SECTION 2
CATTAIL HABITAT IMPROVEMENT PROJECT

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2.1 INTRODUCTION

While Everglades restoration as related to phosphorus (P) has focused on reducing concentrations and loads to the region via the implementation of the stormwater treatment areas (STAs), a significant portion of the Everglades ecosystem remains impacted with high levels of P, readily evidenced by over 11,000 ha of monotypic cattail (*Typha domingensis*) stands. This has resulted in considerable attention on removing cattail as a restoration method, despite the recognition that cattail removal is addressing the symptom as opposed to solving the problem. Large scale intensive restorations efforts (e.g. peat removal or adding chemical amendments) will likely be more disruptive and harmful to the Everglades landscape than allowing the system to recover naturally. Additionally, large-scale removal may be detrimental because the dense cattail areas adjacent to inflow points currently serve an important ecosystem function; protecting downstream pristine areas through their rapid growth and P removal. However, ecosystem function could be enhanced by recognizing the constraints inherent in a monotypic cattail community. That is, there may be some active management strategies that can be implemented in conjunction with a "natural recovery strategy" that may improve ecological function, and thereby contribute to the overall intent of Everglades restoration. A key constraint is the density of the vegetation resulting in net heterotrophic production and limited access by wildlife. Therefore, the first objective in this research project is to *assess whether creating openings in dense cattail areas will sufficiently alter trophic dynamics such that wildlife diversity and abundance is increased*. As the goal of restoration is to return the Everglades to pre-drainage conditions as much as possible, the second objective then becomes, *to what extent does the structure and function of these created open areas compare to those of the natural Everglades*. Combining the answers to these two questions will allow us to make recommendations on this active management strategy as a means to accelerate ecosystem recovery.

Our fundamental hypothesis is that creating openings in P impacted landscapes will cause a shift from the emergent macrophyte-detrital system to one dominated by algae or submerged aquatic vegetation (SAV). In doing so, changes in critical ecosystem processes and food web dynamics will occur. Traditional approaches to examining ecological change can be characterized into two groups; one organism-centric (i.e., community ecology) and the other nutrient-centric (i.e., process level responses; Moore et al. 2004). The community approach emphasizes characteristics such as species composition, density, diversity, and population dynamics. Utilizing this approach in the Everglades, periphyton, macrophyte, and invertebrate communities have been shown to be significantly different in highly enriched densely vegetated areas compared to more open habitats of the unenriched sloughs (McCormick et al. 2001a). In contrast, taking a process level approach we have demonstrated that algal and plant productivity and P content increase in response to P enrichment (Miao and Sklar 1998, McCormick et al.

2001b), resulting in increased peat accumulation and P storage in the soil (Reddy et al. 1993, Craft and Richardson 1993, DeBusk et al. 2001). Both these approaches increase understanding of specific aspects of wetland structure and function, and the parameters we measure in this study will allow us to draw both community and process level interpretations of treatment differences.

The relationships between food web interactions and nutrient supply are often traditionally studied under the guise of "bottom-up" and "top-down" forces. Within this framework, one way to assess how species interact is to measure changes in individual species density or community composition in response to a perturbation (e.g. nutrient enrichment or predator introduction). However, as pointed out by Elser et al. (1998) and Sterner and Elser (2002) these relationship are far more complex than the dichotomous view of "bottom-up" and "top-down". One factor that may account for this greater complexity is the quality of the food available for consumption. That is, consumer taxa may be constrained by the abiotic factors, biotic factors, and feedback mechanisms that regulate the nutrient status and elemental composition of autotrophs (Elser et al. 1998). Recently, multivariate experiments have shown that these patterns of interdependency also apply to ecosystem functions such as C and N storage (Worm et al. 2002). Because of this interdependency of community structure and ecosystem function, it is important to link nutrient supply and food web structure together. Using a stoichiometric approach, it is possible to couple traditional methods of assessing the food web (species composition and density) with the nutrient status and elemental composition of various components of the ecosystem to define food web dynamics.

The objectives of this proposal are to experimentally test: 1) ***whether creating openings within densely vegetated areas will sufficiently alter trophic dynamics such that wildlife diversity and abundance is increased***; and, 2) ***to what extent does the structure and function of these created open areas compare to those of the natural Everglades.*** We will use an ecosystems approach to capture these changes. We will measure *ecosystem structure*, parameters associated with the physiochemical environment and species distributions; e*cosystem function*, parameters associated with nutrient cycling, turnover, and metabolism; and then use *ecological stoichiometry* to discern food web dynamics.

