Tracy L. Buck. HIGH MARSH PLANT COMMUNITY RESPONSE TO SEA-LEVEL INDUCED HIGH MARSH SUBSIDENCE AND ECOSYSTEM STATE CHANGE (Under the direction of Dr. Robert R. Christian) Department of Biology. July 2001.

An observational and experimental study was conducted within a mainland marsh of the Virginia Coast Reserve on the Delmarva Peninsula in Virginia. This study was designed to evaluate the effects of sea-level induced high marsh subsidence and ecosystem state change on the existing high marsh plant community dominated by *Spartina patens* and *Distichlis spicata.* Five sites were chosen based on their apparent stages of progression along a proposed conceptual model of ecosystem state change. The sites were characterized by: (1) a solid turf with very little microtopographic relief, (2) prolonged flooding and development of a hummock and hollow terrain, and (3) an eroding tributary to the major tidal creek within the marsh. Water levels and interstitial water salinity were measured over a period of two years within the five sites chosen for study. Experimental flooding of bordered plots within these sites was conducted in the second year of the study. End-of-year aboveground biomass and aboveground plant adenine nucleotide concentrations and adenylate energy charge (AEC) ratios were measured to assess the effects of state change and experimental flooding on plant community composition and stress levels on individual species.

There was a shift in community structure from *S. patens* dominance to *D. spicata* dominance within areas experiencing state change. While salinity levels differed significantly between sites, recorded values were well within known tolerances of both species, leaving differences in flooding patterns as the most likely cause for community change. Measurements of adenine nucleotides and AEC ratios gave results opposite from

expected, with higher levels recorded in areas undergoing state change. When examined with biomass data, the increased levels of adenine nucleotides and AEC within hummocked areas of the marsh may indicate a stress response caused by plant metabolic acceleration and subsequent increased glucose consumption due to flooding-induced fermentative processes. Experimental flooding produced no measurable effects on plant biomass, and variable results in plant adenylates. Only *S. patens* exhibited a stress response due to flooding, with lowered adenosine triphosphate and total adenine nucleotide concentrations within flooded plots in the non-hummocked sites. While prolonged periods of inundation are stressing both high marsh co-dominants, *D. spicata* and *S. patens*, short-term effects of tidal flooding as a stressor are more evident for *S. patens*. Results support the observed shift in species dominance found along the sequence of state change from organic high marsh to mineral low marsh.

HIGH MARSH PLANT COMMUNITY RESPONSE TO SEA-LEVEL INDUCED HIGH MARSH SUBSIDENCE AND ECOSYSTEM STATE CHANGE

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While my career seems to be physically pulling me farther from home, their selfless support continues to make me a stronger person and strengthen my ties to them.

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1. INTRODUCTION

Salt marsh plant response to stress and disturbances such as flooding (DeLaune *et al.* 1987; Naidoo *et al.* 1992; Broome *et al.* 1995; Gough and Grace 1998; Tolley and Christian 1999), fire (Hackney and de la Cruz 1983; Turner 1987; Schmalzer *et al.* 1991), herbivory (Evers *et al.* 1998; Miller *et al.* 1998), salinity (Parrondo et al 1978; Hester *et al.* 1998; Katembe *et al.* 1998), wrack (Valiela and Rietsma 1995; Brewer *et al.* 1998), and nutrient deficiency (Wilson and Keddy 1986; Ewing *et al.* 1995) has been studied extensively. However, until recently, few studies have focused directly on plant response to sea-level induced ecosystem state change (Brinson *et al.* 1995; Hayden *et al.* 1995; Baldwin and Mendelssohn 1998; Gough and Grace 1998; Christian *et al.* 2000).

Salt marshes may be characterized by ecosystem states arranged according to exposure to tidal inundation, edaphic conditions such as salinity and sulfide concentrations, and competition between plant species (Pennings and Callaway 1992; Streever and Genders 1997; Chambers *et al.* 1998; Bertness 1999). Each ecosystem state is recognized as having distinct plant communities dominated by species adapted to abiotic conditions present within each state. For example, along the mid-Atlantic coast of the United States, a typical gradient would include a *Pinus/Juniperus* mixed upland, an irregularly flooded high marsh dominated by *Spartina patens* (Aiton) Muhl, *Distichlis spicata* (Loisel) Greene, and *Juncus roemerianus* Scheele, and a regularly flooded low marsh dominated by *Spartina alterniflora.* While small-scale disturbances and stresses can create a patchy mosaic within a state (e.g. salt pannes, wrack deposition); (Bertness

and Ellison 1987; Bertness *et al.* 1992), large scale disturbances can initiate a change in state if the stressor is severe enough to drive the existing vegetation beyond their physiological ability to grow there (Warren and Niering 1993; DeLaune *et al.* 1994). For example, a fire in the upland border between transition and forest may open up the canopy enough to allow for the expansion of transition and high marsh species into the once upland area. However, without further fire or tidal inputs, upland species may once again dominate.

This study focuses on the plant community structure within a mainland high marsh on the Delmarva Peninsula within the Virginia Coast Reserve, USA. This portion of the Atlantic coast is experiencing a relative sea-level rise of ~3 mm/yr (Hayden *et al.* 1995, Nuttle *et al.* 1997). I chose to study the two dominant high marsh species, *S. patens* and *D. spicata*, and their levels of stress as measured through aboveground biomass and adenine nucleotides in aboveground plant tissue. Both adenylate concentrations and the adenylate energy charge (AEC) have been used to measure stress in plants due to nutrient deficiency, anaerobiosis, and increased salinity (Mendelssohn and McKee 1992, Ewing *et al.* 1997). Although both *S. patens* and *D. spicata* are common co-dominants in high marshes along the mid to north Atlantic coast (Hinde 1954, Bertness 1999) each species responds differently to varying levels of stress and disturbance. *S. patens* can outcompete *D. spicata* under favorable conditions; however, *D. spicata* is better at colonizing disturbed areas and is more tolerant of elevated salinity and increased levels of flooding (Smart and Barko 1978; Bertness and Ellison 1987; Levine *et al.* 1998).

The high marsh in which this research was done is currently undergoing state change to a mineral low marsh (Brinson *et al.* 1995) by way of increased ponding of water and loss of organic rich soils. This is accompanied by the development of a hummock and hollow terrain in which high marsh species are restricted to hummocks that are surrounded by hollows filled with water for much of the year. In some areas of the marsh these hollows have coalesced to form large potholes that are colonized by *Ruppia maritima* and *S. alterniflora*. It is expected that, with the encroachment of a tidal tributary to Phillips Creek, tidal exchange will allow for mineral sedimentation and colonization by *S. alterniflora*, completing the transition from high to low marsh (Brinson and Christian 1999). Previous studies in this marsh have shown that the response to this transition may be species-specific, having a greater effect on the *S. patens-D. spicata* community and less on the adjacent *J. roemerianus* community (Brinson *et al.* 1995; Brinson and Christian 1999; Christian *et al*. 2000). Brinson and Christian (1999) found that within this transitional area of the high marsh, intact turf of permanent plots of the *S. patens-D. spicata* community decreased to between 25-50% of its original surface area within 6 yr., while the surface area of plots in the *J. roemerianus* community showed more resistance to fragmentation. However, *J. roemerianus* is more affected by disturbances such as wrack deposition (Tolley and Christian 1999). The mechanisms for this transition have been proposed (Brinson *et al*. 1995), but research is still needed on the factors influencing this process. Not knowing what initiates this subsidence and increase in surface microtopography, I was interested in the difference in community structure between areas of intact high marsh and areas that are subsiding.

My objective was to evaluate the response of both *S. patens* and *D. spicata* to increased ponding of water due to sea-level induced high marsh subsidence. I measured both aboveground biomass and tissue adenylate concentrations in plants that were subjected to both natural flooding due to high marsh subsidence and experimental flooding through pumping of tidal creek water onto designated plots. These measurements were compared to measurements taken from areas not yet experiencing high marsh subsidence as well as samples from non-flooded control plots. By measuring plant tissue adenylate energy charge ratios and adenylate concentrations, I wanted to detect the short-term response of the plant community to experimental flooding. Measurement of end-of-year aboveground biomass and plant species composition were made to detect the long-term effects of prolonged flooding on the high marsh community and to contribute to the long-term data set that is being established within Upper Phillips Creek marsh.

My hypotheses were:

1. Areas within the high marsh showing evidence of hummocking and extended periods of standing water (Sites 3 & 5) will have lower total plant biomass (*D. spicata* + *S. patens*) than those areas showing no evidence of hummocking or extended periods of standing water (Sites 2 & 4).

2. Areas within the high marsh showing evidence of hummocking and extended periods of standing water (Sites 3 & 5) will have a greater proportion of *D. spicata* to *S. patens* biomass, while areas showing no evidence of hummocking or extended

periods of standing water (Sites 2 & 4) will have a greater proportion of *S. patens* to *D. spicata* biomass.

3. Areas within the high marsh that show evidence of hummocking and extended periods of standing water (Sites 3 & 5) will show greater levels of stress as indicated by lower adenylate energy charge ratios (AEC) and adenosine triphosphate (ATP) concentrations within both *D. spicata* and *S. patens* plant tissue, than those areas which show no evidence of hummocking or extended periods of standing water (Sites 2 & 4).

4. Within experimentally flooded plots, *D. spicata* and *S. patens* plant tissue will have lower AEC ratios and ATP concentrations than *D. spicata* and *S. patens* plant tissue within control or subsurface control plots.

2. LITERATURE REVIEW

2.1 Salt Marshes

Salt marshes are halophytic grasslands that develop along intertidal shores from mid to high latitudes (Niering and Warren 1980; Pomeroy and Wiegert 1981; Haines and Dunn 1985; Mitsch and Gosselink 1993; Bertness 1999). While salt marsh formation is controlled through the interaction of sea level and coastal shoreline structure, marshes must have adequate protection from direct wave energy to enable the establishment of halophytic vegetation and ensure their stability (Pomeroy and Wiegert 1981; Gammill and Hosier 1992; Mitsch and Gosselink 1993). Maintenance of salt marshes depends not only on sediment supplies (Kastler and Wiberg 1996; Reed *et al.* 1997; Christiansen 1998; Reed 1988; Schmitt *et al*. 1998; Cahoon *et al*. 1999), but also on the stabilization and/or creation of substrate through biogenic controls (Chapman 1974; Nyman *et al*. 1993c; Brinson *et al*. 1995; Pennings and Bertness 1999). Deficits in either soil supply or biogenic accretion in conjunction with a rise in relative sea level can lead to the deterioration and loss of the marsh community (Baumann *et al*. 1984; Reed 1988; Bricker-Urso *et al*. 1989; Reed 1989; DeLaune *et al*. 1990; Nyman and DeLaune 1991, 1999; Boumans and Day 1993; DeLaune *et al*. 1994; Cahoon *et al*. 1995).

2.2 Sea-Level Rise

Estimates of eustatic (global) sea-level rise range from 2.5 to 5 mm/yr. Nyman *et al*. (1993) and Schmitt (1998) estimated the rate to be 2.4 mm/yr, and Michener *et al*.

(1997) estimated it as 3 mm/yr. The Intergovernmental Panel on Climate Change predicts a 49 cm rise in eustatic (global) sea level by the year 2100 (Houghton *et al.* 1996), while the U. S. Environmental Protection Agency estimates a eustatic sea-level rise of 15 cm by 2050, with an increase in the rate of rise to 4.2 mm/yr by the year 2100 (Cahoon *et al.* 1999). These rates are attributed to global warming and do not take into account the relative sea level rise (RSLR) along coastlines such as the Mississippi delta that are experiencing land subsidence (Cahoon *et al*. 1999), or the Canadian coast where post-glacial isostatic uplift results in 100-200 m of new shoreline every 10 years (Bazely and Jefferies 1986). Estimates of relative sea-level rise range from 1.6 to 4.0 mm/yr along the mid to south Atlantic coast (Michener *et al*. 1997; Nuttle *et al*. 1997), 0.71 to 2.5 mm/year for the north Atlantic coast (Warren and Niering 1993; Kelley *et al*. 1995), and 9.0 to 10.0 mm/year (Michener *et al*. 1997) or greater (Day *et al*. 1995) along the Gulf Coast of the United States.

2.3 Ecosystem State Change in Response to Sea-Level Rise

The response of the coastline to relative sea-level rise varies with geographical setting and is dependent on the marshes' ability to accrete and the availability of land for marsh transgression (Brinson *et al*. 1995; Hayden *et al*. 1995; Ricker 1999). The response to rising sea level of a Gulf coast marsh experiencing subsidence due to deltaic sediment deficits and compaction (Baumann *et al.* 1984) varies greatly from a New England marsh in the North Atlantic that is underlain with erosion-resistant glacial deposits and is experiencing post-glacial rebound (Kelley *et al*. 1995). Subsidence is

defined as decrease in elevation of the marsh surface and can be caused by several factors such as accretion deficits, compaction of sediments, plant root growth practices, and land management practices such as water withdrawal (Cahoon *et al*. 1999).

Brinson *et al*. (1995) proposed a transgression model for marshes along the mainland of the mid-Atlantic coast experiencing sea-level rise, based on research of marshes within the Virginia Coast Reserve-Long Term Ecological Research (VCR-LTER) program. This transgression model describes the dynamics of sea-level controlled ecosystems and their response to sea-level driven disturbances such as salt water intrusion, sediment redistribution and wrack deposition (Brinson *et al*. 1995; Hayden *et al*. 1995). Modeling these dynamics within the VCR includes both successional dynamics within an ecosystem state, as well as ecosystem state change. Ecosystem state change is defined by Hayden *et al*. (1995) and Brinson *et al*. (1995) as a transformation from one ecosystem class to another resulting from disturbance and non-autogenic change in the physical environment. Ecosystem state change differs from ecological succession in that the controls on state change are disturbance-driven, initiating an alternate path of progression, while succession involves a natural progressive sequence without the requirement of disturbance to initiate the change. Within the state change model, ecosystem classes such as *S. alterniflora*-dominated low marshes or *Pinus*-dominated forests are replaced by another ecosystem class if they are unable to sustain themselves in response to rising sea level. For example, without sufficient sedimentation, the *Spartina*-dominated low marsh will be replaced by a subtidal system, while the *Pinus*-dominated forest will be replaced by high marsh species with increasing stress from

saltwater intrusion. Although a change in state involves numerous changes in ecosystem function such as altered hydrology and sediment dynamics (Brinson *et al*. 1995; Nyman and DeLaune 1999); the most readily studied change is that of dominant vegetation patterns (Hayden *et al*. 1995; Brinson and Christian 1999). Studies of plant community change in response to rising sea level have been conducted for northeast tidal marshes (Warren and Niering 1993; Rogers *et al*. 1998), mid-Atlantic tidal marshes (Brinson and Christian 1999; Hayden *et al*. 1995; Tolley 1996; Ricker 1999), and Gulf Coast tidal marshes (Pezeshki *et al*. 1987; Baldwin and Mendelssohn 1998), as well as European salt marshes (Olff *et al*. 1997).

2.4 Ecosystem State Change Within Upper Phillips Creek Marsh

The typical change in state from organic high marsh to mineral low marsh as proposed by Brinson *et al*. (1995) may involve the redistribution of mineral sediments over organic soils through sea-level induced tidal deposition. This is not the case, however, in Upper Phillips Creek Marsh, Virginia. It has been proposed that state change from high marsh in Upper Phillips Creek marsh involves an unstable state in which the organic rich soils are removed prior to tidal deposition of mineral sediments (Brinson *et al*. 1995, Christian *et al*. 2000). This stage of transformation is common in marshes that have relatively flat topography and are distant from or inadequate in tidal supply of sediments such as those along the Gulf of Mexico (Turner 1997). Studies within the marsh surrounding Upper Phillips Creek have shown that this marsh receives relatively little sedimentation, is relatively protected from erosion at the lagoon-edge by Fowling

Point to the southeast, and is able to transgress upland area due to its low upland slope of 0.4-0.9^{o} (Kastler and Wiberg 1996). This subsiding or degrading state is characterized by the loss of the organic-rich soil layer overlying the antecedent mineral surface (Brinson *et al*. 1995, Christian *et al*. 2000). The initiating factor for this subsidence is not yet known, but it has been hypothesized that decreased plant productivity due to increased ponding in consort with disturbance (Tolley and Christian 1999) may initiate the exposure and eventual loss of the organic sediment (Christian *et al*. 2000). The terrain created in this stage of change is characterized by hummocks of organic soil vegetated by the high marsh dominants, *D. spicata, S. patens* and *J. roemerianus.* These hummocks are surrounded by barren channels or hollows that may coalesce to form marsh potholes (Brinson *et al*. 1995; Christian *et al*. 2000). The formation of these channels and their connection to form larger potholes may be aided by the grazing activity of muskrats (Christian *et al*. 2000; personal observation). The encroachment of a tidal tributary to Phillips Creek into the organic high marsh may improve drainage through steepening of hydraulic gradients, leading to more rapid oxidation of organic soils and tidal supply of sulfate for increased decomposition through sulfate reduction (Brinson *et al*. 1995). Studies of plant community change within this area by Brinson and Christian (1999) reveal that while the *D. spicata* and *S. patens* community may be vulnerable to the loss of intact turf in this unstable zone, *J. roemerianus* is more resistant to the fragmentation of the marsh surface.

2.5 Other Studies Within Upper Phillips Creek Marsh

Upper Phillips Creek marsh, a mainland marsh on the Eastern Shore of Virginia, is part of the Virginia Coast Reserve-Long Term Ecological Reasearch (VCR-LTER) project. Research at the VCR-LTER is centered on ecosystem, landscape, community and population response to disturbance and physiological stresses (Hayden *et al.* 1995). Several studies have included or focused exclusively on these processes within Upper Phillips Creek marsh. Hmieleski (1994) measured abiotic and biotic variables along transects from high marsh to forest and found that the degree of slope affects the size and position of the transition zone within the marsh. Brinson and Christian (1999) measured the stability of the boundary between stands of *J. roemerianus* and the adjacent high and low marsh communities including *S. patens*, *D. spicata* and *S. alterniflora*, as affected by natural flooding and wrack deposition. Similarly, Tolley and Christian (1999) measured the effects of experimental manipulation of flooding and wrack deposition on a high marsh plant community including the boundary between an almost monospecific stand of *J. roemerianus* and its adjacent community of the codominants, *S. patens* and *D. spicata.* Tolley and Christian (2000) found that *S. patens* was more sensitive than *D. spicata* to increased inundation, but that the interaction between flooding and wrack deposition negatively affects both species. Roberts (2000) developed a less destructive method of measuring primary productivity of these two species in response to both natural and experimental flooding. He found negative effects of flooding and marsh deterioration on both *S. patens* and *D. spicata,* but predicts that *D. spicata* will be able to persist and eventually dominate within the high marsh with rising sea level (Roberts 2000).

Dynamics of the belowground aspect of the salt marsh community have been studied by Blum (1993) who measured *S. alterniflora* root and rhizome decomposition and applied these findings to explaining differences in organic matter accumulation in both high and low marsh communities. Blum (1993) found that marsh plant root production and the factors that influence production, rather than root decomposition processes, account for differences in organic matter accumulation between the high and low marsh.

Biogeochemical cycling of nutrients within Upper Phillips Creek marsh have been studied on both small and large scales. Tarnowski (1997) found that dissolved oxygen concentrations had little affect on rates of nitrification within a high marsh pool or Phillips Creek, except during periods of anoxia or extreme hypoxia. Taylor (1995), in conjunction with the previous mentioned study by Tolley and Christian (1999), found that rates of nitrification, denitrification and standing stocks of NO_3^- and NO_2^- were not affected by experimental flooding, but that denitrification rates were increased by wrack deposition. Anderson *et al.* (1997) measured microbial nitrogen cycling rates between vegetated and unvegetated low marsh sites and the adjacent tidal creek, and used their results to develop a process-based nitrogen mass balance model for the *S. alterniflora* low marsh. Their model suggests that within Phillips Creek Marsh, Virginia, ammonium surpluses within the marsh are sequestered within the microbial organic nitrogen pool until required for marsh plant uptake. Using data collected from literature, Thomas (1998) used network analysis to analyze the nitrogen cycle for each zone (creekbank, low marsh, and high marsh) of Upper Phillips Creek marsh, as well as Great Sippewissett marsh in Massachusetts and the marshes of Sapelo Island, Georgia. Her results indicate

that nitrogen cycling may be directly affected by the adjacent slope of the upland and its affect on the ability of the marsh to migrate overland with rising sea level. Her model suggests that nitrogen cycling and marsh "maturity" may increase if the high marsh is able to migrate overland with a low upland slope, or may decrease if marsh migration is stalled with a steep upland slope (Thomas 1998).

Studies of hydrogeomorphology at Upper Phillips Creek marsh and the VCR-LTER have included those of Christiansen (1998) who characterized the processes controlling mineral sedimentation on the surface of the *S. alterniflora* dominated low marsh. She found that sedimentation processes are strongly controlled by meteorological events such as northeasterly storms. Kastler and Wiberg (1996) used sediment collections and GIS analysis of aerial photography to study mainland, lagoon and barrier island marshes, and relate changes in marsh area to sedimentary processes within the VCR-LTER. Their findings show steady lagoonal marsh loss through submergence and recession, as well as mainland marsh expansion through upland transgression (Kastler and Wiberg 1996). Stasavich (1998) analyzed the hydrodynamics over a period of six years of both mainland and barrier island marshes within the VCR in an effort to estimate the capacity of ecological processes within the marshes. Her study showed that marsh ecosystem states are differentially affected by hydrological inputs such as precipitation, water and tide that can directly influence these states' value as aquatic habitat for estuarine organisms. Her results show that, based on hydrological characteristics, the ability of the mainland high marsh to store water through depressional storage for long periods of time, make it a viable area for resident nekton habitat. Finally, using the

model for ecosystem state change proposed by Brinson *et al.* (1995), Ricker (1999) characterized marshes along the mainland fringe of the Virginia Coast Reserve and used the results to predict where ecosystem state change is most likely to occur in response to rising sea level. She found that based on their physical attributes that were likely to affect their resistance to state change, upland areas within the central geographic region of the VCR-LTER study area have the most land area available for conversion from forest to marsh with rising sea-level.

2.6 *Distichlis spicata* and *Spartina patens*

The high marsh of Upper Phillips Creek marsh is characterized by monospecific stands of *J. roemerianus* interspersed amongst large areas co-dominated by *S. patens* and *D. spicata* (Brinson and Christian 1999; Tolley and Christian 1999). These latter two species are common co-dominants within high marshes along the eastern Atlantic from New England to Northern Florida and the Gulf of Mexico (Beal 1977; Silander 1984; Duncan and Duncan 1987; Mitsch and Gosselink 1993; Bertness 1999). Much work has been done on the relationship of these two species and their differential responses to disturbances such as wrack deposition, salinity and flooding (Smart and Barko 1978; Bertness and Shumway 1993; Brewer *et al*. 1998a,b; Levine *et al*. 1998; Tolley and Christian 1999). Differences in stress tolerance and competitive ability between these two species have been attributed to the differences in their root morphologies and aerenchyma development (Bertness 1999). Aerenchyma, a continuous system of air spaces that allows the transport of oxygen from the shoot to the roots, is the most

important adaptation to flooded soils by wetland plants (Lambers *et al*. 1998). These morphological adaptations and their importance for *S. patens* and *D. spicata* in response to flooding are discussed later.

2.7 Effects of Flooding on Plants

As defined by Levitt (1980), flooding is the presence of water in soil in excess of field capacity, and the primary constraint on plants imposed by flooding is the impedance of gas exchange resulting in creation of an anaerobic environment (Kozlowski 1984, Mitsch and Gosselink 1993; Armstrong *et al*. 1994; Blom and Voesenek 1996; Lambers *et al*. 1998). This reduction or loss of oxygen availability has a direct effect on the ability of plant roots to carry out aerobic respiration (Levitt 1980; Mitsch and Gosselink 1993; Armstrong *et al*. 1994). If flooding submerges the plant, it can also severely affect photosynthesis through reduction of available light (Gleason and Zieman 1981; Blom and Voesenek 1996) and flood-induced stomatal closure (Pezeshki 1994). Added stresses to plants exposed to increased inundation in salt marshes are sulfide toxicity (Mendelssohn and Burdick 1988; Mendelssohn and McKee 1988; Koch *et al*. 1990; Pezeshki *et al*. 1991; Chambers *et al*. 1998) and increased soil salinity (Smart and Barko 1978; Bandyopadhyay *et al*. 1993; Pezeshki and DeLaune 1997; Gough and Grace 1998).

It is predicted that with increasing CO_2 levels, climatic warming will lead to an accelerated rise in eustatic sea-level (Houghton *et al*. 1996). This increase in sea-level will in turn increase the frequency and duration of inundation of coastal wetlands (Baldwin and Mendelssohn 1998). While many flood tolerant plants have developed

morphological or anatomical characteristics that allow them to function under flooded conditions (McKee *et al*. 1989), limitations to these adaptations have been shown to retard growth and reproduction in some species of wetland plants (Armstrong *et al.* 1994; Crawford and Braendle 1996; Gough and Grace 1998; Lessman *et al*. 1997) including *S. patens* (Bandyopadhyay *et al*. 1993; Broome *et al*. 1995) and *D. spicata* (Parrondo *et al*. 1978).

2.8 Morphological Adaptations of *Distichlis spicata* and *Spartina patens*

D. spicata and *S. patens* are both perennial, C4, salt marsh grasses with similar distributions along the western Atlantic and Gulf of Mexico, from Nova Scotia to Texas (Duncan and Duncan 1987; Mitsch and Gosselink 1993). Hansen *et al*. (1976) describe *D. spicata* as a pioneer species in early stages of succession (Mitsch and Gosselink 1993), whose aerenchymatous network of rhizomes, leaf sheath and roots facilitate its development in heavily inundated soils. This is supported by Bertness (1999) but is in contrast to Anderson (1974) who reported no aerenchyma development for *D. spicata* in North Carolina marshes. Both Bertness (1999) and Haines and Dunn (1985) report poor or reduced aerenchyma development in *S. patens*. Naidoo *et al.* (1992), Pezeshki *et al.* (1991) Burdick and Mendelssohn (1987), Bandyopadhyay *et al*. (1993) and Gleason and Zieman (1981) all support these findings, reporting that although *S. patens* has the ability to produce aerenchymatous spaces within root tissue in response to low soil redox potential, this development is inhibited by the lack of oxygen and is insufficient to sustain the plant under prolonged periods of reducing conditions.

In addition to morphological adaptations to flooding, both *S. patens* and *D. spicata* can actively excrete salt through salt glands on the leaves (Naidoo *et al*. 1992; Hansen *et al*. 1976, Kemp and Cunningham 1981) or exclude salt uptake at the roots (Li *et al*. 1995, Smart and Barko 1978) when presented with excess concentrations of salt. The differential levels of adaptation to flooding and salinity stresses within the salt marsh directly affect the distribution of these two grasses.

2.9 Adenylate Energy Charge Ratio

A widely used method for measuring stress in environmental systems is that of the adenylate energy charge or AEC ratio (Burdick *et al*. 1989; Ewing *et al*. 1997; Mendelssohn and McKee 1981; Shafer and Hackney 1987). Daniel Atkinson developed this ratio in 1963, in conjunction with work by Charles Krebs who discovered the importance of AMP in regulating glycolysis and gluconeogenesis. Atkinson proposed that the balance among the concentrations of the adenine nucleotides could be a regulatory factor in instances where metabolites are partitioned between energy-yielding, energy-demanding or energy-storing processes (Atkinson 1968). Atkinson (1968) defined the adenylate energy charge as half of the average number of anhydride-bound phosphate groups per adenine moiety and developed the equation for its calculation as:

$$AEC = ([ATP] + \tfrac{1}{2}[ADP]) / ([ATP] + [ADP] + [AMP]).$$

This definition can therefore be used as a parameter to furnish a quantitative estimate of the energy status of the living cell (Atkinson 1968; Bostick and Ausmus 1978; Cann-Moisan *et al*. 1989; Leach 1981) or used to measure the physiological health of a

particular community, population or organism (Burdick *et al*. 1989; Christian *et al.* 1978; Ewing *et al*. 1997; Mendelssohn and McKee 1992; Mitsch and Gosselink 1993).

Since ATP is either consumed or regenerated in most metabolic sequences, and provides the energy necessary to make growth possible, it is considered the most important biochemical substance produced within living cells (Atkinson 1968; Garrett and Grisham 1995; Leach 1981) and is a direct indication of life (Bostick and Ausmus 1978). The absolute amount of ATP within a cell is important, however, in healthy cells with intact metabolic functions, ATP is rapidly synthesized (Leach 1981). The energy charge, however, is a linear measure of the metabolic energy stored in the adenylate system (Leach 1981). The rapid turnover of ATP within the cell can make detection of abnormal metabolic function difficult if relying on absolute concentration alone (Leach 1981). However, it has been found that the cellular energy charge parallels increases or decreases in metabolic function by regulating the processes that control ATP regeneration and consumption (Atkinson 1968; Leach 1981). Therefore, AEC is considered a better measure of the overall fitness of living cells than ATP concentration alone (Atkinson 1968; Leach 1981).

