.30	5.72	2.35
July	2006	Maples	Root Dup	51.00	5.20	
July	2006	Maples	Root	58.30	4.06	28.01
July	2006	Pearl	Stol Dup	54.50	9.75	
July	2006	Pearl	Stol	53.80	8.53	14.29
August	2006	Maples	Emer Dup	55.60	18.75	
August	2006	Maples	Emer	56.00	18.59	0.88
August	2006	Doyle	Stol Sup	53.60	3.28	
August	2006	Doyle	Stol	54.80	3.66	10.26
August	2006	Pearl	Stol Dup	55.10	20.74	
August	2006	Pearl	Stol	54.60	22.30	7.00
August	2006	Lake	Root Sup	59.00	3.14	
August	2006	Lake	Root	55.10	3.75	16.38
September	2006	Maples	Stol Dup	57.60	15.23	
September	2006	Maples	Stol	55.30	13.22	15.24
September	2006	Lake	Root Dup	52.80	4.11	
September	2006	Lake	Root	55.70	4.12	0.28
September	2006	Pearl	Stol Dup	57.10	9.68	
September	2006	Pearl	Stol	56.70	10.25	5.61
September	2006	Doyle	Emer Dup	53.20	0.69	
September	2006	Doyle	Emer	53.70	0.95	27.47
October	2006	Doyle	Stol Dup	57.70	7.03	
October	2006	Doyle	Stol	60.00	7.64	8.03
October	2006	Lake	Stol Dup	58.30	14.08	
October	2006	Lake	Stol	58.80	15.90	11.45
November	2006	Pearl	Stol Dup	56.00	6.01	
November	2006	Pearl	Stol	54.70	5.84	2.98
November	2006	Doyle	Sub Dup	58.30	0.28	
November	2006	Doyle	Sub	59.70	0.30	9.51
November	2006	Lake	Sub Dup	57.80	0.50	

Table F.1 (continued)

Month	Year	Site	Tissue	Mass	Starch	% Difference
November	2006	Lake	Sub	56.40	0.55	9.76
November	2006	Maples	Stol Dup	59.20	3.86	
November	2006	Maples	Stol	59.20	3.79	1.83
December	2006	Lake	Stol Dup	56.30	12.51	
December	2006	Lake	Stol	54.90	12.83	2.51
December	2006	Maples	Stol Dup	55.00	11.65	
December	2006	Maples	Stol	55.60	10.59	10.08
December	2006	Pearl	Root Dup	52.90	1.76	
December	2006	Pearl	Root	54.20	2.03	13.15
January	2007	Doyle	Emer Dup	56.30	0.47	
January	2007	Doyle	Emer	55.80	0.38	21.98
January	2007	Maples	Stol Dup	56.20	1.56	
January	2007	Maples	Stol	54.70	1.66	5.76
February	2007	Doyle	Sub Dup	55.10	0.49	
February	2007	Doyle	Sub	54.20	0.62	21.23
February	2007	Pearl	Emer Dup	56.20	11.16	
February	2007	Pearl	Emer	59.00	10.17	9.74
February	2007	Maples	Stol Dup	54.00	5.71	
February	2007	Maples	Stol	46.00	7.03	18.67
March	2007	Pearl	Stol Dup	55.80	3.72	
March	2007	Pearl	Stol	55.40	2.99	24.30
March	2007	Lake	Sub Dup	59.30	1.24	
March	2007	Lake	Sub	57.00	1.09	14.06
March	2007	Maples	Stol Dup	57.40	12.16	
March	2007	Maples	Stol	55.50	11.98	1.53
March	2007	Lake	Emer Dup	52.30	0.86	
March	2007	Lake	Emer	51.90	0.75	15.14
March	2007	Maples	Sub Dup	58.10	0.73	
March	2007	Maples	Sub	58.10	0.88	17.63
April	2007	Doyle	Sub Dup	55.70	0.54	
April	2007	Doyle	Sub	55.50	0.49	10.61
April	2007	Lake	Emer Dup	56.90	0.90	
April	2007	Lake	Emer	58.10	1.01	10.96
April	2007	Doyle	Stol Dup	53.10	0.56	
April	2007	Doyle	Stol	58.00	0.56	1.36
April	2007	Pearl	Stol Dup	54.60	24.38	

Table F.1 (continued)