2.2 HYPOTHESES

Hypothesis 1. For our first objective, ***whether creating openings within densely vegetated areas will sufficiently alter trophic dynamics such that wildlife diversity and abundance is increased***, we hypothesize that treatments plots (openings) will experience greater nutrient fluxes and be comprised of more nutritional plants (i.e. algae). Therefore, they are expected to lose higher percentages of production to herbivores (invertebrates and cyprinodontoid fish), channel lower percentages of primary production as detritus, experience faster decomposition rates, and, as a result, store less carbon and nutrients – while also supporting higher wading bird foraging. Table 2-1 lists how we predict the treatment (created opening) to differ relative to the control (emergent macrophyte).

Hypothesis 2. For our second objective, ***to what extent does the structure and function of these created open areas compare to those of the natural Everglades***, our hypothesis is that, relative

to the P limited Everglades, openings in P enriched areas will experience greater nutrient fluxes and be comprised of more nutritional plants (i.e. algae). Thus, they are expected to lose higher percentages of production to herbivores (invertebrates and cyprinodontoid fish), channel lower percentages of primary production as detritus, experience faster decomposition rates, and, as consequently store less carbon and nutrients – while supporting higher wading bird foraging. Table 2-2 lists how we predict the treatment (created opening) to differ relative to the control (emergent macrophyte).

Table 2-1. Predicted responses of parameters in Control and Treatment (openings) plots.

	Control	**Treatment**
Sediment P Flux	Low	High
Incident Solar Radiation	Low	High
Autotroph C:Nutrient Ratio	High	Low
Floc C:Nutrient Ratio	High	Low
Refractory Carbon Accumulation	High	Low
Respiration Rates	Low	High
Areal Primary Production (Periphyton)	Low	High
Net N & P Mineralization Rates	Low	High
Net Ecosystem Production	Low	High
Secondary Consumer	Detritivore	Herbivore
Herbivore Growth Rates and Biomass	Low	High
Percent Carbon Consumed by Herbivores	Low	High
Herbivore Carbon Use Efficiency	High	Low
Secretive Bird Usage	High	Low
Wading Bird Foraging	Low	High
Stoichiometric Balanced	No	Yes

Table 2-2. Predicted responses of parameters in Treatment (openings) and unenriched slough (Everglades) plots.

	Enriched	**Transition**	**Everglades**
Sediment P Flux	High	Moderate	Low
Incident Solar Radiation	High	High	High
Autotroph C:Nutrient Ratio	High	Moderate	Low
Floc C:Nutrient Ratio	High	Moderate	Moderate
Refractory Carbon Accumulation	Low	Low	Low
Respiration Rates	High	High	Low
Areal Primary Production (Periphyton)	High	Moderate	Low
Net N & P Mineralization Rates	High	Moderate	Low
Net Ecosystem Production	High	Moderate	Low
Secondary Consumer	Herbivore	Herbivore	Detritivore
Herbivore Growth Rates and Biomass	High	Moderate	Low
Percent Carbon Consumed by Herbivores	High	Moderate	Low
Herbivore Carbon Use Efficiency	Low	Moderate	High
Wading Bird Foraging	High	Moderate	Low
Species Diversity/Richness	Low	Moderate	Low
Stoichiometric Balanced	Yes	Yes	No

2.3 EXPERIMENTAL DESIGN

The experiments will be conducted in Water Conservation Area (WCA) 2A, a large impoundment that has been impacted by agricultural runoff for over 30 years. The net result of this has been the development of a well-established nutrient gradient and a monotypic stand of

cattail (>11,000 ha). The South Florida Water Management District (SFWMD) Everglades Division has monitored two transects (the "E" and "F" transects) along this nutrient gradient, as well as a third (the "U" transect) within a reference region, since 1994 and has collected a wealth of data. The data set includes measures of water quality and biological integrity.

The experiment will consist of creating replicated 6.25 ha openings in the landscape in a region dominated by cattail and a transitional region containing a 50:50 mix of cattail and sawgrass (*Cladium jamaicense*; Figure 2-1). The former region is highly enriched, average surface water