2.10 Effects of Flooding on Adenylates

As stated earlier, the important effects of flooded soils on plants include oxygen deficiency and accumulation of toxins such as hydrogen sulfide (Armstrong *et al.* 1991; Armstrong *et al*. 1994; Levitt 1980). The immediate consequence of this deprivation in oxygen for plants is that ATP cannot continue to be regenerated through aerobic

respiration (Blom and Voesenek 1996; Lambers *et al*. 1998). This cessation of aerobic respiration leads to the induction of ethanolic fermentation to continue glycolysis in higher plants (Blom and Voesenek 1996; Lambers *et al*. 1998). However, ethanolic fermentation produces less ATP than does aerobic respiration (Armstrong *et al*. 1994; Blom and Voesenek 1996; Garrett and Grisham 1995; Lambers *et al*. 1998). Fermentation also produces toxins such as ethanol and acetaldehyde (Blom and Voesenek 1996; Kozlowski 1984; Lambers *et al*. 1998; Levitt 1980) and increases the demand for and mobilization of reserves such as starch to continue fermentative metabolism (Armstrong *et al*. 1994; Crawford 1996; Lambers *et al*. 1998). Although the switch to anaerobic metabolism allows the plant to survive short periods of inundation and oxygen deprivation, this process is not energy efficient. Ethanolic fermentation only produces two molecules of ATP per glucose molecule, whereas aerobic metabolism produces 36 molecules of ATP per glucose molecule (Garrett and Grisham 1995; Lambers *et al.* 1998). Sublethal stress in plants due to soil waterlogging and oxygen deprivation have been shown to elicit measurable reductions in plant adenylate concentrations (Armstrong *et al*. 1994; Koch *et al*. 1990; Ewing *et al*. 1997), adenylate energy charge (Burdick *et al*. 1989; Mendelssohn and Burdick 1988; Müller *et al*. 1994; Sieber and Brändle 1991), and above and belowground productivity (Crawford and Braendle 1996; Koch *et al*. 1990; Levitt 1980; Mendelssohn and McKee 1988; Pezeshki 1994). These principles were applied to this study of aboveground biomass and plant adenylate energy charge as indicators of stress in response to ambient and experimental flooding within Upper Phillips Creek Marsh, Virginia.

3. SITE DESCRIPTION

The study site is located on the mainland of the Delmarva Peninsula, Virginia, USA, within an organic high marsh at the head of Upper Phillips Creek at the Virginia Coast Reserve (37^{o} 27' 38.5"N, 75^{o} 50' 4.96"W) (Fig. 1**)**. This marsh is dominated by *S. patens*, *D. spicata*, and *J. roemerianus*. The average tidal range is 150 cm (Christiansen 1998), with salinity in Upper Phillips Creek ranging from 8 ppt in winter to 30 ppt in late summer during the study period (Appendix A).

Five sites within an approximately 4.5 ha region of high marsh were established on May 15, 1997 (Fig. 2). These sites were chosen based on their distance from a first order tidal tributary to Phillips Creek and visible differences in micro-topographic relief. Site 1 is directly adjacent to the tributary, which is steadily eroding into the organic substrate of the high marsh (Fig. 3). This site was well drained and had only slight micro-topographic relief. Sites 2 and 4, located approximately 45 and 120 m from the tributary, respectively, had solid organic turf with little relief (Fig. 4). Site 3, located approximately 70 m from the tributary, had well developed microtopography of hollows and hummocks (Fig. 5). Site 5, located approximately 250 m from the tributary, had well developed microtopography and was flooded above ground surface for much of the study period (Fig. 6). All sites had comparable elevation of approximately 1.04 m (range = 1.004 - 1.048) above mean sea level (Appendix B). *S. patens* and *D. spicata* were dominant within all five sites. Sites 2 and 4 were grouped as "non-hummocked," and Sites 3 and 5 were grouped as "hummocked." Due to its unique position between the two

hummocked and non-hummocked extremes, site 1 was kept separate for analysis as "creek."

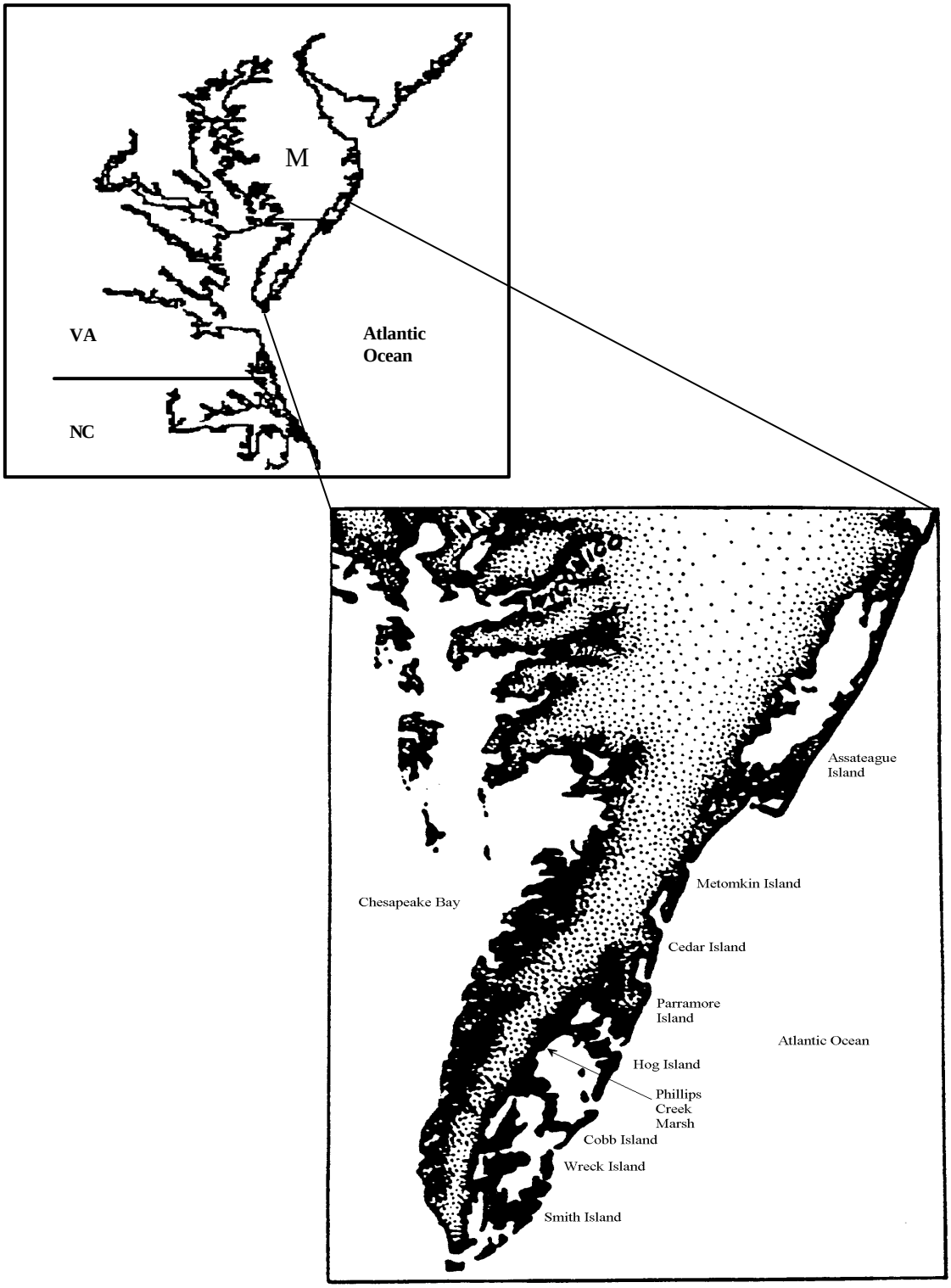


Figure 1. Delmarva Peninsula with indication of the location of Phillips Creek Marsh. Figure modified from Hayden *et al.* 1995.

Figure 2. Photograph of Upper Phillips Creek Marsh with location of individual sites marked by number. The water body to left is Phillips Creek at flood tide. The white line in the center is a boardwalk that now extends to Site 5. The subsiding area is between Sites 3 and 5 with standing water visible. The darker areas are stands of *Juncus roemerianus*.

Figure 3. Photograph of Site 1. The *Spartina patens* and *Distichlis spicata* high marsh community is visible in the foreground. Erosion into the site by a tributary to Phillips Creek is visible in the right side of the picture, with tall *Spartina alterniflora* on the creekbank visible in the background. Three wooden 2x4" boards are visible to the left and were part of the system used to support portable boardwalks at each site.

Figure 4. Photograph of Site 2. Uninterrupted turf of *Spartina patens and Distichlis spicata* with no hummocking present. A portable boardwalk is visible in the lower left and was used to access the center of the plots by supporting a cross-board (bottom center of photo). This site is very similar to Site 4.

Figure 5. Photograph of Site 3. Subsiding area of high marsh with vegetated hummocks dominated by *Distichlis spicata*, surrounded by water-filled hollows. *Spartina patens* becomes subdominant in these areas.

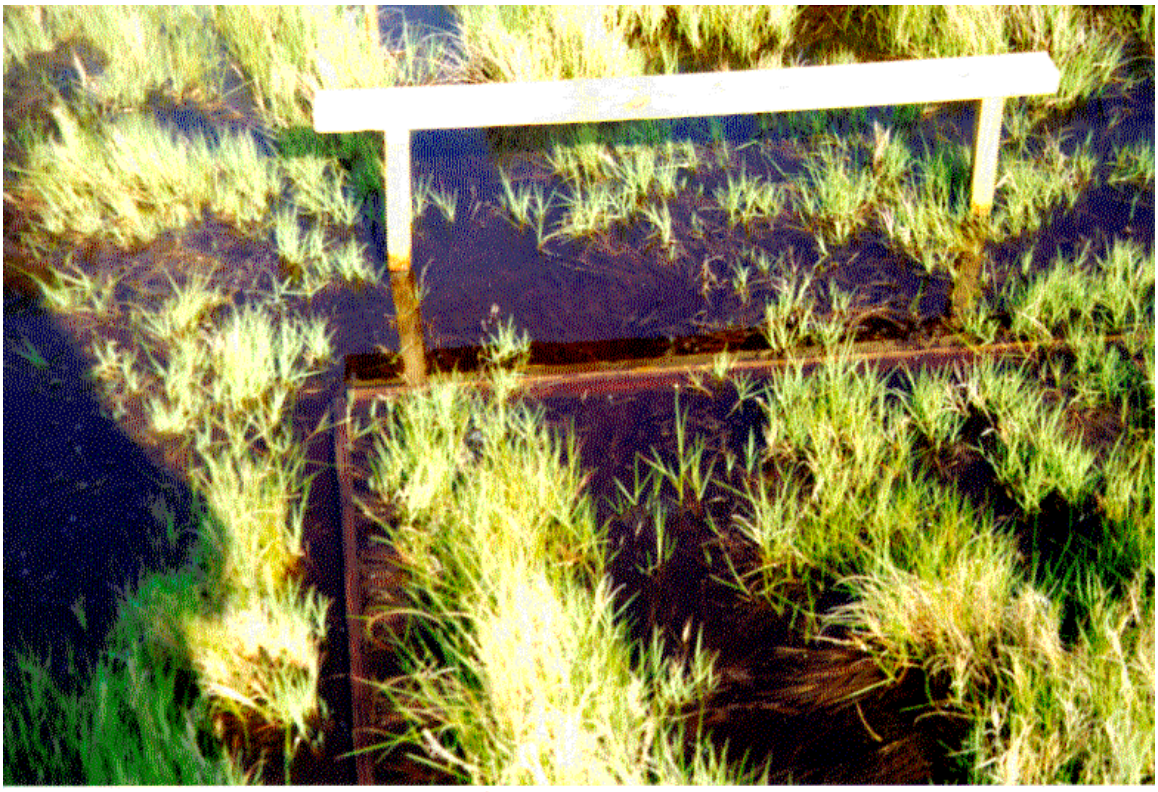


Figure 6. Photograph of Site 5. Vegetated hummocks dominated by *Distichlis spicata* surrounded by water-filled hollows. This site was flooded above ground surface for much of the study period. A ponded border treatment is visible in the bottom center (flooded above 10 cm border). A portable boardwalk is visible in the top center of the picture.

Figure 7. Photograph of marsh potholes in subsiding area of high marsh. Water filled hollows have coalesced to form these large and often times deep (≥1m) potholes that support *Ruppia maritima* (darker green visible in water) and *Spartina alterniflora* (taller plant in bottom right part of the photo). The remaining hummocks are dominated by *Distichlis spicata.* Stands of *Juncus roemerianus* are visible to the left and in the background. A boardwalk is visible in the right of the photo.

4. METHODS

4.1 Experimental Design

Within Sites 2, 4, and 5, six 2.5 x 5-m plots were laid out on May 15, 1997 and designated as two each of PONDED, CONTROL, or SUBSURFACE control (Fig. 8). PONDED plots consisted of a plywood border inserted into the marsh surface to form a border 10 cm belowground and 10 cm aboveground. Borders were installed within experimental sites on April 17-19, and 25, 1998. The wood was waterproofed with Thompson's® Water Sealant and caulked at junctions. SUBSURFACE control plots consisted of a plywood border inserted flush with the marsh surface to form a 10 cm belowground border. This was done to account for any restriction of subsurface flow or vegetative ingrowth by the PONDED borders. SUBSURFACE control plots were not installed at Site 5 until fall of 1998 because of persistent aboveground flooding at that site. CONTROL plots were left undisturbed and marked only at the corners by ½" (1.27 cm) PVC. To simulate tidal flooding by extreme tides, tidal water was pumped onto PONDED plots on June 29, July 25, August 22 and 23, and September 26 and 27, 1998 (Appendix C). Water was pumped using a Briggs and Stratton 5 hp Homelite 2" (5.08 cm) centrifugal pump through flexible 3" (7.62 cm) PVC hose. Flow of water onto the plots was baffled through a capped and slotted section of 4" (10.16 cm) PVC in order to minimize damage to plants. Pumping continued each time until water reached the top of the 10 cm high border. After all plots had been filled, water level and salinity within the borders were measured. Plots were refilled if substantial drawdown had occurred by the

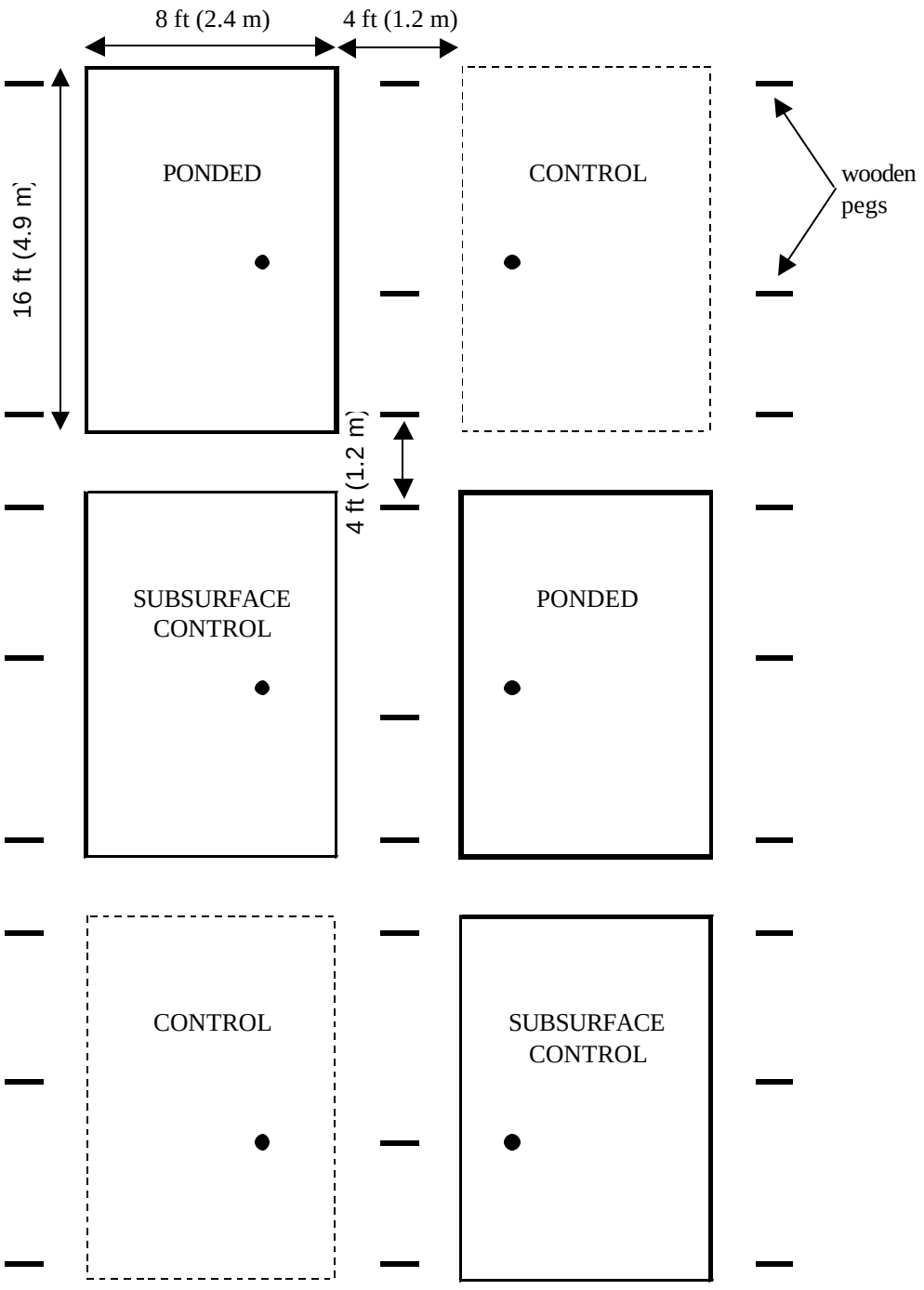


Figure 8. Typical plot layout and dimensions. Wooden pegs were installed to allow portable boardwalks to be used to minimize damage around plots. Black dots represent location of wells. Treatments were randomly assigned to plots at each site.

end of the initial flooding treatment. Four CONTROL plots were established within Sites 1 and 3 in order to sample for differences in subsiding states within the marsh.

4.2 Water Level and Salinity

One ½" (1.27 cm) PVC well was placed within each plot to a depth of 25 cm. Each well had 1/8" (0.32 cm) holes drilled along its length to allow for collection of water from ground-surface to 25 cm belowground. The length of the belowground sampling area of each well was wrapped in glass fiber and covered with 1 mm^2 mesh to slow sediment accumulation within the well reservoir. Water levels were checked approximately every two weeks. After water level was recorded, each well was pumped dry, allowed to refill and then water sampled for measurement of interstitial water salinity using a refractometer. Precipitation data were collected daily from meteorological station VCR-LTER PHCK (http://www.vcrlter.virginia.edu) located approximately 400 m from Site 4. Two WL40 digital water level recorders (Remote Data Systems, Inc., Wilmington, NC) were installed at Site 3 (February 22, 1998) and Site 5 (June 24, 1998) which recorded water levels at 45 minute intervals. Water level recorder data were downloaded monthly and compared to water levels recorded within the plots at these sites.

4.3 Biomass

End-of-year aboveground biomass of plants was collected from each plot using 0.0625-m^2 quadrats in duplicate on September 13, 1997 and in triplicate on September

19, 1998. Plants were returned to the lab and sorted by species, live (all or part green culms + leaves) and dead (all brown culms + leaves). Samples then were dried at 85^{o}C for 72 h and weighed to the nearest 0.01 g.

4.4 Species Composition

On May 21, 1997 (Sites 1-4) and July 9, 1997 (Site 5) species composition and dominance was determined within each plot using a 1-m^2 quadrat sectioned into one hundred 10x10-cm^2 subquadrats. Species present, species dominance and bare area were recorded within each 10x10-cm^2 quadrat for a total of three 1-m^2 quadrats per plot, giving 300 10x10-cm^2 readings per plot. Vegetation classes were determined for each 1-m^2 quadrat as follows: *S. patens* (only), *D. spicata* (only), *S. patens* > *D. spicata*, *D. spicata* > *S. patens*, bare (no vegetation), or other (species other than *S. patens* or *D. spicata*). Total cover for each class was calculated for each site and converted to percent cover of each site.

4.5 Soils

On August 7-8, 1997, three soil cores were collected from each plot to a depth of 30 cm using a 7.5-cm diameter aluminum coring tube. The top 10 cm of each was removed and analyzed for macro-organic matter (n=1) and bulk density (n=2). Bulk densities were dried at 105^{o} C to a constant mass and weighed to the nearest 0.01g. Macro-organic matter was determined by washing each core through a #18 (1mm) USA

Standard Testing Sieve and drying the remaining material at 85^{o}C for 72 h or constant mass (Gallagher 1974).

4.6 Adenylates

Plant samples were collected from each plot 24 h following experimental flooding in July, August and September 1998. June samples were collected prior to flooding, while July, August and September samples were collected after flooding. Six samples were taken per plot, three of *S. patens* and three of *D. spicata.* Each sample consisted of the top 1/3 of approximately eight plants including leaves and culms. Plants sampled later in the growing season were selected for the least amount of dead material per stem. Samples were clipped, placed in Whirlpak bags, sprayed with deionized water and immediately frozen in liquid nitrogen. Immediate freezing of sample tissue in liquid nitrogen is necessary to prevent enzymatic degradation of adenylates within the plant tissue upon separation from the plant (Mendelssohn and McKee 1981) (details of methods are presented in Appendix D). Samples were transferred to dry ice for transport back to the lab where they were freeze-dried, ground in a Wiley Mill to pass a 40-mesh screen, and stored in frozen dessicators until processed. Processing and extraction of plant samples followed the boiling ethylenediaminetetraacetic acid (EDTA) method of Mendelssohn and McKee (1981) excluding the addition of polyvinyl polypropylene (PVPP). Mendelssohn and McKee (1981) added PVPP to samples to stabilize proteins and adsorb phenols to increase light output in the luciferin-luciferase assay (see also Delistraty and Hershner 1983). It was not considered necessary for analysis by high

pressure liquid chromatography (HPLC) (R. Christian, personal communication). Comparison of test samples prepared with and without the addition of PVPP showed no measurable difference in adenylate concentrations (this study, data not shown). Extractant was centrifuged and immediately prepared for analysis. Samples were analyzed using reverse-phase HPLC on a Waters 600 Multisolvent Delivery System, Waters Intelligent Sample Processor and Waters 990 Photodiode Array Detector. Samples were run through a Bio-Sil C_{18} HL 90-5S 250 x 4.6 mm (BioRad) column and Bio-Sil C_{18} HL 90-5 30 x 4.6 mm guard cartridge with both the mobile and stationary phase held at a constant temperature of 28^{o}C using a BioRad column heater. The mobile phase and gradient used for the separation of adenine nucleotides follows that of Cann-Moisan *et al.* (1989) (Appendix D). While awaiting injection, extracted samples were held at a constant temperature of 5^{o}C.

Peak-area integration of adenylate standards were used to calculate standard linear regressions. Time of separation from the column for each standard were used for integration of adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP) and adenosine 5'-monophosphate (AMP) within extracted samples. Standard linear regressions were used to convert integrated area of peaks from extracted sampled to relative concentrations of ATP, ADP, AMP, ATP/ADP, ATP/AMP, AEC and TAN (total adenine nucleotides). AEC was calculated as (Atkinson 1968):

$$AEC = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

4.7 Statistical Analysis

Descriptive and inferential statistics were computed using SAS (SAS Institute, Inc. 1997). Independent variables tested were site (1-5), state of change (hummocked, non-hummocked, creek) and flooding (control, ponded, subsurface control). Dependent variables used to measure effects were aboveground biomass of each *S. patens* and *D. spicata*, leaf tissue adenylate concentrations (ATP, ADP, AMP, TAN) and AEC ratio of both species, as well as soil and water characteristics such as bulk density and salinity. I used General Linear Models (GLM) procedure to evaluate differences between site and state of change. I used repeated measures analysis was used to analyze differences between year (biomass) and month (adenylates). For comparison of biomass differences between species, the ratio of *D. spicata* to *S. patens* was calculated and log transformed (Log_{10}) for GLM analysis. Values were log transformed (Log_{10}) to obtain approximately normally distributed observations since the ratio of *S. patens* to *D. spicata* biomass within sites was not normally distributed.

All monthly adenylate treatment data were analyzed using the MIXED procedure (SAS Institute, Inc. 1997). This treatment allowed for the analysis of the imbalanced treatment design created by the late installation of the subsurface control plots in Site 5. Sidak T-tests were used for multiple comparison of marginal means. In instances where a one-tailed hypothesis was being used, alpha was adjusted to 0.1 for both GLM analysis and comparison of means, and significance was accepted as p-values of 0.1 or less. Acceptance of $P \leq 0.1$ was never used, as all P-values for one-tailed hypotheses were either greater than 0.1 or less than 0.05.

Emphasis was on differences in marsh condition (i.e. hummock vs. non-hummocked vs. creek) rather than site. Therefore, results of statistical analysis for individual site differences will not be presented here unless they provide further insight into confirming or refuting my hypotheses. Full ANOVA tables are given in appendices F-M.

5. RESULTS

5.1 Edaphic Factors

Edaphic factors were different among areas of change in state and individual sites. Differences in bulk density to a depth of 10 cm were significant ($P=0.030$) with the non-hummocked areas having significantly higher mean bulk density (0.1805 g/cm^3) than either the hummocked areas (0.1079 g/cm^3) or the creek site (0.1066 g/cm^3) (Fig. 9a).

As with bulk density, macro-organic matter to a depth of 10 cm was significantly higher ($P=0.0142$) for non-hummocked areas (5.57 kg/m^2) as compared to creek areas (3.27 kg/m^2), however, the difference was not significant for the hummocked areas (4.84 kg/m^2) (Fig. 9b). Percent water content of soils was significantly lower for non-hummocked areas (79.79%) as compared to the creek site (87.05%) or hummocked areas (88.08%) ($P=0.01$) (Fig. 9c).

5.2 Water, Salinity, and Precipitation Patterns

Repeated measures analysis of water levels indicated a significant difference for 1997 and 1998 data (time, $P=0.0001$; site, $P=0.0002$) (Fig. 10). Due to the extensive topographic heterogeneity and distances between sites, water and salinity levels were analyzed as separate sites rather than areas of change. In 1997, water levels showed a significant difference between the two hummocked sites (3 & 5) and the two non-hummocked sites (2 & 4), with the hummocked sites averaging higher water levels (-7.0 cm, Site 3; -2.8 cm, Site 5) than the non-hummocked sites (-10.6 cm, Site 2; -11.4 cm,

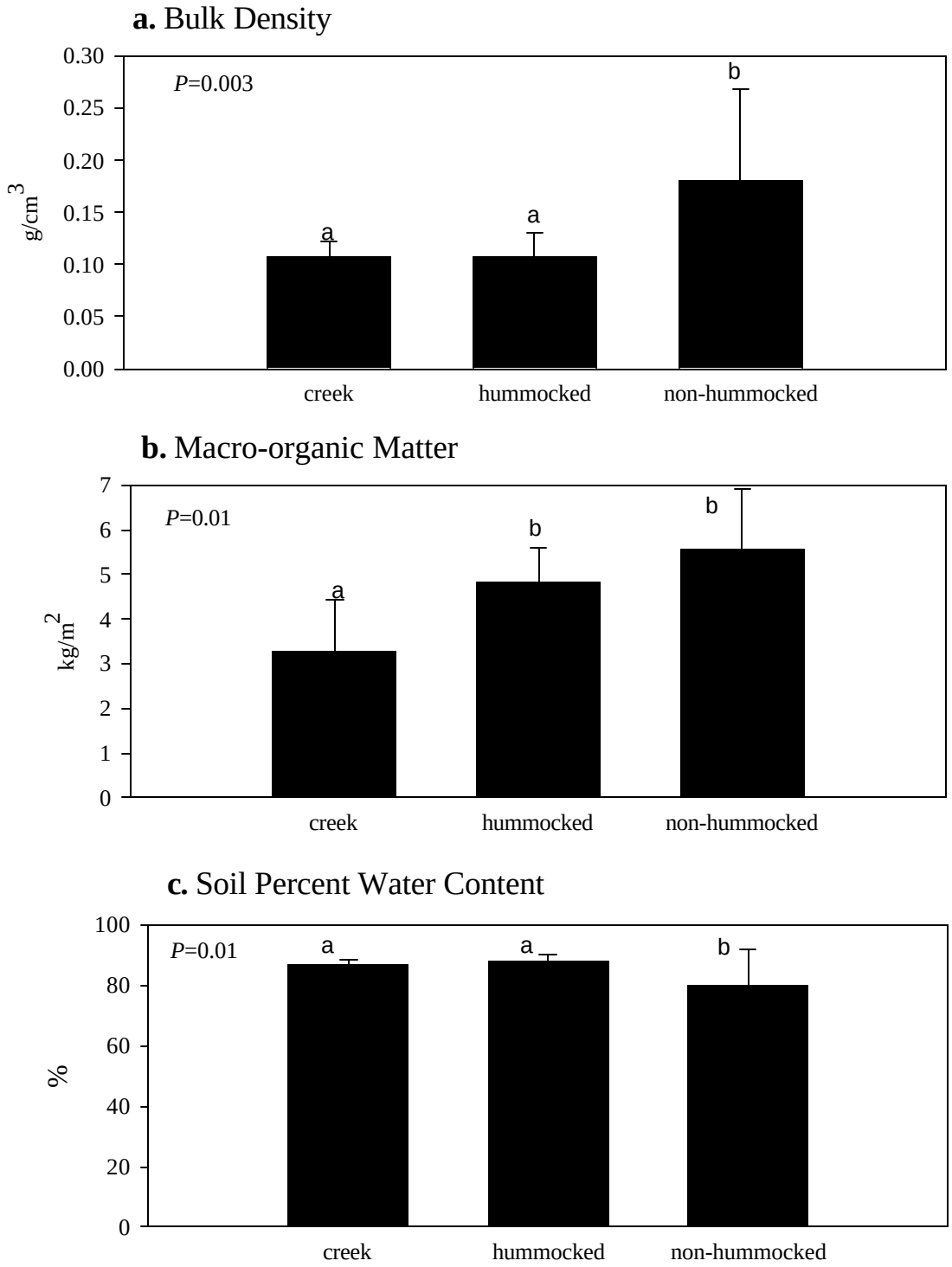


Figure 9. Mean values and standard errors of soil bulk density (a), macro-organic matter (b) and percent water content (c) for differences between states of change. *P*-values are given in top left corner of graph. Same lettered means are not significantly different. Bars represent 1 standard error.

