# YEAR TWO - MONITORING REPORT 2004-2006

# FLOATING MARSH CREATION DEMONSTRATION PROJECT (LA-05)

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# INTRODUCTION

The Coastal Wetlands Planning, Protection, and Restoration Act (CWPPRA) of 28 November, 1990, House Document 646, 101st Congress, provides for the use of federal funds for planning and implementing projects that create, protect, restore, and enhance coastal wetlands of the United States, including Louisiana. The Floating Marsh Creation Demonstration Project (LA-05) was approved for funding and included on the Twelfth Priority Project List which was transmitted to Congress in December 2003.

Land loss in coastal Louisiana has been well documented and related to a variety of causes (Craig et al. 1979, Gagliano et al. 1981, Sasser et al. 1986, Evers et al. 1992, Britsch and Dunbar 1993). This 90 $km^2 yr^{-1}$ (20,000 acres $yr^{-1}$) loss covers all marsh types, including freshwater floating marshes. Even though the remaining marshes in the upper part of the coast have remained fresh since they were first mapped by O'Neil (1949), significant areas of marsh have converted to open water, and vegetation associations have changed from thick-mat maidencane (*Panicum hemitomon*) dominated marsh to thin-mat spikerush (*Eleocharis baldwinii*) dominated marsh (Visser et al. 1999). Visser et al. (1999) identified the following potential causes for the dramatic change in fresh marsh vegetation and land loss: grazing by nutria, increased water levels, hydrologic modifications, and eutrophication. Increased salinity and sulfide concentrations as a result of hydrologic alterations has also been identified as a stressor on *Panicum hemitomon* marshes (Gosselink and Sasser 1995). Sasser et al. (2004) show that grazing by nutria may be the most important of these factors in freshwater marshes. Although the effect of nutria grazing on maidencane marshes has not yet been directly quantified, nutria grazing helps prevent the re-establishment of *P. hemitomon* in spikerush marshes (Visser et al. 2001). In addition, recent research has shown that *P. hemitomon* grows well in the spikerush floating marsh with or without nutrient enhancement when protected from grazing (Sasser et al. 2005). This indicates that no nutrient limitation exists in the maidencane marsh areas that have converted to spikerush marsh and open water.

The belowground structure of *P. hemitomon* is characterized by extensive root and rhizome allocation that results in an organic root mat that is very fibrous and buoyant. *P. hemitomon's* extensive network of fibrous roots and rhizomes is crucial for forming well-integrated floating marsh mats. The ability of other co-dominant or subordinate species (e.g., *Sagittaria lancifolia*, *Eleocharis baldwinii*) to form this type of highly-buoyant floating root mat in the absence of *P. hemitomon* seems improbable based on their respective belowground morphologies and general architecture. Therefore, *P. hemitomon* probably plays a key role in the successful formation and sustainability of healthy (thick mat) freshwater floating marshes (Sasser et al. 1994, Holm et al. 2000), and is the primary plant species utilized in this project.

Wetland plant species typically display aerenchyma (tissue air space) development in their tissues, which facilitates oxygen diffusion to the roots and may also reduce the amount of living, respiring tissue in roots relative to root volume (Armstrong 1979, Jackson et al. 1985, Schussler and Longstreth 1996). Although wetland plants generally form aerenchymatous tissues during their normal development, aerenchyma can also be induced in many wetland plants when subjected to waterlogged or hypoxic conditions (Schat 1984, Burdick 1989, Schussler and Longstreth 1996). Formation of adventitious roots is widespread in grass species regardless of soil conditions, but also occurs in plants subjected to conditions in which the primary root cannot function properly, such as in waterlogged conditions where soil oxygen levels are depleted to the point of inhibiting aerobic metabolism (Jackson and Drew 1984). Flood-induced adventitious roots are typically very porous due to the prevalence of aerenchymatous tissue, which facilitates the diffusion of gases, such as oxygen from shoots to roots, thereby enabling many plants to grow in hypoxic or anoxic soils (reduced soils) that typically form under flooded conditions (Armstrong 1979, Dacey 1980, Jackson et al. 1985; Drew 1992; Naidoo et al. 1992). Therefore, the induction of aerenchyma and the formation of adventitious roots are viewed as mechanisms of facilitating aerobic root respiration under flooded soil conditions and would likely have tremendous implication for root production and mat buoyancy in floating marshes.

## Project Objective

This demonstration project consists of two phases. The first phase, which was performed at Louisiana State University (LSU) and University of New Orleans (UNO), is the development of artificial floating-marsh systems (AFS) and consists of two components. The first component is development of a floating system which would provide the structure to keep the substrate in place and would provide the buoyancy during the period in which *Panicum hemitomon* plants become established. For this component, structures using a variety of mat materials, support structures, and plant materials were evaluated.

The second component of the first phase consists of efforts to understand the plant response to nutrients, flooding, and substrate in order to develop methods to maximize the establishment and growth of *P. hemitomon* in AFS.

Based on the structural integrity, buoyancy, and growth response results from the first phase investigations, two designs were brought forward for deployment in the second phase. The second phase consists of field testing of the two selected designs in a marsh setting, and was initiated in March 2006.

The objective of this demonstration is to develop methods for restoration of open areas within thin and deteriorated mats that once supported thick-mat maidencane marsh and other fresh water areas where establishment of maidencane marsh is desired.

In this report, we present results for years 1 and 2 of the floating marsh creation demonstration project.

## METHODS

### Artificial Floating Marsh Systems

Illustration 1. Testing of Artificial Floating Marsh Systems (August 22, 2005).

We have developed 27 AFS designs that were tested in an outdoor laboratory setting with *P. hemitomon* established from nursery stock and/or plugs harvested from healthy marshes (Appendix A). Dimensions ranged from 1.5-9.3 $m^2$ (16 to 100 $ft^2$), with at least three replicates of each. Frames were constructed using pine wood, PVC, Styrofoam, cedar wood, bamboo, or combinations of these materials (Table 1). Mat materials tested included rope, jute netting, straw-coconut, burlap, coconut, birch, as well as hydroponic growth on chicken wire mats. Hardwood mulch and a peat-bagasse mixture were added to several mats to provide additional substrate. Plants were established using plugs harvested from donor marshes, whole plant fragments (*P. hemitomon* pieces containing both above- and below-ground material), *P. hemitomon* belowground material, or *P. hemitomon* aboveground material (Table 1).

Construction was designed such that each AFS could be assembled in the field. Each design incorporated an anchoring system to minimize horizontal movement, while not hindering vertical movement of the AFS, and we used biodegradable materials where feasible. The AFSs were designed to maintain sufficient structural integrity until the established *P. hemitomon* mat becomes self sustainable. The fabrication of each design is such that multiple units could be attached one to another to create larger areas of floating *P. hemitomon* marsh for field testing. Structures of AFS 1 through 12 were deployed in the ponds in the summer of 2004, structures of AFS 13 through 24 were deployed in the spring of 2005, and structures of AFS 25 through 27 were deployed in summer 2005. Fences were added to structures of AFS 5, 6, 7, and 12 in October 2004 to eliminate grazing. In March 2005, the previously fenced structures were replanted and AFS 3 was replanted and fenced. Structures of AFS 15 through 27 were fenced before deployment in the pond. Buoyancy, structural integrity, and plant cover of each AFS were assessed several times a month.

Table 1. Overview of the 27 Artificial Floating Systems developed during 2004-05.

| AFS | Dimension (ft) | Frame | Mat | Substrate | Plant Source |
|---|---|---|---|---|---|
| 1 | 10 x 10 | pine | rope | none | large plugs |
| 2 | 10 x 10 | pine | jute | hardwood mulch | plugs |
| 3 | 4 x 10 | PVC & pine | straw-coconut | hardwood mulch | plugs |
| 4 | 10 x 10 | Styrofoam & pine | burlap | hardwood mulch | plugs |
| 5 | 10 x 10 | PVC | coconut | hardwood mulch | A & C plugs; B whole plants |
| 6 | 4 x 4 | pine | birch | hardwood mulch | plugs |
| 7 | 4 x 4 | PVC | coconut | hardwood mulch | plugs |
| 8 | 4 x 4 | none | burlap | water-hyacinth | plugs |
| 9 | 4 x 4 | pine & Styrofoam | coconut | hardwood mulch | plugs |
| 10 | 4 x 4 | pine | coconut | hardwood mulch | plugs |
| 11 | 4 x 4 | pine | rope | none | plugs |
| 12 | 4 x 4 | pine | chicken-wire | none | plugs |
| 13 | 4 x 4 | PVC | chicken-wire | none | plugs |
| 14 | 4 x 4 | PVC | chicken-wire | peat & bagasse | whole plants |
| 15 | 4 x 4 | cedar lattice | coconut | none | plugs |
| 16 | 4 x 4 | cedar lattice | coconut | none | plugs |
| 17 | 4 x 4 | cedar lattice | none | none | plugs |
| 18 | 4 x 4 | cedar lattice | coconut | none | plugs |
| 19 | 4 x 4 | pine | chicken-wire | none | A & C whole plants;<br>B plugs |
| 20 | 4 x 4 | pine | birch | peat & bagasse | A & B plugs;<br>C whole plants |
| 21 | 4 x 4 | pine | coconut | peat & bagasse | A & B plugs;<br>C whole plants |
| 22 | 4 x 4 | bamboo | chicken-wire | none | A whole plants;<br>B & C plugs;<br>D, E, & F belowground;<br>G, H, I aboveground |
| 23 | 4 x 4 | bamboo | birch | peat & bagasse | B & C whole plants;<br>A plugs |
| 24 | 4 x 4 | bamboo | coconut | peat & bagasse | A & B whole plants;<br>C plugs;<br>D, E, & F belowground;<br>G, H, I aboveground |
| 25 | 4 x 4 | PVC | none | styrofoam | peat pots |
| 26 | 4 x 10 | PVC | chicken-wire | peat | peat pots |
| 27 | 4 x 10 | PVC | chicken-wire | none | whole plants |

*Plant establishment*

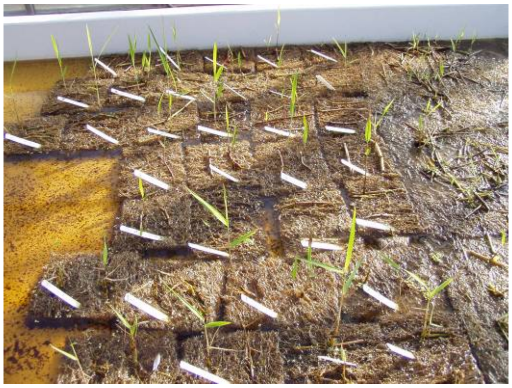

Illustration 2. Testing of Vegetative Establishment

In addition to determining which AFS would be successful in keeping plant material buoyant in a larger scale application for initiating floating marsh development, we also tested which types of plant material could be used to establish *P. hemitomon* under greenhouse conditions. We tested the application of using *P. hemitomon* whole plants, as well as rhizome or aerial stem material. Furthermore, we tested for the smallest portion to consistently result in the growth of new plants. Sizes used included no node, one node, and two node pieces.

Fragment, stem and rhizome pieces were placed on individual squares of coconut mat material. These mats were monitored to determine the number of shoots produced by each different type and size of plant material.

## Optimization of Plant Response

A series of greenhouse and controlled-setting experiments were conducted in an effort to elucidate growth characteristics and patterns of biomass allocation in *P. hemitomon*. More specifically, it is our intent to identify those conditions (i.e., nutrient loading rate, flooding depth, substrate type, mat material, and subordinate plant species) that will not only lead to vigorous growth by *P. hemitomon*, but will facilitate or enhance the overall rate of flotant mat development within the restoration context. These experiments are described in more detail below.

*Experiment-1: Evaluating the effects of nutrient loading rate and flooding depth*

Illustration 3. Experiment-1 (before Katrina): Evaluating the effects of nutrient loading rate and flooding depth

The first version of this experiment was initiated in June of 2004 and was ended prematurely in August of 2005 as a result of extensive flooding and physical damage associated with Hurricane Katrina. The experimental design was a 3 x 3 x 2 completely cross-classified factorial with 3 levels each of both nitrogen and phosphorous and 2 levels of flooding, all replicated in 5 blocks for a total of 90 experimental units (n = 90). Nitrogen was applied at the rates of 2.5, 25, and 50 g N $m^{-2}$ $yr^{-1}$ and phosphorous was applied at a the rates of 0.5, 5, and 10 g P $m^{-2}$ $yr^{-1}$. Additions of N and P were administered weekly while micronutrient solutions were applied monthly, coinciding with

Illustration 4. Experiment-1: Destruction due to Hurricane Katrina

the monthly rinsing and scrubbing of the vessels. Nutrient solutions were completely replaced to minimize algal growth. Flooding treatments were flooded to the surface of the mat (flooding depth of 0 cm) and flooded to a depth of 15 cm. Each experimental unit consisted of two square layers of DuraLast coconut fiber mat (Duralast Products, Memphis, TN) fastened together by plastic tie straps. This was essentially a hydroponic design that did not include a typical substrate material (i.e., the DuraLast coconut fiber served as the planting medium and substrate). Four individual plugs of commercially-grown *P. hemitomon* were planted into the corners of each double-layer mat sandwich, and each vegetated mat was placed in their respective experimental units. Each vessel was filled to near-capacity with deionized water to ensure that manipulations of nutrient availability remained constant. Flooding treatments (0 and 15cm) were maintained by placing each vegetated mat on a segment of 4”PVC pipe that was cut to a pre-determined length.