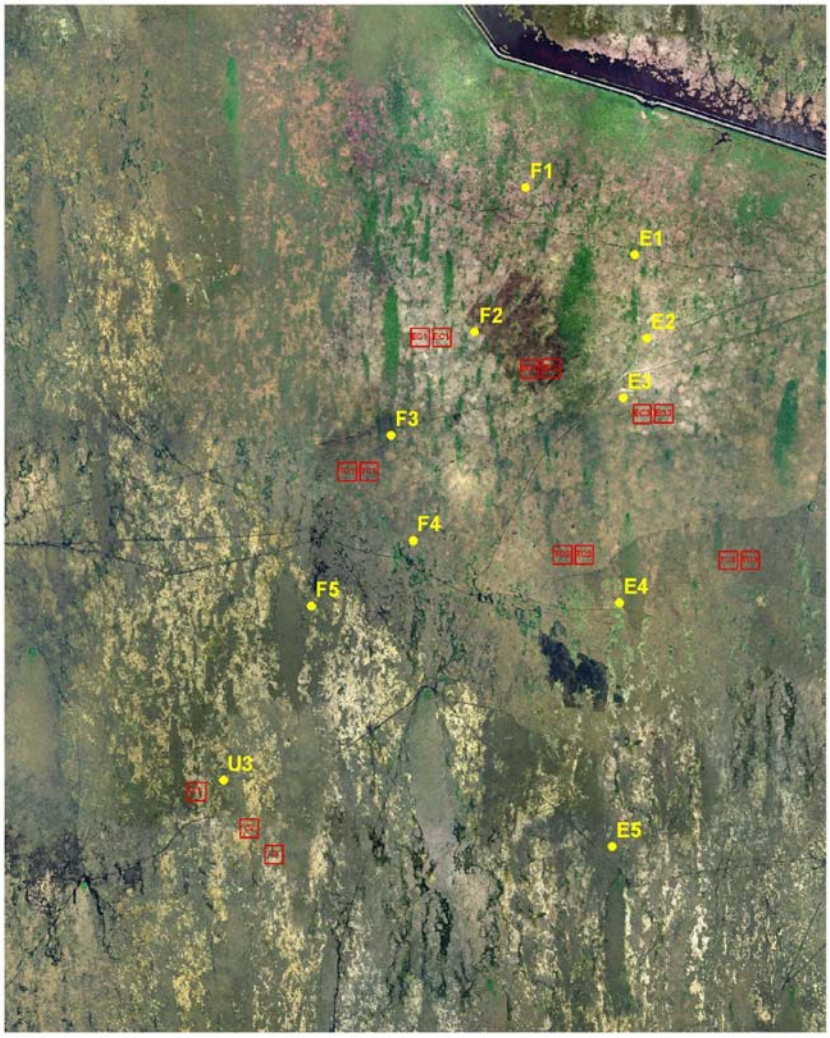

Figure 2-1. Approximate location of 6.25 ha plots to be used in the Cattail Habitat Improvement Project (plots shown to scale). C=control, O=created-opening. Yellow text and circle denote the location of the long-term monitoring sites, along the "E" and "F" transects, as well as reference site "U3".

TP > 50 µg l^{-1} and sediment (floc) TP > 1500 mg kg^{-1}, whereas the latter region is moderately enriched, with average surface water TP > 15 µg l^{-1} and sediment (floc) TP> 900 mg kg^{-1}. The

first hypothesis (Objective 1) will be addressed using a 2×2 factorial design, with 2 treatments (created opening versus control), two locations (enriched and transitional) and 3 replicates. The second hypothesis (Objective 2) will be addressed utilizing the open plots from the experimental set-up in Objective 1, with the addition of a third location (reference) in a region not impacted by P, (near the long term monitoring site U3) with 3 replicates (Figure 2-1).

All plots will be located proximal to the existing transect sites, such that prior history and ongoing monitoring will provide background information for this study (Figure 2-1). For the enriched location, 6 cattail-dominated plots are located near sites F2-E3. For the transitional region, 6 plots with a 50:50 mix of cattail and sawgrass are positioned near sites F4-E4. In addition, 3 slough plots have been selected in the nutrient poor Everglades near U3. All plots are 250 x 250 m in size. In each of these locations, three plots will be treated to remove cattail. To create the openings, we will use a combination of aerial herbicide application; glyphosate and arsenal, followed by burning once the plants have senesced (specific details described below).