Site 4) (Fig. 10). The creek site had the lowest average water levels of 1997 (-12.3 cm). Water levels in 1998 showed a similar trend. Again, hummocked areas had the higher average water levels (-0.6 cm, Site 3; 6.0 cm, Site 5), than the non-hummocked areas (-3.0 cm, Site 2; -1.6 cm, Site 4). The creek site again had the lowest average water level (-9.0 cm). It should be noted that both the hummocked and non-hummocked areas had water levels above ground surface for much of the year in 1998 (Figs. 10, 11, 12). This, as well as variation in water levels between 1997 and 1998, can be attributed to the significant difference in precipitation between the two years (Fig. 13). The year 1997 was dry with 32.3 cm of rain recorded for the year, only 6.5 of which fell between the months of April and August. The year 1998 had three times that amount of precipitation with 106.9 cm recorded for the year, 58.1 cm of which fell between April and August (VCR-LTER http://www.vcrlter.virginia.edu). There was no significant effect of treatment (borders vs. controls) on water levels within or between the sites ($P=0.4038$) (Appendix F).

Repeated measures analysis of interstitial water salinity showed a significant difference for 1997 and 1998 data (Time $P=0.0001$; Site $P=0.0004$) (Fig. 14). Salinity was more variable within and between sites than water levels in that the sites did not group by state of change, as was evident with water levels. Salinity levels did however show similar patterns between the two years. Average salinity was greater in 1997 than 1998 for all sites, which again can be attributed to precipitation differences between years. Site 5 (hummocked) had the highest average salinity of the five sites for both years (24.4 ppt, 1997; 19.2 ppt, 1998), and was the least variable of the five sites. As

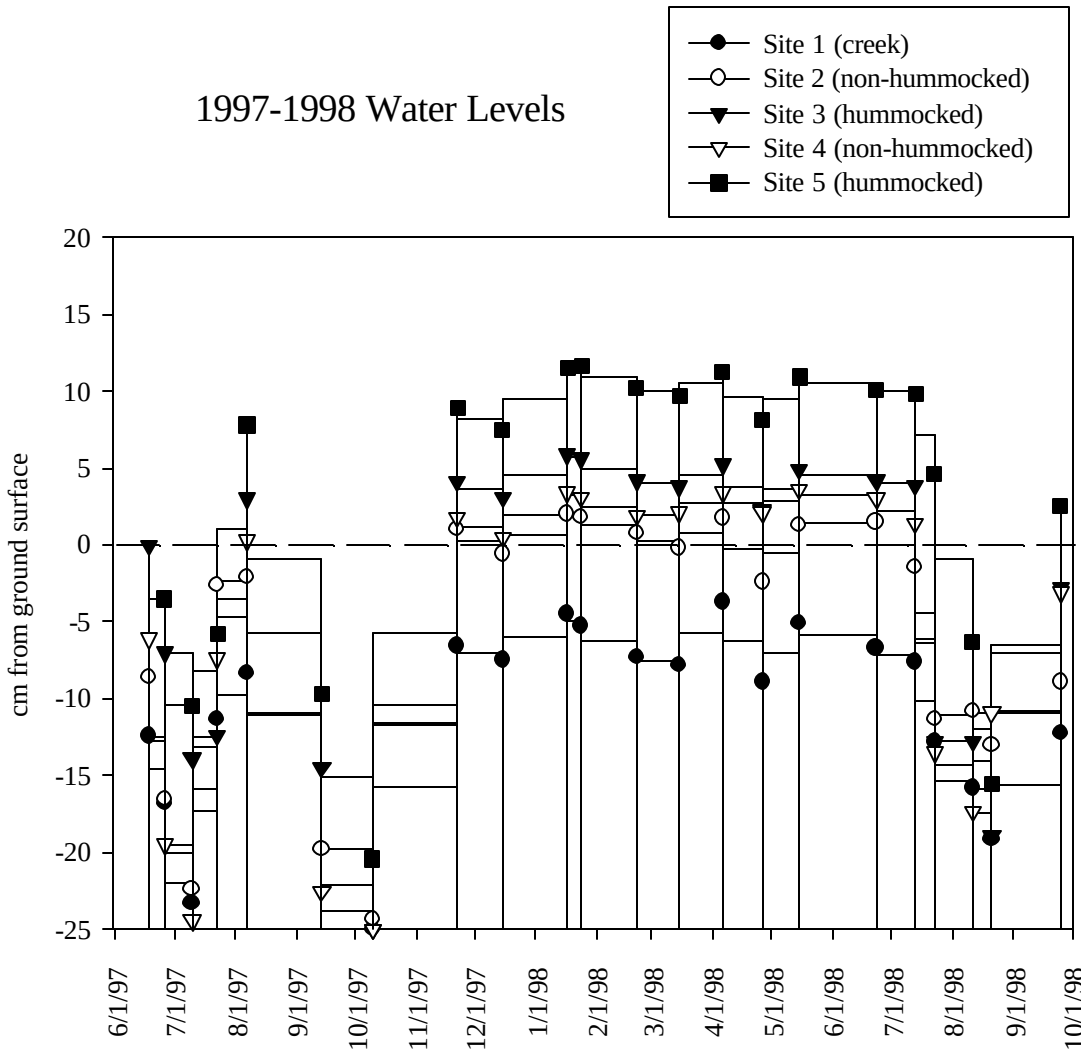


Figure 10. Average water levels for 1997 and 1998 for each site. Dashed line indicates ground surface. Water levels were recorded within individual plots and averaged to get a site mean. Drop lines indicate point in month in which measurements were taken.

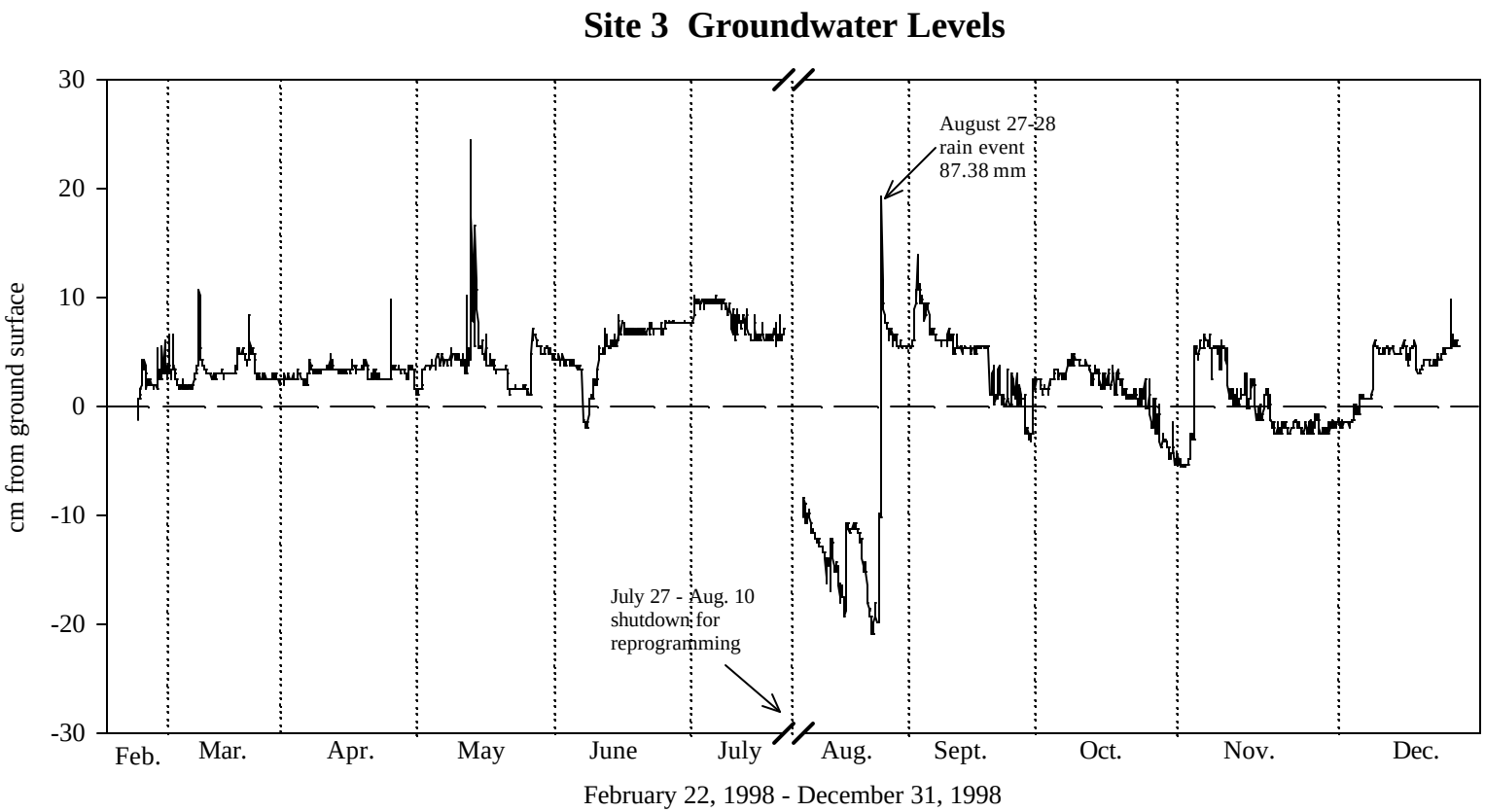


Figure 11. Water levels for 1998 as recorded at Site 3 by digital water level recorder. Dashed line indicates ground surface. Site 3 recordings began on February 22, 1998 and ended December 31, 1998. The break in recording on July 27 indicates a 15-day shutdown and reprogramming event. The sharp spike on August 27-28 indicates an 87.38 mm rainfall event over that two day period.

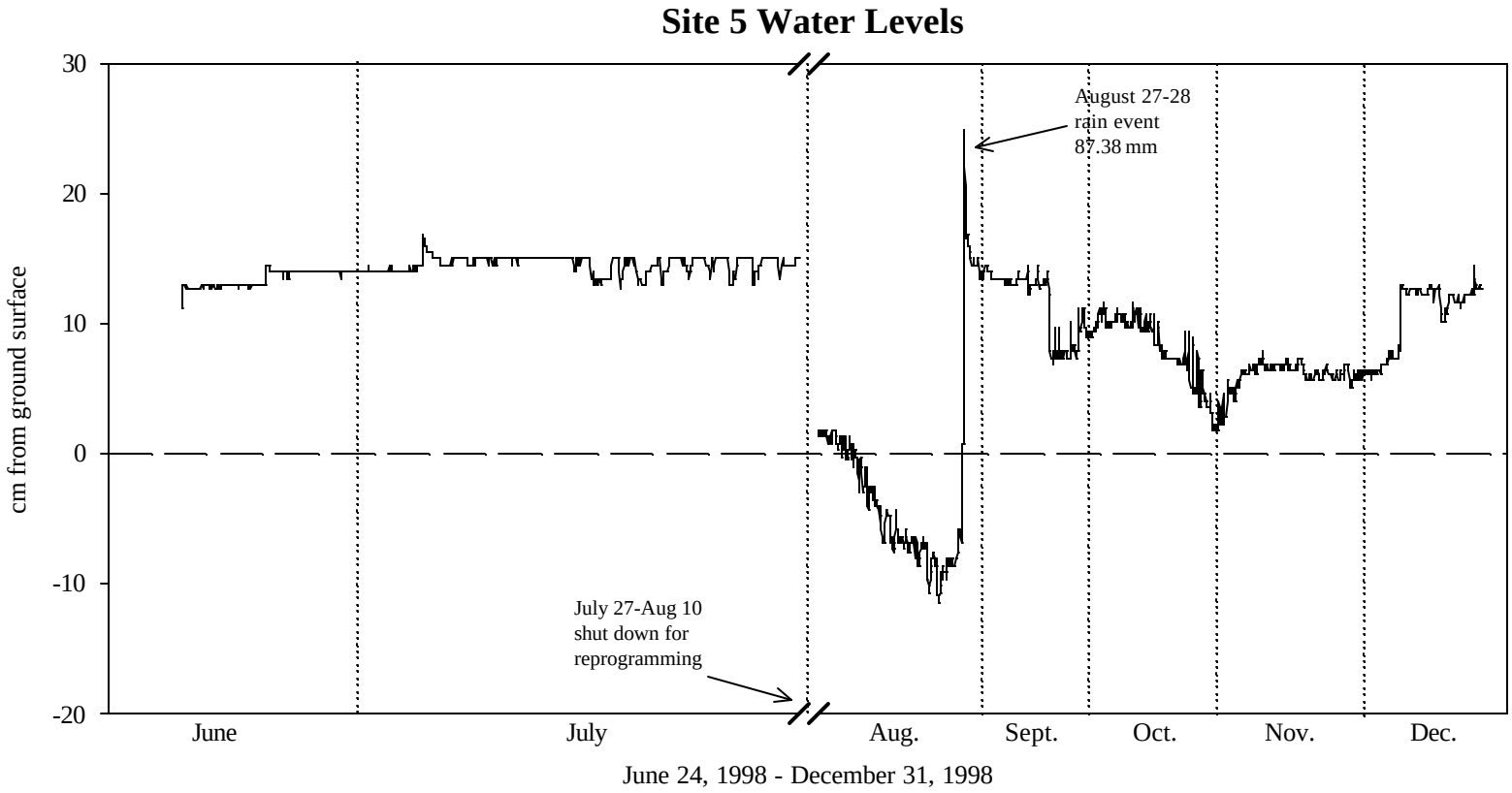


Figure 12. Water levels for 1998 as recorded at Site 5 by digital water level recorder. Dashed line indicates ground surface. Site 5 recordings began on June 24, 1998 and ended December 31, 1998. The break in recording on July 27 indicates a 15-day shutdown and reprogramming event. The sharp spike on August 27-28 indicates an 87.38 mm rainfall event over that two day period.

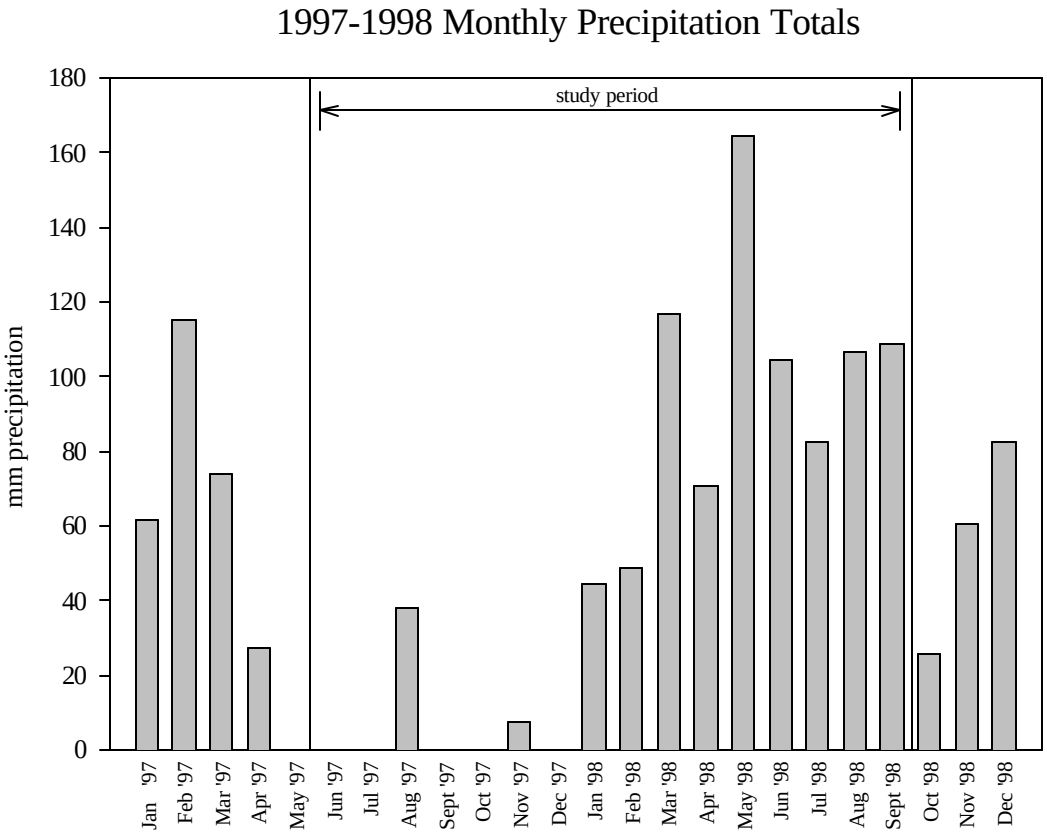


Figure 13. Monthly precipitation totals for January 1997 through December 1998. Precipitation data was collected at the Phillips Creek meteorological station (PHCK). Data provided by the Virginia Coast Reserve-Long Term Ecological Research program. Vertical lines indicate the beginning and end of the study period as determined by salinity and water levels.

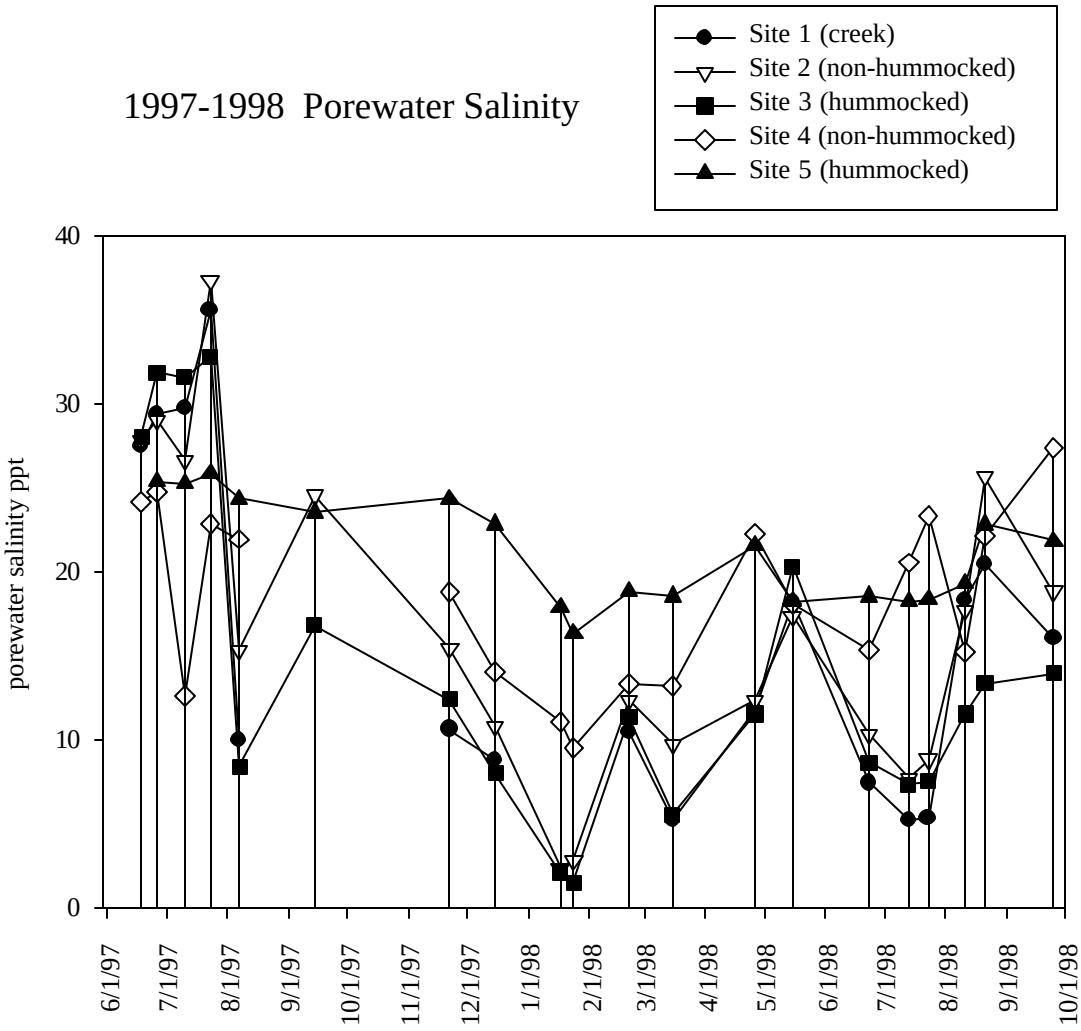


Figure 14. Average porewater salinity for 1997 and 1998 for each site. Salinity was determined for each plot and averaged to get a site mean. Drop lines indicate point in month at which measurement was taken.

shown in Figs. 11 and 13 this site also had the least variable water levels, with levels above ground surface for much of the study period. Sites 1 (creek) and 3 (hummocked) had the lowest average salinity of 1998 (9.8 and 9.5 ppt, respectively). The remaining two sites (non-hummocked) both had intermediate average salinities (12.1 ppt, Site 2; 17.6 ppt Site 4). There was no significant effect of treatment (borders vs. controls) on salinity levels within or between the sites ($P=0.7856$).

5.3 Species Composition and Percent Cover

Results of measurements of species composition and percent groundcover in 1997 indicate the dominance of *S. patens* and *D. spicata* within the three areas of the high marsh community studied, with no other species recorded within the experimental plots in 1997 (Fig. 15). Sites 2 and 4 (non-hummocked) had greater than 50% of the groundcover as the vegetation class *S. patens* > *D. spicata*, with that class occupying 75% and 84% of those sites, respectively (Fig. 15). The remaining area of those sites were covered by the *D. spicata* > *S. patens* vegetation class (25% for Site 2, and 16% for Site 4). Sites 1 (creek) and 3 (hummocked) had greater than 50% of measured groundcover as the vegetation class *D. spicata* > *S. patens*, with that class occupying 79% and 69% of those sites, respectively (Fig. 15). The remaining 21% of Site 1 was covered by *S. patens* > *D. spicata.* The remaining 31% of Site 3 was covered by 3 different classes, 27% *S. patens* >*D. spicata*, 3% bare area, and 1% *D. spicata* only. Site 5 (hummocked) had 50% of its groundcover as monospecific *D. spicata* (Fig. 15). Twenty-four percent of the site

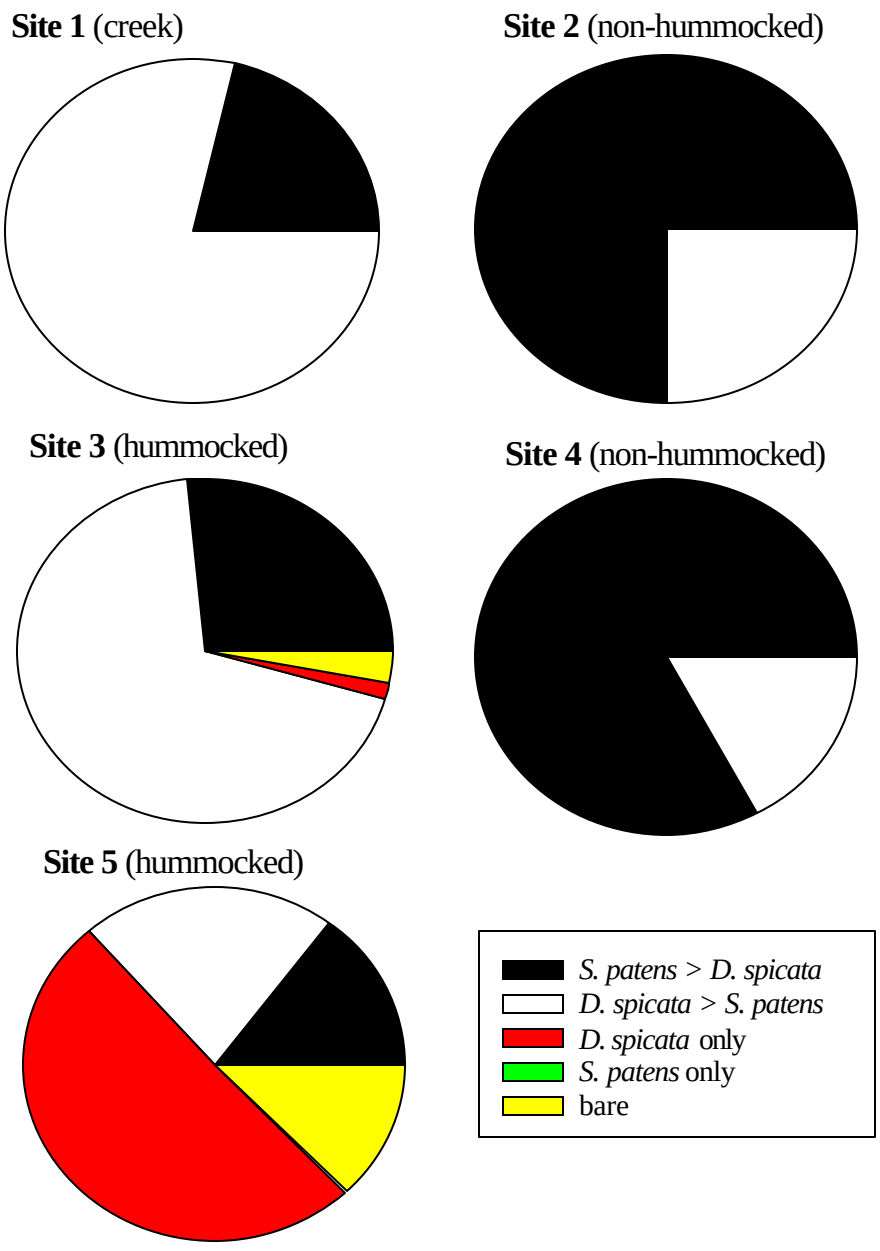


Figure 15. Percent cover and species composition in 1997 at experimental sites.

was covered by *D. spicata* > *S. patens*, while 14% was covered by *S. patens* > *D. spicata* and 12% as bare area.

It should be noted that for 1997, no species other than *S. patens* and *D. spicata* were collected in end-of-year aboveground biomass for any of the sites. However, in 1998, four total species were collected in end-of-year biomass for Site 1, and three total species were collected in end-of-year biomass for Site 2. At Site 1, 128.48 g/m^2 of *Atriplex patula* L. and 29.12 g/m^2 of *Aster tenuifolius* L. were collected. At Site 2, 330.88 g/m^2 of *Fimbristylis spadicea* (L.) Vahl was collected.

5.4 End-of-year Aboveground Biomass

Total end-of-year aboveground biomass (*D. spicata* + *S. patens* , live + dead) within hummocked areas was significantly lower than the creek site for both 1997 (P=0.04) and 1998 (P=0.0001) and significantly lower than the non-hummocked sites in 1998 (Fig. 16a). The difference in total biomass between hummocked and non-hummocked areas was not significant (P>0.05) for 1997 (Fig. 16a). Differences in total biomass between the creek and non-hummocked areas were not significant (P>0.05) for either 1997 or 1998 (Fig. 16a).

Total live aboveground biomass (*D. spicata* + *S. patens*) was significantly greater in the creek site than the hummocked or non-hummocked sites for 1997 (P=0.0023) and 1998 (P=0.0001) (Fig. 16b). Non-hummocked total live biomass (*D. spicata* + *S. patens*) was greater than hummocked for both 1997 and 1998, however, the difference was not significant (P>0.05) in 1997. There was a significant increase (P=0.0001) in total live

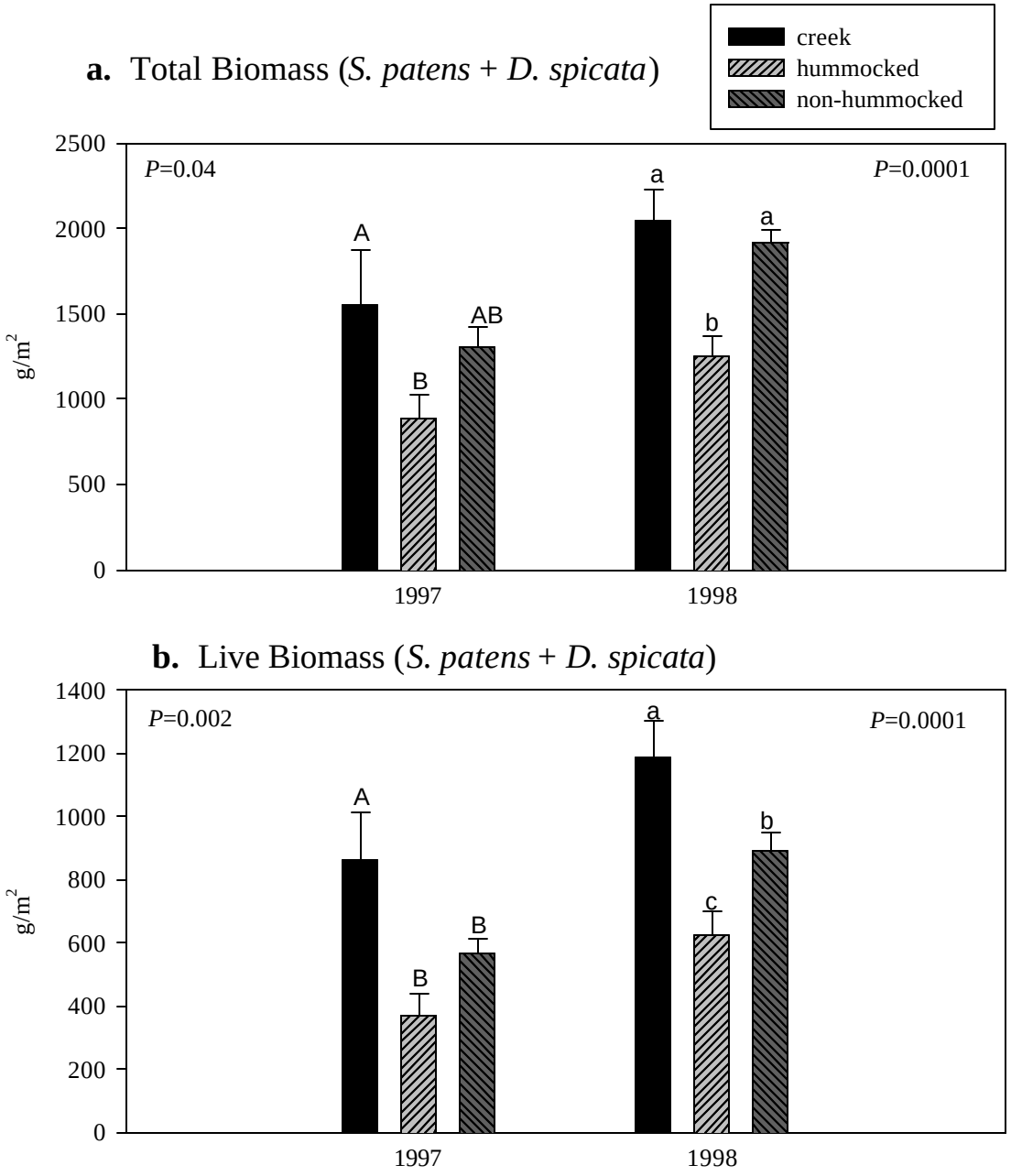


Figure 16. Total (a) and live (b) end-of-year aboveground biomass (*D. spicata* + *S. patens*) between states of change for 1997 and 1998 . *P*-values for 1997 are in upper left corner of graph and *P*-values for 1998 are in upper right. Same lettered means are not significantly different. Bars represent 1 standard error.