Month	Year	Site	Tissue	Mass	Starch	% Difference
April	2007	Pearl	Stol	51.90	21.84	11.62
April	2007	Maples	Root Dup	57.40	0.95	
April	2007	Maples	Root	53.30	0.94	0.95
May	2007	Lake	Root Dup	55.70	1.42	
May	2007	Lake	Root	57.40	1.27	12.17
May	2007	Doyle	Sub Dup	56.30	0.47	
May	2007	Doyle	Sub	56.30	0.48	2.59
May	2007	Pearl	Stol Dup	53.90	21.46	
May	2007	Pearl	Stol	58.30	22.52	4.71
May	2007	Doyle	Stol Dup	53.30	0.23	
May	2007	Doyle	Stol	57.50	0.19	18.94
June	2007	Doyle	Sub Dup	57.20	5.73	
June	2007	Doyle	Sub	58.20	5.42	5.68
June	2007	Maples	Emer Dup	57.70	18.20	
June	2007	Maples	Emer	57.00	16.08	13.20
June	2007	Maples	Stol Dup	52.70	12.82	
June	2007	Maples	Stol	56.60	13.42	4.46
June	2007	Pearl	Emer Dup	54.00	9.38	
June	2007	Pearl	Emer	56.40	8.50	10.36
July	2007	Pearl	Stol Dup	55.80	15.31	
July	2007	Pearl	Stol	54.10	16.56	7.58
July	2007	Pearl	Stol Dup	58.80	9.36	
July	2007	Pearl	Stol	55.00	9.02	3.85
July	2007	Doyle	Stol Dup	59.30	1.45	
July	2007	Doyle	Stol	55.58	1.66	12.86
July	2007	Maples	Emer Dup	59.60	15.95	
July	2007	Maples	Emer	58.80	15.58	2.33
August	2007	Doyle	Stol Dup	53.80	1.94	
August	2007	Doyle	Stol	51.30	1.53	26.77
August	2007	Lake	Emer Dup	58.00	10.01	
August	2007	Lake	Emer	59.70	9.73	2.79
August	2007	Lake	Stol Dup	57.50	8.19	
August	2007	Lake	Stol	57.60	12.11	32.41
August	2007	Pearl	Emer Dup	58.10	10.97	
August	2007	Pearl	Emer	51.10	8.51	28.86
September	2007	Pearl	Stol Dup	58.70	12.18	

Table F.1 (continued)

Month	Year	Site	Tissue	Mass	Starch	% Difference
September	2007	Pearl	Stolon	54.90	12.47	2.34
September	2007	Pearl	Stol Dup	55.50	6.47	
September	2007	Pearl	Stolon	55.60	6.90	6.14
September	2007	Doyle	Stol Dup	55.20	7.80	
September	2007	Doyle	Stol	57.50	8.40	7.17
September	2007	Doyle	Stol Dup	59.70	5.02	
September	2007	Doyle	Stol	52.80	4.65	7.77
October	2007	Pearl	Root Dup	56.30	1.07	
October	2007	Pearl	Root	57.10	1.44	25.44
October	2007	Pearl	Emer Dup	52.40	0.80	
October	2007	Pearl	Emer	57.20	0.72	10.18
October	2007	Doyle	Stol Dup	52.90	3.71	
October	2007	Doyle	Stol	58.60	4.12	9.91
October	2007	Doyle	Stol Dup	52.80	5.69	
October	2007	Doyle	Stol	53.60	6.05	5.91
November	2007	Pearl	Stol Dup	54.50	3.84	
November	2007	Pearl	Stol	55.20	4.27	10.08
November	2007	Lake	Emer Dup	56.50	1.95	
November	2007	Lake	Emer	54.20	1.88	4.02
November	2007	Lake	Stol Dup	56.70	13.83	
November	2007	Lake	Stol	53.30	13.93	0.68
November	2007	Doyle	Sub Dup	56.10	3.85	
November	2007	Doyle	Sub	55.20	4.48	14.03
December	2007	Doyle	Stol Dup	56.20	8.13	
December	2007	Doyle	Stol	54.50	7.75	4.96
December	2007	Lake	Stol Dup	56.70	3.64	
December	2007	Lake	Stol	57.10	3.51	3.60
December	2007	Pearl	Stol Dup	53.90	1.43	
December	2007	Pearl	Stol	51.60	1.42	1.13
December	2007	Pearl	Emer Dup	57.10	0.53	
December	2007	Pearl	Emer	56.60	0.55	4.35
Mean (± 1 SE)						10.60 ± 0.88
95% CI						8.84 < > 12.36