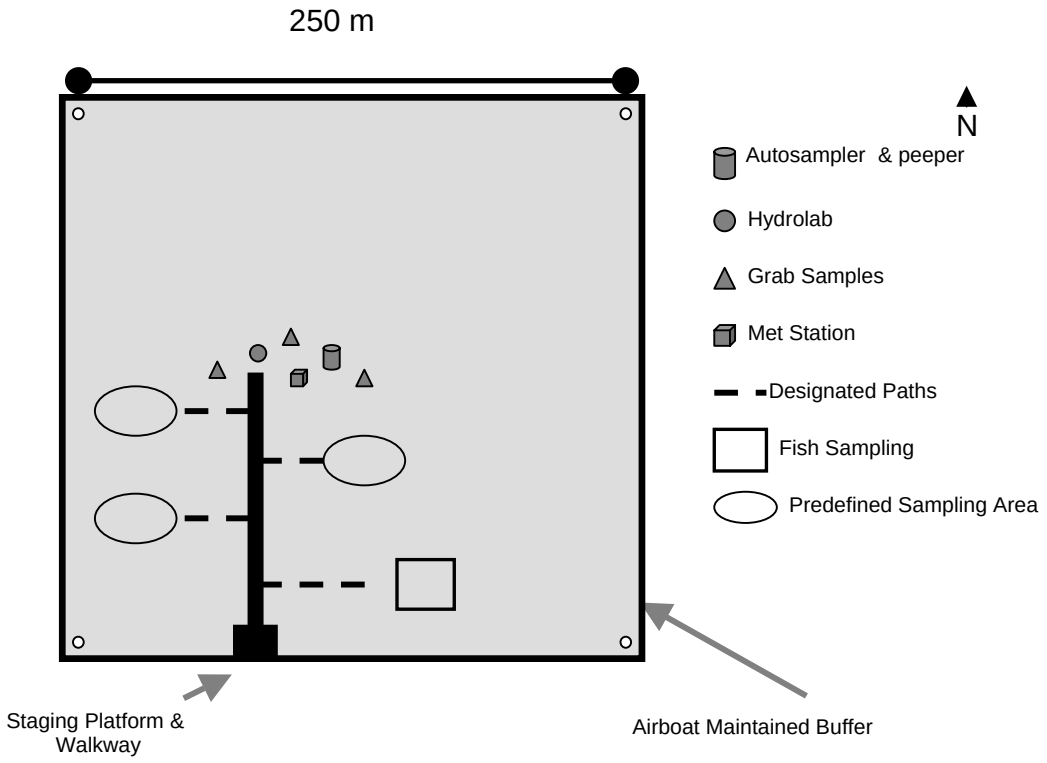

Figure 2-2. Experimental layout of plots used in the Cattail Habitat Improvement Project. Each plot is 250 x 250 m and will contain similar instrumentation and sampling platforms.

Openings will be created in spring, just prior to the onset of the rainy season, such that increasing water levels will increase the efficacy of the treatment. Control plots (untreated) will be co-located as pairs, so each location has control and treated plots in close proximity. Because rapid cattail regrowth via rhizome expansion from the edges is anticipated, a buffer will be actively maintained through a combination of airboat flattening and herbicide application in order to retain the interior plot size of 250 x 250 m (Figure 2-2). The interior of the plots will also be maintained via herbicide application as necessary to keep cattail cover at <10%. This is

particularly important during the dry season when cattail may reestablish from seed. Frequency and amount of herbicide applied will be documented. The herbicide will not be applied to slough plots (i.e. plots near U3).

While the absolute size of the plots is somewhat arbitrary, the intent is to have the plots large enough to ensure we capture both community and biogeochemical processes inherent to the ridge and slough landscape features of the Everglades. Through previous experiments we have learned if the plot size is too small (e.g. 10 x 10 m), maintenance of the treatment is difficult because of rapid cattail invasion from the edges (Newman, unpublished data). The decision to maintain open plots versus plots with a mixture of open-water and cattail (greater within plot heterogeneity) reflects the landscape scale of the ridge and slough, but also allows us to test main effects of the treatments that are currently not well understood. It is also hoped that this approach will minimize herbicide application.

To minimize plot disturbance, sampling platforms will be constructed within each plot (Figure 2-2). Metal walkways will be placed in the center of each plot via helicopter or airboat, prior to the implementation of any treatment. A staging platform will be placed at the bottom of each plot, from which a boardwalk will extend to the center metal platform. Samples that will be collected frequently and are sensitive to floc disturbance, e.g., water quality, will be collected from locations adjacent to the boardwalk (Figure 2-2). Other samples will be collected from pre-determined minimally walked trails from the platform. Several 1.0 m^2 plots will be identified at these pre-determined locations to avoid any potential for sample overlap. All airboat traffic will approach each plot from the south (downstream) to avoid establishing a hydrologic path to the plot.

This project is intended to be a 3 year study, during which time we will collect samples at two frequency levels to address specific questions. The first level is designed to primarily capture the flux of nutrients between the sediments and surface water. Nutrient flux in the Everglades is typically associated with rewetting (the shift from the dry season to wet season) and major disturbances like fire. Therefore, the measurement of nutrient fluxes will occur during a 2 week interval immediately following the treatment burn and following the onset of the wet season in years 1, 2, and 3. The second level is associated with determining how ecosystem structure and function differ among treatments and locations, this phase of the project is termed the "Food Web Study". The major sample events will occur in the 2nd and 3rd year. Periodic measurement of some parameters will be made in the first year, but we use this period of time for the ecosystems to recover from the disturbance. Samples will be collected 4 times a year during year 2 and 3. Sampling is centered around the dry and wet season, so that two events occur in each. The duration between sampling events within a season is approximately 4-6 weeks. This 4-6 week interval in the dry season will straddle the period when foraging bird activity is greatest; hence, the first sampling event is pre-bird foraging (Pre) and the second event is post-bird foraging (Post). We anticipate that Pre and Post sampling will occur in March and April/May, respectively. For the wet season sampling, Pre and Post sampling will occur in July and August/September, respectively. Note that since no disturbance event (wading bird foraging) is anticipated during the wet season, the Pre and Post designation during this time is for convenience and consistency only. The sampling design is outlined in Table 3 and the frequencies of sampling for each parameter are detailed in the sections below.