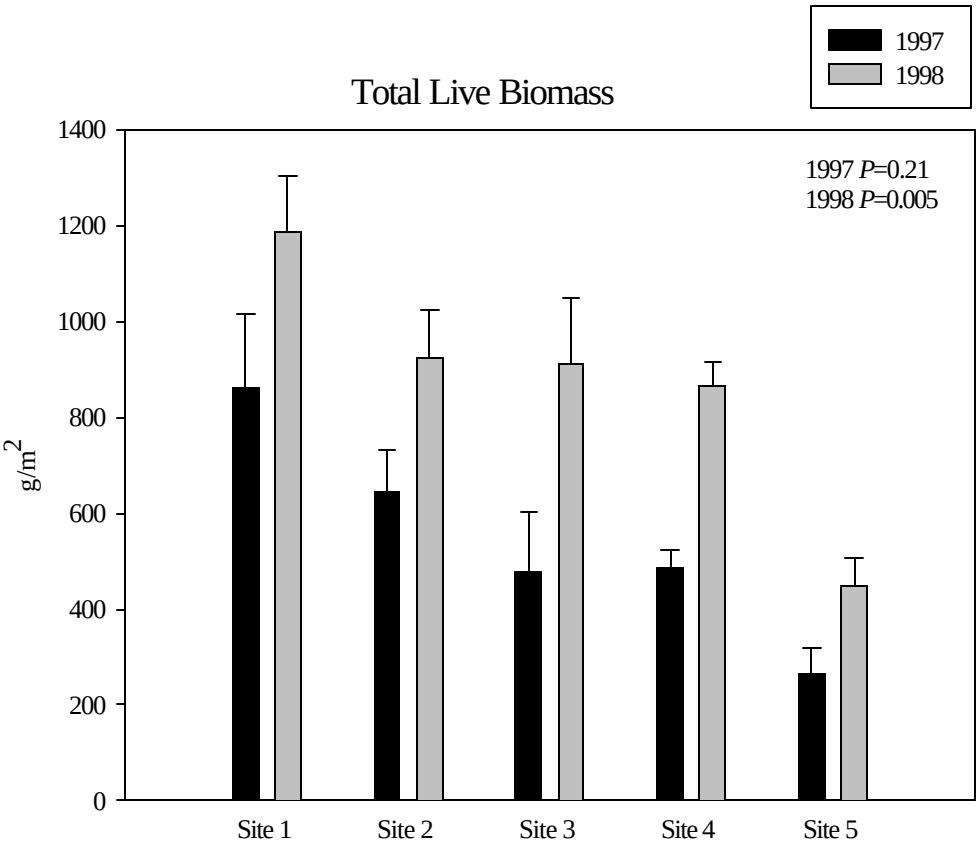


Figure 17. Total live biomass (*D. spicata* + *S. patens*) between sites for 1997 and 1998. Site 1 = creek, Sites 2,4 = non-hummocked, Sites 3,5 = hummocked. *P*-values for analysis by year given in upper right corner of graph. Bars represent 1 standard error.

biomass from 1997 to 1998 within all sites (Fig. 17). This increase may be attributed to the difference in rainfall between the two years (Fig. 13).

Ratios of total biomass (live + dead leaves & culms) *D. spicata* to *S. patens* showed a significantly greater proportion of *S. patens* to *D. spicata* in 1997 ($P=0.003$) and 1998 ($P=0.0009$) in non-hummocked areas as compared to both creek and hummocked areas (Fig. 18a). Ratios of *D. spicata* to *S. patens* between creek and hummocked areas were not statistically different for either 1997 or 1998 ($P>0.05$). Comparison of the ratio of live *D. spicata* to live *S. patens* between areas shows that for both 1997 ($P=0.0248$) and 1998 ($P=0.0045$) hummocked areas had a significantly greater ratio of *D. spicata* to *S. patens* than non-hummocked areas (Fig. 18b). Hummocked areas had proportionately more *D. spicata* biomass than *S. patens* biomass for both years, while non-hummocked areas had proportionately more *S. patens* biomass than *D. spicata* biomass. The ratio of *D. spicata* to *S. patens* for the creek site was not significantly different from either the hummocked or non-hummocked sites in 1997, however, in 1998 the creek site was significantly different from the non-hummocked sites. In 1997, the creek site had a greater proportion of both live and total *S. patens* to *D. spicata*, while in 1998 there was a greater proportion of both live and total *D. spicata* to *S. patens* (Fig. 18a & b).

Analysis of 1998 biomass within experimental treatment sites for effects of flooding showed no significant treatment effects on live or total biomass for *D. spicata* (Fig. 19). There was, however, a significant treatment effect ($P=0.0266$) for *S. patens* live biomass (Fig. 19a). Comparison of biomass within treatment sites showed that

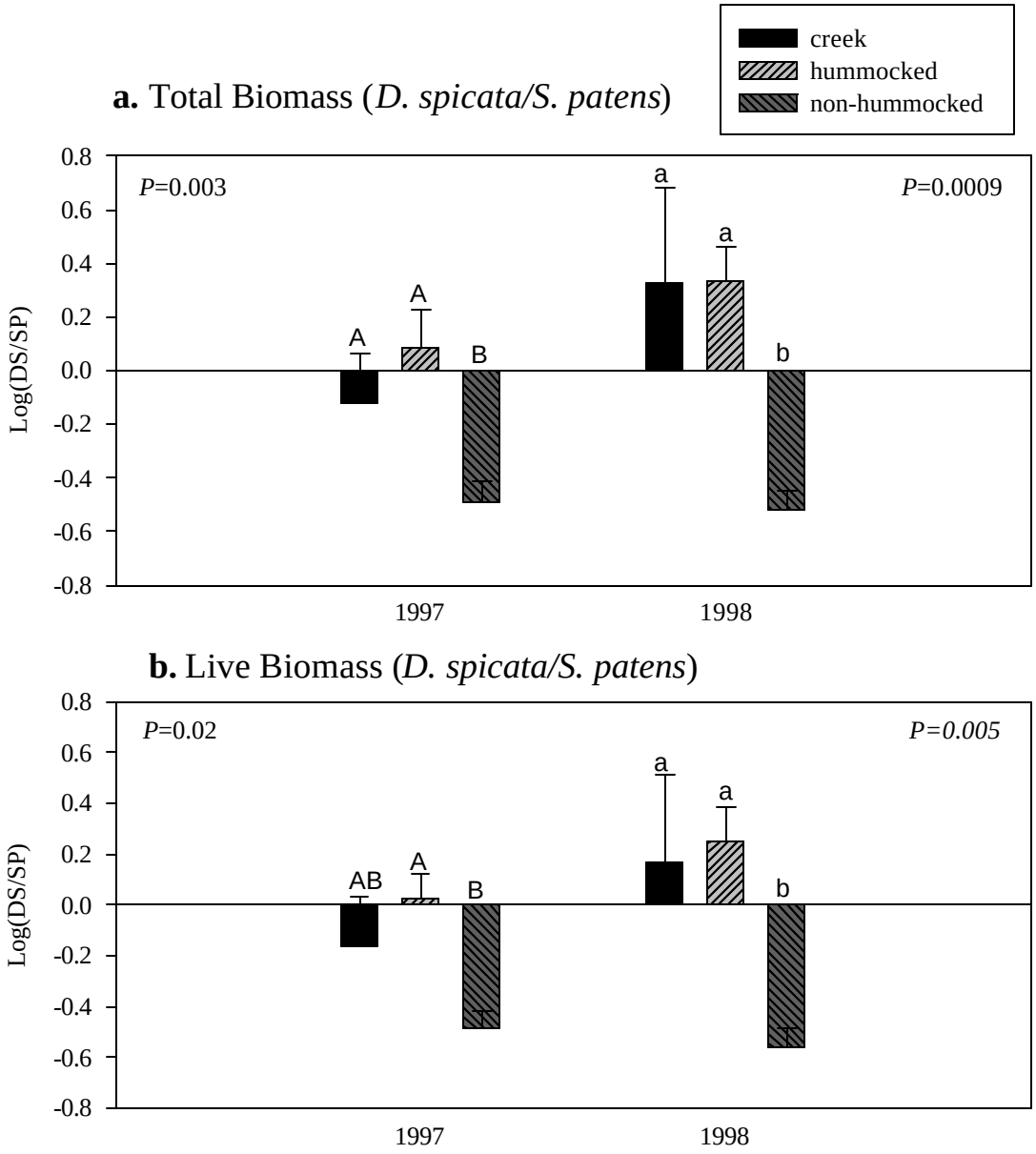


Figure 18. Log transformed ratios of *D. spicata* to *S. patens* total (a) and live (b) end-of-year aboveground biomass for 1997 and 1998. *P*-values for 1997 are in upper left corner of graph and *P*-values for 1998 are in upper right. Same lettered means are not significantly different. Positive values indicate proportionately more *D. spicata*, while negative values indicate proportionately more *S. patens.* Bars represent 1 standard error.

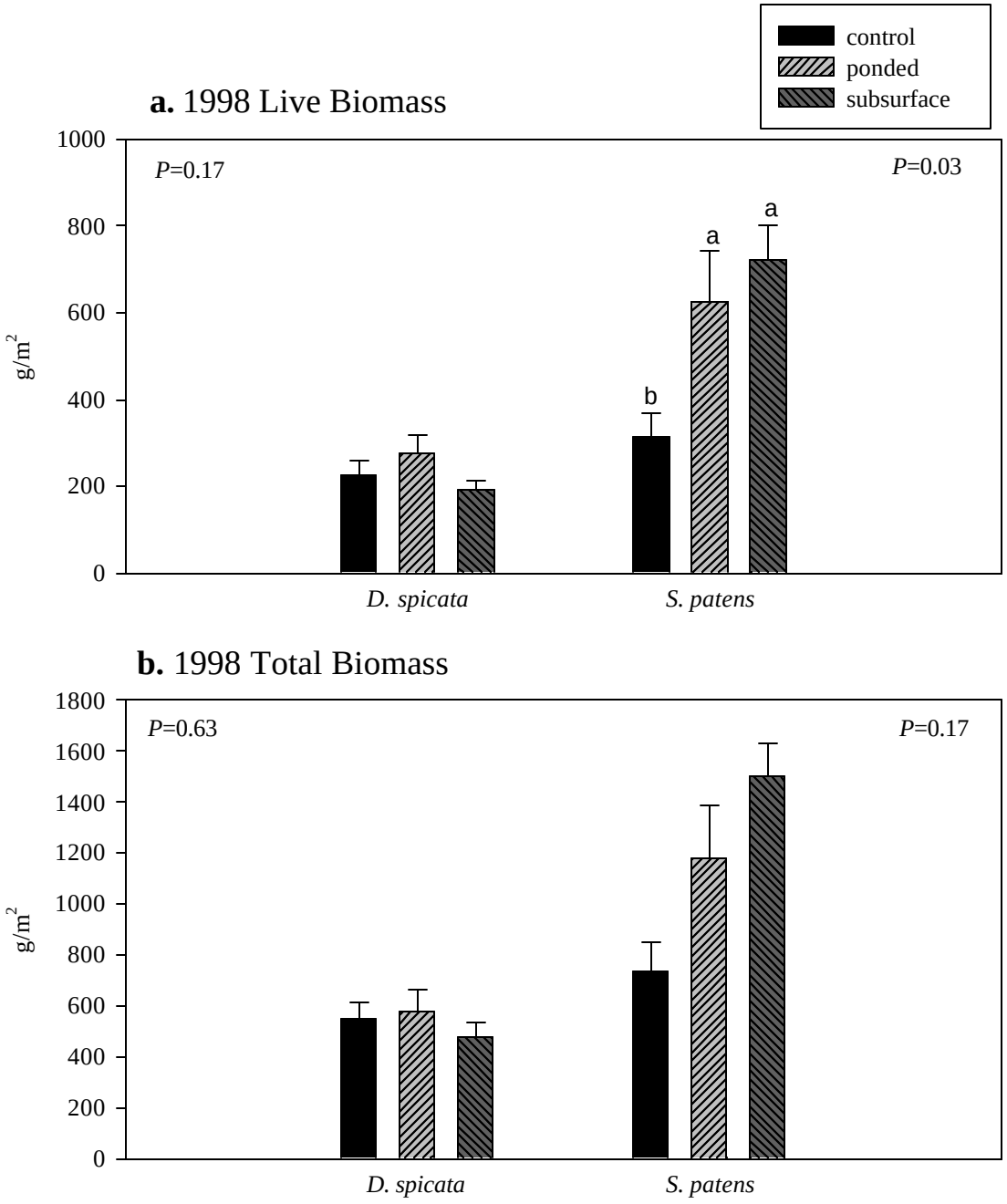


Figure 19. Live (a) and total (b) end-of-year aboveground biomass for individual species between treatments for 1998. *D. spicata P*-values are in upper left corner of graph and *S. patens P*-values are in upper right. Same lettered means are not significantly different. Bars represent 1 standard error.

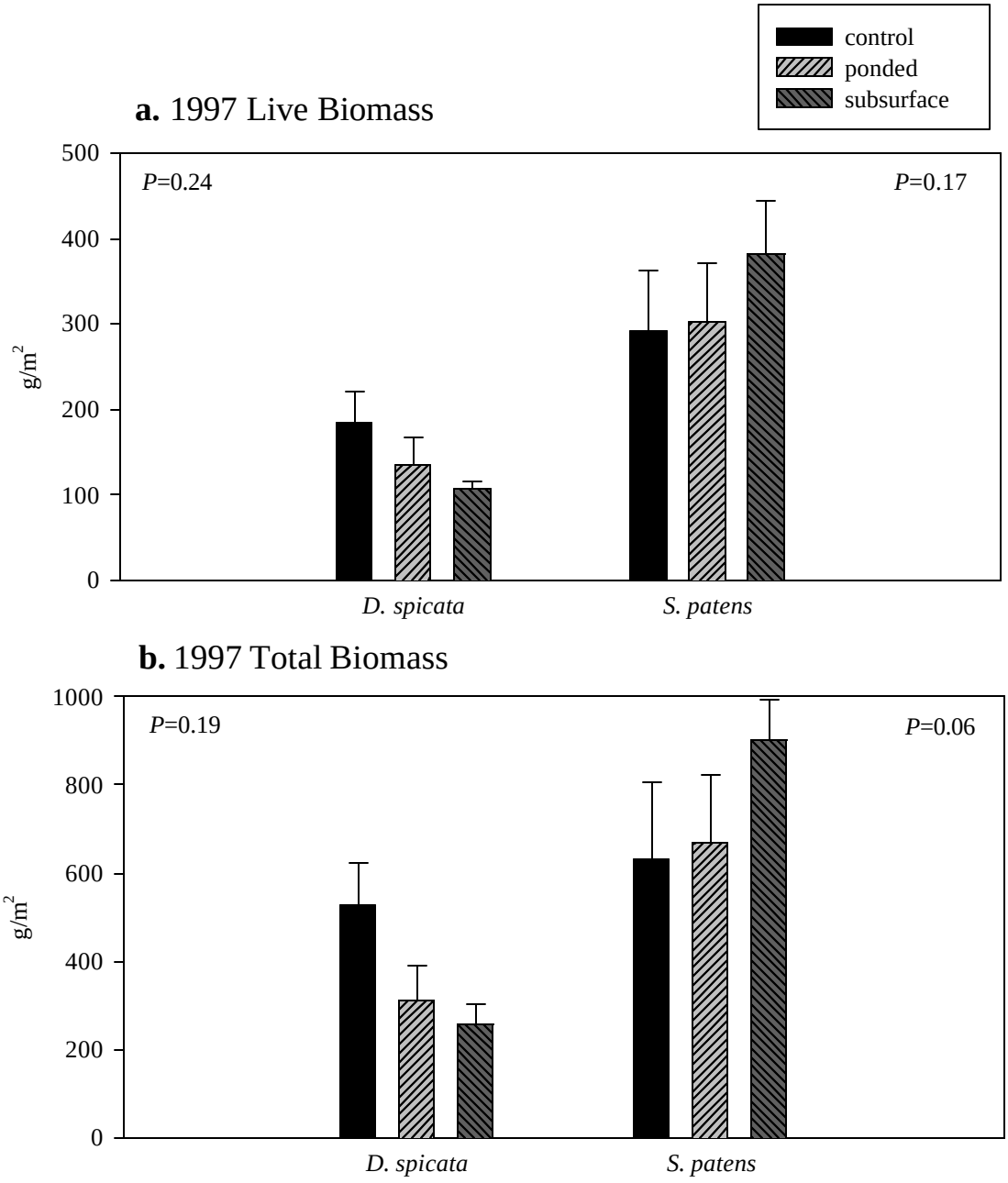


Figure 20. Live (a) and total (b) end-of-year aboveground biomass for individual species between treatments for 1997. *D. spicata P*-values are in upper left corner of graph and *S. patens P*-values are in upper right. Bars represent 1 standard error.

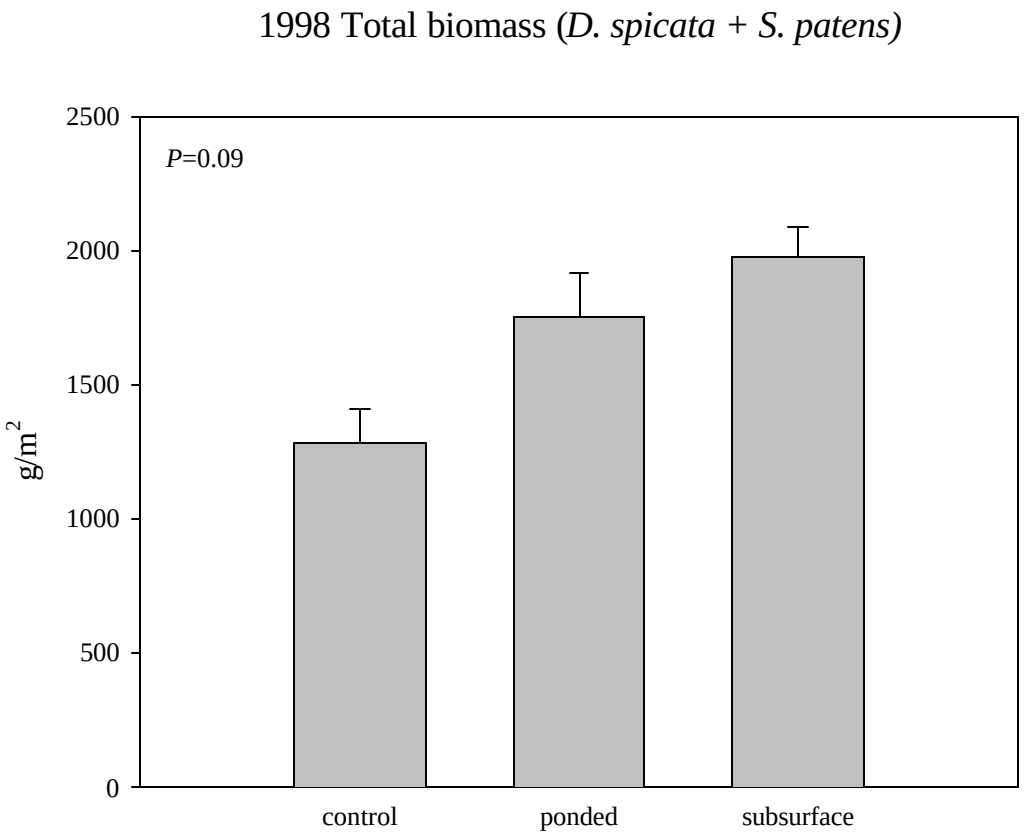


Figure 21. Total biomass (*D. spicata* + *S. patens*) between treatments for 1998. Bars represent 1 standard error.

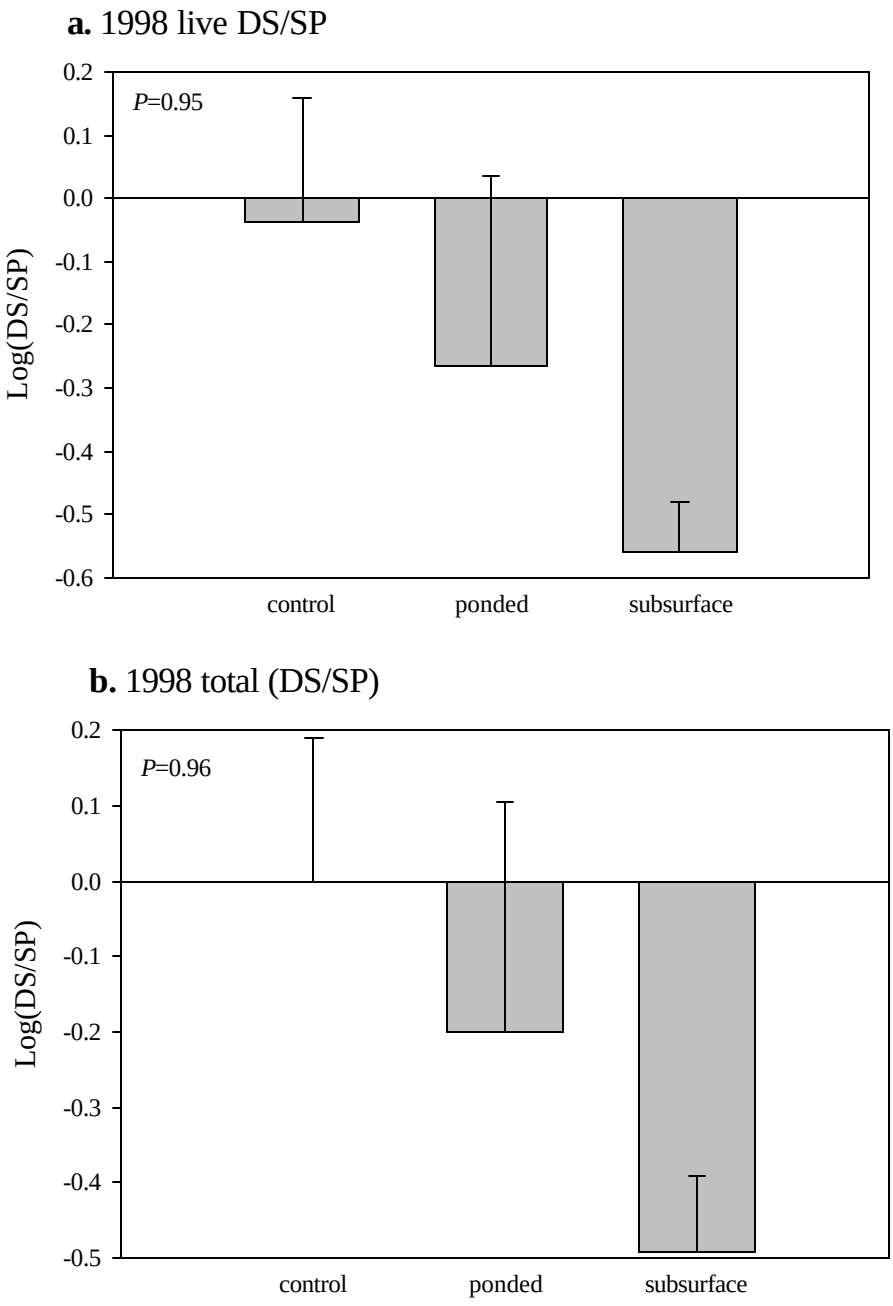


Figure 22. Log transformed ratios of *D. spicata* to *S. patens* live (a) and total (b) biomass between treatments for 1998. Bars represent 1 standard error.

CONTROL plots had significantly lower live *S. patens* biomass than either PONDED or SUBSURFACE control plots (Fig. 19). However, this same trend can be seen in the 1997 biomass data although the effect is not significant (live *S. patens* $P=0.1717$, total *S. patens* $P=0.0585$) (Fig. 20). In 1998, total biomass (*D. spicata* + *S. patens*) shows the same trend ($P=0.0854$) with CONTROL plots having lower biomass than the PONDED or SUBSURFACE plots (Fig. 21). Ratios of *D. spicata* to *S. patens* within treatment sites indicate no significant treatment effects (live $P=0.9459$, total $P=0.9625$) (Fig. 22).

5.5 Adenylates

Analysis of adenine nucleotides showed significant variation between states of change within the marsh for both species, as well as significant variation between species and season. Effects of experimental treatment on nucleotide concentrations were not as apparent. Summer averages showed a significant seasonal effect with a dramatic decrease in AEC, ATP and TAN concentrations for August and September as compared to June and July ($P<0.05$) for both species (Fig. 23). Secondly, there was a significant species effect for both month and season; *D. spicata* had significantly higher average AEC, ATP, and TAN concentrations than *S. patens* for each month.

Differences in ATP concentrations between states varied between species. *S. patens* showed significant differences between states for June, July and August (Fig. 24), while *D. spicata* showed significant differences for July and September only (Fig. 24). For June, July and August, *S. patens* ATP concentrations in non-hummocked areas were significantly lower ($P<0.05$) than in hummocked areas. This same trend was found for

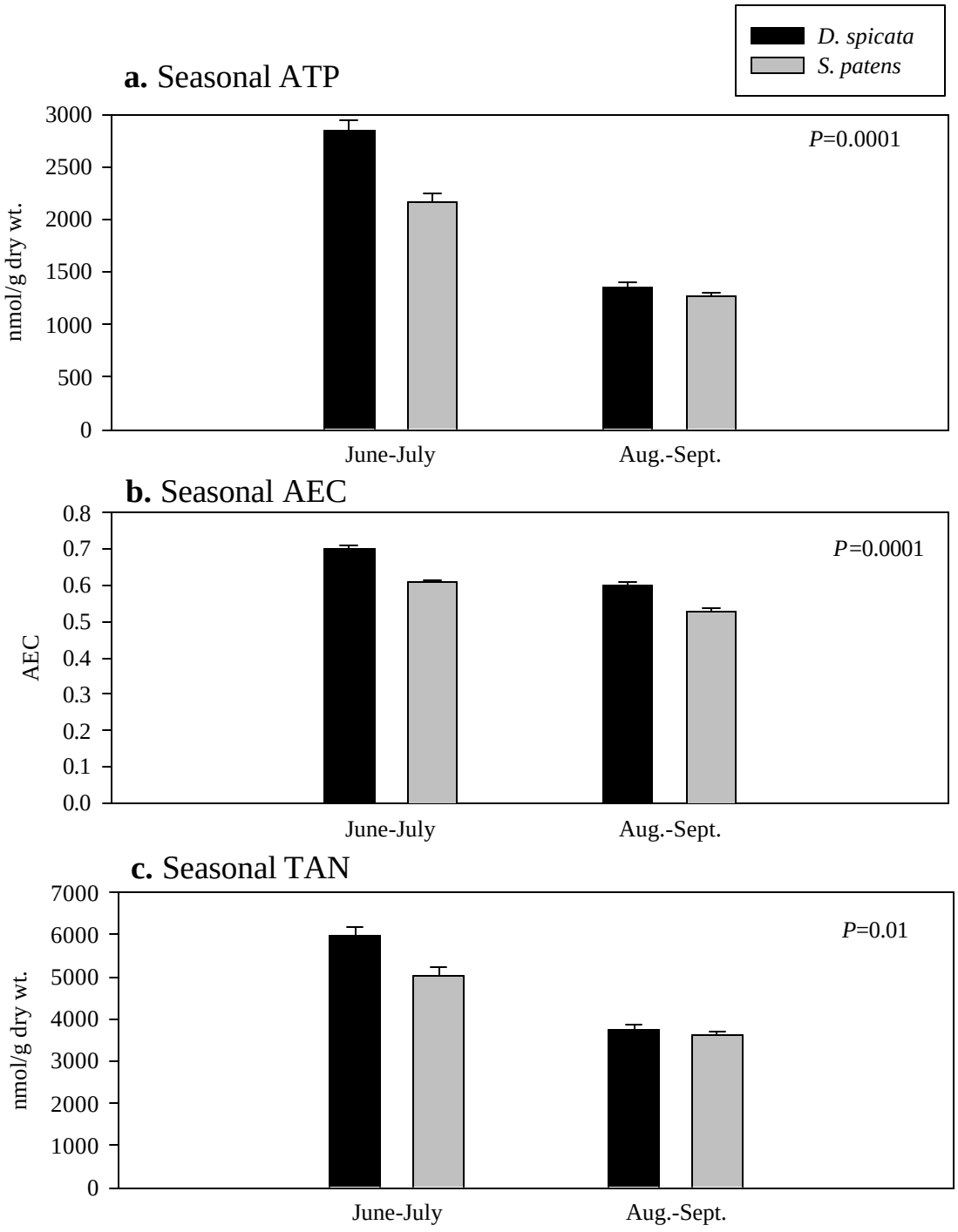


Figure 23. Seasonal differences in ATP (a), AEC (b), and TAN (c) concentrations for each species. *P*-values indicate significance between early summer and late summer adenylate concentrations for both species. Bars represent 1 standard error.