Data collection included bi-weekly (initially) and then monthly measurements of cumulative stem height. Maximum root length and percent root coverage were measured and/or estimated bi-monthly, although these assessments did not occur until several months after the initiation of the experiment. Maximum root length represented the distance from the bottom of the vegetated mat to the tip of the longest root, while percent root coverage represented the percentage of area of the bottom of the vegetated mat with root tissue protruding from it. Net photosynthesis and stomatal conductance were measured at peak standing crop (after 12 months of growth) using a Li-Cor 6400 portable photosystem. Net photosynthesis and stomatal conductance were measured on a single leaf, second from the terminal leaf of each plant in each experimental unit. Each leaf was then clipped, dried and ground for CHN analysis. Root specific gravity was measured in June of 2005. Live root samples were clipped from each vegetated mat and taken to the Coastal Plant Sciences Laboratory for analysis. Root specific gravity was computed using the formula: $SG = R/ (P + R - PR)$, where R = mass of roots, P = mass of water-filled pycnometer, and PR = mass of pycnometer with roots and water. Biomass was measured on 62 of the 90 experimental units post-Katrina. The remaining 28 units were not identifiable, and consequently not recoverable. Biomass was divided into dead above- and belowground. Separating biomass into live and dead was not possible because the majority of the vegetated mats succumbed to desiccation stress. All biomass was oven-dried at 60˚C until a constant mass was attained.

The version of experiment-1 after Katrina, initiated in March of 2006, was designed much like the first version in terms of the experimental design and the expected outcomes. The experimental design was a 2 x 2 x 2 completely cross-classified factorial with 2 levels of both N and P loading, and two levels of flooding, replicated in 5 blocks for a total of 40 experimental units (n = 40). The two nitrogen loading rates were 25 and 50 g N $m^{-2}$ $yr^{-1}$ and phosphorous loading rates were 5 and 10 g P $m^{-2}$ $yr^{-1}$. As in the first version, flooding depths were either flooded to the surface (0 cm) of the mat or flooded to a depth of 15 cm. The primary difference

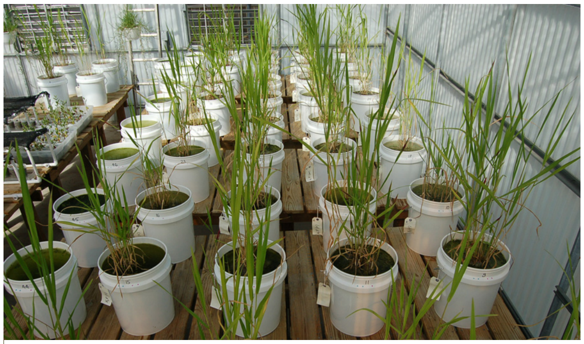
Illustration 5. Experiment-1 (after Katrina): Evaluating the effects of nutrient loading rate and flooding depth

between the experiment before and after Katrina was the addition of a true substrate to the double-layer DuraLast coconut fiber configuration. Sphagnum peat served as the substrate and was placed between the two layers of coconut fiber. Plastic tie straps were once again used to fasten the materials together. A single plug of *P. hemitomon* was planted in the center of each DuraLast coconut fiber and peat sandwich. Plant material for Experiment-1 after Katrina was collected from a non-cultivated population of *P. hemitomon* on the USDA Golden Meadow Plant Materials Center property in Galliano, LA. Root stock was brought back to the UNO greenhouse facility and propagated until planting occurred.

Data collection followed a similar protocol as before Katrina, although several modifications were made to the sampling regime. Cumulative stem height was measured monthly over the 4 month course of the experiment. In an effort to minimize disturbance of each experimental unit, maximum root length and percent root coverage were not measured. On the other hand, substrate redox potential was measured in a bi-monthly fashion. As in the first version, root specific gravity was assessed at peak standing crop (June 2006), as was net $CO_2$ assimilation and stomatal conductance. Leaf samples were clipped, dried and ground for CHN analysis. Additional leaf material was collected for determining the relationship between leaf mass and leaf area. The most significant changes in the methods for Experiment-1 after Katrina occurred at harvest. Each vegetated mat underwent a complete census which not only included the separation of biomass into above- and belowground components, but the separation of belowground biomass into roots and rhizomes. Each vegetated mat was disassembled and all biomass recovered. Aboveground biomass was clipped, although not separated into live and dead because all material was live at the time of harvest. The length of all rhizomes was measured, and all root tissue was set aside for further analyses. Complete root systems were scanned and quantified in terms of volume using an Epson 10000XL high-resolution scanner and Whin-Rhizo Pro-Version root imaging software (Regent Instruments, Quebec, Canada). Furthermore, 5 individual root samples from each root system were reserved in order to assess individual root morphology, once again using Whin-Rhizo Pro-version root imaging software. All above- and belowground biomass was oven-dried at 60˚C until a constant mass was attained.

### *Experiment-2: Evaluating the effects of substrate type*

Experiment-2 was initiated in October of 2004 and was ended prematurely in August of 2005 due to Hurricane Katrina. In preparation for the eminent landfall of Hurricane Katrina this experiment was relocated from an outdoor setting to a semi-protected greenhouse setting where it was spared physical damage (although all plant material subsequently died due to desiccation because the UNO Campus was inaccessible for over one month post Katrina).

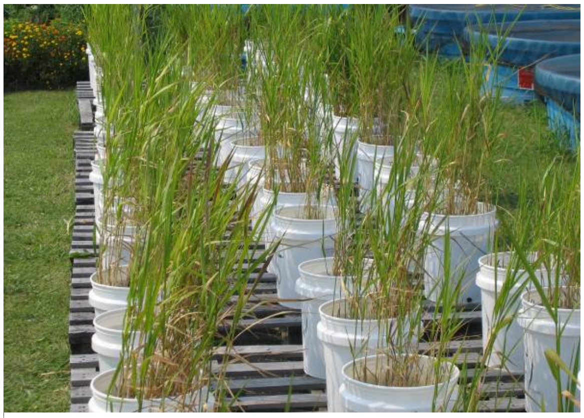

Illustration 6. Experiment-2: Evaluating the effects of substrate type.

The experimental design included one mat material (DuraLast coconut fiber), multiple substrate types (7 individual types and 5 blends), all replicated in 5 blocks for a total of 60 experimental units (n = 60). Each experimental vessel contained a layer of mat material followed by a layer of substrate, culminating with another layer of mat material in that order from bottom to top. Individual substrate types included sphagnum peat, bagasse, sugarcane leaf strippings, pine shavings, cypress mulch, hardwood mulch, and pine bark mulch. Substrate blends included sphagnum peat x bagasse, sphagnum peat x hardwood mulch, sphagnum peat x cypress mulch, cypress mulch x bagasse, and hardwood mulch x sugarcane leaf strippings. The fertilization regime, applied once every three months, was uniform across all treatments (23.4 g N $m^{-2}$ $yr^{-1}$ and 2.0 g P $m^{-2}$ $yr^{-1}$). Flooded conditions were maintained at 10 cm above the mat's surface in all treatments. A single plug of *P. hemitomon* was planted in the center of each DuraLast coconut fiber and substrate sandwich. Plant material was collected from a non-cultivated population of *P. hemitomon* on the USDA Golden Meadow Plant Materials Center property in Galliano, LA. Root stock was brought back to the UNO greenhouse facility and propagated until planting occurred.

Cumulative stem height was measured monthly over the duration of the experiment, while interstitial metrics such as pH, conductivity and substrate redox potential were measured bi-monthly. Net carbon dioxide ($CO_2$) assimilation and stomatal conductance were measured on a single leaf, second from the terminal leaf of each plant in each experimental unit. These measurements were done using a Li-Cor 6400 portable photosystem. Each leaf was clipped, dried and ground for CHN analysis. Because all experimental units were not living at the time of salvage, harvest followed a different protocol than was originally intended. Above- and belowground biomass was separated but root and rhizome components were not distinguishable, nor were they separable from the substrate itself. Because of this, all belowground biomass had to be described in terms of a total change value. Pre-weights were determined at the outset of the experiment, and when combined with dry weights at the time of harvest, allowed for the calculation of a total change value. All biomass was oven-dried at 60˚C until a constant mass was attained.

A supplemental study to experiment-2 designed to elucidate substrate chemical oxygen demand (COD) as a function of substrate type was conducted between February 2006 and September 2006. The original 7 individual substrates, along with two controls (water only and water x DuraLast coconut fiber) served as the 9 treatments. Each treatment was replicated 5 times for a total of 45 experimental units (n = 45). Interstitial samples were withdrawn throughout the study and sent to Nichols State University in Thibodaux, LA for processing and analysis. Interstitial pH was measured at each sampling, and substrate redox potential was measured twice over the 20-week period. Pre-weights were determined for all experimental units at the outset of the experiment, and final weights were determined at completion. This allowed for the calculation of substrate mass loss due to decomposition.

### *Experiment-3: Evaluating the effects of mat type*

Illustration 7. Experiment-3: Evaluating the effects of mat type.

Experiment-3 was initiated in May of 2005 and, like experiment-2, was ended prematurely in August of 2005 due to Hurricane Katrina. Experiment-3 was also relocated from an exposed outdoor setting to a semi-protected greenhouse setting where it was spared physical damage (but as with experiment-2, all plant material died due to desiccation stress). The overall objective of this experiment was not only to compliment the results of the substrate experiment (experiment-2), but to specifically assess *P. hemitomon* growth responses when grown in conjunction with different commercially available mat or containment materials.

The experimental design included 5 mat materials and 1 substrate material (sphagnum peat), all replicated 5 times for a total of 25 experimental units (n = 25). Mat materials included two composed of coconut fiber (i.e., DuraLast coconut fiber and plain coconut fiber with plastic mesh) and one each of burlap, shredded birch and wheat straw. Each sphagnum peat and mat combination was housed in a 7.5-L container and flooded to a depth of approximately 10 cm above the surface of the mat. Fertilization, applied once every three months, equaled 23.4 g N $m^{-2}$ $yr^{-1}$ and 2.0 g P $m^{-2}$ $yr^{-1}$. A single plug of *P. hemitomon* was planted in the center of each mat and sphagnum peat sandwich. Plant material was collected from a non-cultivated population of *P. hemitomon* on the USDA Golden Meadow Plant Materials Center property in Galliano, LA. Root stock was brought back to the UNO greenhouse facility and propagated until planting occurred.

Cumulative stem height was measured monthly over the course of the experiment, while interstitial pH and substrate redox potential were measured bi-monthly. Similar to that of experiment-2, harvest of this experiment did not proceed as planned due to Hurricane Katrina. All biomass was dead and therefore only separable into dead above- and belowground components. Above- and belowground biomass was separated but root and rhizome components were not distinguishable, nor where they separable from the substrate itself. All biomass was oven-dried at 60˚C until a constant mass was attained.

### *Experiment-4: Evaluation of edge-expansion species*

Experiment-4 was designed to incorporate aspects of previous experiments, along with an evaluation of additional plant species and growth supporting structures. This experiment also allowed for the elucidation of inter-specific competition among common thick-mat plant species, as well as a more detailed assessment of different *P. hemitomon* growth supporting structures that could have flotant creation potential.

This experiment was initiated in July of 2005 at an off-campus site owned and managed by the University of New Orleans. After a brief acclimation period, and before data collection could begin, this experiment was totally destroyed in August of 2005 by flooding and storm surge associated with Hurricane Katrina. Because of the severity of the impact and the early termination of the study, no further details are provided.


Illustration 8. Experiment-4: Evaluating the effects of mat expansion species.

The initial experiment was replaced in March of 2006 and was completed in September of 2006. It was conducted in an outdoor setting within the greenhouse complex at the University of New Orleans. The experimental design included 7 plant combinations and 5 growth supporting structures, each replicated 4 times for a total of 48 experimental units (n=48). The 7 plant combinations included: *P. hemitomon* only, *P. hemitomon* x *Althernanthera philoxeroides* (alligator weed), *P. hemitomon* x *Hydrocotyle ranunculoides* (floating pennywort), *P. hemitomon* x *Ludwigia peploides* (floating swamp primrose), *P. hemitomon* x *Sagittaria lancifolia* (bull tongue), *P. hemitomon* x all edge species (excluding *S. lancifolia*), and *P. hemitomon* x all plant species (including *S. lancifolia*). Each treatment received 9 plugs of *P. hemitomon* planted in a 3 x 3 grid fashion. *P. hemitomon* plant material for experiment-4 was also harvested from the USDA Golden Meadow Plant Materials Center property in Galliano, LA. Root stock was brought back to the UNO greenhouse facility and propagated until planting occurred. All other species were wild-harvested from various road-side wetlands in Orleans, Lafourche, Jefferson, or St. John the Baptist Parishes. Moreover, none of the non-maidencane species were propagated, they were gathered and planted within a 48 hour period. In all plant combinations DuraLast coconut fiber served as the mat material and sphagnum peat served as the substrate material.

In regards to the 5 growth supporting structures, *P. hemitomon* served as the only plant species considered. Growth supporting structures included: *P. hemitomon* x chicken wire (in a hydroponic setting), *P. hemitomon* x chicken wire x humic acid amendment (also in a hydroponic setting), *P. hemitomon* x bagasse x DuraLast coconut fiber, *P. hemitomon* x DuraLast coconut fiber x peat x canvas underpinning, and *P. hemitomon* x DuraLast coconut fiber x peat x humic acid amendment. As in the planting treatments, 9 *P. hemitomon* plugs were planted in a 3 x 3 grid fashion. All experimental units were housed in 1330-L livestock watering tanks filled to capacity with a combination of tap and rain water. Buoyancy was maintained in each treatment by a rigid PVC support structure. Additional support was provided by nylon rope fastened to the PVC structure. A fertilization regime of 23.4 g N $m^{-2}$ $yr^{-1}$ and 2.0 g P $m^{-2}$ $yr^{-1}$ was applied every three months.

Data collection included monthly aerial photographs of each experimental unit in order to assess vegetative spread of each mat, as well as to estimate percent cover by species in the multi-species treatments. This was accomplished by constructing a large tripod that rested on the rim of each tank. An infra-red remote shutter release was used to ensure a clear photograph of each tank was obtained. Each digital picture will be overlain by a grid of known size in order to determine plant species cover (these analyses are not yet completed).

Illustration 9. Experiment-4: Example of aerial photograph.