2.4 MATERIALS AND METHODS

2.4.1 Herbicide Accumulation

A combination of 7.5 pts/acre AquatNet™ (aquatic labeled glyphosate), 1 qt/acre Habitat™ (aquatic labeled arsenal), 1 qt/acre SunWet™ (mentholated seed oil) and 4 oz/acre NuFilm will be applied via helicopter to create the open plots. The helicopter will apply the herbicide at an altitude of 25ft, with an airspeed of 50 mph using a 40 ft boom.

The application of herbicides to any environment is always of concern. Thus, while the soil half-life for glyphosate is documented to be approximately one week, under both aerobic and anaerobic (warm climate) environments (Pfeuffer, pers commn) we will, nonetheless, monitor glyphosate to determine if it accumulates in the environment. In addition, we will monitor concentrations of aminomethyl phosphoric acid (AMPA) which is the primary soil degradate. Both compounds will be measured in floc samples for the buffer zone (Figure 2-2) of each plot twice per year (Table 2-3). In addition, we will also test for AMPA in floc, soil, plants, periphyton and fish within interior of the plots during the Post dry season sampling event.

2.4.2 Ecosystem Structure and Function

P and N flux – Vegetation removal is anticipated to result in increased nutrient release to the overlying water in both enriched and transitional plots, with the magnitude of flux determined by the degree of soil enrichment (Fisher and Reddy 2001). Nutrient release into the overlying water will be measured using 3 methods; autosamplers, grab samples and porewater equilibrators (i.e., peepers; Hesslein 1976). Once the open sites become vegetated by algae and/or SAV, nutrient fluxes are predicted to decrease.

Autosamplers will be centrally located in each plot (Figure 2-2). Autosamplers will collect water samples every hour and composited daily for two weeks to capture the anticipated intensive nutrient fluxes associated with the fire treatment. Samples will be analyzed for total P (TP) and total Kjeldahl nitrogen (TKN). Autosamplers will also be deployed at the onset of seasonal rewetting. Grab samples, composited from three locations around the sampling platform, will be collected immediately after fire treatment and immediately following rewetting at the onset of the wet season, 1 week, 2 weeks, 4 weeks, 2 months, 6 months, and in conjunction with the food web study (4 times per year) thereafter. Grab samples will be analyzed for TP, TKN, soluble reactive P (SRP), total dissolved P (TDP), total dissolved Kjeldahl N (TDKN), ammonium (NH_4^+-N), nitrate and nitrite (NO_x-N), dissolved organic carbon (DOC), sulfate (SO_4^{2-}), pH, and specific conductivity. A single peeper will be located in each plot to capture nutrient flux following the fire treatment, during the dry season, and following rewetting (Table 2-3). Peeper samples will be collected at 2.0 cm increments from +10.0 cm above the soil-water interface to –10.0 cm into the soil, resulting in 10 samples per plot. Each sample will be analyzed for SRP, NH_4^+-N, NO_x-N and DOC. Flux calculations will use Fick's First Law:

$$F_i = -Ø\ D_i\ q^{-2}\ dc_i/dz$$

where: F_i=flux of dissolved species *i* (µg cm^{-2} d^{-1}), Ø=porosity of the soil (0.97 cm^3 cm^{-3}), D_i=diffusion coefficient of species *i* (0.683 and 1.71 cm^2 d^{-1} for SRP, and NH_4-N, respectively; Li and Gregory 1974), q=tortuosity factor that is assumed to be unity based on the low bulk density of soils (Moore et al. 1991), c_i=concentration of species $_i$ (µg cm^{-3}), and *z*=depth (cm). The concentration gradient dc_i/dz will be calculated by the least square fit of the data to a regression between depth and porewater/water concentration.

Periphyton composition and primary productivity – Periphyton composition will be quantitatively assessed for each plot in association with the food web sampling events and during the first wet season (Table 2-3). Species composition for each periphyton type (metaphyton, epiphyton, and epipelon) will be determined, if present. Taxonomic analysis will be performed by the Biological Laboratory, Florida Department of Environmental Protection (FDEP). Biomass per unit area will also be determined following the methods of McCormick et al. (1998). Total carbon (TC), total N (TN), TP, total organic carbon (TOC), and total calcium (TCa) will be determined for each periphyton type collected on each event. Analyses will be performed by DB Labs (Rockledge, FL) using standard methods.