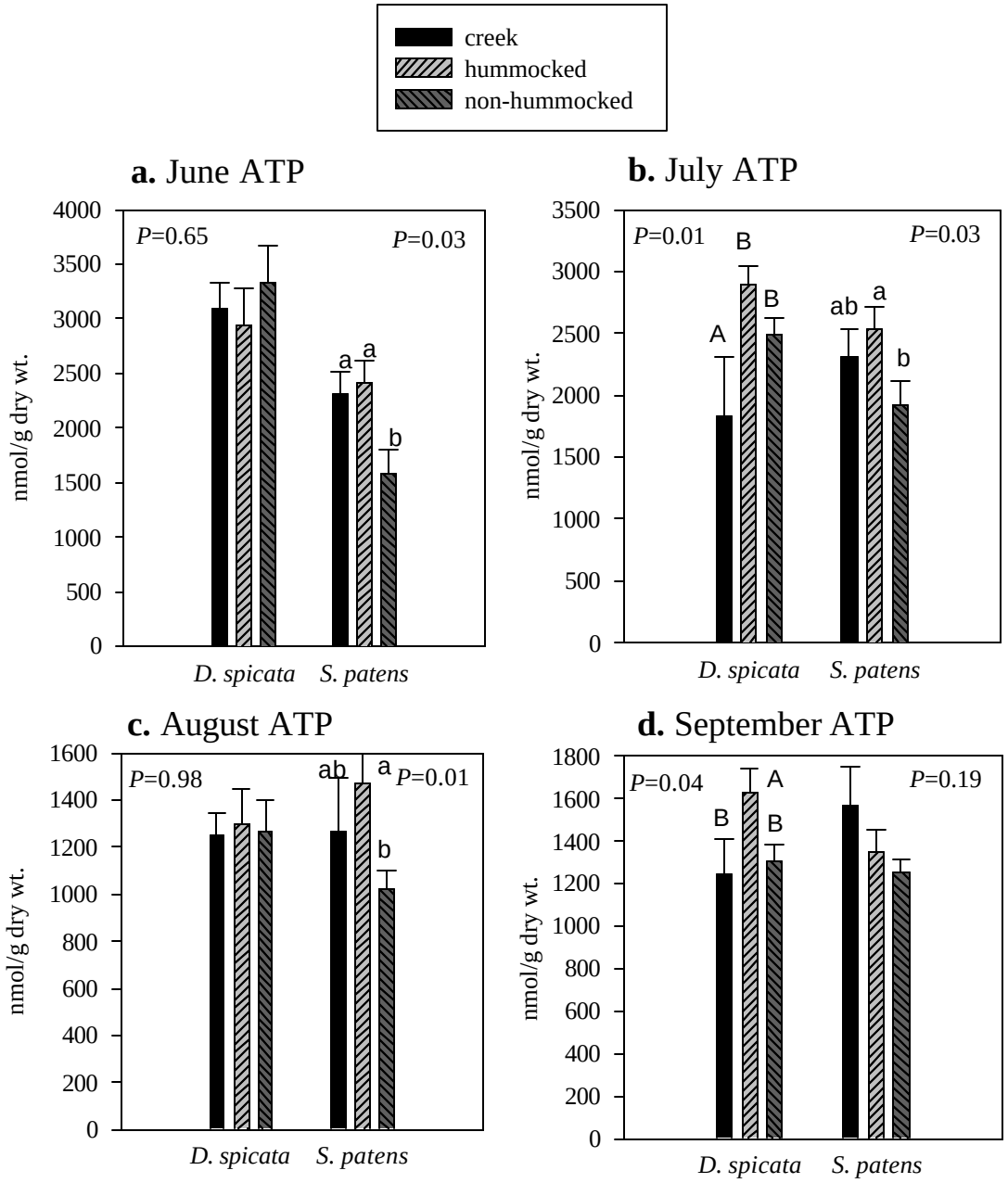


Figure 24. *D. spicata* and *S. patens* ATP concentrations between states of change for June (a), July (b), August (c), and September (d) 1998. *D. spicata P*-values are in upper left corner of graph and *S. patens P*-values are in upper right. Same lettered means are not significantly different. Bars represent 1 standard error.

September, however, the effect was not significant. It should be noted that the hummocked areas had higher ATP concentrations than the creek site also for those same months, although the difference was not significant at the 0.05 level.

For *D. spicata*, the results were more variable. Both July and September ATP concentrations were significantly higher in hummocked areas than in the creek site. ATP concentrations in hummocked areas also were higher than in non-hummocked areas, however, the difference was only significant for September. *D. spicata* ATP concentrations for June and August showed no significant difference between the three areas.

AEC ratios for both *D. spicata* and *S. patens* showed very little response to differences between states of change during the study period, June through September. *D. spicata* showed no significant monthly effects for the AEC ratio among states of change (Fig. 25). *S. patens* showed significant effects between areas for AEC in August and September only (Fig. 25). *S. patens* AEC ratios in August showed significantly higher ratios in hummocked areas than in non-hummocked areas. Creek AEC ratios in August were lowest, however the difference was not significant from hummocked or non-hummocked areas. September AEC ratios showed a different trend, with the creek site having significantly higher AEC than hummocked or non-hummocked sites.

The relationship between TAN (total adenine nucleotide) concentrations and areas of state change varied between months as did ATP concentrations and AEC ratios for both species. *S. patens* showed significant effects among areas for TAN concentrations in June and July, while *D. spicata* showed significant differences among areas in TAN

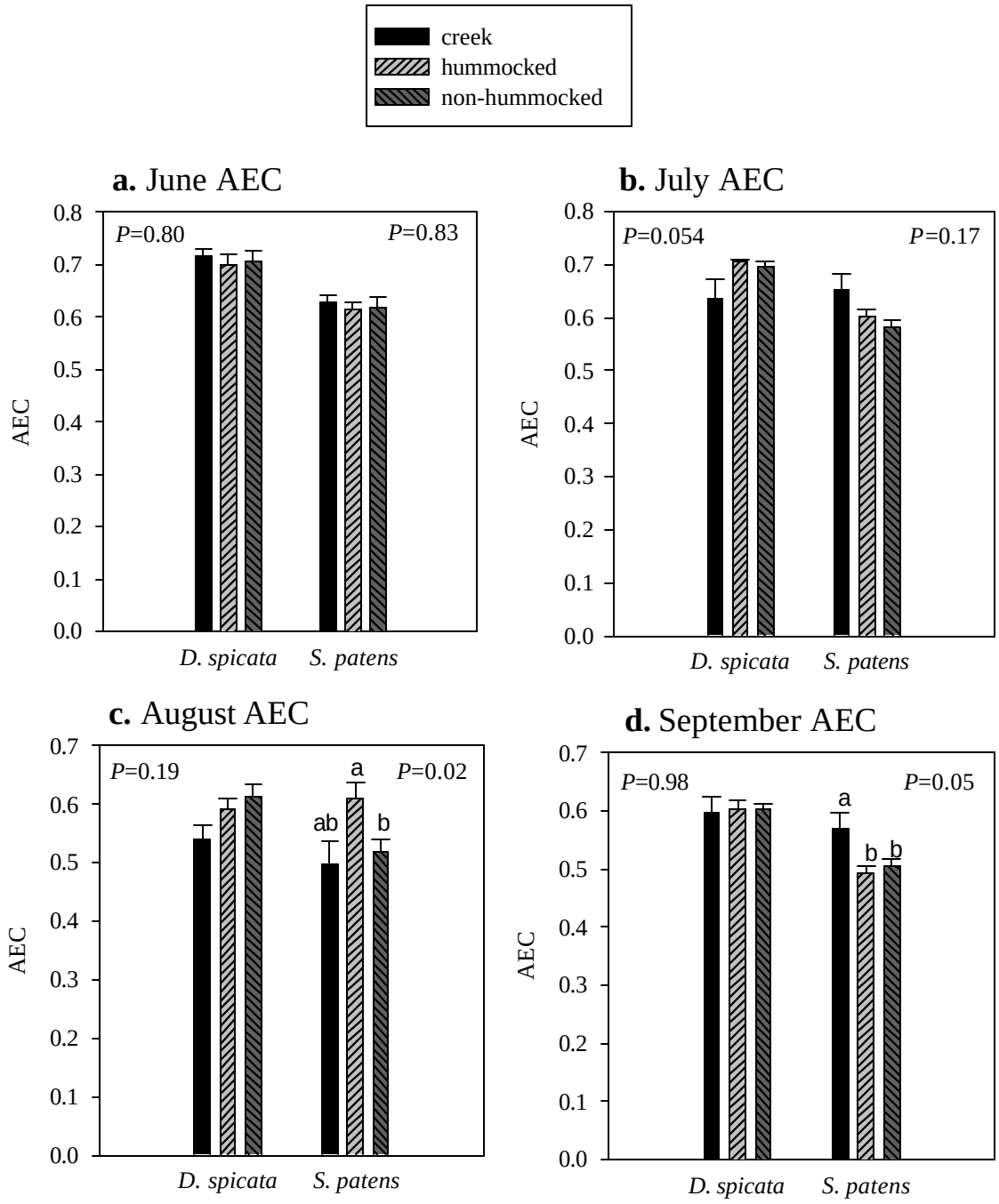


Figure 25. *D. spicata* and *S. patens* AEC ratios between states of change for June (a), July (b), August (c), and September (d) 1998. *D. spicata P*-values are in upper left corner of graph and *S. patens P*-values are in upper right. Same lettered means are not significantly different. Bars represent 1 standard error.

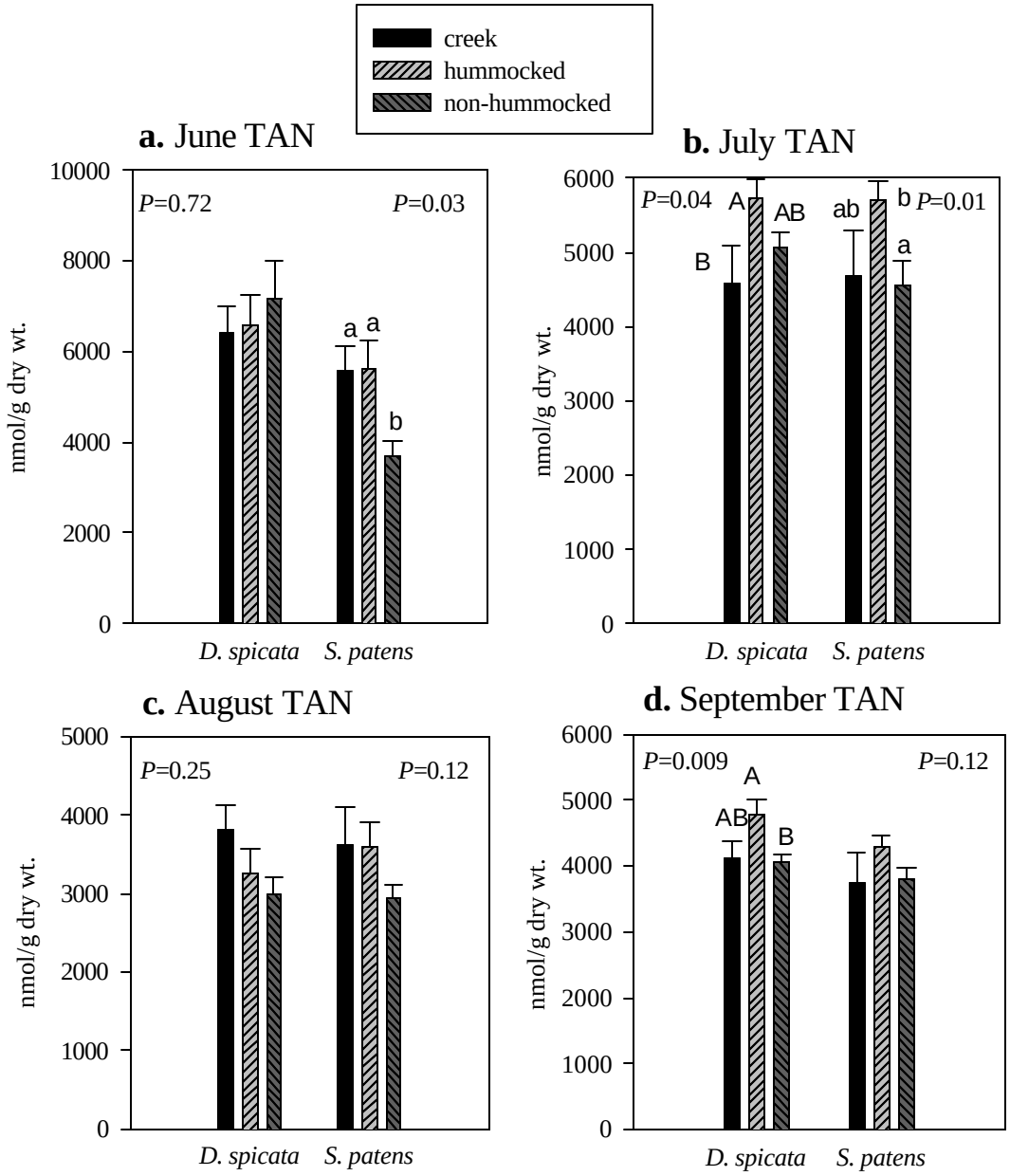


Figure 26. *D. spicata* and *S. patens* TAN concentrations between states of change for June (a), July (b), August (c), and September (d) 1998. *D. spicata* *P*-values are in upper left corner of graph and *S. patens* *P*-values are in upper right. Same lettered means are not significantly different. Bars represent 1 standard error.

concentrations for July and September (Fig. 26). For *S. patens*, TAN concentrations in June were significantly lower in non-hummocked areas than the hummocked or creek areas (Fig. 26). *S. patens* TAN concentrations for July were still significantly lower in non-hummocked areas compared to hummocked areas, however, while still intermediate, the creek site was not significantly different from either of the other two states. *D. spicata* TAN concentrations in July showed hummocked areas having higher concentrations than either the creek or non-hummocked areas. This difference was only significant, however, between the hummocked and creek areas. The same trend was evident for September, however, the significant difference was between the hummocked and non-hummocked areas.

Only July showed significant treatment effects in adenine nucleotide variables (Fig. 27-29). ATP concentrations in July were significantly lower in PONDED plots than CONTROL or SUBSURFACE plots for *S. patens* (Fig. 27a). *D. spicata* showed no significant difference in ATP concentrations between treatments (Fig. 27). There were no significant treatment effects on AEC ratio for either species (Fig. 28). Both species showed a significant treatment effect for TAN concentrations. *S. patens* had significantly lower TAN concentrations in PONDED plots than CONTROL or SUBSURFACE plots (Fig. 29a). *D. spicata* had significantly lower TAN concentrations in PONDED and SUBSURFACE as compared to the CONTROL plots (Fig. 29). There were no significant treatment effects for August or September.

An analysis of significant treatment results by site is shown in Figure 30. The interaction between site and treatment was not significant for either of the three

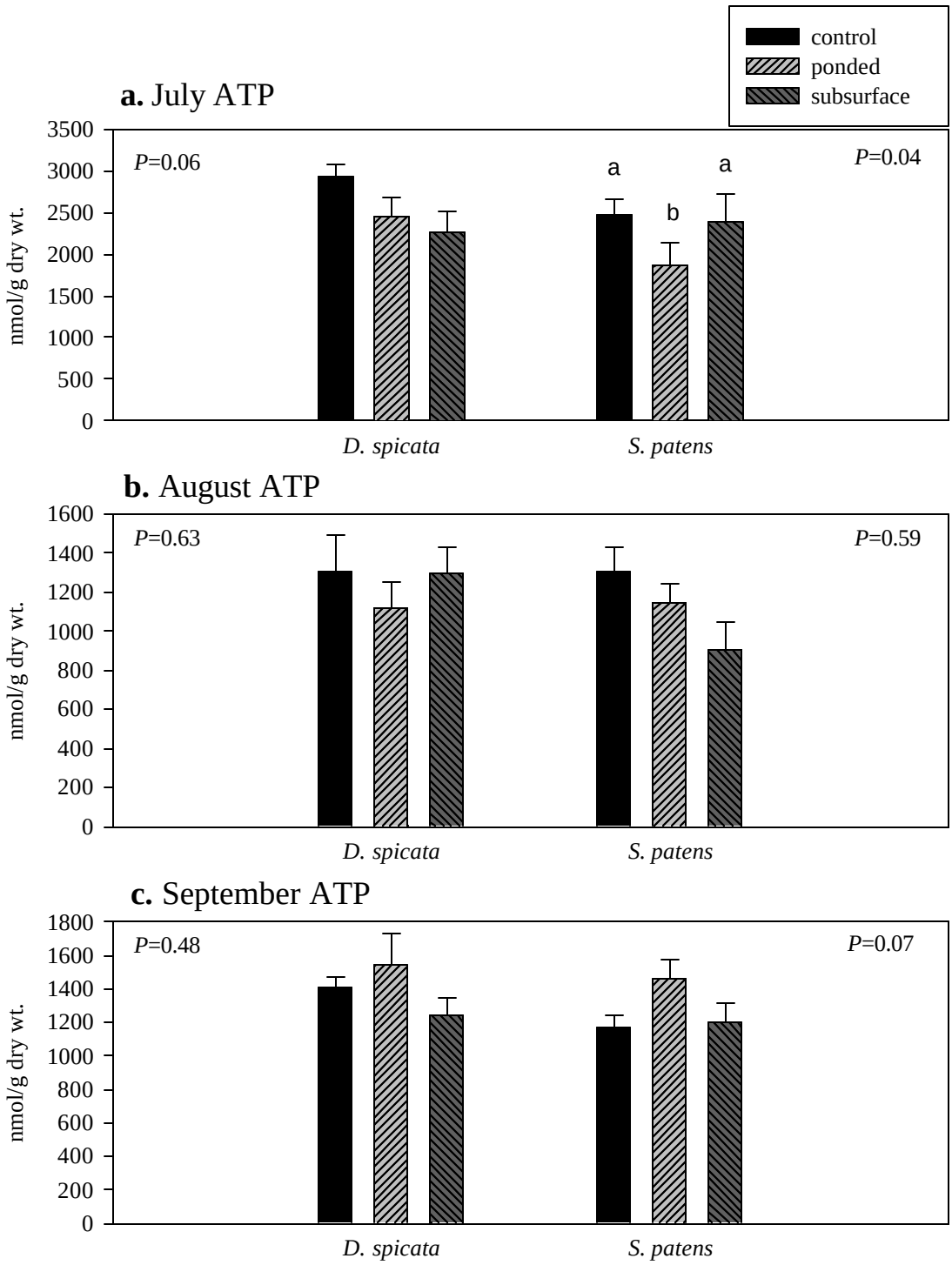


Figure 27. *D. spicata* and *S. patens* ATP concentrations between flooding treatments for July (a), August (b), and September (c) 1998. *D. spicata P*-values are in upper left corner of graph and *S. patens P*-values are in upper right. Same lettered means are not significantly different. Bars represent 1 standard error.

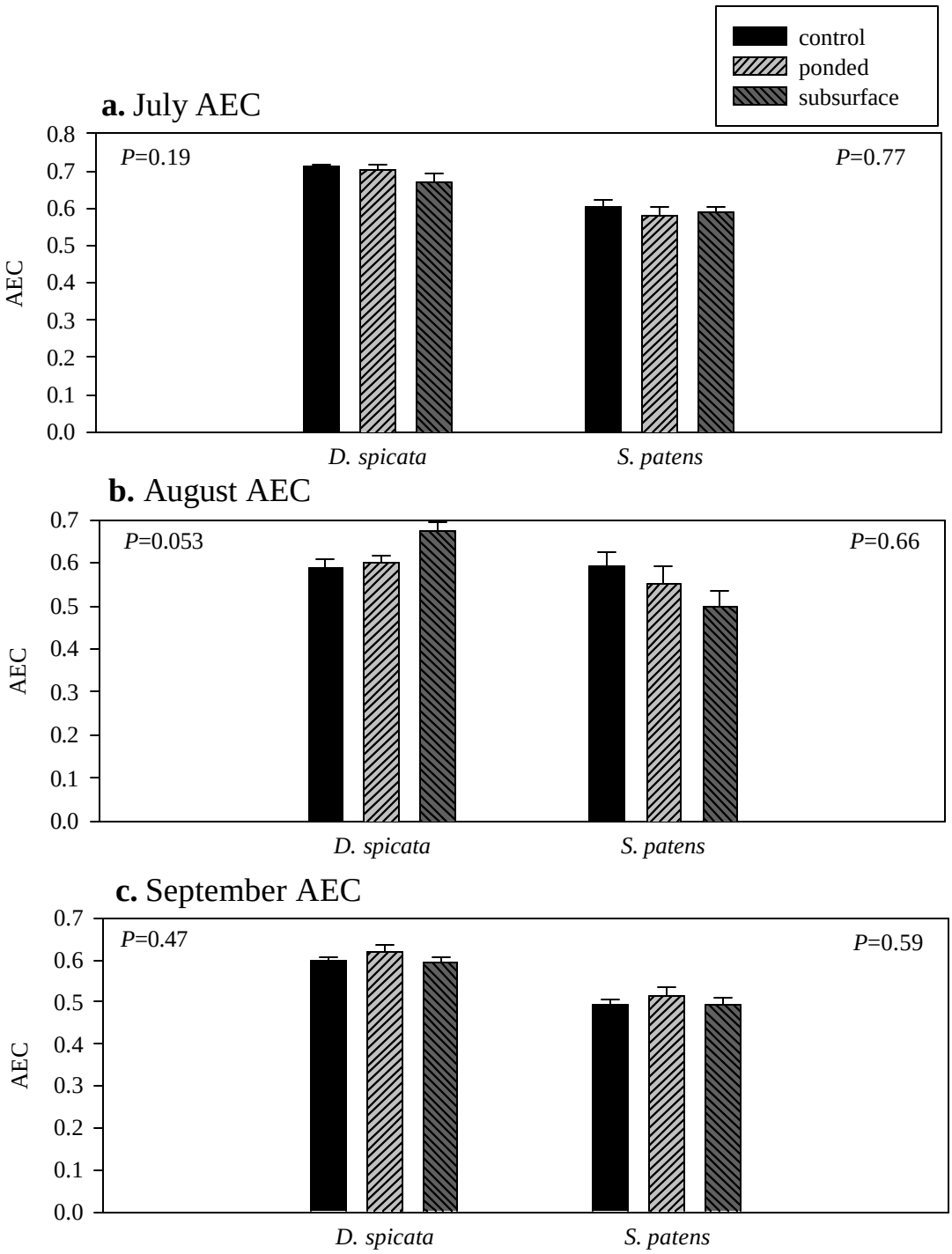


Figure 28. *D. spicata* and *S. patens* AEC ratios between flooding treatments for July (a), August (b), and September (c) 1998. *D. spicata P*-values are in upper left corner of graph and *S. patens P*-values are in upper right. Bars represent 1 standard error.

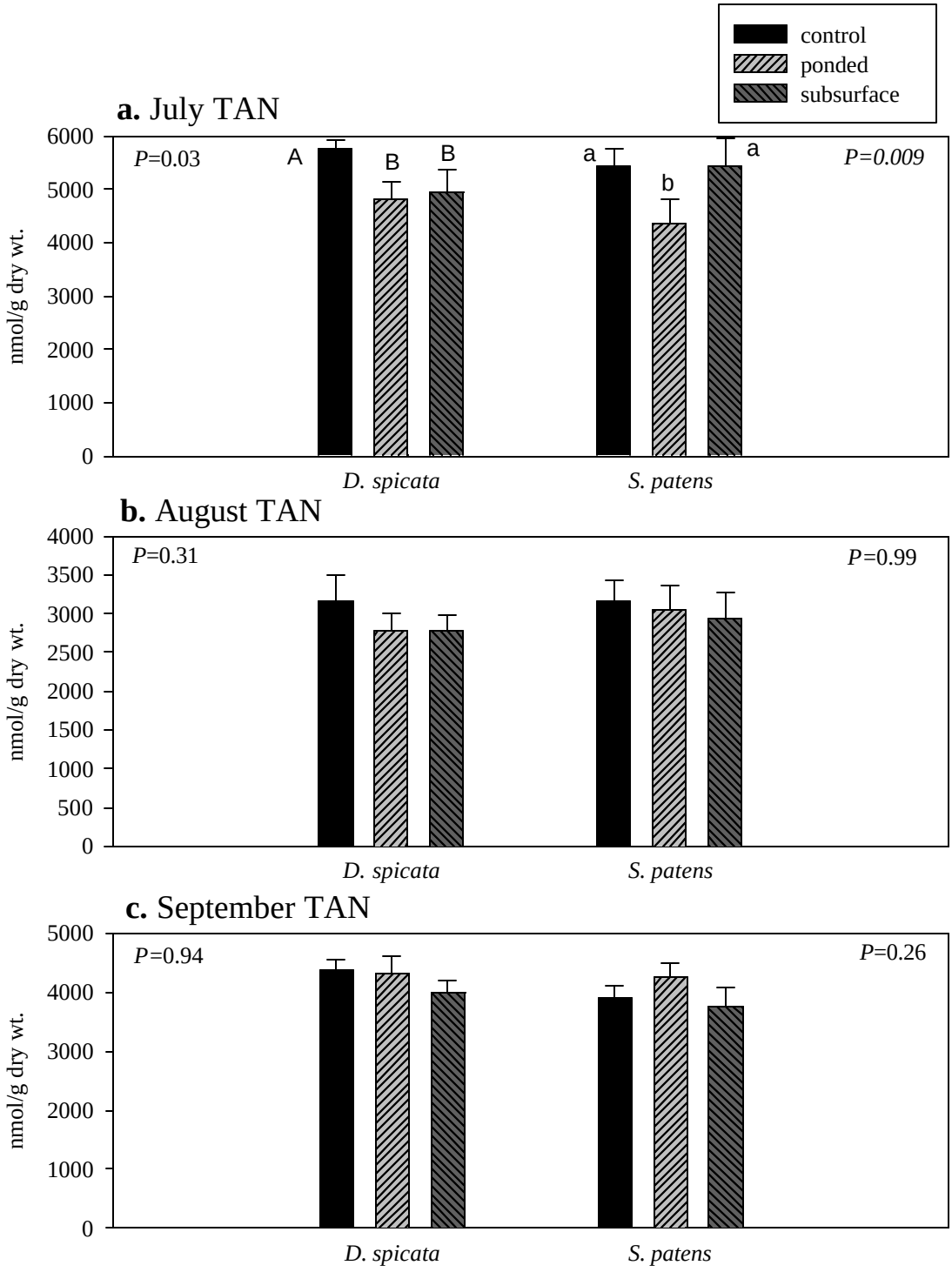


Figure 29. *D. spicata* and *S. patens* TAN concentrations between flooding treatments for July (a), August (b) and September (c) 1998. *D. spicata P*-values are in upper left corner of graph and *S. patens P*-values are in upper right. Same lettered means are not significantly different. Bars represent 1 standard error.