At harvest each mat was removed from its tank and support structure and completely dissembled much like experiment-1 after Katrina. *P. hemitomon* aboveground biomass was separated from belowground biomass, which was further partitioned into roots and rhizomes. All belowground biomass was hand-picked from both the top and bottom layers of each mat. Moreover, in those treatments that contained substrate (i.e., non-chicken wire treatments), all substrate was washed and fine root tissue removed. The length of each rhizome segment was measured to estimate a total rhizome length per mat. In this way total rhizome length provided a metric for inferring the lateral spreading potential of *P. hemitomon* within each treatment. In multi-species treatments, all biomass was separated by species, including both above-and belowground components. However, above- and belowground biomass are reported together as total biomass for non-maidencane species. Live root samples, 5 per treatment, were taken from each species in order to determine root specific gravity. Root specific gravity was determined using the same formula used in experiment-1. All biomass, regardless of species, was oven-dried at 60˚C until a constant mass was attained.

### *Experiment-5: Evaluation of Panicum hemitomon seed production and germination potential.*

Experiment-5 has not been undertaken at this point and its feasibility remains uncertain. *P. hemitomon* is considered to be an extremely poor seed producer even in favorable years, not to mention in years with above average tropical storm and/or hurricane activity.

### *Statistical Analyses*

All analyses were performed using SAS statistical software, version 9.1 (SAS Institute Inc., Cary, NC). In most cases either a one or two-way ANOVA was used to test for differences in particular variables. When specific variables were measured multiple times over the course of a given experiment (i.e., cumulative stem height), a repeated measures ANOVA was used. Statistical significance is set at an alpha level of 0.05 unless specified otherwise. All figures were created using Delta Graph software, version 5.6 (Red Rock Software Inc, Salt Lake City, UT).

## Field Deployment

Mandalay National Wildlife Refuge (MNWR) in Terrebonne Parish Louisiana was selected as the location for field deployment. The MNWR is located within the historical area that once supported a large expanse of thick-mat floating maidencane marsh that in recent decades has undergone extensive conversion to open water and/or thin-mat floating marsh. The U.S. Fish and Wildlife Service has issued a Special Use Permit for full implementation of the project. MNWR utilizes trappers supported by the Coastwide Nutria Control Program to control nutria.

Within MNWR, four deployment sites were identified (Figure 1). Two deployment sites are in large open water bodies, and two deployment sites are in small open water bodies. The small open water bodies are small enough that the deployed structures occupy greater than 50% of the available space. Sites 2 and 4 are located in a large open water area and are open to wind fetch. Sites 1 and 3 are in small ponds and are protected from wind fetch in most directions.

Figure 1. Location of sites within Mandalay National Wildlife Refuge.

After deployment it became evident that the pond at site 1 changes in size, due to the movement of the thin-mat marsh that surrounds it. In contrast, the pond at site 3 maintains its shape, because the surrounding marsh is an attached marsh dominated by *Sagittaria lancifolia*.

Based on the structural integrity, buoyancy, and growth response results from the first phase investigations, two successful AFS designs (see results section) were brought forward for field deployment in a marsh setting. The first design uses PVC for buoyancy and is based on AFS 26 (Table 1, Appendix A). The AFS 26 design was modified by adding two additional spacers in between the PVC tubes (Figure 2). This improved PVC design was repeated 50 times at each deployment site (Figure 3) for a total of 200 structures. The second design uses bamboo for buoyancy and is the same as the AFS 22 design (Table 1, Appendix A, Figure 2). This bamboo design was repeated 25 times at each deployment site (Figure 3) for a total of 100 structures.

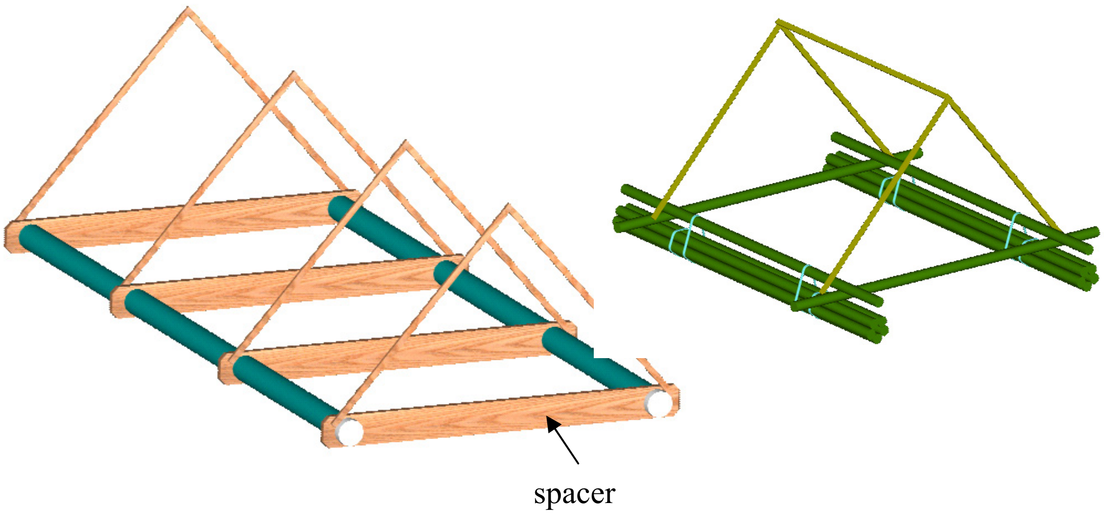

Figure 2. Structure designs deployed in the field. PVC structure on left, bamboo structure on right. Diagram does not include chicken-wire basket and fencing for clarity.

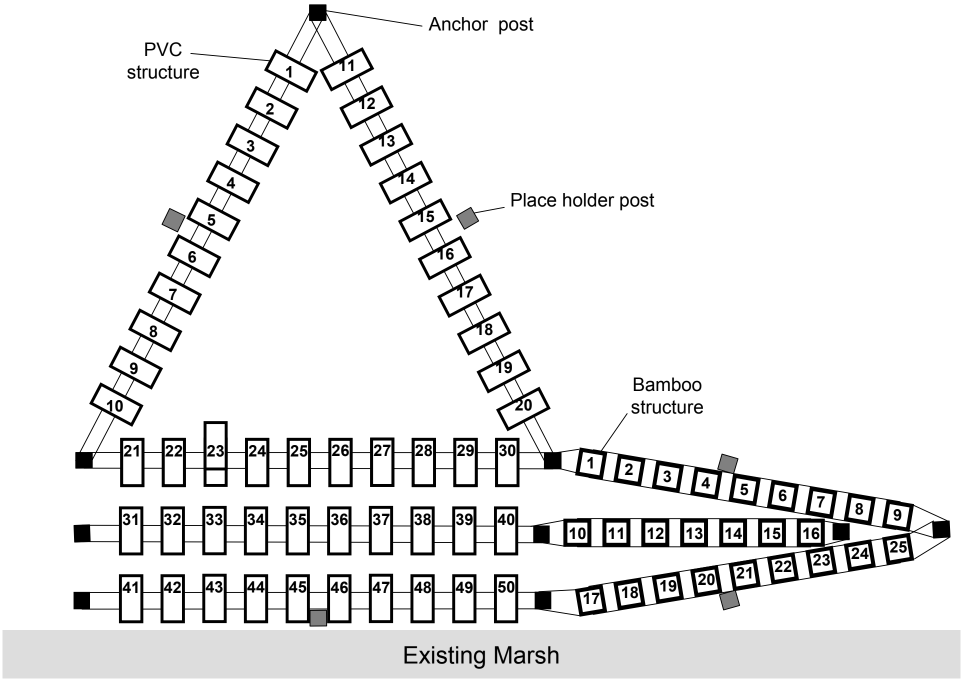

Figure 3. General layout of structures at each site. Drawing is not to scale.

Structure construction started at LSU in February 2006 and continued at the field location. The PVC designs were deployed first. The sites were completed in the following order Site 1, Site 2, Site 4, and Site 3. Site 1 received the first structures in early April, 2006 and Site 3 received the final PVC structure on May 11, 2006. Bamboo structures were constructed and deployed from May 16 through June 1, 2006 with the same order of sites.

Two methods for plant establishment were used with each structure design:

1. *P. hemitomon* in peat pots.
2. *P. hemitomon* stems

The structures were planted with a number of pots that provided an average density of 1 pot per square feet of structure. For the PVC design we used 30 pots per structure, and for the bamboo design we used 10 pots per structure. For the stem planted structures we used aerial stems from the number of pots that would be used if the structure was planted with pots (approximately 30 stems in the bamboo structures and 90 stems in the PVC structures). In half of the PVC structures *P. hemitomon* was established in pots and in the other half it was established from stems. All PVC structures were fenced, except for five replicates of each establishment technique at each site. Nine of the bamboo structures at each site utilized stem establishment and 16 used pot establishment. None of the bamboo structures were left unfenced.

Monitoring parameters were obtained from 5 replicates of each treatment combination at each site. Treatment combinations were:

| **Structure type** | **Establishment technique** | **Grazing treatment** |
|---|---|---|
| PVC | Pots | Fenced |
| PVC | Pots | Unfenced |
| PVC | Stems | Fenced |
| PVC | Stems | Unfenced |
| Bamboo | Pots | Fenced |
| Bamboo | Stems | Fenced |

We monitored vegetation, structural integrity, and buoyancy on July 11, August 9, September 11 and October 24, 2006. Vegetation cover within each structure was estimated to the nearest 5% by species. Farthest spread of *P. hemitomon* was measured to the nearest cm from each of the four sides of the structure. Structural integrity was determined by answering the following questions:

- Is the shape maintained?
- Do the fasteners show signs of wear and tear?
- Does the containment fabric show signs of wear and tear?

Where possible the source of wear and tear was identified.

To determine the buoyancy of the structure we used the assignment to one of three buoyancy classes:

1. floating at or above the water surface
2. submerged floating (0 -15 cm below the water surface)
3. submerged non-floating (>15 cm below the water surface)

If the structure was tilted (i.e. with one side of the structure floating and the other side submerged), the buoyancy class of the majority of the structure's surface was assigned.

We collected 3 replicate surface water samples at each site in July, September, and October. Samples were preserved on ice in the field and were frozen at LSU until analysis. Inorganic nutrient concentrations of ammonium, nitrate-nitrite, and phosphate were determined with the standard methods of the LSU Coastal Ecology Institute Analytical Laboratory.

The monitoring plan calls for measurement of root mat depth in October of each year. We attempted to measure this parameter on October 24, however due to the incredible growth of *P. hemitomon* the structures were extremely heavy and very difficult to lift. Lifting the structures from the water to reveal the root mat causes concern that this procedure could damage the structures. For the five structures that were measured the average mat depth was 40 cm. We recommend that the root mat development be measured by coring through the mats of 1 or 2 structures of each treatment combination at each site, near the end of the project life.

Illustration 10. Measurement of root mat depth.

# RESULTS AND DISCUSSION

## Artificial Floating Marsh Systems Evaluation in Aquaculture Ponds

### *Performance*

Twenty-seven AFS designs were constructed and tested at the LSU AgCenter research ponds (Table 1). Below we describe designs that performed well. We have also had several structures that lost buoyancy and/or suffered from mat disintegration. We will describe these failures first, and end with those structures that have performed well for at least three months of the testing. The first AFS that failed was AFS 4. The Styrofoam billets became a favorite spot for nutria and muskrat and these animals damaged the burlap mat. These structures were removed from the ponds in October 2004. The second failing design was AFS 2. The jute mat used in this design disintegrated within a few months after deployment, and one of the structures lost buoyancy as the pine frame became water logged. This design was removed from the pond in early March 2005. AFS 8 submerged in November 2004. After submergence these structures completely disintegrated and no remains of them were found even after one of the ponds was drained. Four of the five replicates of AFS 10 lost buoyancy as both the coconut mat and the pine frame became waterlogged. These structures were removed from the ponds between November 2004 and March 2005. All structures were heavily grazed by both nutria and muskrat, and it became apparent that these grazers needed to be excluded from the structures. Exclusion was not possible for AFS designs 1 and 11, which were heavily grazed and in addition the burlap bags in these designs and some of the ropes had deteriorated. Therefore, AFS 1 and 11 were removed from the ponds in early March 2005. AFS 9 remained buoyant until grazers damaged the fasteners that attached the Styrofoam, so these structures were also removed in early March 2005. Both the cedar lattice structures (AFS 15, 16, 17, and 18) and the wood gabion structures (AFS 19, 20, and 21) lost buoyancy within several weeks after deployment. Buoyancy was increased by adding bamboo pieces to these structures.

Two of the original pine frames (AFS 6 and 12) were fenced with vinyl coated chicken-wire (crab pot wire) in October 2004, which added a significant amount of weight and required the addition of Styrofoam to increase the buoyancy of these structures when they were fenced. The designs that incorporated either PVC (AFS 3, 5, and 7) or bamboo (AFS 22, 23, and 24) were successful in maintaining buoyancy on their own.

Nutrient levels in the ponds were relatively low with Nitrate-Nitrite averaging 0.02 mg/l, Ammonium averaging 0.01 mg/l and Phosphate averaging 0.04 mg/l. These values are much lower than nutrient concentrations of surface water at Lake Boeuf, which has average concentrations for Nitrate-Nitrite at 0.07 mg/l, Ammonium averaging 4.2 mg/l and phosphate averaging 0.31 mg/l (Sasser et al. 1991). These low nutrient concentrations at the ponds necessitated fertilization of the AFS designs. Structures planted with plugs as well as those structures with coconut or birch mats were fertilized in April 2005. Applying fertilizer to the chicken-wire mat structures (AFS 19 and 22) required an innovative solution. At the end of June 2005, these chicken-wire mat structures were fertilized with Osmocote® suspended in nylon bags.

We present the analysis of the *P. hemitomon* cover and species diversity as observed in the AFS designs on August 17, 2005 (the last observation before the hurricanes disturbed a few of the structures) using only those structures that were deployed throughout the 2005 growing season (AFS 3, 6, 7, 12, and 15 through 25). When grouped by frame type, the cedar designs tended to have the greatest cover of *P. hemitomon* (Figure 4). However, these structures also had the highest cover immediately after planting (28%). The other structures had an average initial cover of 10%.