With the removal of a large area of vegetation, we expect that periphyton primary production will increase in response to increased light availability. Biomass-specific periphyton productivity will be assessed on an areal basis using a modification of standard light and dark BOD bottle technique (Wetzel and Likens 1991, McCormick et al. 1998). An independent estimate of areal primary production for metaphyton will be made using a diffusion-reaction model to describe steady state oxygen microprofiles (Epping and Jørgensen 1996, Epping et al. 1999). Each plot will be measured in the first year one month after the burn and towards the end of the wet season. For the second and third year, each plot will be measured during the main sampling events.

Filamentous cyanobacteria are the dominant periphyton species in both the oligotrophic and eutrophic regions of the Everglades. Many species of cyanobacteria produce toxins that make them unpalatable for herbivores. Consequently, since toxin production may be an important factor regulating herbivory, we will quantitatively measure, during the dry and wet season of the 2nd year only (Table 2-3), total microcystins, anatoxin-a, and cylidrospermopsin concentrations in periphyton collected from each site. Toxicity screening will be performed by GreenWater Laboratories, Palatka, FL. Total microcystins will be analyzed using an enzyme linked immunosorbent assay (ELISA) method. Anatoxin-*a* and cylidrospermopsin will be analyzed using a liquid chromatography-mass spectrometry (LC-MS) method.

Heterotrophic respiration – Floc samples will be collected 4 times per year in association with the food web study (Table 2-3). Three floc samples will be collected from each plot using a 10 cm stainless steel soil corer. The thin-walled soil corer, with a sharpened edge, will be inserted 10 cm into the soil. The surface floc layer will be poured off, compositing the three within plot replicates. The surface 0-5 cm soil layers will also be extruded and composited within each plot. Both floc and surface soils will be analyzed for TP, TN, TC, TOC and ash content. Deeper soil cores will be collected once during the wet season each year and in addition to the parameters listed above, floc, 0-5, 5-10, 10-20 and 20-30 cm depth increments will be analyzed for P speciation through a combination of P fractionation (Ivanoff et al. 1998) and ^{31}P-NMR (Turner

and Newman 2005). In addition, floc samples will be analyzed for microbial biomass C, N and P using the chloroform fumigation technique (Brookes et al. 1982, Hedley and Stewart 1982). Microbial respiration will be determined via CO_2 generation (Wright and Reddy 2001).

A recent study suggests that the C produced in benthic layers of open-water areas is less recalcitrant than that produced in vegetated areas, regardless of degree of enrichment (Penton and Newman In Review). Thus, microbially mediated nutrient regeneration is predicted to be higher in open-water plots. Microbial activity, expressed via enzymatic hydrolysis of C (ß-glucosidase), N (leucine aminopeptidase), P (phosphomonoesterase and phosphodiesterase) and oxidation of more lignified structures (phenol oxidase and peroxidase) will be determined using methylumbelliferyl and amidomethylcoumarin substrates (Table 2-3).

Decomposition – Increased microbial respiration will result in more rapid decomposition of the plant material. Thus, in addition to heterotrophic respiration, C:N:P turnover of plant material will be documented by following plant litter decomposition over time. Standing dead litter, collected from emergent macrophytes in enriched, transition and unenriched, will be collected, placed in litterbags and subsequently redeployed in plots of the same nutrient status. Three replicate litterbags will be collected from each plot every 6 months (Table 2-3). Each bag will be analyzed for mass loss per unit time, TP, TN, TOC, TC, microbial biomass C, N and P, microbial respiration, and hydrolytic and oxidative enzymes; ß-glucosidase, leucine aminopeptidase, phosphomonoesterase phosphodiesterase, phenol oxidase and peroxidase, using methods described above.

Macrophyte composition and productivity – While control plots are expected to remain monotypic cattail stands throughout the duration of the study, increased light availability in the maintained open plots is predicted to increase the abundance of SAV. Percent cover, species composition, and specific biomass per m^2 will be determined once per year via destructive sampling (Table 2-3). Percent cover for each plot will be estimated via aerial observation and digital photography. Emergent and/or SAV biomass will be determined in each plot by harvesting all the above-ground material contained within 0.5 x 0.5 m quadrat (Orth and Moore 1983, Miao and Sklar 1998), randomly selected from the 3 designated sampling areas. Below-ground biomass will be estimated once per year by collecting 10 cm diameter soil cores following removal of above-ground biomass. Both above and belowground material will analyzed for TP, TN, TOC, TC, TCa, and ash content. The tissue nutrient content (TC, TP, TN) of dominant macrophytes will be measured in conjunction with the food web sampling (Table 2-3) by collecting 5 leaves of each species from each plot,