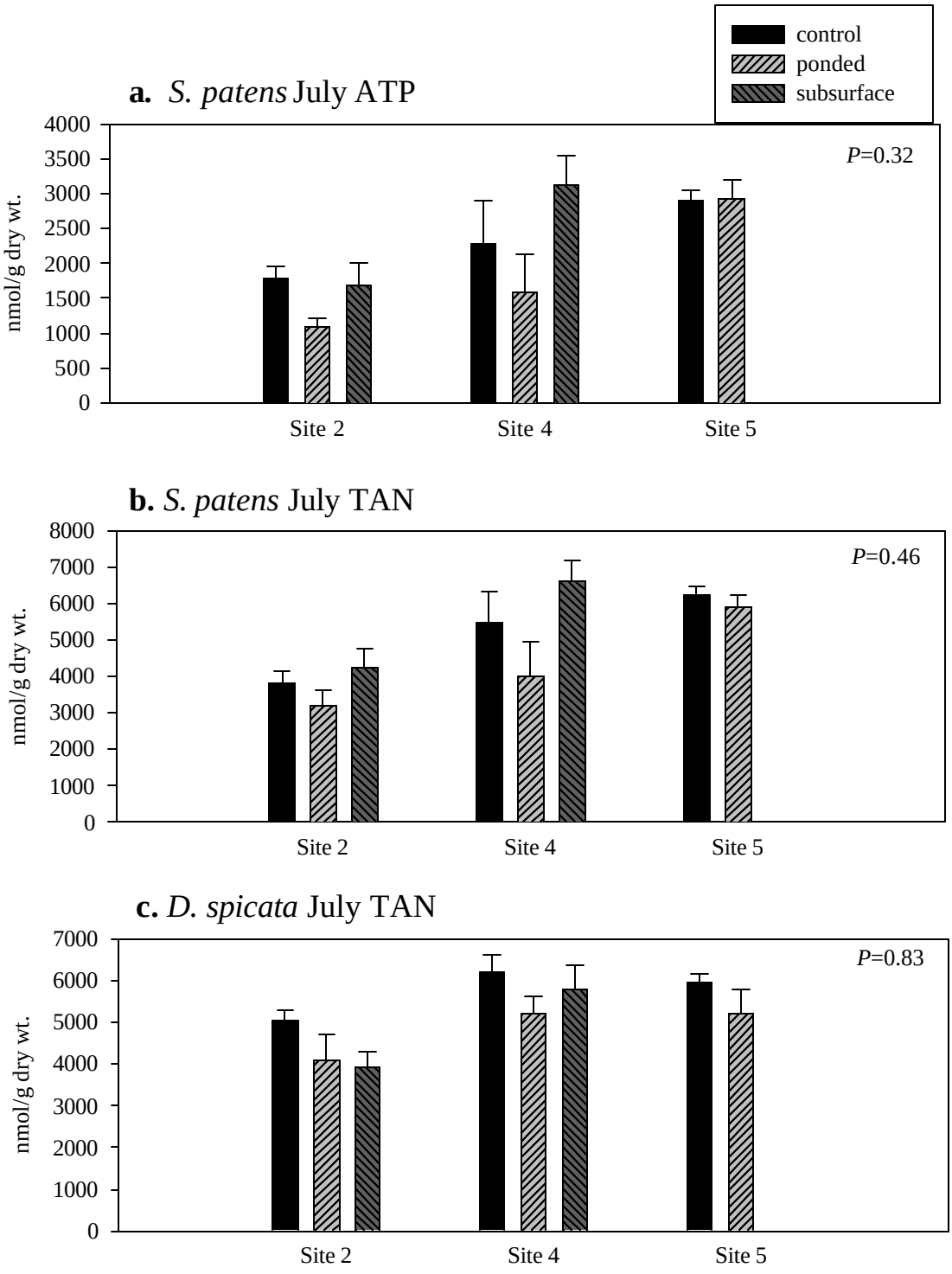


Figure 30. *S. patens* July ATP (a), July TAN (b), and *D. spicata* July TAN (c) concentrations between sites and flooding treatments in 1998. *P*-values given are for site*treatment interaction. Bars represent 1 standard error.

significant flooding effects measured in July (Fig. 30a, b, and c). However, it should be noted that reductions in ATP or TAN concentrations within ponded treatments are more evident for the two non-hummocked sites as compared to the hummocked site. It is possible that with greater replication of states of change and larger sample size, this interaction could have been significant. This would indicate that non-hummocked areas could be more responsive to flooding stress than hummocked areas that are already impacted by flooding.

6. DISCUSSION

This study was designed to assess the effects of sea-level induced state change on the high marsh plant community within Upper Phillips Creek marsh. Plant community aboveground biomass and adenine nucleotide concentrations were used to measure the effects of both the conditions found within natural states of progression along the state change model as well as experimental flooding of the plant community within two of these states: hummocked and non-hummocked. Within this transitional sequence of state change, it is proposed that the extant high marsh community dominated by *S. patens* and *D. spicata* will give way to a low marsh community dominated by *S. alterniflora*. This transition will occur in response to increased inundation due to rising sea level. The transition is characterized by a shift from a solid organic turf to an increasingly hummocked terrain surrounded by water-filled hollows (Brinson *et al*. 1995; Christian *et al*. 2000). It is proposed that this hummocked area will eventually become a mineral low marsh as the existing tidal tributary to Phillips Creek erodes into the hummocked area and connects the network of hollows and marsh potholes to a tidal source.

In the following discussion, I will address each of the four hypotheses proposed at the beginning of this study, providing both statistical evidence from this study as well as data from the literature to evaluate these statements. I will first give a description of each of the three areas in question: hummocked, non-hummocked and creek, using both edaphic and hydrological data gathered from this and previous studies within this marsh. Next I will address each hypothesis individually with the addition of issues that arose

aside from the proposed hypotheses. These include the effects of plant morphology on sampling for plant adenylates, the unexpected effects of precipitation patterns on plant production, the unintended effects of experimental design on plant production, and finally seasonal differences in plant metabolism and their consequences for accurate interpretation of experimental results.

6.1 Characterization of Sites Undergoing State Change Within Upper Phillips Creek Marsh

Measurements of water levels, interstitial water salinity, and edaphic conditions support the classification of the five sites chosen for study into the three intermediary states of change in which they were grouped: creek, hummocked, and non-hummocked. The hummocked sites, characterized by a broken landscape of vegetated hummocks surrounded by water filled hollows, were found to have lower soil bulk density, lower macro-organic matter and increased water levels as compared to the non-hummocked sites (Figs. 9, 10, 11, 12). Salinity patterns were not as distinct between states of change within the marsh as were other edaphic characteristics (Fig. 14). The higher salinity averaged within Site 5 (hummocked) was unexpected since it was flooded for much of the year, but was rarely flooded by a tidal source. However, the greater microtopography and position within the marsh of this site could enable the trapping of brackish storm tides. Such tides, combined with the increased exposure of the soil surface from lack of vegetative cover, could allow for the evaporative buildup of salts in times of drought. The lower average porewater salinity within the creek site is likely a consequence of frequent tidal flushing and drainage.

Water level data from within hummocked sites indicate that these sites are flood dominated with water levels at or above ground surface for much of the year (Figs. 10, 11, 12). This persistent flooding of hummocked sites combined with a lack of tidal signal in continuous water level records indicates that precipitation controls the frequency of flooding over the year within these sites. This is in support of Stasavich (1998) who found this high marsh to be precipitation driven, with infrequent tidal exchanges and a high potential for flood stress.

The seasonality and influence of precipitation on the water and salinity levels can be seen in comparisons of individual site water and salinity measurements made between 1997 and 1998 (Figs. 10, 14). There is an inverse relationship between salinity and water levels, with initially high salinities at a time of low (belowground) water levels. The salinities measured at the beginning of the study in June through August of 1997 were the highest recorded throughout the study period. Both the elevated salinity and lower belowground water levels recorded during this time period can be explained from precipitation data during that time period (Fig. 13). There was no recorded rainfall within Upper Phillips Creek marsh during the months of May, June or July of 1997. The first visible spike in water levels occurred in August of 1997 and was accompanied by a drop in salinity within each site. These two events were in response to a rainfall event that occurred in August of 1997. This pattern can be seen throughout the remainder of the study period, with measurable rainfall leading to spikes in water levels and depressed salinity levels. The high amounts of precipitation that fell from January through September of 1998 held water levels at or above ground surface for all but the creek site

and kept salinity levels depressed below 25 ppt. In late July of 1998 we observed a drop in water levels and a concomitant increase in salinity levels. This is most likely due to extended periods of high temperature and evapotranspiration. This period ended, however, with a large rainfall event in late August (Fig. 13), which again resulted in elevated water levels and reductions in salinity levels within the sites.

The hummocked stage of development in Upper Phillips Creek marsh may appear very similar to that described by Baumann *et al*. (1984) and Nyman *et al*. (1993b,c, 1994, 1995) for the rapidly subsiding micro-tidal deltaic marshes of southern Louisiana. They attribute the subsidence of these marshes to sediment starvation from management of the Mississippi River, changing hydrological patterns due to canal dredging and dredge spoil, as well as deep subsidence and sediment compaction enabled through manmade perturbations such as petroleum withdrawal (Baumann *et al*. 1984; Cahoon *et al*. 1999; Nyman *et al*. 1994). The resultant marsh within this deltaic system is "broken" into a hummock and hollow terrain in which prolonged periods of waterlogging stress vegetation, while low mineral sedimentation controls plant productivity and substrate building through decreased organic matter formation. A conceptual model for the initiation of subsidence and ecosystem state change within Upper Phillips Creek marsh has been proposed (Brinson *et al*. 1995; Brinson and Christian 1999; Christian *et al*. 2000). While superficial characteristics such as increased microtopographic relief and increased flooding are shared by both the systems within Louisiana and that of Upper Phillips Creek, the underlying mechanisms for this change are substantially different with

the lack of these manmade perturbations noticeably absent within Upper Phillips Creek marsh.

Within this study, the end-of-year aboveground biomass measured for *S. patens* was lower than that of marshes in Louisiana studied by Hopkinson *et al.* (1978) and Nyman *et al.* (1994). My production values are comparable to marshes in North Carolina (Waits 1967), Virginia (Wass and Wright 1969), and Louisiana (McKee and Mendelssohn 1984), and higher than a Virginia barrier island dune system (Dilustro and Day 1997) (Table 1). *D. spicata* aboveground biomass for this study was lower than that of some marshes in Louisiana (White *et al.* 1978), North Carolina (Bellis and Gaither 1985) and Florida (Kruczynski *et al.* 1978), higher than a Louisiana marsh studied by Nyman *et al.* 1984, and comparable to marshes in New Jersey (Good 1972), Louisiana (McKee and Mendelssohn 1984) and a previous study within this marsh (Tolley and Christian 1999) (Table 1). While the dominance and distribution of these two species differs within their ranges along the western Atlantic, the similarity of my measurements of biomass to those in other studies along the coast enable us to compare our findings with those marshes that are experiencing similar stresses (LA) as well as those not exposed to these conditions (NC, NJ, FL). The comparisons are variable, but indicate that the values of aboveground biomass of both species measured within this study are comparable to other marshes along the Atlantic and Gulf coasts. The effects of the natural progression of state change on the production of aboveground biomass within Upper Phillips Creek high marsh will be discussed next.

Table 1. Annual mean biomass (g/m^2) of *Distichlis spicata* and *Spartina patens* from literature.

Species	live	dead	total	Location - condition	Source
Distichlis spicata			670	New Jersey	Good (1972)
	960			North Carolina	Bellis & Gaither (1985)
	970			Florida	Kruczynski *et al*. (1978)
	740			Louisiana	White *et al*. (1978)
	620			Louisiana	White *et al*. (1978)
	13			Louisiana – broken marsh	Nyman *et al.* (1994)
	2			Louisiana – unbroken marsh	Nyman *et al.* (1994)
	560	1143		Louisiana	Hopkinson *et al.* (1978)
	300			Louisiana	McKee & Mendellson (1984)
	90-99		131-237	Virginia - flooded	Tolley & Christian (1999)
	103-188		173-358	Virginia - nonflooded	Tolley & Christian (1999)
	176-360		**453-788**	**Virginia – hummocked**	**This study**
	148-190		**350-453**	**Virginia - nonhummocked**	**This study**
			256-413	Virginia – hummocked	Roberts (2000)
			130-152	Virginia - nonhummocked	Roberts (2000)
Spartina patens			805	Virginia	Wass & Wright (1969)
			640	North Carolina	Waits (1967)
	1097			Louisiana– broken marsh	Nyman *et al.* (1994)
	1013			Louisiana– unbroken marsh	Nyman *et al.* (1994)
	900	1530		Louisiana	Hopkinson *et al.* (1978)
	600			Louisiana	McKee & Mendellson (1984)
			115	Virginia – barrier island dune	Dilustro & Day (1997)
	209-289		620-953	Virginia – flooded	Tolley & Christian (1999)
	349		803-885	Virginia - nonflooded	Tolley & Christian (1999)
	196-264		**432-462**	**Virginia – hummocked**	**This study**
	418-704		**955-1458**	**Virginia - nonhummocked**	**This study**
			130-150	Virginia – hummocked	Roberts (2000)
			437-489	Virginia - nonhummocked	Roberts (2000)

6.2 Effects of High Marsh Subsidence and Ecosystem State Change on Plant Community Biomass

My first hypothesis was that the high marsh community composed of *D. spicata* and *S. patens* responds to the flooded conditions within hummocked areas with lower total plant biomass than those sites that have a solid organic turf and lower water levels. It was thought that this reduction in biomass would be a result of stress to both *D. spicata* and *S. patens* from exposure to the hypoxic/anoxic conditions and soil phytotoxins created during periods of prolonged flooding (Armstrong *et al*. 1994; Mendelssohn and Burdick 1988). Examination of this hypothesis would be accomplished through measurement of end-of-year aboveground biomass within these sites.

End-of-year biomass is used as an estimate of net primary production when it is the measure of the maximum amount of biomass at the end of the growing season (Milner and Hughes 1968). This is a singular measurement of plant production within the growing season and does not account for losses due to early mortality and turnover or herbivory, and does not account for further plant production which might have taken place after the time of harvest (Milner and Hughes 1968). End-of-year biomass was used within this study for its ease in measurement and for comparison to previous studies within Upper Phillips Creek marsh that also utilized this method of measurement. There are limitations in using this method of measurement for estimating annual plant production. Roberts (2000) found that much of the total end-of-year biomass within these plots includes dead material that likely came from the previous year's production. Therefore, live end-of-year biomass underestimates production while total end-of-year biomass overestimates production. Roberts (2000) found relatively high turnover rates

for *D. spicata* as well as peak production for both *D. spicata* and *S. patens* being reached in August, a month earlier than when samples for this study were collected. This may also factor into interpreting adenylate data presented later within this discussion.

Measurement of end-of-year aboveground biomasses for both 1997 and 1998 support the hypothesis of decreased plant community biomass in areas experiencing subsidence. There was significantly less total and live aboveground biomass in the two sites exposed to increased inundation with hummock and hollow formation as compared to the non-hummocked sites (Fig. 16). Considering salinity pattern as a possible explanation for decreased plant production within hummocked sites, it was found that Site 5, one of the hummocked sites, averaged the highest salinity of the five sites (Fig. 14). However, the second hummocked site (Site 3) had average salinities well below the two non-hummocked sites, and all salinity measurements were well below known harmful concentrations for both *D. spicata* and *S. patens* (Baldwin and Mendelssohn 1998; Bertness 1991; Niering and Warren 1980). This reduces consideration of salinity as a possible factor influencing plant production within these sites.

Mineral matter content (bulk density) of the soils could be another factor influencing plant production within these sites, in addition to the different flooding regimes. Edaphic data showed that non-hummocked sites had significantly higher mean bulk density than hummocked sites. In studies of Louisiana *S. alterniflora* and *S. patens* marshes, positive relationships between plant productivity and soil bulk density have been found, demonstrating the importance of mineral nutrients in plant production (Nyman *et al.* 1994, 1995, 1993b,c). Nyman *et al.* (1993c) found that marsh loss in these

fragmented areas could be attributed to inadequate vertical accretion of mineral and organic material due to low rates of sedimentation as well as low plant production and plant dieback from flooding stress caused by the decrease in vertical accretion.

In summary, the persistent aboveground flooding of hummocked sites within Upper Phillips Creek marsh negatively affects the existing plant community, leading to a reduction of biomass within these areas as compared to non-hummocked areas that do not experience long periods of flooding. Flooding causes a rapid depletion in soil oxygen leading to anoxic conditions and the accumulation of harmful phytotoxins such as hydrogen sulfide (Armstrong *et al.* 1994; Blom and Voesenek 1996; Levitt 1980; Pezeshki 1994). As a consequence of these flooding stresses, both shoot and root growth in plants is reduced (Kozlowski 1984; Levitt 1980) since anaerobic fermentation requires the mobilization of large amounts of glucose reserves within the plant (Lambers *et al*. 1998). Within Upper Phillips Creek Marsh, the stress from flooding as well as the deficit in mineral nutrients as suggested by lower soil bulk density within hummocked sites leads to an overall reduction in plant community biomass within these areas. Loss of biomass within hummocked areas may be attributed to both stress due to extended flooding as discussed above, as well as loss of surface area for growth due to increased microtopography in these areas. This has been noted for marshes in Louisiana, in which subsiding areas of the marsh flood more frequently, causing erosion and subsequent loss of the substrate, leading to reductions in plant production (Nyman *et al.* 1993; DeLaune *et al*. 1994). While expansion of these areas has not been measured within Upper Phillips Creek Marsh, it is possible that the increased flooding within hummocked areas

may be contributing to the loss of substrate through erosion, allowing for expansion of hollows and eventual formation of marsh potholes (Nyman *et al.* 1993; DeLaune *et al.* 1994; Roberts 2000).

6.3 Effects of High Marsh Subsidence and Ecosystem State Change on Ratios of *D. spicata* to *S. patens*

My second hypothesis proposed a shift in species dominance between hummocked and non-hummocked areas within the marsh. I hypothesized that a shift from non-hummocked to hummocked areas would be followed with a corresponding shift from proportionately more *S. patens* biomass to proportionately more *D. spicata* biomass. Both biomass and species composition data support this hypothesis. These data indicate that the increased hydroperiod experienced by the plant community in areas of high marsh deterioration may be associated with a decrease in plant community biomass and a shift from *S. patens* dominated non-hummocked areas to *D. spicata* dominated hummocked areas (Fig. 18). This switch in species dominance within areas experiencing fragmentation and prolonged flooding was expected due to the different capabilities of these two species to adapt to flooding and disturbance. The dominance of *S. patens* in non-hummocked areas is most likely a function of the ability of this species to outcompete *D. spicata* under favorable conditions (Bertness and Ellison 1987; Bertness and Shumway 1993). The shift in dominance within hummocked areas to *D. spicata* dominance, is most likely due to the inability of *S. patens* to persist under prolonged flooding conditions. Another causative factor could be the subsequent removal of this interspecific competition which limits the production of *D. spicata* in non-disturbed

areas. Together with total biomass data, these results indicate that while both species are inhibited by prolonged flooding within hummocked areas, *D. spicata* is more tolerant of these conditions, allowing it to persist within the hummocked areas while *S. patens* is more negatively affected by the conditions present there. This is supported by Roberts (2000), who showed that flooding negatively affects productivity of *S. patens* in both hummocked and non-hummocked areas of the marsh. His results indicate that within hummocked areas of the marsh, decreases in *S. patens* productivity were coupled with increased productivity of *D. spicata.* This further supports the idea that competition with *S. patens* limits *D. spicata* production within non-hummocked areas.

This shift in species dominance is similar to and supported by the work of Warren and Niering (1993) who found shifts in vegetation patterns in some New England marshes due to increasing rates of sea-level rise. Within these New England marshes, areas of the high marsh that have experienced an increased hydroperiod showed a shift in plant community structure from *S. patens* dominated to a community dominated by stunted *S. alterniflora, D. spicata,* and various forbs. In a previous study within Upper Phillips Creek marsh, Tolley and Christian (1999) found that *D. spicata* biomass increased under both flooding and wrack treatments within a *J. roemerianus* community, and dominated recolonization within bare patches in both the *S. patens-D. spicata* and *J. roemerianus* dominated communities.

The response of *D. spicata* and *S. patens* to flooded conditions has been studied most extensively in Gulf Coast (Baldwin and Mendelssohn 1998; Ewing *et al*. 1997; Gough and Grace 1998; Pezeshki and DeLaune 1996) and New England marshes

(Bertness 1991, Niering and Warren 1980; Warren and Niering 1993). These studies have shown that *S. patens* is less tolerant than *D. spicata* of prolonged flooding due to its lack of aerenchyma and inability to adequately oxygenate its roots (Bertness 1991; Gleason and Zieman 1981). *D. spicata* is more tolerant of flooding with a well developed aerenchymatous network that may provide sufficient oxygen for aerobic respiration in flooded conditions (Allison 1996; Baldwin and Mendelssohn 1998; Hansen *et al.* 1976).

To summarize, *D. spicata* is able to outcompete *S. patens* within sites experiencing subsidence and prolonged flooding as well as within the creek site which is transitionary between the two states. This shift in dominance is most likely attributed to the different morphologies (aerenchyma) and growth practices (guerilla vs. phalanx) between the two species. The reduction in *S. patens* production within flooded areas alleviates the interspecific competition which limits *D. spicata*'s growth under favorable conditions, while the more rapid *D. spicata* guerilla growth form enables it to outcompete the slower turf growth of *S. patens* under disturbed conditions.

6.4 Plant Community Dynamics Within the Creekside Community

The unique characteristics of the creek site are best discussed separately from the hummocked and non-hummocked sites. Both water levels and salinity values were lowest within this site in comparison to the other sites throughout the two years of study. This may be a result of a hydrological gradient along the creekbank allowing for improved drainage and periodic flushing of soils by daily tidal fluxes and precipitation as

compared to the non-tidal hummocked and non-hummocked areas. Aboveground surface water levels were never recorded within this site during the study period. Bulk density of soils within the creek site were significantly lower than the non-hummocked areas, and lower than the hummocked areas, although this difference was not significant. Macro-organic matter was significantly lower within the creek site than both the hummocked and non-hummocked areas.

Within this study, there was significantly greater live aboveground biomass at the creek site than in both the hummocked and non-hummocked areas of the marsh. Total biomass was also higher within the creek site, however the difference was not significant between the creek and non-hummocked areas (Fig. 16). The combination of better drainage and more frequent tidal influence at this site relative to the others is most likely enabling the higher plant production inferred from end-of-year biomass (Howes *et al*. 1981; Pennings and Bertness 1999).

The measured change in species dominance from proportionately more *S. patens* in 1997 to proportionately more *D. spicata* in 1998 (Fig. 18) may be attributed to the ability of *D. spicata* to better tolerate stressful environments. This site was in the intermediate stage of change from a fully intact turf to the fragmented hummock and hollow terrain that may be attributed to several factors including headward creek erosion, muskrat tunneling, and ponding of water on the interior or upland portion of the site. I have personally observed muskrat activity within these sites. This activity resulted in large holes leading to belowground tunnels that were easily broken through to with foot traffic around the site. These disturbances, in conjunction with *D. spicata's* more rapid

growth (Roberts 2000) and ability to colonize disturbed areas more rapidly (Bertness 1991) than *S. patens*, could have enabled the switch in dominance within this site from 1997 to 1998. Roberts (2000) cites salinity as a potential stress within this site due to the low water levels and frequent tidal inundation. However, during this study, the combination of improved drainage coupled with tidal flushing and high precipitation could have worked to prevent buildup of flood-induced soil phytotoxins as well as stressful salinity levels (Baldwin and Mendelssohn 1998; Bertness 1991; Bertness *et al.* 1992; Niering and Warren 1980; Smart and Barko 1980).

6.5 Effects of Precipitation Patterns on Aboveground Production of *S. patens* and *D. spicata*

An unexpected effect measured within Upper Phillips Creek marsh within the two years of this study was the significant increase in aboveground biomass within all sites from 1997 to 1998 (Fig. 17). I had expected a decrease in biomass within those areas of the marsh that were subsiding and exposed to the extended periods of flooding, however, each of the three states, hummocked, non-hummocked and creek, showed an increase in biomass for both species from 1997 to 1998. I attribute this increase to the difference in precipitation between the two years. Rainfall during the growing season of 1998 was three times that in 1997 (Fig. 13). In a 13-year study of salt marsh production in the Dutch Friesian Islands, DeLeeuw and Bakker (1990) found that production of aboveground biomass was directly related to precipitation, with rainfall deficits during the growing season causing reduced aboveground biomass. Miller *et al.* (1988) found similar results, with increases in precipitation associated with increased production of *D.*

spicata and faster rates of growth into disturbed areas. This beneficial effect of precipitation within Upper Phillips Creek marsh is further supported by Roberts (2000) who found decreases in *D. spicata* and *S. patens* productivity with a decrease in rainfall between the 1998 and 1999 growing seasons within these same plots. Upper Phillips Creek high marsh has a very low slope (Hmieleski 1994) with nearly all below and aboveground water inputs to the marsh during the summer originating from precipitation (Stasavich 1998). Hydrological and precipitation data from this study and that of Stasavich (1998), as well as the biomass data from 1997, 1998, and 1999 (Roberts 2000), indicates that precipitation may be a controlling factor of aboveground production of both *S. patens* and *D. spicata* within Upper Phillips Creek marsh. This enhanced production in response to increased precipitation could be a result of reduction in salinity as well as flushing of soil toxins (DeLeeuw and Bakker 1990; Miller *et al.* 1988).

6.6 Effects of Experimental Flooding on Plant Community Biomass

There was no measurable effect of experimental flooding on aboveground biomass of either *D. spicata* or *S. patens* in 1998. Since the experimental flooding was to be done in one short sequence each month for 4 months, long-term effects on plant community biomass were not expected, but rather short term stress responses were measured using plant adenylate concentrations. These results will be discussed later.

The experimental flooding results of Roberts (2000) within these same plots prompts me to briefly discuss my results in relation to this aspect of the experiment. Roberts (2000) found reductions in productivity for both *D. spicata* (ponded and

subsurface) and *S. patens* (ponded) in response to experimental flooding with tidal water for 1999 but not for 1998. He attributed these responses to differences between species as well as precipitation patterns between the two years. Biomass data within this study were collected in 1998 as well, and show no flooding effects on either species. Roberts (2000) attributes the flood response of *S. patens* in 1999 to salinity stress caused by the pumping of tidal water onto plots in combination with low precipitation levels during the growing season. Hydrological and biomass data from 1998 (this study) as well as productivity data (Roberts 2000) indicate that salinity was less of a limiting factor in 1998, most likely due to the heavy rainfall recorded during that growing season. The reduction in *D. spicata* productivity within ponded and subsurface plots for 1999 (Roberts 2000) was attributed to disturbance from the belowground plywood borders required for both treatments. While this effect was not seen for biomass data within this study in 1998, adenylate data support this "border" effect on *D. spicata* and will be discussed in detail later.

6.7 Adenylate Concentrations: Effects of Sampling and Analysis Methods

It should be noted that values for ATP within both *S. patens* and *D. spicata* within this study were much higher than values found in the literature for similar species (Table 2). There were two differences in methodology used in this study as compared to other studies of plant nucleotides that could have caused such a difference in recovered concentrations of adenine nucleotides. Within all of the studies used for comparison of plant ATP values, plant leaf material was sampled, and the luciferin luciferase assay was

Table 2. Adenylate values (nmol/g dry wt) and AEC ratio (unitless) of different plant species from literature.

Species	**ATP**	**AEC**	**TAN**	**Location - condition**	**Source**
S. alterniflora (roots)	225	0.68	350	Louisiana – streamside	Mendelssohn *et al.* (1981)
S. alterniflora (roots)	350	0.75	580	Louisiana – inland	Mendelssohn *et al.* (1981)
S. alterniflora (leaves)	304	0.75	536	greenhouse	Mendelssohn & McKee (1981)
S. patens (leaves)	1065	0.80	1620	greenhouse	Mendelssohn & McKee (1981)
S. alterniflora (leaf sheath)	1172	0.81	1763	greenhouse	Mendelssohn & McKee (1981)
S. alterniflora (roots)	1913	0.77	2756	greenhouse	Mendelssohn & McKee (1981)
S. patens (leaves)	90	0.58	225	greenhouse – low nutrients	Ewing *et al.* (1995)
S. patens (leaves)	375	0.65	900	greenhouse – high nutrients	Ewing *et al.* (1995)
S. patens (roots)		0.77		Louisiana – marsh	Burdick & Mendelssohn (1987)
S. patens (leaves)		0.73		Louisiana - marsh	Burdick & Mendelssohn (1987)
S. patens (leaves)		0.83		Louisiana – marsh	McKee & Mendellsohn (1984)
D. spicata (leaves)		0.78		Louisiana - marsh	McKee & Mendellsohn (1984)
S. patens (leaves)	392	0.69	829	Louisiana – unburned	Ewing *et al.* (1997)
S. patens (leaves)	307	0.69	676	Louisiana - burned	Ewing *et al.* (1997)
S. patens (leaves)	302	0.75	534	Louisiana – berm	Burdick *et al.* (1989)
S. patens (leaves)	254	0.67	538	Louisiana - inland	Burdick *et al.* (1989)
J. roemerianus (leaves)		0.78	500	North Carolina – undisturbed	Shafer & Hackney (1987)
J. roemerianus (leaves)		0.68	300	North Carolina - disturbed	Shafer & Hackney (1987)
S. alterniflora (leaves)		0.80	200	North Carolina – undisturbed	Shafer & Hackney (1987)
S. alterniflora (leaves)		0.62	550	North Carolina - disturbed	Shafer & Hackney (1987)
***S. patens* (leaf + stem)**	**2405**	**0.61**	**5612**	**Virginia –hummocked**	**This study**
***S. patens* (leaf + stem)**	**1584**	**0.62**	**3670**	**Virginia – nonhummocked**	**This study**
***D. spicata* (leaf + stem)**	**2937**	**0.70**	**6570**	**Virginia –hummocked**	**This study**
***D. spicata* (leaf + stem)**	**3332**	**0.71**	**7167**	**Virginia – nonhummocked**	**This study**

used for determining adenine nucleotide concentrations. Within my study, plant leaf, leaf sheath, and stem material were pooled within a single sample, thus, the top 1/3 of the plant was used, and adenine nucleotide concentrations were determined using reverse-phase high pressure liquid chromatography.

In a study comparing extractable adenine nucleotides in different plant organs, Mendelssohn and McKee (1981) found that for both *S. alterniflora* and *S. cynosuroides*, adenylate concentrations were much higher when extracted from the leaf sheath as compared to leaf blade material alone. They attribute this to the greater amount of meristematic tissue per unit area within the leaf sheath as compared to the leaf blade. They also found that organs with more meristematic tissue (roots, leaf sheath) could exhibit changes in the AEC ratio more rapidly than those with relatively less meristematic tissue (leaf). The values of ATP they obtained from *S. alterniflora* leaf sheath material were comparable to ATP concentrations found within this study (Table 3).

D. spicata averaged higher adenylate values and AEC ratios than *S. patens* within all treatments in most instances (Figs. 23, 27, 28, 29). Although not one of my original hypotheses, this would seem to contradict what you would expect to see in those areas of the marsh where *S. patens* production is much greater than that of *D. spicata* (non-hummocked). I at first thought that these higher values for *D. spicata* indicated that indeed this species was better equipped to tolerate the "stressful" conditions present in these areas of change within the marsh. However, while *D. spicata's* reproductive morphologically may make it better equipped to deal with the stresses from prolonged

flooding and degrading soil stability present in the hummocked areas, the higher values of adenine nucleotides measured for this species may also be a result of the different growth morphologies of the two species and the sampling technique used.