By August 2005, a noticeable difference in species diversity was observed among the different frames (Figure 5). Structures that were planted with marsh plugs had a significantly higher number of species than those planted with whole plant, belowground, or aboveground material (Figure 5). This is not surprising, since the plugs were obtained from mature natural marshes and contained multiple species at the time they were added to the structure. In contrast, species could only establish on the other AFS designs from local seed sources. Of the structures planted with plugs, the more buoyant frames (PVC and bamboo) had higher species diversity than the less buoyant frames.

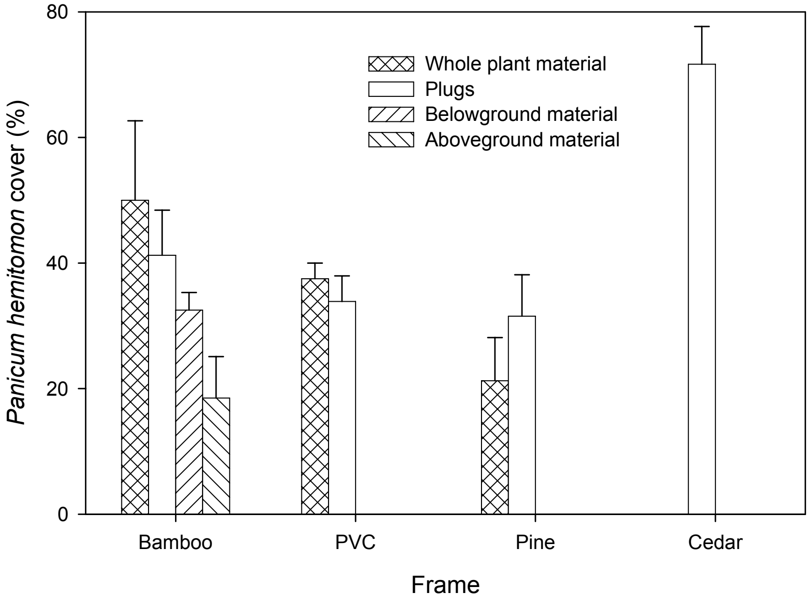

Figure 4. Effect of frame type on *P. hemitomon* cover on August 17, 2005. Only the bamboo frames were planted with aboveground material or belowground material.

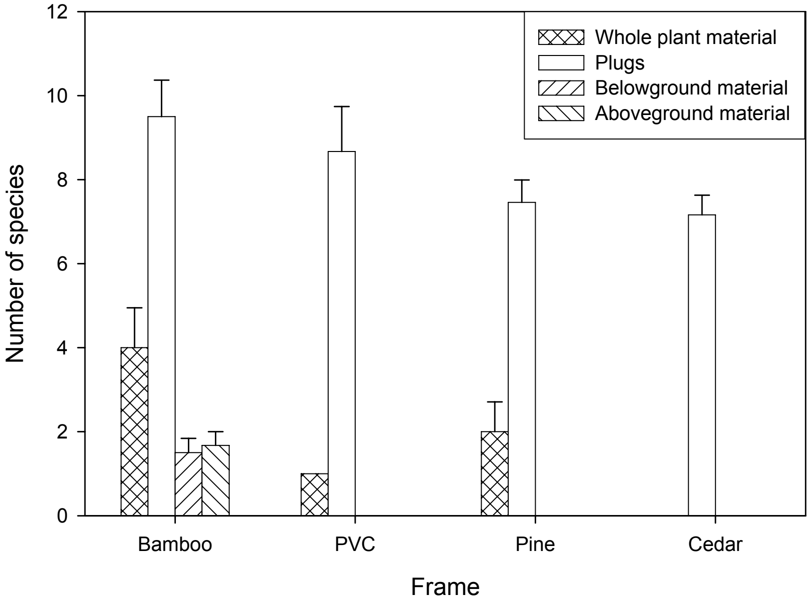

Figure 5. Effect of frame type on the number of species on August 17, 2005. Only the bamboo frames were planted with aboveground material or belowground material.

*P. hemitomon* cover was highest when plant establishment occurred with plugs and the structure contained either coconut, chicken-wire or no mat (Figure 6). The relatively low cover on the straw-coconut mat, may be the result of the lower planting density on these mats (initial cover 5%). The lower performance of plugs on birch mats may be due to the establishment of *Ludwigia* sp. which outcompeted *P. hemitomon* late in the growing season as well as reduced the buoyancy of these structures. When established from whole plant materials, the highest *P. hemitomon* cover occurred on structures with birch mats, followed by coconut mats, and chicken wire mats (Figure 6). Establishment from belowground material resulted in similar cover to those established from whole plant material irrespective of mat type. On the coconut mat, establishment from aboveground material resulted in the same *P. hemitomon* cover reached by whole plant or belowground material. In contrast, establishment from aboveground material failed while grown under hydroponic conditions on chicken-wire mats.

The poor performance of *P. hemitomon* on chicken-wire mats, when established from sources other than plugs, may have resulted from our inability to fertilize these structures in April. Plugs were fertilized and in addition plugs provide their own substrate. We fertilized the chicken-wire mat structures (AFS 19 and 22) at the end of June 2005, because we devised a method for application of fertilizer to these structures at that time. However, this fertilizer application was too late to have the aboveground material catch up to the whole plants and belowground material planted structures.

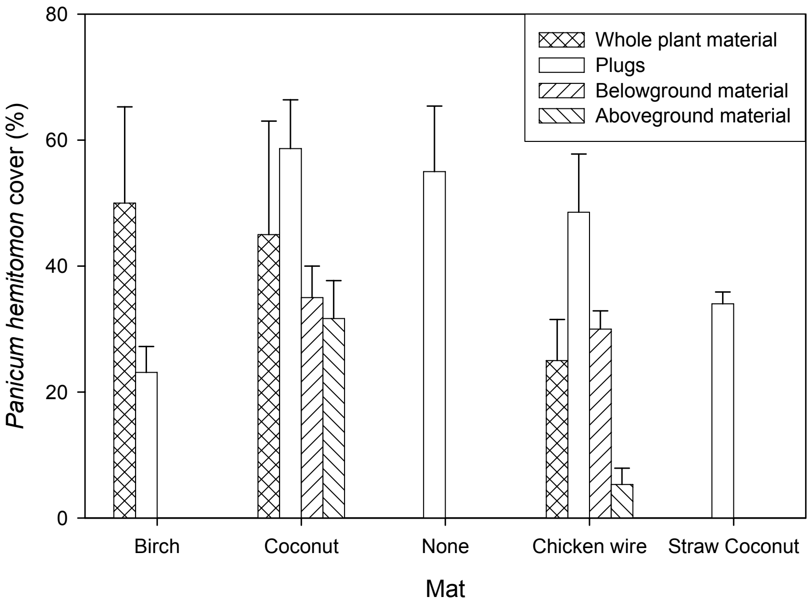

Figure 6. Effect of mat type on *P. hemitomon* cover on August 17, 2005. Only the coconut and chicken wire mats were planted with aboveground material and belowground material.

Substrate had some effects on *P. hemitomon* performance (Figure 7). Plugs performed best without any substrate, but this may be driven by the denser planting of the lattice designs (AFS 15, 16, 17, and 18), which make up the majority of the structures without substrate. The other structures without substrate are AFS 12, 19, and 22. The performance of plugs was similar for hardwood mulch and peat-bagasse substrates. When established from whole plant, belowground, and aboveground material cover was larger on the peat-bagasse substrate than when grown under hydroponic condition. This is probably related to our inability to properly fertilize the hydroponic structures early in the growing season.

The substrate also affected species diversity (Figure 8). When established from plugs, highest species diversity occurred on the hardwood mulch, followed by hydroponic and the lowest diversity on peat-bagasse. In contrast, when established from whole plant, aboveground, or belowground material no other species besides *P. hemitomon* established under hydroponic conditions, with a few more species establishing from seed when a substrate was available.

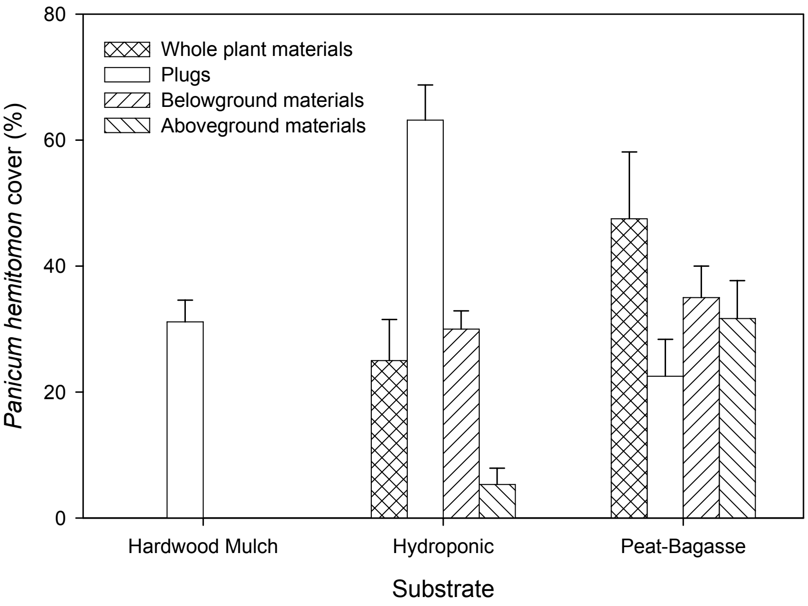

Figure 7. Effect of substrate type on *P. hemitomon* cover on August 17, 2005. Only the peat-bagasse treatments and the treatments without substrate were planted with aboveground material or belowground material.

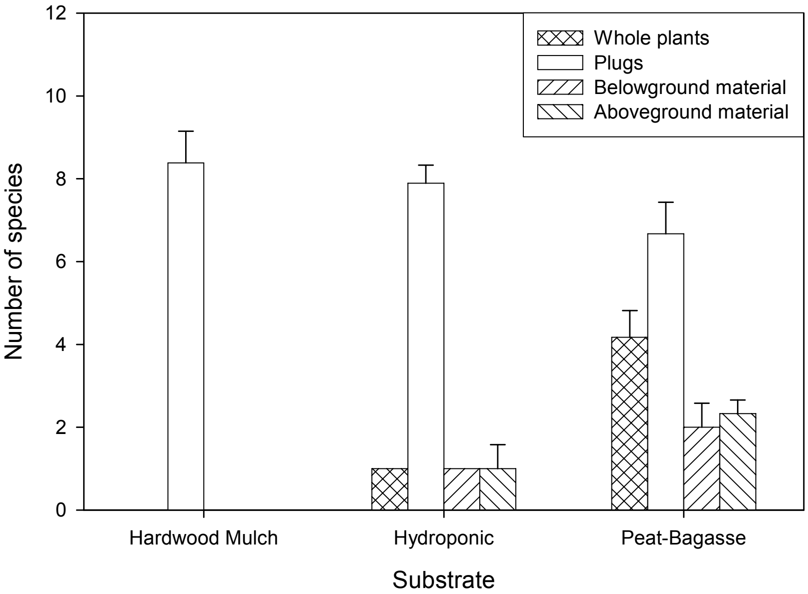

Figure 8. Effect of substrate type on the number of species on August 17, 2005. Only the peat-bagasse treatments and the treatments without substrate were planted with aboveground material or belowground material.

### *Plant Establishment*

We found highest shoot production in the greenhouse when using whole plants compared to rhizome and aerial stem material. Almost all whole plants produced shoots, while nearly 75% of belowground material with either 1 or 2 nodes yielded shoots after 28 days (Figure 9). Shoot production from stem pieces was lowest, with 50% of stems producing shoots after 28 days.

We also evaluated the effect of fertilization on shoot production of *P. hemitomon* stem and rhizome material (Figure 10). In this trial, we used randomly chopped stem or rhizome material, with variable number of nodes per piece. We spread those pieces evenly across six 2-inch thick coconut fiber squares that were saturated with water. Half of the coconut fiber squares were fertilized and the other half were left unfertilized. In the unfertilized treatment, rhizome pieces outperformed stem pieces throughout the experiment (Figure 10).

However, we found that the addition of fertilizer greatly improved shoot production in both stem pieces and belowground material. Thus, using randomly chopped stem or rhizome material could be a viable way to propagate *P. hemitomon*, and nutrient availability in the water will improve shoot production.

Illustration 11. Root mat formation from discarded stems

In the process of growing plants for the field deployment stage of the project, plants were regularly cut to promote root development. Some of the cut stems were left in a tray with water and developed a vigorous root mat. This observation combined with the shoot formation experiment as well as the success of growing *P. hemitomon* from stem pieces in the floating structures contributed to our conclusion that plant establishment from aerial stems is an option that should be tested under field conditions.

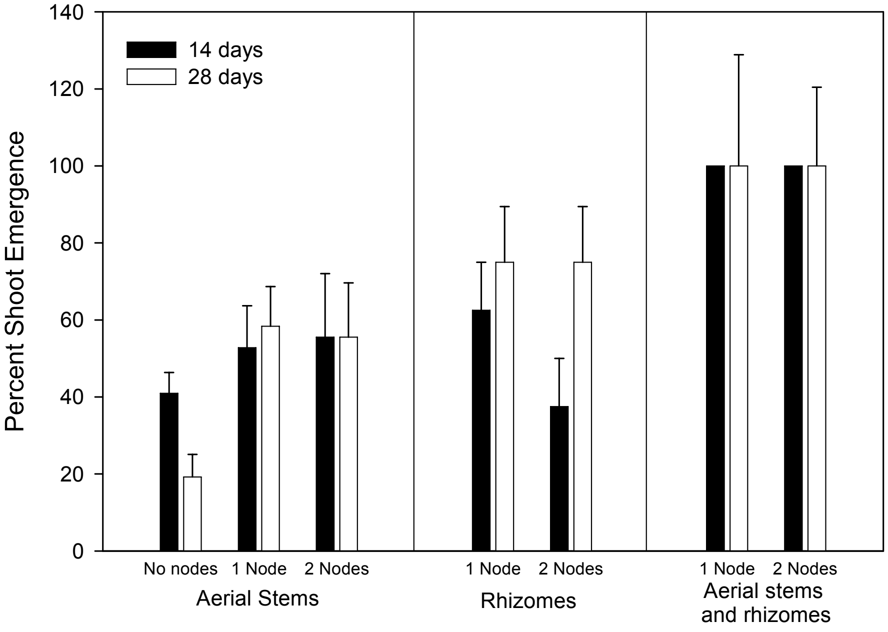

Figure 9. Effect of plant material on shoot development.