Secondary productivity – Species composition, abundance and functional differences of macroinvertebrates and fish will be assessed in relationship to bird feeding and periphyton abundance (Table 2-3). With the creation of open areas, we expect that secondary production will increase as a result of increased primary production and higher nutrient content food resources. Secondary production estimates will be obtained using the increment-summation method (Benke 1984, 1993, 1996, Meyer and Poepperl 2003). Secondary production for invertebrates is defined as the increase in biomass per square meter per time and calculated as:

$$P=\sum N\ \Delta W$$

where P=production (mg m^{-2}) for the time span of interest, N=mean abundance of the taxon for a given sampling date (individuals m^{-2}), and ΔW=difference of mean individual biomass between two consecutive sampling dates (mg). Invertebrates will be collected four times per year in association with the food web study; thus, invertebrate production estimates will reflect the 4-6 week period prior to sampling the food web. One throw trap per plot will be used to collect and enumerate the number of invertebrates per unit area (Turner and Trexler 1997). Each sample will be washed through nested sieves (1.6 mm and 500 µm). In densely vegetated areas, the vegetation will be clipped several days prior to throw trapping. Funnel traps will be used to capture transient macro- and meiofauna and obtain biomass and production estimates. Invertebrates will be placed on pre-weighed filters, dried, and reweighed to determine total biomass in terms of dry weight and mean individual biomass in terms of dry weight per animal. The mean individual biomass will be used to calculate the percent total biomass of the main invertebrate groups that occur within the Everglades. The groups are: the insects, sub-divided into dipterans, ephemeropterans, trichopterans, and odonates; oligochaetes; gastropods; and amphipods (McCormick et al. 2004).

Population estimation for common fish and crayfish species will be determined using mark-recapture and/or removal methods (see Otis et al. 1978 and Williams et al. 2002 for comprehensive reviews). In short, animals are caught in an enclosed trapping grid of known area, individually marked for future identification, returned to the population and recaptured over a series of discrete sampling episodes. The resulting data is a series of individual capture histories, vectors of 1s and 0s that represent the sequence of captures over all sampling events. Population estimators are based on probabilistic models of events giving rise to the data in the capture histories. The computer program CAPTURE (Otis et al. 1978, Rexstad and Burnham 1991) is used to compute population estimates for each of a series models with different assumptions about sources of variation in capture probability. Model selection procedures are then used to select the model that best fits the data.

To obtain reliable estimates of population size, a sufficiently large number of individuals must be captured and recaptured. The factors that control number of captures include the number of traps in a given area, capture probabilities and number of trapping episodes. Since little is known of the aquatic community of P enriched cattail stands, a pilot study is needed to identify the appropriate focal species for study and the capture efforts required for reliable estimates of population sizes. The pilot study will employ the mark-recapture method described above and consist of a pair of enclosed trapping grids within cattail habitat to examine the community and population characteristic of common small (< 10 cm) and large (>10cm) fish species. Small fish and crayfish will be marked and recaptured using 100 minnow traps set in a 10 x 10 m square grid, with each trap placed 1.5 m from its nearest neighbor. Large fish will be sampled using a series of ten fyke nets set within a 30 x 30 m trapping grid. All traps will be sampled once every 24 h for approximately five to ten days depending on numbers of captures. The utility of the pilot study design for population estimation in cattail habitat will be examined using the program CAPTURE. If data are insufficient for reliable estimation, simulation studies based on the pilot data will be conducted using CAPTURE to determine the appropriate capture effort.

Population estimation will occur at the height of the wet season to measure secondary production and community structure, and again during the dry season to examine consumption of secondary production by foraging wading bird (Table 2-3). During the wet season, we will employ a two stage sampling regime wherein the first sampling episode will provide a reference measure of population size and overall biomass for each target species. The second sampling episode, approximately 30 days after the end of the first, will be used to calculate the change in biomass and thus provide a measure of secondary productivity/time. A similar two sampling strategy will be employed in the dry season to quantify removal of secondary production by wading birds, but the time period between sampling episodes will be dependent on the foraging duration of wading birds (see wading birds below).

Time constraints will limit sampling to only 5 of the 15 plots on any one day. Thus, during a given sampling event, a control and an open plot from the same block at both the enriched and transitional locations and a natural plot will be sampled. This process will be repeated until all plots have been sampled. To minimize temporal differences between samples, all plots will be completed within 4 weeks.

For the stoichiometric analysis, C, N, and P content will be determined for the most common invertebrate taxa obtained from the 1.0 m^2 throw traps using a method similar to Elser et al. (1998) and Cross et al. (2003). Fish nutrient content will be measured following the method of Sterner and George (2000). Phosphorus will be determined using the acid-molybdate technique following persulfate oxidation or HNO_3 acid-hydrolysis for inverts and fish, respectively. Carbon and N will be determined using a CHN elemental analyzer.