D. spicata is a relatively short perennial grass (usually <40 cm in height) that has many short (<10 cm) leaves, aligned in two ranks along the stem with heavily overlapped leaf sheaths (Radford *et al.* 1968; Godfrey and Wooten 1979; Duncan and Duncan 1987). *S. patens* is a taller (60-150 cm) grass that has fewer and longer leaves (to 60 cm) (Duncan and Duncan 1987; Godfrey and Wooten 1979; Radford *et al.* 1968). Table 2 gives morphological characteristics of *D. spicata* and *S. patens* as measured in Upper Phillips Creek Marsh for 1999 (Roberts 2000). Since the top 1/3 of each plant was sampled for this study, there may have been a greater amount of meristematic tissue per unit area of stem sampled for *D. spicata* as compared to *S. patens*. This could significantly affect the amount of extractable nucleotides and lead to higher values for *D. spicata* as compared to *S. patens* (Mendelssohn and McKee 1981; Shafer and Hackney 1987).

6.8 Effects of High Marsh Subsidence and Ecosystem State Change on Plant Community Adenylates

Hypothesis 4 predicted greater levels of stress for both *D. spicata* and *S. patens* within hummocked sites as measured through reductions in plant adenylate concentrations (ATP, TAN) and AEC ratios. Significant differences in plant adenylates were found between states of change with a trend toward higher concentrations of ATP and TAN within hummocked areas of the marsh as compared to the non-hummocked

areas (Figs. 24, 26). As for the AEC ratio, the results for both species were highly variable with little significant differences between states of change (Fig. 25). However, the trend again was toward higher values in the hummocked areas than in the non-

Table 3. Average values of morphological characteristics for *D. spicata* and *S. patens* for each month of experimental sampling in 1999 (Roberts 2000). Standard deviations are given in parentheses.

	culm height (cm)	**height to uppermost leaf (cm)**	**number of green leaves**	**number of brown leaves**
D. spicata				
June	29 (7.9)	19.6 (5.9)	6.8 (2)	0.7 (0.8)
July	29.1 (8.6)	21.9 (7.2)	6.4 (2.8)	1.9 (1.5)
August	32 (8.3)	25.1 (7.5)	7.1 (2.6)	3.3 (1.8)
September	35.4 (9.3)	29.1 (9)	5.6 (3.1)	6.5 (3.3)
S. patens				
June	34 (9.6)	16.2 (4.8)	3.4 (0.8)	0.6 (0.7)
July	39 (9.2)	19.8 (5.8)	3.2 (0.9)	1.3 (0.9)
August	40.6 (9.3)	21 (6.2)	3.4 (4.3)	2 (1)
September	38.9 (11.5)	21.7 (7.4)	2.6 (1.2)	2.9 (1.3)

hummocked areas. This contradicts the prediction of lowered adenylate concentrations due to stress within areas experiencing state change. However, comparison of both adenylate and biomass results from this study to similar studies of Gulf Coast marshes indicate that the plants within the hummocked areas are indeed stressed relative to those in non-hummocked areas. Roberts (2000) found that productivity for *S. patens* was reduced in hummocked areas as compared to non-hummocked areas within Upper Phillips Creek Marsh. His findings for *D. spicata* show that while *D. spicata* is more productive within hummocked areas, this is most likely a result of competitive release from *S. patens* (Roberts 2000). *D. spicata* shows stress from conditions present in the hummocked areas through increased turnover rates rather than reduced productivity (Roberts 2000). I have already shown that aboveground production for both species is reduced within hummocked areas that experience prolonged flooding, and that competitive differences between species may result in a shift in species dominance between non-hummocked and hummocked areas. So what could cause the apparent enhanced metabolic state in these areas in which that plants are obviously stressed?

In a study of *S. alterniflora's* metabolic adaptation to anoxia, Mendelssohn *et al.* (1981) found that when comparing the plant community between zones along a transect from highly drained creekside to increasingly waterlogged inland, both ATP and AEC values for the anoxic, waterlogged inland site were similar to those of the less reduced creekside community. Only in inland areas of obvious plant dieback did adenylate concentrations and the AEC ratio decline in response to the stress of prolonged waterlogging. They attributed the high ATP and AEC values in the highly reduced

inland zone to maintenance of energy status of the plant through increased alcoholic fermentation and the induction of the Pasteur effect in which ATP yield is increased at the expense of considerable glucose consumption. By this method, plants accelerate rates of glycolysis to maintain high ATP concentrations or energy status, but do so at the ultimate expense of plant production (Mendelssohn and Burdick 1988; Mendelssohn *et al.* 1981; Pradet and Bomsel 1978). Within Upper Phillips Creek Marsh, productivity is reduced for *S. patens* within hummocked areas as compared to non-hummocked areas (Roberts 2000). However, *D. spicata* responds with increased turnover rather than a decrease in plant production within hummocked areas (Roberts 2000).

It is possible then that the observed increases in both ATP and AEC values within the hummocked areas of the marsh are actually a stress response created by an increase in plant alcoholic fermentation in response to anoxic to hypoxic conditions caused by prolonged flooding within these sites. This switch from aerobic to anaerobic metabolism could account not only for the increase in plant adenine nucleotide concentrations, but also for the reduction in biomass through increased carbohydrate consumption for maintenance of cellular energy status. While neither measurements of soil redox or indicators of anaerobic root metabolism were made within these sites, the maintenance of aboveground water levels within these hummocked areas, with only brief periods of drawdown, may indicate that the plants within these areas could be exposed to long periods of anoxic to hypoxic conditions.

6.9 Effects of Season on Adenine Nucleotide Concentrations

A significant trend of lower adenine nucleotide concentrations and AEC ratios was found for both species late in the growing season as compared to early in the growing season (Fig. 23). Experimental manipulation of flooded plots within this study was carried out from June through September of 1998, with a significant drop in adenylate concentrations and AEC ratios after July. Very little information is available for the temporal aspects of adenine nucleotides in vascular plant communities, however, a few studies have focused on or detected seasonal differences in several aquatic plants including *S. cynosuroides*, *S. alterniflora*, *S. patens*, *D. spicata* (Burdick *et al.* 1989; McKee and Mendelssohn 1984), *J. roemerianus* (Shafer and Hackney 1987) and *Zostera marina* (Delistraty and Hershner 1983). In general, it has been found that concentrations of extractable nucleotides and AEC ratios tend to be lower later in the growing season after peak biomass has been attained (Burdick *et al.* 1989; McKee and Mendelssohn 1984; Shafer and Hackney 1987). The seasonal differences are attributed to higher levels of adenylate and AEC during periods of rapid growth rate and metabolism, followed by reduced adenylate concentrations and AEC during and after periods of flowering and reproduction but before senescence (Burdick *et al.* 1989; McKee and Mendelssohn 1984; Shafer and Hackney 1987).

The variability in and lack of significant differences in adenylates between treatments for August and September within this study is most likely due to the overall depression of plant metabolism through a cessation or slowing of plant productivity late in the growing season (Roberts 2000). Both McKee and Mendelssohn (1984) and Shafer

and Hackney (1987) found that measurable adenylates were greatly affected by season in several species of vascular plants. They stress the importance of understanding the influence of both season and extraneous environmental variables on both AEC and adenylate production before using them as a measure of stress within plant communities. This is especially important in areas where prolonged flooding, elevated salinity or nutrient limitation may be controlling plant productivity. There can be interactions between these variables as well as unintended influences on the production of adenine nucleotides in the plants exposed to these variables (Burdick *et al.* 1989; Ewing *et al.* 1997; McKee and Mendelssohn 1984; Mendelssohn *et al.* 1981; Shafer and Hackney 1987).

These findings would help to explain the seasonal differences found within this study. Roberts (2000) showed that within these plots, peak aboveground biomass, density and culm height were achieved by both *D. spicata* and *S. patens* in August. Thus, sampling to evaluate plant metabolism would be most useful during the earlier months of the growing season before growth has slowed or stopped, as was the case with these species in late August and September. During the course of my study, *S. patens* was observed in bloom as early as June 1998, while *D. spicata* was in bloom in July 1998 (personal observation). Plant metabolic responses measured later in the growing season may be masked by the transition through flowering and reproduction into senescence.

6.10 Effects of Experimental Flooding on Plant Community Adenylates

My final hypothesis addresses the effects of experimental flooding on plant community adenylates, with an expected drop in plant adenylate concentrations and AEC ratios for both *D. spicata* and *S. patens* within plots experimentally flooded with tidal water. Based on recent use of the adenylate energy charge as a measure of plant stress within coastal wetlands (Burdick *et al.* 1989; Ewing *et al.* 1997; Mendelssohn *et al.* 1981; Shafer and Hackney 1987), this method was chosen to measure stress to the plant community within this study. If the initiating factor of state change within this system is more frequent flooding of the high marsh community due to sea-level rise, the monthly flooding of selected sites and measurement of the physiological response of the plants to this flooding seemed sufficient to determine if indeed experimental flooding was a stressor to the high marsh vegetation.

Experimental flooding of bordered treatment plots produced significant changes in plant adenine nucleotide concentrations in July of 1998 only, with lower concentrations of ATP and TAN in *S. patens* tissue within ponded plots (Figs. 27, 29), and lowered TAN concentrations for *D. spicata* within ponded and subsurface control plots (Fig. 29). These responses are opposite that measured for the hummocked vs. non-hummocked sites. In these sites, we interpreted higher levels of adenylates within hummocked sites as a stress response. The difference in these responses, however, is that the flooding effects were significant only in the two non-hummocked sites (Fig. 30a & b). A comparison of flooding effects within individual sites shows that while Sites 2 and 4 showed reduced ATP and TAN concentrations in ponded plots, there was no difference

between control and ponded plots in Site 5 (Fig. 30 a & b). Site 5 also averaged higher ATP and TAN concentrations than Sites 2 and 4 in July (Fig. 30a & b). Natural hydrological differences between sites could be a contributing factor the experimental flooding effects measured.

During the month of July 1998, Site 5 was flooded aboveground surface to a depth of ~15 cm , while water levels within Sites 2 and 4 averaged 7 cm belowground surface (Fig. 11 & 12). The prolonged flooding within Site 5 may have been producing a stress response which could account for the higher average adenylate concentrations as compared to Sites 2 & 4. The existing flooded conditions during this time period could also prevent any measurable effect of experimental flooding within the ponded plots in Site 5.

There was a significant decrease in TAN concentrations in July for *D. spicata* in the ponded and subsurface control plots (Fig. 29). This decrease was evident in August as well, although not statistically significant. The reduction in adenylates for *D. spicata* in the two treatments that had belowground borders indicates that the severing of *D. spicata's* rhizomes may have been a source of stress (Roberts 2000). This effect can be seen in the individual site analysis of July TAN concentrations in which *D. spicata* TAN levels were consistently lower in ponded and border control plots within each site (Fig. 30c).

In a study of dune colonization at Mono Lake, California, Brotherson and Rushforth (1985) found that *D. spicata* was able to rapidly colonize and stabilize recently exposed beach through aggressive production of rhizomes, some measured as long as 20

m. Both Hansen *et al.* (1978) and Pennings and Callaway (2000) showed that the in areas where clonal ramets experience contrasting environments (saline vs. non-saline; flooded vs. non-flooded), the rhizome network can provide limiting resources (freshwater, oxygen) to the clone experiencing the stressor. Such may be the case within this study, with the severing of rhizomes causing an unintended effect in those plots that had belowground borders. The same effect would not be evident for *S. patens*, as its growth form is that of dense turf roots rather than long invasive rhizomes (Bertness 2000).

In summary, both species elicited differential responses to flooding treatment with *S. patens* showing a stress response through reduced metabolic function following tidal flooding within non-hummocked sites only. *D. spicata*'s response to experimental flooding appears to be, a least partly, a response to a disruption in normal root growth practices as a byproduct of the installation of belowground borders necessary to accomplish aboveground flooding within this study. These findings are further supported by productivity data from within these plots for 1998 and 1999 in Roberts (2000).

6.11 Implications of Results for Ecosystem State Change Within Upper Phillips Creek Marsh

The transformation of this high marsh from stable organic turf to a broken mosaic of vegetated hummocked surrounded by water-filled hollows has been proposed by Brinson *et al.* (1995), Brinson and Christian (1999) and Christian *et al.* (2000). Within this study, results of both aboveground biomass and adenine nucleotide concentrations and AEC ratios indicate that both *D. spicata* and *S. patens* are stressed by conditions present with the hummock and hollow stage of transformation from organic high marsh

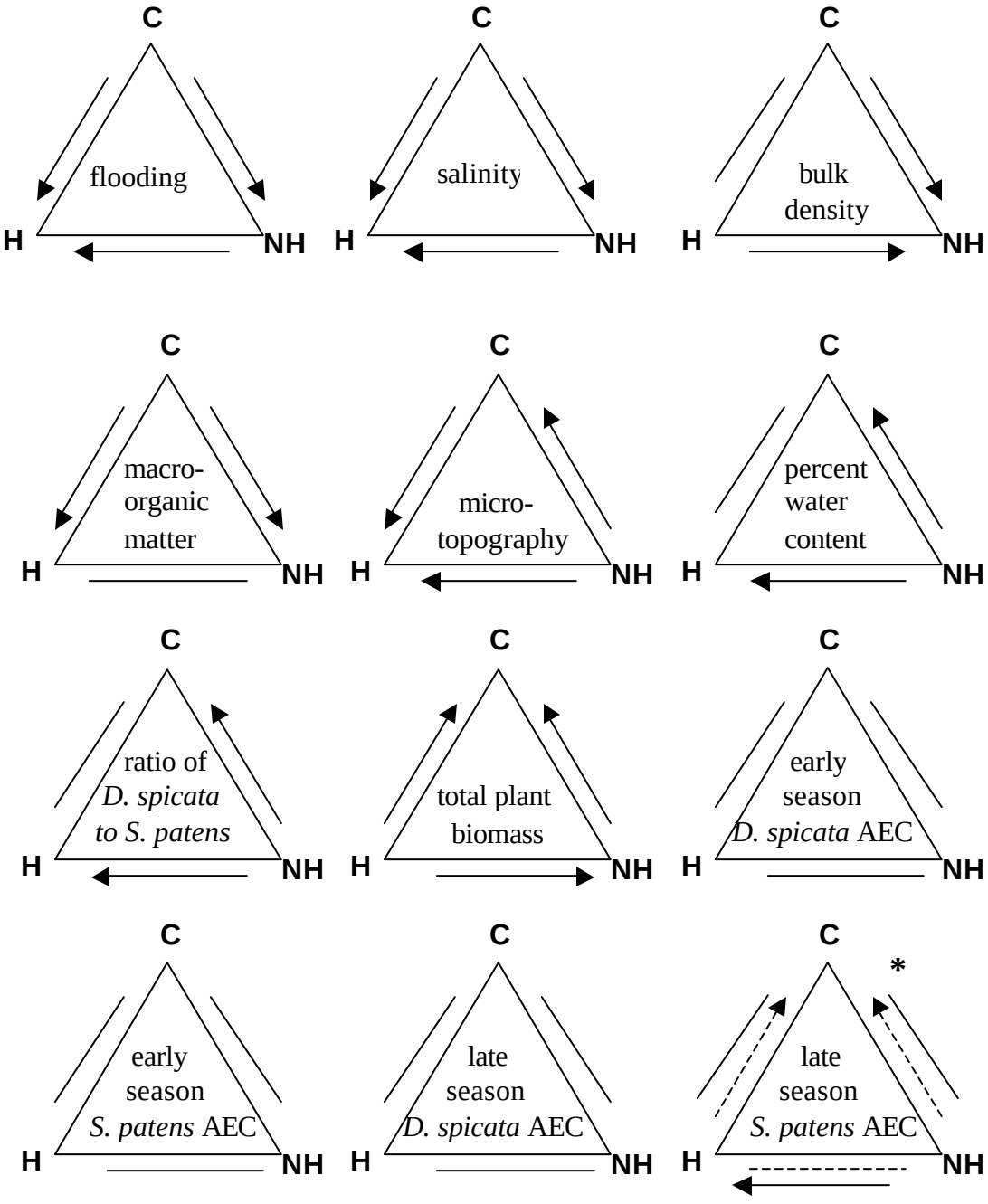
to mineral low marsh. These species respond to these conditions (loss of substrate, prolonged flooding) with decreases in aboveground biomass production in association with decreased metabolic function. The conditions encountered within this transitional sequence do not act equally on both species, however. Based on the results of this study as well as that of Roberts (2000), we have found that *S. patens* is the first to respond to the changing conditions within the transitional sequence, reacting negatively to increased flooding. In turn, the depressed production of *S. patens* alleviates the pressure of interspecific competition present within non-hummocked sites, allowing *D. spicata* to dominate areas that have become fragmented and flood-dominated. These results have been synthesized in Figure 31.

In the long term, however, depression of aboveground biomass within hummocked areas, as well as evidence of flooding stress through the disruption of normal metabolic functions for both *S. patens* and *D. spicata* indicates that *D. spicata*'s dominance in hummocked areas may be a short-lived phenomenon. Prolonged flooding and loss of substrate may eventually lead to complete vegetative dieback and the eventual formation of marsh potholes. Within Upper Phillips Creek high marsh, this transition may be facilitated by both the tunneling activity of muskrats as well as the ability for greater tidal influence with the encroachment of the tidal tributary to Phillips Creek present in this area of the marsh.

Continuation of experiments and long term monitoring within this area of Upper Phillips Creek marsh will allow substantiation of the final phase of the proposed transition sequence (Brinson *et al*. 1995; Christian *et al.* 2000) from a fragmented organic

high marsh to an intertidal mineral low marsh environment. Experiments such as this and those of Roberts (2000), Tolley and Christian (1999) and Brinson and Christian (1999) can provide useful insight into the factors influencing the changes taking place within Upper Phillips Creek marsh, as well as help in determining the best methods to use for assessing the impact these changes are having on the existing plant community within this high marsh. In keeping with the goals of the Long Term Ecological Research network of which this study is a part, these findings could be applied on a broader scale for comparison to and help in understanding the processes driving similar conditions within marshes such as those along the Mississippi Delta and New England.

Figure 31. Synthesis of variables observed within Upper Phillips Creek Marsh. States of change are given as C = creek, NH = non-hummocked, and H = hummocked. Arrows indicate direction of increase in variable, while a straight line indicates no difference between states.



* The solid line represents August results. The dashed line represents September results. There were no other significant effects in late or early season AEC.

7. CONCLUSIONS

The results of this study support the conceptual model that increased flooding associated with high marsh subsidence is eliciting both a community-level response and a species-specific response on the dominant high marsh community within Upper Phillips Creek (Brinson *et al*. 1995; Christian *et al*. 2000). The decrease in plant community aboveground biomass in areas of high marsh subsidence and increased ponding of water indicate that these conditions are having a negative impact on the extant, high marsh plant community. The loss of biomass and continued stress to the exisiting plant community may be facilitating the transition from organic high marsh to mineral low marsh through formation of a transitionary open-water environment. Within this transitional sequence, the two high marsh co-dominants, *S. patens* and *D. spicata* are differentially affected. There was a measured shift in species dominance from *S. patens* in areas of solid turf and no ponding of water, to *D. spicata* domination in areas of increased microtopography and flooding.

Measured results for the detection of physiological stress due to subsidence within this study were not as conclusive. It appears, however, that increased inundation with rising sea level could have a species-level affect within the high marsh community. *S. patens* appears to be much more sensitive to increased flooding within its environment than does *D. spicata.* The loss of *S. patens* through stress-induced decreases in production and biomass, may create a feedback loop in which the loss of its turf-root morphology allows for further deflation and/or erosion of the organic high marsh

substrate. This, in turn, would enable more frequent and prolonged flooding from either tidal and/or precipitation sources. While the more flood tolerant *D. spicata* may benefit briefly from the loss of *S. patens* as a competitor, the more frequent and prolonged flooding within the high marsh can eventually stress this species beyond its capacity to support itself. The loss of both of these species and their supportive root structure within the organic matrix of the high marsh, may be the final step in the transition towards an open pothole environment.

These results and those of previous studies within this marsh (Brinson and Christian 1999; Roberts 2000) indicate that within Upper Phillips Creek Marsh, a physiologically healthy high marsh community may be needed to maintain the stability of the organic substrate. Increased flooding with rising sea level creates conditions that may expedite the state change from organic high marsh to mineral low marsh. These findings may be applied at a broader scale to offer a possible explanation or contributing factor towards marsh loss in other areas of the United States and abroad with similar characteristics.

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APPENDIX A. DATES AND SALINITY OF TIDAL WATER SAMPLED FROM TRIBUTARY TO PHILLIPS CREEK.

Date	**Salinity (ppt)**
7/9/97	32
7/10/97	32
7/11/97	33
7/23/97	30
8/7/97	17
8/8/97	18
9/14/97	26
10/10/97	20
11/22/97	14
12/15/97	17
1/17/98	13
1/24/98	10
2/21/98	8
3/15/98	9
4/6/98	9
4/26/98	10
5/15/98	13
6/23/98	12
6/24/98	15
7/13/98	18
7/25/98	16
7/26/98	15
8/22/98	30
8/23/98	30
9/26/98	20

APPENDIX B. INDIVIDUAL PLOT ELEVATIONS RELATIVE TO MEAN SEA LEVEL.

On January 6 and 26, 1999 a global positioning system (GPS) unit was used to determine the elevation of each plot in relation to mean sea level (MSL). Elevations were determined at the corner of each plot and directly beside each well giving a total of 140 data points. The GPS receiver used was a Trimble 4000 SE unit (L1 only), and the software used to process the GPS data was GPSurvey 3.20a. A permanent benchmark (BRNV) located within 500 m of each site was used to relate elevations to MSL. These elevations were tied into MSL according to the 1929 National Geodetic Survey, using a permanent benchmark (VCR1, 1.225 m MSL) at that has been established as a cignet global network tie (Ricker 1999). The high precision of the VCR1 benchmark allows for GPS elevations accurate to the nearest centimeter. All elevation points recorded during this survey were found to be within this level of accuracy (VCR-LTER http://www.vcrlter.virginia.edu).

Latitude, longitude and elevation (m) above mean sea level of experimental plots at Upper Phillips Creek, Virginia. C=control, SC=subsurface control, P=ponded.

Site	Plot	Rep	Point #	Latitude	Longitude	Elevation (m)	Plot mean	Site mean
1	C	1	1	37.46093	-75.8355	0.957	0.975	1.032
1	C	1	2	37.46091	-75.8355	0.903		
1	C	1	3	37.46092	-75.8356	0.975		
1	C	1	4	37.46094	-75.8355	1.031		
1	C	1	5	37.46092	-75.8355	1.011		
1	C	2	1	37.46095	-75.8354	1.104	1.086	
1	C	2	2	37.46094	-75.8354	1.031		
1	C	2	3	37.46096	-75.8355	1.104		

1	C	2	4	37.46098	-75.8354	1.076		
1	C	2	5	37.46096	-75.8354	1.117		
1	P	1	1	37.46091	-75.8355	1.048	0.999	
1	P	1	2	37.46088	-75.8354	1.047		
1	P	1	3	37.4609	-75.8355	0.914		
1	P	1	4	37.46092	-75.8355	0.975		
1	P	1	5	37.46091	-75.8354	1.011		
1	P	2	1	37.46098	-75.8355	1.077	1.047	
1	P	2	2	37.46096	-75.8355	1.083		
1	P	2	3	37.46098	-75.8355	0.886		
1	P	2	4	37.461	-75.8355	1.07		
1	P	2	5	37.46098	-75.8355	1.12		
1	S	1	1	37.46092	-75.8354	1.077	1.056	
1	S	1	2	37.46091	-75.8354	1.019		
1	S	1	3	37.46093	-75.8355	1.017		
1	S	1	4	37.46095	-75.8355	1.09		
1	S	1	5	37.46093	-75.8354	1.078		
1	S	2	1	37.46094	-75.8355	1.016	1.030	
1	S	2	2	37.46093	-75.8355	1.04		
1	S	2	3	37.46095	-75.8355	1.033		
1	S	2	4	37.46097	-75.8355	1.012		
1	S	2	5	37.46095	-75.8355	1.048		
2	C	1	1	37.46116	-75.8358	1.064	1.045	1.033
2	C	1	2	37.46114	-75.8358	1.051		
2	C	1	3	37.46119	-75.8358	1.039		
2	C	1	4	37.46118	-75.8358	1.029		
2	C	1	5	37.46114	-75.8358	1.041		
2	C	2	1	37.46114	-75.8359	1.024	1.019	
2	C	2	2	37.46113	-75.8359	0.995		
2	C	2	3	37.46117	-75.8359	1.033		
2	C	2	4	37.46115	-75.8359	1.039		
2	C	2	5	37.46112	-75.8359	1.006		
2	P	1	1	37.4611	-75.8359	1.037	1.028	
2	P	1	2	37.46108	-75.8359	1.006		
2	P	1	3	37.46112	-75.8359	1.024		
2	P	1	4	37.46111	-75.8358	1.043		
2	P	1	5	37.46107	-75.8359	1.029		
2	P	2	1	37.46121	-75.8359	1.042	1.049	
2	P	2	2	37.46119	-75.8359	1.05		
2	P	2	3	37.46124	-75.8359	1.047		
2	P	2	4	37.46123	-75.8359	1.071		
2	P	2	5	37.46119	-75.8359	1.034		

2	S	1	1	37.46112	-75.8359	1.026	1.025	
2	S	1	2	37.46111	-75.8359	1.015		
2	S	1	3	37.46115	-75.8359	1.026		
2	S	1	4	37.46114	-75.8359	1.032		
2	S	1	5	37.4611	-75.8359	1.024		
2	S	2	1	37.46119	-75.8358	1.021	1.030	
2	S	2	2	37.46117	-75.8359	1.017		
2	S	2	3	37.46121	-75.8359	1.045		
2	S	2	4	37.46121	-75.8358	1.039		
2	S	2	5	37.46117	-75.8358	1.029		
3	C	1	1	37.46138	-75.8351	1.101	1.0618	1.048
3	C	1	2	37.4614	-75.8351	1.057		
3	C	1	3	37.46138	-75.835	1.075		
3	C	1	4	37.46136	-75.835	1.08		
3	C	1	5	37.46138	-75.8351	0.996		
3	C	2	1	37.4614	-75.8351	0.959	1.0374	
3	C	2	2	37.46142	-75.8351	1.061		
3	C	2	3	37.4614	-75.835	0.991		
3	C	2	4	37.46138	-75.835	1.067		
3	C	2	5	37.4614	-75.8351	1.109		
3	C	3	1	37.46137	-75.835	1.065	1.0412	
3	C	3	2	37.46139	-75.835	0.993		
3	C	3	3	37.46137	-75.835	1.086		
3	C	3	4	37.46135	-75.835	1.089		
3	C	3	5	37.46138	-75.835	0.973		
3	C	4	1	37.46135	-75.835	1.04	1.0548	
3	C	4	2	37.46136	-75.835	1.061		
3	C	4	3	37.46134	-75.835	1.046		
3	C	4	4	37.46132	-75.835	1.022		
3	C	4	5	37.46135	-75.835	1.105		
4	C	1	1	37.46094	-75.8343	1.082	1.0426	1.085
4	C	1	2	37.46091	-75.8343	1.097		
4	C	1	3	37.46096	-75.8343	0.81		
4	C	1	4	37.46096	-75.8343	1.127		
4	C	1	5	37.46091	-75.8343	1.097		
4	C	2	1	37.46106	-75.8342	1.094	1.0826	
4	C	2	2	37.46104	-75.8343	1.081		
4	C	2	3	37.46108	-75.8342	1.069		
4	C	2	4	37.46108	-75.8342	1.107		
4	C	2	5	37.46104	-75.8342	1.062		
4	P	1	1	37.461	-75.8343	1.105	1.1088	
4	P	1	2	37.46098	-75.8343	1.123		

4	P	1	3	37.46102	-75.8343	1.122		
4	P	1	4	37.46103	-75.8343	1.108		
4	P	1	5	37.46098	-75.8343	1.086		
4	P	2	1	37.46094	-75.8342	1.079	1.0894	
4	P	2	2	37.46092	-75.8342	1.059		
4	P	2	3	37.46096	-75.8342	1.12		
4	P	2	4	37.46096	-75.8342	1.113		
4	P	2	5	37.46092	-75.8342	1.076		
4	S	1	1	37.46106	-75.8343	1.094	1.0828	
4	S	1	2	37.46104	-75.8343	1.105		
4	S	1	3	37.46108	-75.8343	1.082		
4	S	1	4	37.46108	-75.8343	1.065		
4	S	1	5	37.46104	-75.8343	1.068		
4	S	2	1	37.461	-75.8342	1.111	1.1092	
4	S	2	2	37.46098	-75.8343	1.098		
4	S	2	3	37.46102	-75.8342	1.103		
4	S	2	4	37.46102	-75.8342	1.13		
4	S	2	5	37.46098	-75.8342	1.104		
5	C	1	1	37.46272	-75.8354	1.02	1.0122	1.004
5	C	1	2	37.4627	-75.8354	1.024		
5	C	1	3	37.46274	-75.8354	1.004		
5	C	1	4	37.46274	-75.8354	0.998		
5	C	1	5	37.4627	-75.8354	1.015		
5	C	2	1	37.46278	-75.8354	1.055	1.025	
5	C	2	2	37.46277	-75.8354	1.009		
5	C	2	3	37.46281	-75.8354	1.037		
5	C	2	4	37.4628	-75.8354	1.019		
5	C	2	5	37.46276	-75.8354	1.005		
5	P	1	1	37.46273	-75.8354	1.007	0.9996	
5	P	1	2	37.46271	-75.8355	0.966		
5	P	1	3	37.46275	-75.8354	1.018		
5	P	1	4	37.46275	-75.8354	1.009		
5	P	1	5	37.4627	-75.8354	0.998		
5	P	2	1	37.46285	-75.8354	0.976	0.9896	
5	P	2	2	37.46283	-75.8354	0.998		
5	P	2	3	37.46286	-75.8355	0.99		
5	P	2	4	37.46287	-75.8354	0.994		
5	P	2	5	37.46286	-75.8355	0.99		
5	S	1	1	37.46278	-75.8354	1.008	1.0292	
5	S	1	2	37.46276	-75.8354	1.03		
5	S	1	3	37.4628	-75.8354	1.077		
5	S	1	4	37.4628	-75.8354	1.003		

5	S	1	5	37.46276	-75.8354	1.028		
5	S	2	1	37.46283	-75.8355	0.951	0.9696	
5	S	2	2	37.46281	-75.8355	0.972		
5	S	2	3	37.46284	-75.8355	1.025		
5	S	2	4	37.46285	-75.8355	0.953		
5	S	2	5	37.46282	-75.8354	0.947		

APPENDIX C. DATE AND AMOUNT OF WATER PUMPED ONTO INDIVIDUAL PLOTS FOR FLOODING TREATMENT.