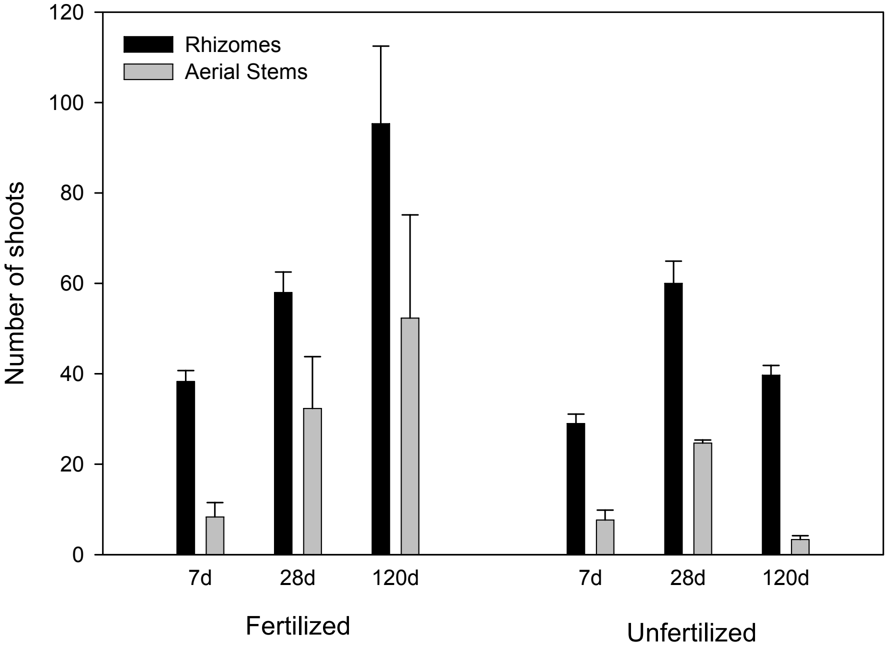

Figure 10. Effect of plant material and fertilization on shoot development.

## Optimization of Plant Response

Graphs and discussion of results for this section have been limited to main features and primary results of experiments. Monthly monitoring assessments and spot-checks are intentionally not included for brevity and conciseness. Results from Experiment-1 before Katrina are not repeated in this report since they were in the 2004-05 Annual Report (Visser et al. 2005) and the experiment was re-conducted this year. The findings presented in this report represent preliminary results to date, with a significant amount of data from experiments carried out in 2006 to be forthcoming.

### *Experiment-1: Evaluating the effects of nutrient loading rate and flooding depth*

Based on findings from experiment-1 (after Katrina), *P. hemitomon* aboveground biomass (Figure 11; Table 2) responded favorably to increased nutrient availability ($F_{3,32} = 31.13$, $p = 0.0001$), but unfavorably to flooded conditions ($F_{1,32} = 16.35$, $p = 0.0003$). The nutrient x flooding interaction term was not statistically significant ($F_{3,32} = 1.34$, $p = 0.2788$). Aboveground biomass responded more to elevated N treatments (NN,P and NN,PP) than when N was not elevated (N,P and N,PP; Table 2). The positive response observed under elevated N availability occurred under both non-flooded and flooded conditions.

Similar to the aboveground biomass findings, *P. hemitomon* belowground biomass (Figure 12; Table 2), including both root and rhizome tissue, exhibited greater production under increased nutrient availability, particularly N availability ($F_{3,32} = 8.64$, $p = 0.0002$), but reduced production when subjected to flooded conditions regardless of nutrient treatment ($F_{1,32} = 119.88$, $p < 0.0001$). The nutrient x flooding interaction term for belowground production was not statistically significant ($F_{3,32} = 1.65$, $p = 0.1964$).

Rhizome length (Figure 13) followed suit with most other biomass metrics from experiment-1 (after Katrina) in that total length was significantly greater in the presence of elevated nutrient availability ($F_{3,32} = 4.04$, $p = 0.0153$), although it was unclear which nutrient, N or P, was more influential (Figure 13). Flooded conditions resulted in significantly reduced overall rhizome length ($F_{1,32} = 78.72$, $p < 0.0001$), while there was a slight trend for greater length under high N treatments (NN,P and NN,PP). The nutrient x flooding interaction term was not statistically significant ($F_{3,32} = 1.21$, $p = 0.3229$).

Much like individual biomass components, total biomass (Figure 14) exhibited statistically significant differences in response to both nutrient ($F_{3,72} = 29.09$, $p < 0.0001$) and flooding regime ($F_{1,72} = 60.19$, $p < 0.0001$). However, the interaction of nutrient x flooding was not statistically significant ($F_{3,72} = 1.92$, $p < 0.1456$).

Illustration 12. Experiment-1 (after Katrina). Left plant represents low N and P x flooding. Right plant represents low N and P x no flooding.

Illustration 13. Experiment-1 (after Katrina). Left plant represents high N and P x no flooding. Right plant represents high N and P x flooding.

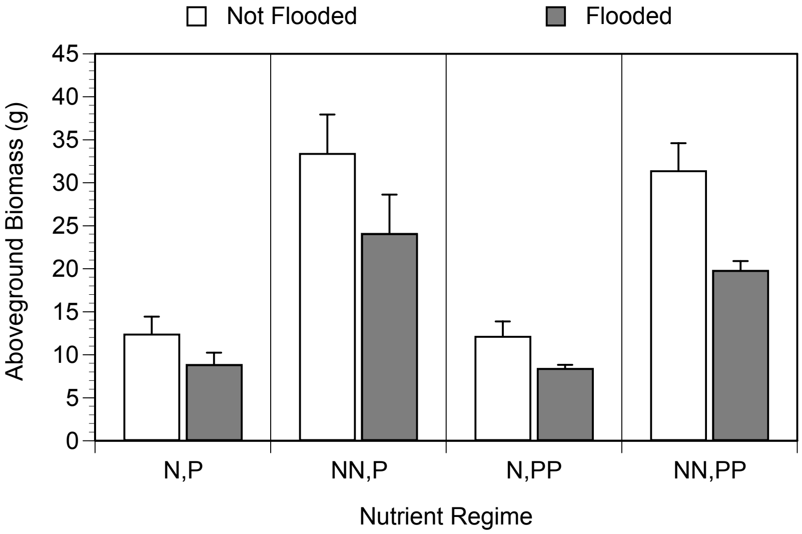

Figure 11. The effect of nutrient loading rate and flooding depth on *P. hemitomon* aboveground biomass. N,P = low N and low P; NN,P = high N and low P; N,PP = low N and high P; NN,PP = high N and high P. Nutrient: $F_{3,32} = 31.13$, $p < 0.0001$; Flooding: $F_{1,32} = 16.35$, $p = 0.0003$; Nutrient x Flooding: $F_{3,32} = 1.34$, $p = 0.2788$.

Table 2. Table of means for *P. hemitomon* biomass allocation and morphometric response metrics from experiment-1 (after Katrina). Values are means ± SE.

| Nutrient (Flooding) | Biomass (g) | | | | Root | | Root | Root/shoot | |
|---|---|---|---|---|---|---|---|---|---|
| Regime | Aboveground | | Belowground | | Volume (cm3) | | Specific Gravity | Ratio | |
| N,P (nf) | 12.44±1.98 | c | 16.08±2.20 | b | 50.31±17.83 | cd | 0.56±0.04 | 1.12±0.15 | c |
| N,P (f) | 8.891.36 | c | 8.14±1.32 | dc | 22.56±10.51 | ed | 0.75±0.06 | 1.42±0.14 | cb |
| NN,P (nf) | 33.44±2.94 | a | 22.40±1.57 | a | 91.31±4.34 | b | 0.54±0.07 | 2.13±0.18 | b |
| NN,P (f) | 24.13±4.49 | b | 10.58±1.88 | c | 28.28±15.49 | ed | 0.67±0.05 | 3.28±0.31 | a |
| N,PP (nf) | 12.21±1.67 | c | 16.70±0.56 | b | 64.84±15.02 | cb | 0.58±0.07 | 1.16±0.09 | c |
| N,PP (f) | 8.46±0.35 | c | 6.16±0.60 | d | 12.18±1.59 | e | 0.67±0.16 | 1.84±0.22 | cb |
| NN,PP (nf) | 31.42±3.16 | a | 24.38±1.58 | a | 131.68±19.21 | a | 0.71±0.07 | 2.10±0.35 | b |
| NN,PP (f) | 19.82±1.03 | a | 10.16±0.76 | dc | 17.15±1.16 | ed | 0.83±0.06 | 3.21±0.40 | a |
| F value (7,32df) | 16.25** | | 21.52** | | 10.92** | | 1.32NS | 7.30** | |

means with same letter are not statistically different ($P < 0.05$) based on LSD procedure; ** Highly significant difference ($P < 0.01$); NS Non-significant difference ($P > 0.05$). Treatment codes are as follows, in all cases nf = not flooded and f = flooded: N,P = low N and low P; NN,P = high N and low P; N,PP = low N and high P; NN,PP = high N and high P.

Illustration 14. Two examples of root and rhizome production from experiment-1 (after Katrina). Left frame shows belowground production under high N and P x no flooding, right frame shows belowground under low N and P x flooding.

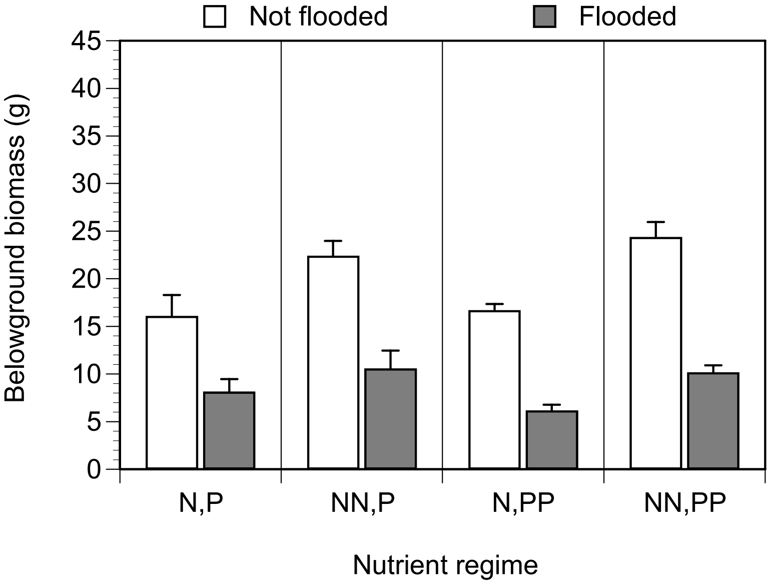

Figure 12. The effect of nutrient loading rate and flooding depth on *P. hemitomon* belowground ground biomass. N,P = low N and low P; NN,P = high N and low P; N,PP = low N and high P; NN,PP = high N and high P. Nutrient: F3,32 = 8.61, p = 0.0002; Flooding: F1,32 = 119.88, p ≤ 0.0001; Nutrient x Flooding: F3,32 = 1.65, p = 0.1964.

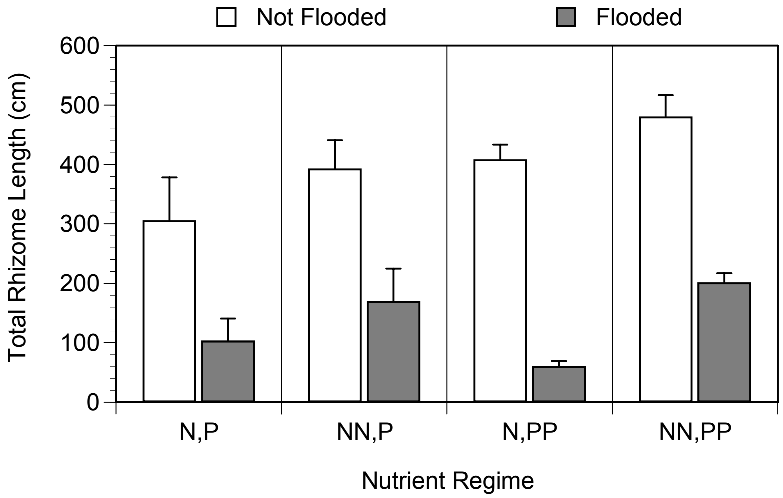

Figure 13. The effect of nutrient loading rate and flooding depth on *P. hemitomon* total rhizome length. N,P = low N and low P; NN,P = high N and low P; N,PP = low N and high P; NN,PP = high N and high P. Nutrient: F3,32 = 4.04, p = 0.0153; Flooding: F1,32 = 78.72, p ≤ 0.0001; Nutrient x Flooding: F3,32 = 1.21, p = 0.3329.

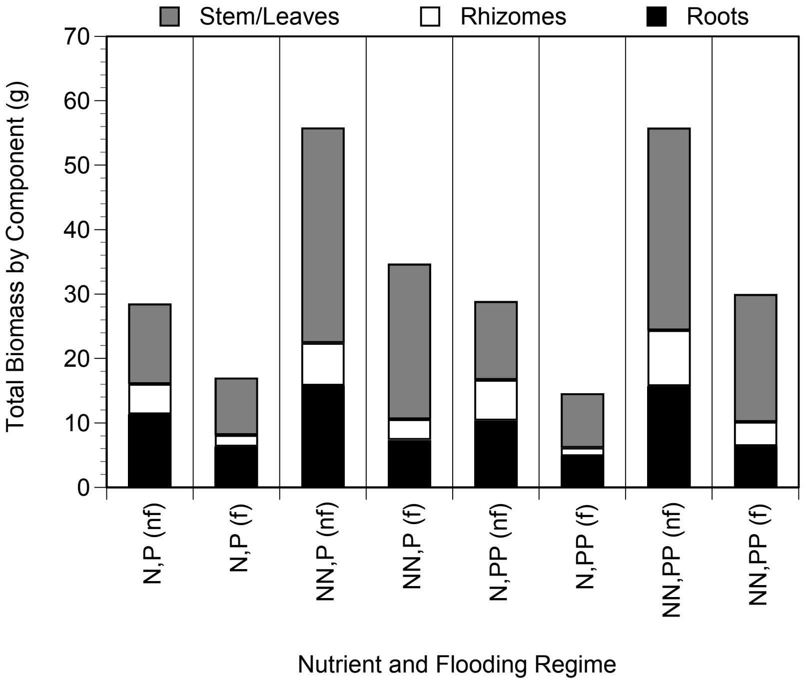

Figure 14. The effect of nutrient loading rate and flooding depth on *P. hemitomon* total biomass by plant component. nf = not flooded and f = flooded: N,P = low N and low P; NN,P = high N and low P; N,PP = low N and high P; NN,PP = high N and high P. Nutrient: F3,32 = 29.09, p < 0.0001; Flooding: F1,32 = 60.19, p ≤ 0.0001;Nutrient x Flooding: F3,32 = 1.92, p = 0.1456.

*P. hemitomon* net $CO_2$ assimilation (Figure 15) displayed statistically significant responses to both nutrient (F3,72 = 3.69, p = 0.0156) and flooding regimes (F3,72 = 20.28, p < 0.0001), although the interaction of nutrient x flooding was not significant (F3,72 = 0.60, p = 0.6140).

Root specific gravity (Figure 16; Table 2) failed to exhibit a statistically significant nutrient effect (Figure D, $F_{3,32}$ = 1.41, p = 0.2576). However, a significant flooding effect was observed ($F_{1,32}$ = 4.67, p = 0.0382). The nutrient x flooding interaction was not statistically significant ($F_{3,32}$ = 0.11, p = 0.9521).

Root volume mirrored belowground biomass with the exception of the nutrient x flooding interaction term (Figure 17; Table 2). Root volume was strongly influenced by increased nutrient availability ($F_{3,32}$ = 4.09, p = 0.0145), with the greatest volumetric measures being attained under high N treatments (NN,P and NN,PP). The effect of flooding on root volume was also statistically significant, ($F_{1,32}$ = 51.71, p < 0.0001), as was the nutrient x flooding interaction term ($F_{3,32}$ = 4.14, p = 0.0138).

Root/shoot ratios (Figure 18; Table 2) were calculated to better understand the relative contribution of above- and belowground biomass to total biomass. Root/shoot ratio exhibited a statistically significant nutrient effect (Figure D, $F_{3,32}$ = 12.31, p < 0.0001), as well as a significant flooding effect ($F_{1,32}$ = 13.41, p = 0.0009), whereas the interaction of nutrient x flooding interaction term was not statistically significant ($F_{3,32}$ = 0.24, p = 0.8650).

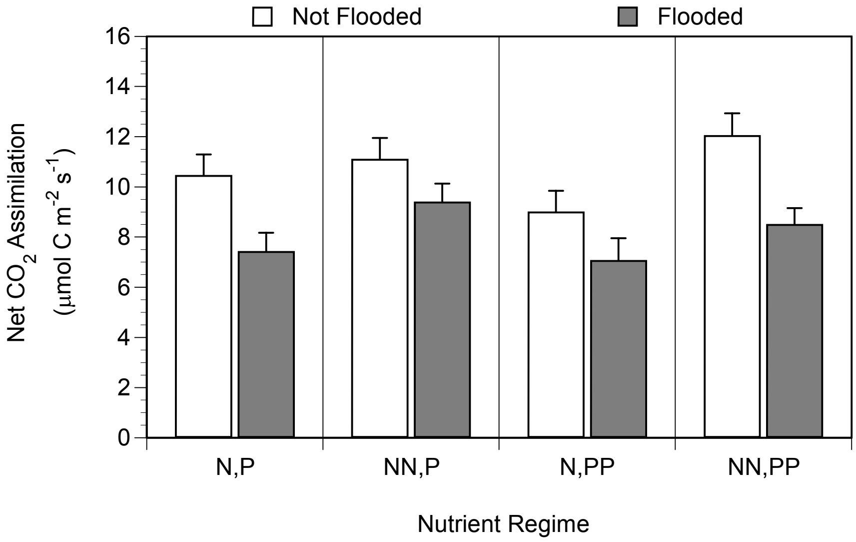

Figure 15. The effect of nutrient loading rate and flooding depth on *P. hemitomon* net CO2 assimilation. N,P = low N and low P; NN,P = high N and low P; N,PP = low N and high P; NN,PP = high N and high P. Nutrient: F3,72 = 3.69, p = 0.0156; Flooding: F1,72 = 20.28, p ≤ 0.0001; Nutrient x Flooding: F3,72 = 0.60, p = 0.6140.

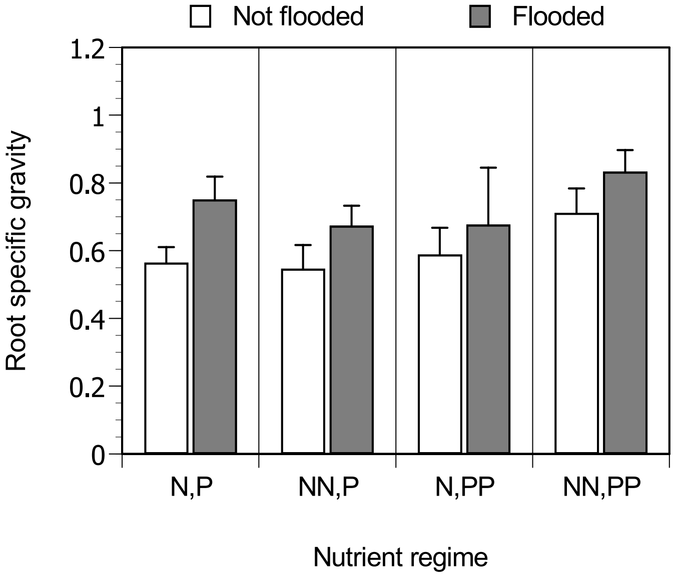

Figure 16. The effect of nutrient loading rate and flooding depth on *P. hemitomon* root specific gravity. N,P = low N and low P; NN,P = high N and low P; N,PP = low N and high P; NN,PP = high N and high P. Nutrient: F3,32 = 1.41, p = 0.2576; Flooding: F1,32 = 4.67, p = 0.0382; Nutrient x Flooding: F3,32 = 0.11, p = 0.9521.

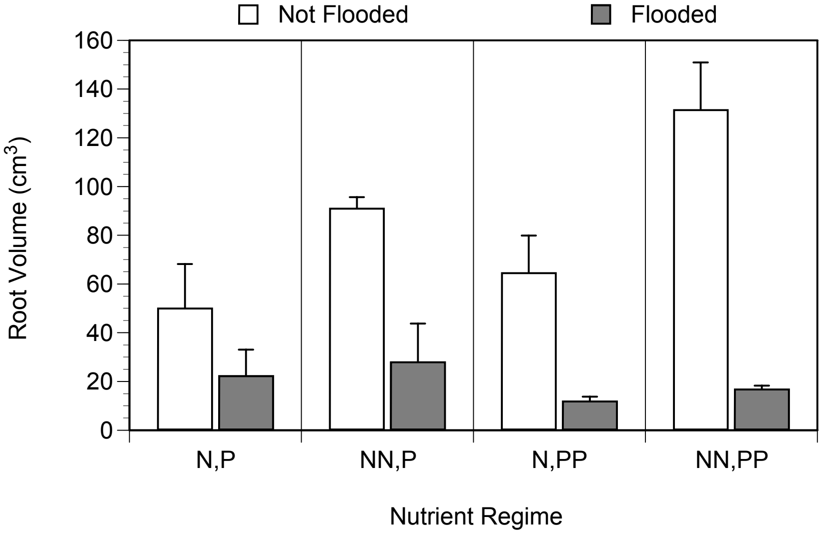

Figure 17. The effect of nutrient loading rate and flooding depth on *P. hemitomon* root volume. N,P = low N and low P; NN,P = high N and low P; N,PP = low N and high P; NN,PP = high N and high P. Nutrient: $F_{3,32} = 4.09$, p = 0.0143; Flooding: $F_{1,32} = 31.71$, $p \leq 0.0001$; Nutrient x Flooding: $F_{3,32} = 4.14$, p = 0.0138.

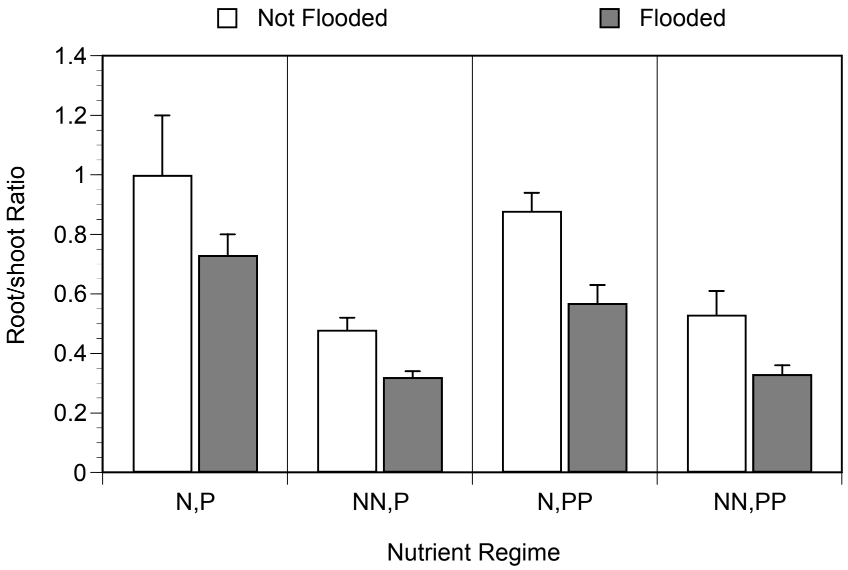

Figure 18. The effect of nutrient loading rate and flooding depth on *P. hemitomon* root/shoot ratio. N,P = low N and low P; NN,P = high N and low P; N,PP = low N and high P; NN,PP = high N and high P. Nutrient: $F_{3,32} = 12.31$, $p \leq 0.0001$; Flooding: $F_{1,32} = 13.41$, $p = 0.0009$; Nutrient x Flooding: $F_{3,32} = 0.24$, $p = 0.8560$.

In terms of biomass production, whether above- or belowground, it seems clear that N, not P, is the key limiting nutrient (Figure 11). Above- and belowground biomass production were greater under high N treatments when compared to low N treatments. Furthermore, high N and non-flooded conditions lead to the greatest biomass production (Table 2; Figures 8, 9 and 11). Total rhizome length exhibited a slightly different trend, with total rhizome length increasing with greater N and P loading, as opposed to solely high N loading (Figure 13). Net $CO_2$ assimilation (Figure 15) also exhibited statistically significant differences according to nutrient availability, supporting those effects observed in above- and belowground biomass production. Flooding, a known stressor even for wetland-adapted plants, resulted in reduced net $CO_2$ assimilation and biomass production under all scenarios. Root specific gravity was rather uniform across nutrient treatments with the exception of a slight trend for greater specific gravity (less buoyant tissue) under the highest N and P loading rate (Figure 16). Furthermore and somewhat unexpected, flooding tended to have a negative effect on root specific gravity. Flooding typically enhances aerenchyma formation, leading to decreased root specific gravity (more buoyant tissue). A possible explanation regarding the trend that was observed is that root tissue in the flooded treatments became waterlogged toward the end of the study when sampling occurred. Root volume (Figure 17) clearly demonstrates the combined effect of nutrient loading and flooding. A significant increase in root volume was observed under both high N treatments, but the greatest root volume was attained under the high N and P treatment (Figure 17). Moreover, root volume is the only variable in which the nutrient x flooding regime interaction was significant. Flooding stress suppressed root volume across all treatments, with only minimal nutrient effects under flooded treatments. A final metric stemming from experiment-1 was root/shoot ratio. Root/shoot ratio represents the amount of root biomass per unit of shoot biomass. Therefore, greater values represent more root biomass relative to shoot biomass. Root/shoot ratios in this study tended to be greater under lower nutrient loading rates, and smaller under higher loading rates (Figure 18). However, total above and belowground biomass was significantly greater under higher loading rates. Because of this, the root/shoot ratio observed in experiment-1 must be interpreted in the context of total biomass production. Furthermore, the fact that root/shoot ratio increased as root specific gravity exhibited a decreasing trend under high N and P loading is an interesting finding. We anticipate that forthcoming analyses on individual root attributes will build upon this finding.

### *Experiment-2: Evaluating the effects of substrate type*

Despite the fact that experiment-2 was ended prematurely, statistically significant differences in *P. hemitomon* biomass change values were observed (Figure 19) for both above- ($F_{11,48} = 6.29$, $p < 0.001$) and belowground production ($F_{11,48} = 45.85$, $p < 0.0001$). Overall, peat substrates, either individually or in a blend with another substrate material, were most favorable for both above- and belowground production (Table 3). However, it should be noted that when peat was blended with substrate materials such as bagasse or hardwood mulch, those substrate blends negatively influenced net belowground production (hence the negative change in biomass values; Figure 19; Table 3). The same held true for aboveground production, albeit to a slightly lesser degree.

Substrate COD (chemical oxygen demand), measured sequentially over the 20 week course of the experiment, exhibited statistically significant differences in week-1 ($F_{8,36} = 38.79$, $p < 0.0001$), week-4 ($F_{8,36} = 81.12$, $p < 0.0001$), and week-8 ($F_{8,36} = 22.93$, $p < 0.0001$), but only a marginally significant difference in week-20 ($F_{8,36} = 2.19$, $p = 0.0517$). As would be expected considering the level of statistical significance associated with each individual time interval, the overall effect of time, taking into account the duration of the experiment, was statistically significant ($F_{3,34} = 1388.25$, $p <0.0001$), as was the time x treatment interaction ($F_{3.34} = 10.73$, $p < 0.0001$). Ultimately, those substrate materials that exhibited the highest COD values (Figure 20) were essentially the same individual substrate materials that were least favorable for *P. hemitomon* growth (Figure 20).