Liters of water pumped is an approximation based on time required to fill plot and average pumping capacity of pump as determined by measuring the amount of time required to fill a 50 gallon tub. The actual amount of water pumped per minute varied with distance from pump. N/A indicates measurements were not taken for that time. Plot: Site number / P=ponded / replicate number.

Date	Plot	Duration (min)	Amount (L)	Surface water salinity (ppt)	Creek salinity (ppt)
6/29/98	2P1	N/A	N/A	N/A	N/A
	2P2	N/A	N/A	N/A	
	4P1	N/A	N/A	N/A	
	4P2	N/A	N/A	N/A	
	5P1	N/A	N/A	N/A	
	5P2	N/A	N/A	N/A	
7/24/98	2P1	5	1900	24	15
	2P2	6	2280	24	
	4P1	7	2660	22	
	4P2	7	2660	21	
	5P1	6	2280	18	
	5P2	5	1900	20	
8/22/98	2P1	4	1520	32	30
	2P2	4	1520	32	
	4P1	6	2280	30	
	4P2	4	1520	30	
	5P1	7	2660	30	
	5P2	6	2280	31	

8/23/98	2P1	7.5	2840	32	30
	2P2	6.25	2375	33	
	4P1	5	1900	33	
	4P2	4	1520	32	
	5P1	8.5	3230	27	
	5P2	7.5	2840	32	
9/26/98	2P1	4	1520	N/A	N/A
	2P2	5	1900	N/A	
	4P1	5	1900	N/A	
	4P2	3	1140	N/A	
	5P1	5	1900	N/A	
	5P2	4	1520	N/A	
9/27/98	2P1	4	1520	N/A	20
	2P2	3.5	1330	N/A	
	4P1	5	1900	25	
	4P2	5	1900	24	
	5P1	4	1520	23	
	5P2	4	1520	24	

APPENDIX D. DETAILED METHODS OF ADENYLATE ANALYSIS.

Plant collection and extraction of adenine nucleotides from plant samples were a modification of the methods of Mendelssohn and McKee (1981). Plant samples were collected from each plot 24 h following experimental flooding in July, August and September. June samples were collected prior to flooding. Six samples were taken per plot, three of *S. patens* and three of *D. spicata.* Each sample consisted of the top 1/3 of eight plants including leaves and culms. Plants sampled were chosen to be as similar in height as possible. Samples were clipped, placed in Whirlpak® bags, sprayed with deionized water and frozen in liquid nitrogen within at least 3 min of excision. In most cases, samples were frozen less than 1 min after excision. Mendelssohn and McKee (1981) found significantly higher retention of adenylate concentrations in plant tissue samples frozen in liquid nitrogen within 3 min of excision as compared to tissue samples frozen on dry ice. Samples then were transferred to dry ice for transport back to the lab. The addition of deionized water to the samples was done to prevent the samples from thawing during transfer from one storage unit to another, as well as transfer to the freeze-dryer (Mendelssohn and McKee 1981). In the lab, samples were held on dry ice while each bag was opened for ventilation. Bags then were placed inside a Labconco 16 port chamber (Model 75229), which was sealed immediately and vacuum applied. Samples were freeze-dried for 72 hr using a Labconco FreeZone® 6-L freeze dry system (Model 77535). The samples then were removed, resealed, and placed in dessicators. Each freeze-dried sample was then ground in a Wiley Mill to pass a 40 mesh screen, placed in a scintillation vial and stored in a dessicator at 0^{o}C until processed.

The extractant used was 1 mM ethylenediaminetetraacetic acid (EDTA) at pH 7.4 (Sigma Chemical Co.). All solvents were prepared using fresh deionized water, buffered with 1N H_2SO_4 and 50% NaOH and autoclave-sterilized prior to use. Twenty milliliters of EDTA extractant were measured into Kjeldhal digestion tubes and heated to 100^{o}C in a digestion block. In separate digestion tubes, enough sample plant material was measured initially to give a weight:volume ratio of plant:extractant between 1 and 1.5% (~0.26g dry plant material for 20 mL extraction). This ratio was adjusted to 1.7% later in the season as the samples begin to show a decrease in measurable adenylates. After reaching a temperature of at least 100^{o}C, the EDTA extractant was transferred to the digestion tubes containing the dry plant material. This mixture was vortexed briefly to ensure wetting of all plant material then returned to the digestion block for 30 s. After 30 s, the samples were briefly vortexed again, then transferred to 50 mL centrifuge tubes. The tubes were sealed and the samples centrifuged using a Sorvall General Purpose RC3 Automatic Refrigerated Centrifuge at 5^{o}C for 15 min (HG4 Sorvall head, 3,200 rpm). After centrifugation, samples were placed in a refrigerator for immediate processing.

Each sample was directly filtered through a 0.2 μm membrane filter into sterile 250 μL conical glass HPLC vial inserts. Four blanks, which consisted of 1 mM EDTA extracted and filtered as described above, also were prepared. A sample run consisted of 27 samples, four blanks, two sets of four standards, and two samples prepared with internal standards.

While awaiting injection, samples were placed in a Waters Intelligent Sample Processor (WISP 712) and held at a constant temperature of 5^{o}C. Samples were analyzed

using reverse-phase high-pressure liquid chromatography on a Waters 600 Multisolvent Delivery System, Waters Intelligent Sample Processor and Waters 990 Photodiode Array Detector. Samples were separated through a Bio-Sil C_{18} HL 90-5S 250 x 4.6 mm (BioRad) column and Bio-Sil C_{18} HL 90-5 30 x 4.6 mm guard cartridge with both the mobile and stationary phase held at a constant temperature of 28^{o}C using a BioRad column heater. The mobile phase and gradient used for the separation of adenine nucleotides follows that of Cann-Moisan *et al*. (1989). Mobile phase A was KH_2PO_4 0.1 M pH 6.35 and mobile phase B was KH_2PO_4 w/ 10% Methanol 0.1 M pH 6.0. The gradient used for introduction and proportioning of mobile phases is shown in Table 2. This gradient was adjusted as needed to account for differences in sample strength and HPLC performance over time. Peaks were measured at 254 nm wavelength using a Waters 990 Photodiode Array Detector, while all detectable peaks were integrated individually using Waters 990 software. Both the column and precolumn were routinely cleaned with 5 full sample loop injections of 20% dimethyl sulfoxide (DMSO) in a flow of 100% acetonitrile for 0.5 to 1 hr at a flow rate of 0.3 ml/min (BIO-RAD cleaning procedures for reverse-phase columns). The Waters HPLC system was routinely cleaned with lukewarm, degassed, deionized water followed by 100% acetonitrile in order to remove buildup of salts which might effect pump efficiency, flow rates and sample retention times.

Table 4. High Pressure Liquid Chromatography gradient used for analysis of adenine nucleotides. Times and curves were periodically adjusted to give peak resolution of compounds. Curve values represent predetermined rates at which the two mobile phases are introduced into the flow rate. Adjustment of this curve allows for some control of when compounds come off of the column and allows for better resolution of peaks.

Time (min)	**Flow Rate (ml/min)**	**% Mobile Phase A**	**% Mobile Phase B**	**Curve**
0	0.8	100	0	*
4.50	0.8	100	0	6
10.00	0.8	75	25	7
13.00	0.8	10	90	6
18.00	0.8	0	100	6
27.00	0.8	100	0	6

Analysis of standards for regression and peak identification allowed for integration of ATP, ADP and AMP. Integrated data were used to calculate relative concentrations of ATP, ADP, AMP, ATP/ADP, ATP/AMP, AEC and TAN (total adenine nucleotides).

Standards

Adenine nucleotide standards of Adenosine 5'-Triphosphate (ATP), Adenosine 5'-Diphosphate (ADP), and Adenosine 5'-Monophosphate (AMP) (Sigma Chemical Co.) were prepared in concentrations of 1 mM each. Set volumes of each standard were then combined and diluted with 1 mM EDTA pH 7.4 to produce mixed standards at concentrations of 25, 50, 75, 100, and 150 μM. Each standard was then divided into 1 ml aliquots, flame sealed in Kimble ampules, and frozen until use. Two sets of four standards (25, 50, 75, 100 μM) were run with each separation, one set each at the beginning and end of each separation run. This was to allow accurate identification of peaks due to shifting in retention times during sample runs. Peak retention times of standards were used to identify sample nucleotide peaks within each separation. After integration, standard values were used to generate a regression of concentration vs. peak area for each nucleotide, ATP, ADP, and AMP. These regressions were used to calculate sample values of individual sample separations. As sample nucleotide concentrations decreased with season, standard concentrations were adjusted accordingly.

Internal standards were used to test for percent recovery of adenine nucleotides. For each separation, 2 samples were randomly chosen for addition of internal standard.

900 µL of plant extract was combined with 100 µL of 75, 100 or 150 µM mixed standard (ATP + ADP + AMP). Percent recovery of added standards averaged between 68 and 100%, with an overall average percent recovery of 93 ± 7.8% for the experiment.

APPENDIX E. MEAN CONCENTRATIONS, STANDARD ERRORS AND P-VALUES FOR ADENYLATE SPECIES BETWEEN STATES OF CHANGE, AND FLOODING TREATMENT.

P-values for significant effects are in bold type. Different lettered means within rows are significantly different. Values for ATP, ADP and AMP are given in nmol/g dry weight.

Month	Variable	Species	Creek	Creek SE	Hummocked	Hummocked SE	Non-hummocked	Non-hummocked SE	*P*-value
6/98	ADP	DS	2749	268	3104	471	3059	391	0.75
		SP	2164 a	205	1818 ab	254	1411 b	117	**0.05**
	AMP	DS	574	149	530	148	776	237	0.61
		SP	1106 a	179	1389 ab	199	675 b	112	**0.03**
	ATP/ ADP	DS	1.29	0.09	1.33	0.20	1.39	0.19	0.91
		SP	1.27	0.19	1.68	0.18	1.23	0.16	0.19
	ATP/ AMP	DS	12.29	1.54	11.25	1.78	12.20	2.28	0.91
		SP	3.93	0.60	2.44	0.40	3.50	0.64	0.16
7/98	ADP	DS	2343	209	2286	134	2089	138	0.51
		SP	1309	81	1825	143	1578	110	0.15
	AMP	DS	416	64	529	43	484	32	0.42
		SP	1055	379	1345	73	1061	86	0.06
	ATP/ ADP	DS	0.82	0.23	1.34	0.09	1.42	0.15	0.17
		SP	1.79	0.18	1.63	0.15	1.30	0.12	0.12
	ATP/ AMP	DS	7.01	0.68	5.81	0.86	4.38	0.41	0.53
		SP	3.23	0.63	1.98	0.16	2.08	0.22	0.06

Month	Variable	Species	Creek	Creek SE	Hummocked	Hummocked SE	Non-hummocked	Non-hummocked SE	*P*-value
8/98	ADP	DS	1561	159	1270	161	1128	86	0.20
		SP	1221	187	1170	136	1017	116	0.59
	AMP	DS	1004 a	121	675 b	77	592 b	61	**0.02**
		SP	1146	116	956	137	894	56	0.51
	ATP/ ADP	DS	0.88	0.16	1.08	0.09	1.35	0.15	0.20
		SP	1.04	0.20	1.90	0.47	1.47	0.21	0.45
	ATP/ AMP	DS	1.37	0.26	2.49	0.38	3.32	0.57	0.21
		SP	1.09	0.21	6.13	1.88	2.38	0.83	0.05
9/98	ADP	DS	2482	158	2493	213	2296	76	0.57
		SP	1167	344	1482	161	1305	89	0.45
	AMP	DS	383 ab	79	653 a	59	453 b	33	**0.005**
		SP	1003 b	49	1457 a	71	1252 ab	94	**0.02**
	ATP/ ADP	DS	0.49	0.06	0.80	0.09	0.61	0.06	0.11
		SP	2.21	0.77	1.30	0.21	1.30	0.23	0.28
	ATP/ AMP	DS	3.90	0.71	3.29	0.55	4.58	1.06	0.63
		SP	1.61	0.24	0.97	0.08	2.42	1.35	0.65

Month	Variable	Species	Control	Control SE	Ponded	Ponded SE	Subsurface Control	Subsurface Control SE	*P*-value
7/98	ADP	DS	2310	99	1871	174	2122	314	0.51
		SP	1712	141	1529	148	1657	136	0.63
	AMP	DS	502	41	488	52	553	51	0.45
		SP	1266 a	89	966 b	100	1381 a	165	**0.001**
	ATP/	DS	1.34	0.09	1.63	0.26	1.19	0.17	0.24
	ADP	SP	1.61	0.15	1.23	0.16	1.52	0.20	0.16
	ATP/	DS	7.14	0.79	6.24	1.07	4.38	0.41	0.09
	AMP	SP	2.10	0.17	2.26	0.38	1.78	0.19	0.45
8/98	ADP	DS	1172	118	1084	80	1035	127	0.54
		SP	1004	121	1024	137	1074	285	0.92
	AMP	DS	688	82	567	69	438	66	0.06
		SP	846	129	880	149	967	96	0.82
	ATP/	DS	1.18	0.14	1.12	0.15	1.70	0.32	0.18
	ADP	SP	1.80	0.47	1.43	0.21	1.79	0.55	0.70
	ATP/	DS	2.52	0.43	2.61	0.45	5.04	1.39	0.06
	AMP	SP	5.52	1.75	4.47	1.92	1.25	0.40	0.79
9/98	ADP	DS	2391	142	2326	224	2255	133	0.96
		SP	1455	162	1408	117	1311	165	0.99
	AMP	DS	587	52	467	62	493	61	0.44
		SP	1291	85	1388	152	1251	147	0.54

Month	Variable	Species	Control	Control SE	Ponded	Ponded SE	Subsurface Control	Subsurface Control SE	*P*-value
	ATP/	DS	0.68	0.08	0.79	0.14	0.56	0.05	0.49
	ADP	SP	1.38	0.34	1.24	0.22	1.05	0.15	0.64
	ATP/	DS	2.90	0.32	4.87	1.06	5.41	2.89	0.46
	AMP	SP	0.99	0.09	3.88	2.78	1.02		0.35

APPENDIX F. ANOVA RESULTS FOR REPEATED MEASURES ANALYSIS OF WATER LEVEL AND POREWATER SALINITY.

Statistic used for ANOVA results is Wilks' Lambda.

Source of variation	d.f.	MS	F	P
a. *water level*				
time	64	2.5E-06	13.5732	0.0001
time*site (all sites)	16	0.0011	453.876	0.0001
time*treatment (treatment sites only)	32	4.53111	1.22	0.2148
b. *salinity*				
time	15	0.00243	246.122	0.0001
time*site (all sites)	60	1.1E-06	20.1406	0.0001
time*treatment (treatment sites only)	30	8.7982	0.81	0.7423

APPENDIX G. ANOVA RESULTS FOR SOIL BULK DENSITY, MACRO-ORGANIC MATTER, AND PERCENT WATER CONTENT.

Source of variation	d.f.	MS	F	P
a. *bulk density*				
site	4	0.0338	26.9677	<0.0001
change	2	0.025	6.8899	0.003
b. *macro-organic matter*				
site	4	4.7306	4.3391	0.0172
change	2	7.0541	5.6215	0.0142
c. *percent water content*				
site	4	397.9978	10.3254	<0.0001
change	2	292.562	4.4933	0.0183

APPENDIX H. ANOVA FOR 1997 ABOVEGROUND BIOMASS OF *D. SPICATA* AND *S. PATENS*.

Change (creek, hummocked, non-hummocked); site (1-5); treatment (control, ponded, subsurface); species (*D. spicata, S. patens*).

	Distichlis spicata				*Spartina patens*			
Source of variation	d.f.	MS	F	P	d.f.	MS	F	P
a. *live biomass*								
change	2	149426.51	5.01	0.0132	2	300366.68	5.58	0.0087
site	4	79849.16	2.68	0.0506	4	189043.4	3.51	0.0182
treatment	2	15214.79	1.55	0.2426	2	33868.8	1.97	0.1717
site*treatment	3	2138.57	0.22	0.8826	3	1609.65	0.09	0.9625
b. *total biomass*								
change	2	417337.56	2.95	0.0679	2	1138101.1	6.1	0.006
site	4	213535.69	1.51	0.2251	4	815308.13	4.37	0.0067
treatment	2	131597.72	1.86	0.1875	2	166016.92	3.41	0.0585
site*treatment	3	9020.91	0.13	0.9423	3	98845.89	2.01	0.1528
c. *live biomass* (*D. spicata* + *S. patens*)								
species	1	382399.51	9.59	0.0029				
change	2	647404.07	7.46	0.0023				
site	4	143900.6	1.66	0.2073				
d. *total biomass* (*D. spicata* + *S. patens*)								
species	1	1228840.3	7.74	0.0071				
change	2	1383301.8	3.74	0.0354				
site	4	949146.31	2.57	0.0582				

APPENDIX I. ANOVA RESULTS FOR LOG TRANSFORMED RATIOS OF *D. SPICATA* TO *S. PATENS* ABOVEGROUND BIOMASS.

Change (creek, hummocked, non-hummocked); site (1-5); treatment (control, ponded, subsurface).

	1997				1998			
Source of variation	d.f.	MS	F	P	d.f.	MS	F	P
a. *live biomass*								
change	1	0.5283109	5.48	0.0248	2	2.1178883	7.39	0.0045
site	1	0.1328014	1.38	0.2965	1	0.3198429	1.12	0.3491
treatment	2	0.0108621	0.15	0.8633	2	0.0066388	0.06	0.9459
change*treatment	1	0.0415374	0.39	0.5643	1	0.2394692	2.02	0.186
b. *total biomass*								
change	1	0.6720502	9.68	0.0031	1	2.48341	10.06	0.0009
site	1	0.4089979	5.89	0.0165	1	0.2378234	0.96	0.3987
treatment	2	0.0214173	0.49	0.6327	2	0.0034837	0.04	0.9625
change*treatment	1	0.1881135	4.34	0.0823	1	0.2353683	2.59	0.1334

APPENDIX J. ANOVA RESULTS FOR 1997-1998 COMPARISONS OF END-OF-YEAR ABOVEGROUND BIOMASS USING THE MIXED PROCEDURE.

	Distichlis spicata				*Spartina patens*			
Source of variation	d.f.	MS	F	P	d.f.	MS	F	P
a. *live biomass*								
treatment	2	n/a	0.42	0.6588	2	n/a	0.32	0.7249
year	1	n/a	11.63	0.0011	1	n/a	14.37	0.0003
treatment*year	2	n/a	2.62	0.0804	2	n/a	3.48	0.0366
b. *total biomass*								
treatment	2	n/a	0.98	0.3805	2	n/a	0.03	0.9665
year	1	n/a	7.28	0.0089	1	n/a	13.96	0.0004
treatment*year	2	n/a	2.51	0.0893	2	n/a	2.67	0.077
c. *total biomass (D. spicata + S. patens)*								
treatment	2	n/a	0.27	0.7606				
year	1	n/a	23.16	0.0001				
treatment*year	2	n/a	5.2	0.008				

APPENDIX K. ANOVA RESULTS FOR SEASONAL DIFFERENCES IN ADENYLATES.

Source of variation	d.f.	MS	F	P
a. *ATP*				
season	1	186355043	250.55	0.0001
season*change	2	573024.76	0.77	0.4634
season*site	2	2522747.7	3.39	0.0344
season*species	1	11152907	14.99	0.0001
b. *TAN*				
season	1	435888483	127.86	0.0001
season*change	2	2105409.9	0.62	0.5397
season*site	2	1947961.8	0.57	0.5651
season*species	1	20302111	5.96	0.015
c. *AEC*				
season	1	1.0394858	134.61	0.0001
season*change	2	0.008029	1.04	0.3543
season*site	2	0.0056663	0.73	0.4806
season*species	1	0.013845	1.79	0.1812

APPENDIX L. ANOVA RESULTS FOR ADENYLATES BY MONTH (SPECIES SEPARATE).

	Distichlis spicata				*Spartina patens*			
Source of variation	d.f.	MS	F	P	d.f.	MS	F	P
a. *June ATP*								
change	2	741506.9	0.43	0.6508	2	4295595.9	3.85	0.0264
site	2	10621158	6.2	0.0036	2	20443.511	0.02	0.9819
b. *June AEC*								
change	2	0.0016467	0.22	0.8406	2	0.0011334	0.18	0.8324
site	2	0.003994	0.53	0.5918	2	0.0025729	0.42	0.6604
c. *June TAN*								
change	2	3139616	0.33	0.7209	2	26622012	3.87	0.0258
site	2	25210491	2.64	0.0793	2	334769.32	0.05	0.9526
d. *July ATP*								
change	2	2943460.9	4.78	0.0122	2	2727712	3.57	0.0347
site	2	3719606.7	6.04	0.0043	2	7989545.5	10.46	0.0001
treatment	2	1591487.3	3.03	0.0578	2	2867862.5	3.54	0.0372
site*treatment	3	212903.69	0.41	0.7495	3	979486.12	1.21	0.3172
e. *July AEC*								

change	2	0.0118091	3.08	0.054	2	0.0140881	1.86	0.1652
site	2	0.0036268	0.95	0.3945	2	0.0097194	1.28	0.2852
treatment	2	0.0061608	1.73	0.1892	2	0.00178	0.26	0.7712
site*treatment	3	0.0018917	0.53	0.6638	3	0.0140994	2.07	0.1173
		Distichlis spicata				*Spartina patens*		
Source of variation	d.f.	MS	F	P	d.f.	MS	F	P
f. *July TAN*								
change	2	4762919.6	3.5	0.037	2	9462519.8	4.89	0.011
site	2	7821112.9	5.74	0.0054	2	17258388	8.92	0.0004
treatment	2	4332750.7	3.65	0.0338	2	9846900.5	5.25	0.0088
site*treatment	3	344025.92	0.29	0.8325	3	1662073.4	0.89	0.4555
g. *August ATP*								
change	2	9165.4409	0.02	0.9799	2	1493624.8	4.76	0.0123
site	2	609400.28	1.35	0.268	2	67005.747	0.21	0.8084
treatment	2	198923.24	0.47	0.6254	2	179509.54	0.53	0.5938
site*treatment	3	2513120.5	5.99	0.0016	3	2918.1449	0.01	0.9989
h. *August AEC*								
change	2	0.0153768	1.7	0.1922	2	0.0676158	4.11	0.0217
site	2	0.0104944	1.16	0.3211	2	0.0231014	1.4	0.2543
treatment	2	0.0303583	3.09	0.0553	2	0.0075494	0.41	0.6649
site*treatment	3	0.0035944	0.37	0.7784	3	0.006793	0.37	0.7746
i. *August TAN*								

change	2	1915291.3	1.41	0.2523	2	3726402.6	2.24	0.116
site	2	8473577	6.25	0.0036	2	3102260.9	1.86	0.1645
treatment	2	1385402.7	1.21	0.3075	2	6109.0422	0	0.9964
site*treatment	3	8471582	7.4	0.0004	3	1453302.2	0.85	0.4718

	Distichlis spicata				*Spartina patens*			
Source of variation	d.f.	MS	F	P	d.f.	MS	F	P
j. *September ATP*								
change	2	881841.39	3.38	0.0411	2	286695.21	1.7	0.193
site	2	169901.86	0.65	0.5252	2	63274.202	0.37	0.6895
treatment	2	208530.96	0.74	0.4812	2	474800.04	2.8	0.0716
site*treatment	3	303525.84	1.08	0.3663	3	220321.77	1.3	0.2866
k. *September AEC*								
change	2	7.052E-05	0.02	0.9804	2	0.0137885	3.17	0.0499
site	2	0.0001396	0.04	0.9617	2	0.0023332	0.54	0.5879
treatment	2	0.0027367	0.76	0.4714	2	0.0023332	0.53	0.5924
site*treatment	3	0.0037135	1.04	0.3849	3	0.0044551	1.01	0.3966
l. *September TAN*								
change	2	3946663.9	5.08	0.0094	2	1812062.3	2.17	0.1238
site	2	543587.33	0.7	0.5013	2	1680945.4	2.01	0.1433
treatment	2	50084.287	0.06	0.9374	2	1092119.4	1.37	0.2641
site*treatment	3	935883	1.21	0.3167	3	1922204.4	2.42	0.0791

APPENDIX M. ANOVA RESULTS FOR ADENYLATES BY MONTH (SPECIES COMBINED).

Source of variation	d.f.	MS	F	P
a. *June ATP*				
change	2	1175413	0.84	0.4351
site	2	4958560	3.53	0.0322
species	1	33889477	24.16	0.0001
change*species	2	3243878	2.31	0.1033
site*species	2	5754181	0.61	0.5449
b. *June AEC*				
change	2	0.003264	0.48	0.6211
site	2	0.00324	0.47	0.6232
species	1	0.273742	40.11	0.0001
change*species	2	0.000312	0.05	0.9553
site*species	2	0.005972	0.87	0.4194
c. *June TAN*				
change	2	7724758	0.95	0.3908
site	2	13501041	1.65	0.1953
species	1	91718781	11.24	0.0011
change*species	2	21827375	2.67	0.0728
site*species	2	10384149	1.27	0.2837
d. *July ATP*				
change	2	4181983	6.05	0.0032
site	2	10275757	14.88	0.0001
species	1	4727275	6.84	0.0101
change*species	2	1429050	2.07	0.1311
site*species	2	1563397	2.26	0.1088
treatment	2	2184104	3.27	0.0424
treatment*species	2	2334547	3.5	0.0343

Source of variation	d.f.	MS	F	P
e. *July AEC*				
change	2	0.003084	0.54	0.5848
site	2	0.010321	1.8	0.1694
species	1	0.316429	55.31	0.0001
change*species	2	0.022026	3.85	0.0242
site*species	2	0.005059	0.88	0.4159
treatment	2	0.004481	0.86	0.4251
treatment*species	2	0.004728	0.91	0.4057
f. *July TAN*				
change	2	13425602	8.14	0.0005
site	2	22163068	13.43	0.0001
species	1	2477422	1.5	0.223
change*species	2	1000237	0.61	0.5471
site*species	2	2807263	1.7	0.187
treatment	2	9414043	6.15	0.0031
treatment*species	2	2614473	1.71	0.1871
g. *August ATP*				
change	2	849482.2	2.22	0.1135
site	2	520646.8	1.36	0.261
species	1	165501.4	0.43	0.5123
change*species	2	653308	1.71	0.1863
site*species	2	155759.2	0.41	0.6668
treatment	2	251867.3	0.66	0.5178
treatment*species	2	126565.5	0.33	0.7176
h. *August AEC*				
change	2	0.037384	2.93	0.0575
site	2	0.015973	1.25	0.2899

species	1	0.078061	6.12	0.0149
change*species	2	0.045609	3.57	0.0313
site*species	2	0.017623	1.38	0.2555
treatment	2	0.005623	0.4	0.672
treatment*species	2	0.032284	2.29	0.1068

Source of variation	d.f.	MS	F	P
i. *August TAN*				
change	2	4909259	3.25	0.0424
site	2	10911304	7.22	0.0011
species	1	214597.5	0.14	0.7069
change*species	2	732434.6	0.48	0.617
site*species	2	828079.8	0.55	0.5795
treatment	2	749370.5	0.53	0.5925
treatment*species	2	685614.2	0.48	0.6193
j. *September ATP*				
change	2	684190.5	3.17	0.0458
site	2	106898.5	0.5	0.6106
species	2	312883.3	1.45	0.2311
change*species	2	460315	2.13	0.1233
site*species	2	116340.6	0.54	0.5847
treatment	2	676646.3	2.99	0.0553
treatment*species	2	40823.56	0.18	0.8352
k. *September AEC*				
change	2	0.005593	1.42	0.2472
site	2	0.011838	3	0.0541
species	1	0.30293	76.65	0.0001
change*species	2	0.007859	1.99	0.1418
site*species	2	0.014842	3.76	0.0264
treatment	2	0.005603	1.41	0.2502
treatment*species	2	3.13E-06	0	0.9992

l. *September TAN*

change	2	5582075	6.93	0.0015
site	2	1805946	2.24	0.111
species	1	3859635	4.79	0.0307
change*species	2	169477.2	0.21	0.8106
site*species	2	473220.7	0.59	0.5574
treatment	2	743959.6	0.95	0.3911
treatment*species	2	589613.5	0.75	0.4745