Experiment-2 provided key information regarding which substrate materials are most favorable regarding maidencane above- and belowground production. As is evident in Figure 19, peat and peat-blended substrates in general were more favorable, than non–peat substrates (i.e., bagasse and hardwood mulch). However, when peat was blended either with bagasse or hardwood mulch, overall production was significantly reduced (Figure 19; Table 3). The negative amount-change values evident in Figure 19 can be attributed to the combination of substrate decomposition and marginal plant growth.

Table 3. *P. hemitomon* biomass production statistics from experiment-2. Aboveground values represent total dry mass. Belowground values represent mean change in belowground biomass and substrate material over the course of the experiment. Means are based on 5 replications per substrate material. Values are means ± SE.

| Substrate material | Aboveground biomass (g) | | Change in Belowground biomass (g) | |
|---|---|---|---|---|
| Bagasse | 22.3±3.49 | ef | -213.91±12.37 | g |
| Bagasse xSphagnum Peat | 130.7±29.45 | a | -180.42±34.18 | gf |
| Cane Leaf Strippings | 73.7±14.97 | bdc | -0.05±24.05 | ced |
| Cypress Mulch | 18.2±3.09 | f | 39.85±14.39 | cbd |
| Cypress Mulch x Bagasse | 70.2±3.08 | bac | 81.55±10.78 | b |
| Hardwood Mulch | 39.1±4.67 | edf | -132.58±12.52 | f |
| Hardwood Mulch x Cane Strippings | 92.1±11.6 | bac | -22.13±23.86 | ed |
| Hardwood Mulch x Sphagnum Peat | 93.6±4.66 | bac | -39.66±20.53 | e |
| Pinebark Mulch | 62.1±12.23 | edc | 52.72±25.27 | cb |
| Sphagnum Peat | 115.9±7.19 | a | 267.97±38.73 | a |
| Sphagnum Peat x Cypress Mulch | 107.2±8.77 | ba | 240.92±11.59 | a |
| Pine Shavings | 44.6±31.41 | edf | 54.12±7.78 | cb |
| F value (11,48df) | 6.29** | | 45.86** | |

Means with same letter are not statistically different ($P < 0.05$) based on LSD procedure;
** Highly significant difference ($P < 0.01$).

The additional COD results of experiment-2 support differences observed in *P. hemitomon* growth in relation to different substrate materials (Figure 20). More specifically, substrates with higher COD values were the same substrates in which *P. hemitomon* grew poorly. COD refers to the amount of oxygen needed to chemically oxidize organic matter in a given solution. Generally, COD is greater than biological oxygen demand (BOD), suggesting that higher COD values infer a more stressful environment for organisms that respire aerobically. Despite the fact that *P. hemitomon* possess both physiological and anatomical adaptations for dealing with low-oxygen conditions, such conditions nevertheless incur stress and are therefore detrimental to overall plant growth.

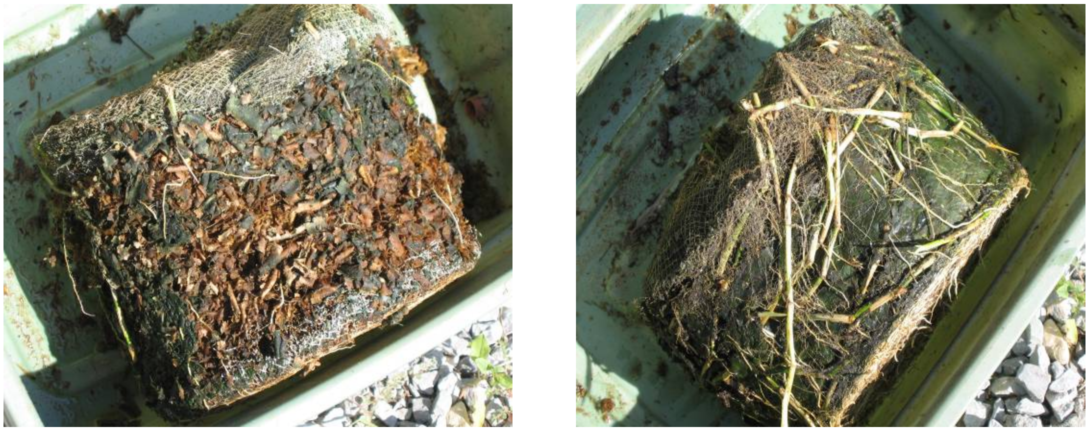

Illustration 15. Effect of substrate material on root and rhizome production. Left frame is a pine shaving treatment and right frame is a sphagnum peat treatment.

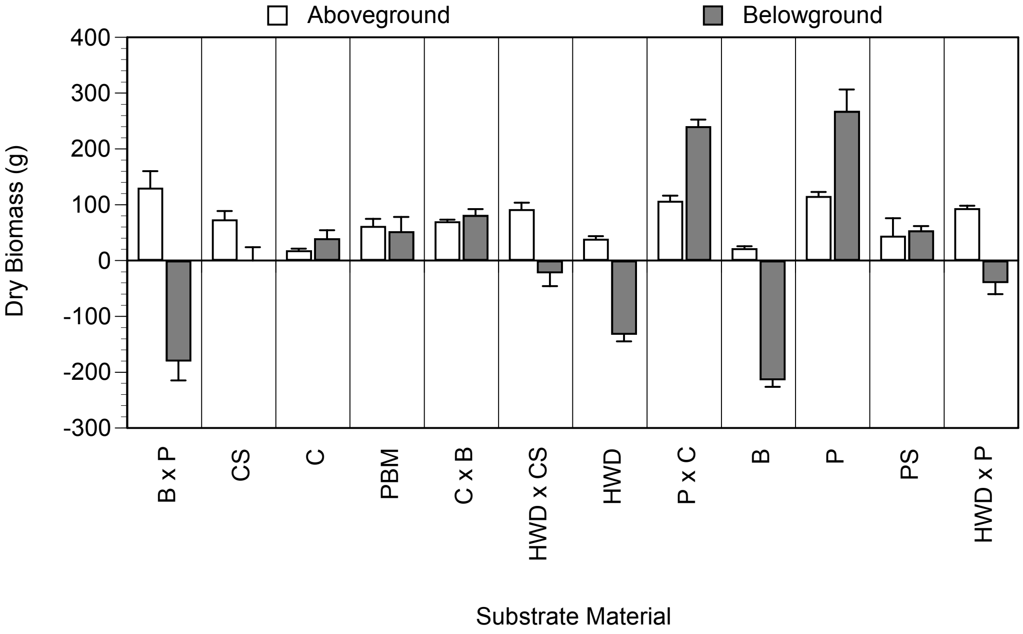

Figure 19. The effect of substrate material on amount biomass change in *P. hemitomon* above- and belowground production. Negative change values represent decomposition of substrate material that overshadowed belowground (root and rhizome) production. Treatment codes are as follows: B x P = bagasse x sphagnum peat; CS = cane leaf strippings; C = cypress mulch; PBM = pine bark mulch; C x B = cypress mulch x bagasse; HWD x CS = hardwood mulch x cane leaf strippings; HWD = hardwood mulch; P x C = sphagnum peat x cypress mulch; B = bagasse; P = sphagnum peat; PS = pine shavings; HWD x P = hardwood mulch x sphagnum peat. Statistical significance for aboveground biomass: $F = 6.29$, $p \leq 0.001$; belowground biomass: $F_{11,48} = 45.86$, $p \leq 0.0001$.

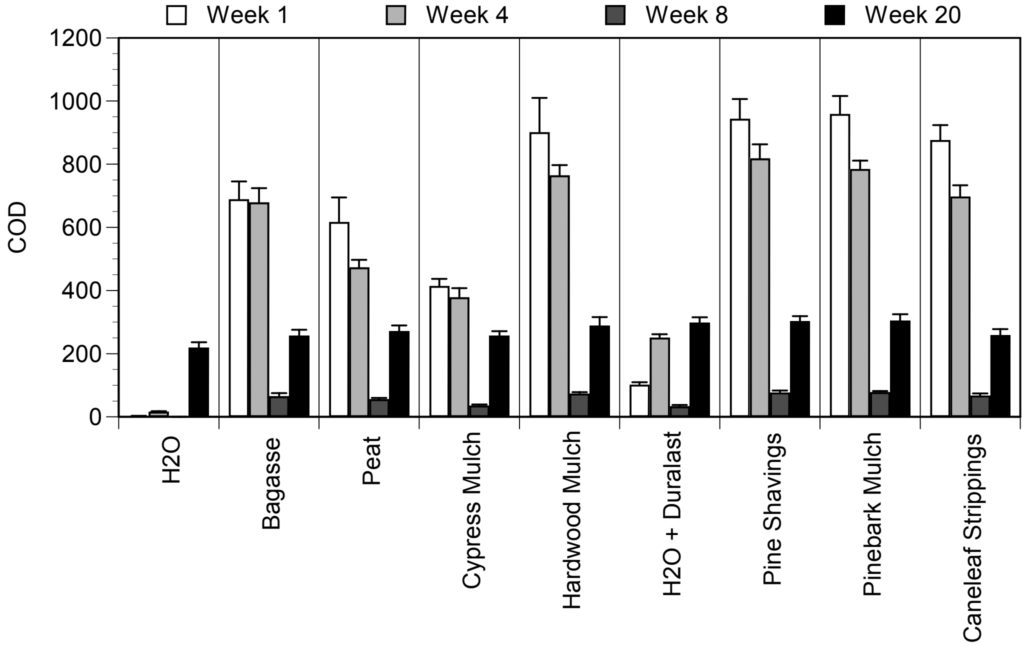

Figure 20. The effect of substrate type on interstitial chemical oxygen demand (COD). Treatment codes are as follows: B x P = bagasse x sphagnum peat; CS = cane leaf strippings; C = cypress mulch; PBM = pine bark mulch; C x B = cypress mulch x bagasse; HWD x CS = hardwood mulch x cane leaf strippings; HWD = hardwood mulch; P x C = sphagnum peat x cypress mulch; B = bagasse; P = sphagnum peat; PS = pine shavings; HWD x P = hardwood mulch x sphagnum peat. Statistical significance for week-1: $F_{8,36} = 38.79$, $p \leq 0.0001$; week-4: $F_{8,36} = 81.12$, $p \leq 0.0001$; week-8: $F_{8,36} = 22.93$, $p \leq 0.0001$; week-20: $F_{8,36} = 2.19$, $p \leq 0.0517$.

*Experiment-3: Evaluating the effects of mat type*

Experiment-3 was an evaluation of *P. hemitomon* growth and production in relation to mat or containment material. After only three months of growth, statistically significant differences in above ($F_{4,20} = 34.47$, $p < 0.0001$) and belowground ($F_{8,36} = 3.52$, $p = 0.0250$) production were observed (Figure 21; Table 4). In general, above- and belowground production were relatively uniform across all mat or containment materials, with the exception of those treatments that included shredded birch. *P. hemitomon* above- and belowground growth was significantly less in the presence of shredded birch.

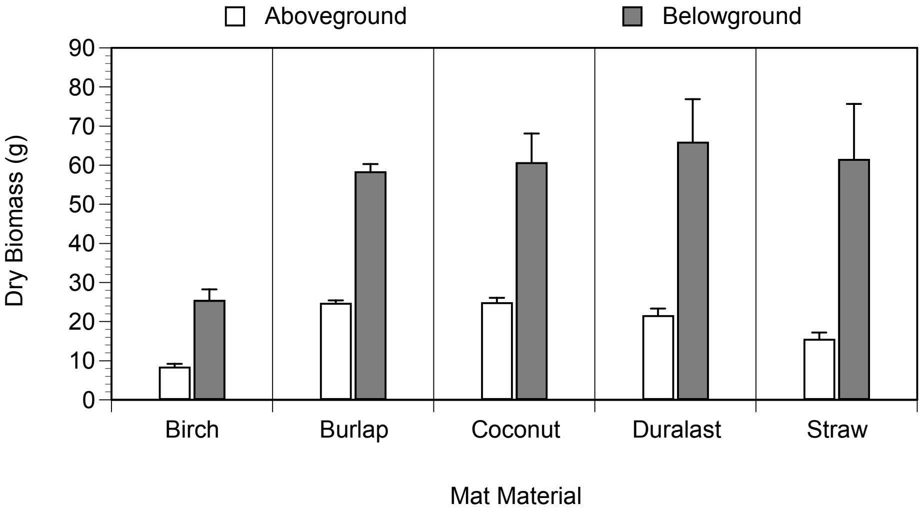

Figure 21. The effect of mat material on *P. hemitomon* above- and belowground production. Statistical significance for aboveground biomass: $F_{4,20} = 34.47$, $p \leq 0.0001$; belowground biomass: $F_{4,20} = 3.52$, $p = 0.0250$.

Table 4. *P. hemitomon* biomass production statistics from experiment-3. Aboveground values represent total dry mass. Belowground biomass values represent mean change in root and rhizome biomass over the course of the experiment. Means are based on 5 replications per containment material. Values are means ± SE.

| Containment material | Aboveground biomass (g) | | Change in Belowground biomass (g) | |
|---|---|---|---|---|
| Birch | 8.54±0.67 | a | 25.49±2.65 | a |
| Burlap | 24.75±0.57 | b | 58.49±1.81 | b |
| Coconut | 24.99±1.24 | b | 60.75±7.34 | b |
| Duralast | 21.70±1.63 | b | 65.95±10.87 | b |
| Straw | 15.57±1.56 | c | 61.59±14.16 | b |
| F value (4,20) | 34.47** | | 3.52* | |

Means with same letter are not statistically different (P<0.05) based on LSD procedure; **Highly significant difference (P<0.01).

Different mat or containment materials were evaluated in experiment-3, and it became clear after only 4 months of growth that Duralast coconut fiber was most accomodating, and shredded birch least accommodating for vigorous *P. hemitomon* growth (Figure 21; Table 4). While the differences in above- and belowground production between Duralast, straw, burlap, and coconut are not significant, it possess other qualities that makes it a better choice. In particular, its rigid construction enhances the structural integrity of the mat. Although not scientifically demonstrated, the density of the Duralast mat aids in sphagnum peat retention, an important quality that all other mat materials did to a lesser degree.

### *Experiment-4: Evaluation of edge-expansion species*

Preliminary analyses from experiment-4 revealed statistically significant differences across several components of *P. hemitomon* growth and biomass allocation in relation to growth supporting structures and plant species combination (Table 5).

*P. hemitomon* aboveground dry biomass (Figure 22; Table 5) exhibited statistically significant differences across growth supporting structures and plant species combinations ($F_{11,36} = 13.89$, $p < 0.0001$). Considering all treatments, substantially more variability existed across growth supporting structures than did across plant species combination. In general, the greatest aboveground biomass was attained by the PDH (*Panicum* in Duralast mat and peat with humic acid) and PDC (*Panicum* in Duralast mat and peat with canvas underpinning) treatments. On the contrary, the least aboveground biomass was associated with PDB (*Panicum* in Duralast mat with bagasse), PC (*Panicum* in chicken wire support), and PCH (*Panicum* in chicken wire support with humic acid) treatments.

Dry root biomass (Figure 23; Table 5) displayed a similar pattern of significance ($F_{11,36} = 9.29$, $p < 0.0001$). For dry root biomass, PDH, PD (*Panicum* in Durlast mat with peat), and, and PDC treatments yielded the highest amounts, while treatments such as PDB, PC, and PCH were associated with the lowest biomass production (Figure 23). Total rhizome length (Figure 24; Table 5) also exhibited statistically significant differences according to growth supporting structures and plant species combination ($F_{11,36} = 21.66$, $p < 0.0001$), with trends paralleling those observed in aboveground and root biomass in terms of which treatments were most favorable.

Experiment-4 evaluated a suite of different floating marsh creation or restoration-oriented designs, including 5 growth supporting structures and 7 plant species combinations. At this point data analysis for this study has yet to be completed, although interesting trends are apparent in preliminary analyses. *P. hemitomon* aboveground biomass exhibited significant differences across both growth supporting structure and plant combination (Figure 22; Table 5). The greatest aboveground biomass was observed in the PDC treatment (*P. hemitomon* x Duralast x peat x canvas underpinning), whereas the least was observed in the PC treatments (*P. hemitomon* x chicken wire with or without humic acid). *P. hemitomon* aboveground response was rather uniform across all plant species combinations (Figure 22). Results for root production were similar to that of aboveground production for both growth supporting structure and plant species

Table 5. *P. hemitomon* biomass production statistics from experiment-4. Aboveground values represent total dry mass. Root biomass represents only dry root biomass (rhizome dry mass not included). Means are based on 4 replications per treatment. Values are means ± SE. Means with same letter are not statistically different ($P < 0.05$) based on LSD procedure. Treatment codes for growth supporting structures and planting combinations are as follows (in all cases P = *P. hemitomon* and unless otherwise stated, sphagnum peat served as the substrate): PDH = Duralast x humic acid; PDB = Duralast x bagasse; PDLp = Duralast x *L. peploides*; PC = chicken-wire; PDAp = Duralast x *A. philoxeroides*; PDall = Duralast x all species; PD = Duralast; PCH = chicken wire x humic acid; PD all edge = Duralast x all edge species (excluding S. lancifolia); PDHr = Durslast x *H. ranunculoides*; PDC = Duralast x canvas underpinning; PDSl = Duralast x *S. lancifolia*.

| Planting combination/ Supporting structure | Aboveground biomass (g) | | Root biomass (g) | | Rhizome length (m) | |
|---|---|---|---|---|---|---|
| PDH | 123.15±9.00 | ba | 114.24±14.49 | ba | 56.70±5.53 | a |
| PDB | 28.63±6.04 | e | 32.01±12.62 | d | 10.75±3.92 | f |
| PDLp | 88.31±1.90 | dc | 87.56±4.56 | bc | 28.92±2.60 | e |
| PC | 23.22±5.58 | e | 20.75±5.17 | d | 5.71±0.99 | f |
| PDAp | 90.59±15.48 | dc | 94.54±16.34 | bac | 35.46±5.83 | ecd |
| PDall | 78.77±7.23 | d | 84.86±5.60 | bc | 28.47±2.07 | e |
| PD | 111.71±11.45 | bc | 87.36±17.36 | bc | 45.52±4.07 | bc |
| PCH | 20.28±4.54 | e | 20.43±4.29 | d | 4.72±1.13 | f |
| PD all edge | 88.62±9.13 | dc | 90.82±6.51 | bac | 34.83±1.16 | ed |
| PDHr | 83.27±7.08 | dc | 78.12±6.69 | c | 32.56±3.27 | ed |
| PDC | 152.39±25.50 | a | 122.32±12.64 | a | 49.04±4.08 | ba |
| PDSl | 90.41±4.91 | dc | 97.16±15.64 | bac | 39.80±3.92 | bcd |
| F value (11,36df) | 13.89** | | 9.29** | | 21.66** | |

**Highly significant difference ($P < 0.01$).

combination (Figure 23). Interestingly, total rhizome length did exhibit a positive response to humic acid amendment (Figure 24). However, in terms of species interactions in the multi-species mats, *P. hemitomon* production was decreased slightly when other species were present. In terms of total biomass per mat, any decrease in production by *P. hemitomon* was likely compensated for by the total biomass of the additional species. For example, *P. hemitomon* production in the PDLp treatment was less than the PD, PDC, and PDH treatments. However, once the *L. peploides* biomass is accounted for, the mean total biomass of the PDLp treatment was significantly greater. Such effects are important, especially if total biomass is one of the criteria used for evaluating marsh creation success.

Illustration 16. Experiment-4 showing aboveground growth of P. hemitomon after 1.5 months of growth. Note the new tillers emerging from the surface of the mat.

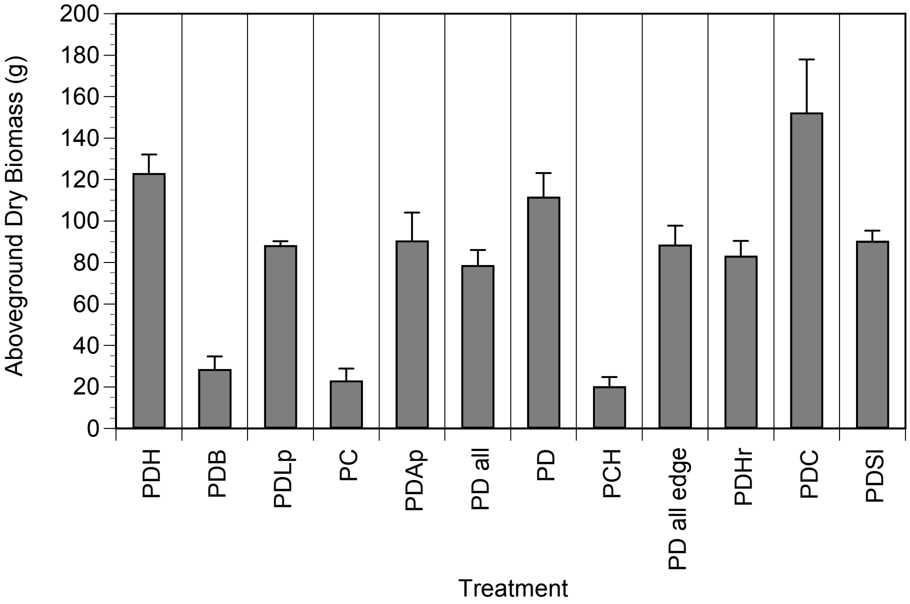

Figure 22. The effect of growth supporting structures and plant species combination on *P. hemitomon* aboveground production. Treatment codes for growth supporting structures and planting combinations are as follows (in all cases P = *P. hemitomon* and unless otherwise stated, sphagnum peat served as the substrate): PDH = Duralast x humic acid; PDB = Duralast x bagasse; PDLp = Duralast x *L. peploides*; PC = chicken-wire; PDAp = Duralast x *A. philoxeroides*; PDall = Duralast x all species; PD = Duralast; PCH = chicken wire x humic acid; PD all edge = Duralast x all edge species (excluding *S. lancifolia*); PDHr = Durslast x *H. ranunculoides*; PDC = Duralast x canvas underpinning; PDSl = Duralast x *S. lancifolia*.

Illustration 17. Experiment-4 showing belowground P. hemitomon root growth after 5 months of growth (treatment is PD). Note new rhizomes emerging from the bottom of the mat.

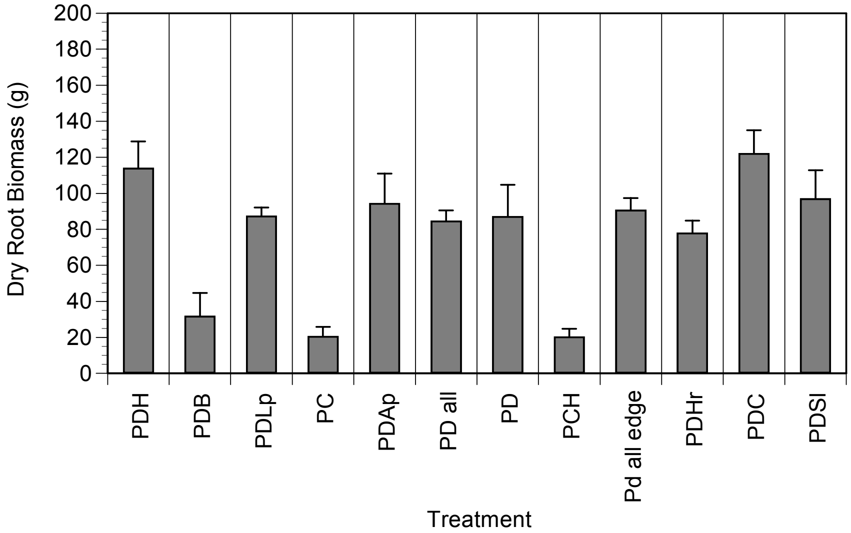

Figure 23. The effect of growth supporting structures and plant species combination on *P. hemitomon* root production. Treatment codes for growth supporting structures and planting combinations are as follows (in all cases P = *P. hemitomon* and unless otherwise stated, sphagnum peat served as the substrate): PDH = Duralast x humic acid; PDB = Duralast x bagasse; PDLp = Duralast x *L. peploides*; PC = chicken-wire; PDAp = Duralast x *A. philoxeroides*; PDall = Duralast x all species; PD = Duralast; PCH = chicken wire x humic acid; PD all edge = Duralast x all edge species (excluding *S. lancifolia*); PDHr = Duralast x *H. ranunculoides*; PDC = Duralast x canvas underpinning; PDSl = Duralast x *S. lancifolia*. Statistical significance for differences in *P. hemitomon* belowground biomass: $F_{11,36} = 9.29$, $p \leq 0.0001$.

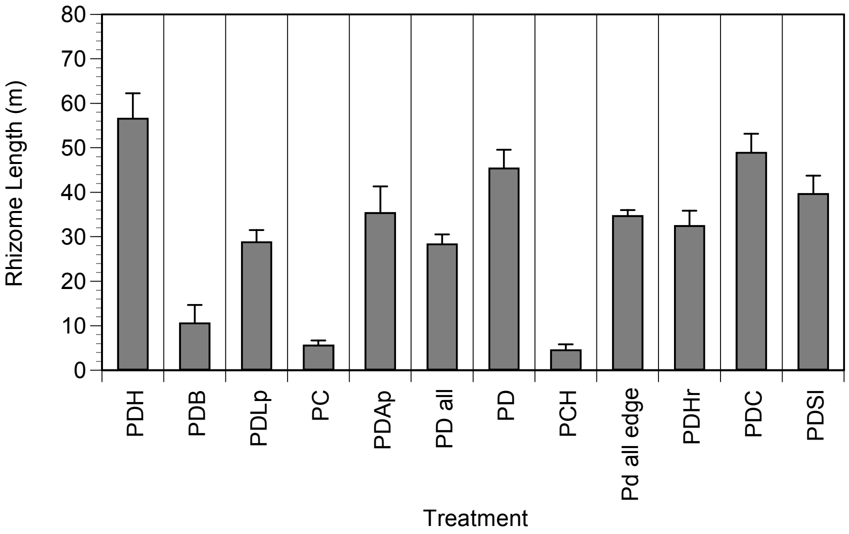

Figure 24. The effect of growth supporting structures and plant species combination on *P. hemitomon* total rhizome length. Treatment codes for growth supporting structures and planting combinations are as follows (in all cases P = *P. hemitomon* and unless otherwise stated, sphagnum peat served as the substrate): PDH = Duralast x humic acid; PDB = Duralast x bagasse; PDLp = Duralast x *L. peploides*; PC = chicken-wire; PDAp = Duralast x *A. philoxeroides*; PDall = Duralast x all species; PD = Duralast; PCH = chicken wire x humic acid; PD all edge = Duralast x all edge species (excluding *S. lancifolia*); PDHr = Duralast x *H. ranunculoides*; PDC = Duralast x canvas underpinning; PDSl = Duralast x *S.* lancifolia. Statistical significance for differences in *P. hemitomon* total rhizome length by treatment: $F_{11,36} = 21.66$, $p \leq 0.0001$.

A final aspect of experiment-4 which has yet to be completely analyzed is that of lateral spread and percent cover by species. However, there were several interesting trends that became apparent over the 5-month course of the experiment. Of the 4 additional species evaluated, *L. peploides* performed best in terms of lateral spread and total biomass. Furthermore, it was the easiest to transplant and most resilient to transplant shock. Conversely, *H. ranunculoides* was especially susceptible to transplanting and appeared to take a very long time to acclimate once transplanted. *A. philoxeroides* was the second best performer in terms of lateral spread and total biomass, but exhibited rather large variance in terms of plant vigor. Although not directly assessed here, *A. philoxeroides* may ultimately prefer less flooded, drier site conditions. *S. lancifolia* faired poorly overall, exhibiting very little growth over the course of the study.