Fish movement behavior – To understand treatment effects on food web dynamics and community structure it is necessary to account for movement of aquatic organisms in and out of study plots. Movement patterns of fish and crayfish will be assessed using a series of traps placed along the edge of each plot. Dispersal of small fish and crayfish will be sampled using a drift net array. This trapping system consists of two drift fences positioned in an 'X' shape, with a series of minnow traps placed at the center of the array at the apex of each of the four compartments. Two minnow traps will be placed in each compartment, one at the surface and one at the base of the water column, to capture top and bottom dwelling organisms. The relative number of organisms caught in each quarter of the array indicates direction of movement, which is movement into or out of plots or along plot edges (modified from Loftus et al. 2003). Movement behaviors of large fish will be quantified using 100 m gill nets set parallel to the edge of the plot. Movement into or out of plots will be established from the relative number of fish caught on each side of the net.

A single gill net and drift net array will be positioned along one randomly chosen edge of each study plot. Sampling will occur twice per year in conjunction with secondary production fish sampling. Traps will be sampled once every 12 hours at each site over a 5 day period or until a sufficient sample size is reached. This will provide information both on overall movements of fish in and out of plots and diurnal movement patterns of fish.

Community structure – A number of approaches will be employed to measure aquatic faunal community characteristics, such as the Simpson Index and Shannon-Weiner Index to measure

species diversity (Simpson 1949, Shannon and Weaver 1949), the guild structure of functional groups (Root 1967, Simberloff and Dayan 1991), null model analysis of species co-occurrence (Gotelli 2000, Kobza 2001, Sanders et al. 2003), indicator species analysis (Dufrene and Legendre 1997), and non-metric multidimensional scaling (Beals 1984, Kobza et al. 2004).

Wading birds – Wading bird use of plots will be surveyed during the dry season when water levels are receding and potentially good foraging conditions are available (Table 2-3). Plots will be surveyed daily for approximately one month when water depth is about $\leq$ 20 cm. Data from the Systematic Reconnaissance Flight (SRF) surveys and water levels within the plots will be used to determine when the hydrology is suitable for the plots to be surveyed (typically around March or April). In each plot, approximately five decoys will be placed to attract wading birds to the sites (G. Herring, personal communication). Decoys in cattail plots will be placed on PVC poles so that the cattail plots are not biased by vegetation obscuring the decoys. Each day, observations will begin at sunrise and continue for approximately 1 hour. The number of flocks landing in each plot (as well as the species composition of the flock, number of individuals, and subsequent behavior of the birds) will be recorded by either human observer or video camera. Observations are likely to be made from makeshift towers or the top of an airboat at the corner of each plot. A short pilot study will be conducted in the spring of 2005 to determine the suitability of using video cameras, and to compare the relative effectiveness of observer and video data.

At open sites, greater accessibility and higher expected secondary production is likely to support increased wading bird foraging. The relative profitability of open and reference plots for wading bird foraging is difficult to predict. Open plots are likely to have high secondary production and thus support greater wading bird foraging than nutrient poor reference plots. Indeed, a recent study has shown that nutrient enriched sites have greater numbers of foraging wading birds than similar unenriched sites (Crozier and Gawlik 2002). Alternatively, we hypothesize that if open enriched plots are dominated with high densities of SAV and a deep layer of residual floc, prey may not be available to wading birds. Prey intake rates of individual birds will be estimated using video cameras and related to prey densities, submerged aquatic vegetation density and water depth. It is important to note that we will not be estimating population responses of wading birds to treatment effects.

Secretive birds – With the loss of emergent cover, it is probable that secretive bird (rails, bitterns, etc.) usage in the open plots will be less than in the control plots. This will be assessed in winter and spring via a point-count method that uses recorded bird calls of the target species (Table 2-3). Based upon recent discussion of the benefits and detriments of call-broadcast surveys for secretive marsh birds, the survey will include both passive periods and call-broadcast periods (Conway and Gibbs 2005). Surveys will be conducted 30 minutes before sunrise to 4 hours after sunrise from the monitoring platform of each plot.

Ecosystem production – Net ecosystem production (NEP) is the difference between gross primary production (GPP) and community respiration (R). In the Everglades, water column GPP is regulated primarily by periphyton and the contribution from emergent macrophytes is negligible (McCormick et al. 1997, McCormick and Laing 2003). McCormick et al. (1997) report that in pristine regions of the Everglades, production to respiration ratios (P/R) are near unity indicating that periphyton contribute significantly to ecosystem metabolism. In contrast, in

nutrient impacted regions, P/R are less than 1 indicating that water column respiration is sustained by organic matter decomposition derived from emergent macrophytes (i.e., cattail). With the large scale removal of emergent macrophytes, we expect that NEP will be greater in the open than the control plots because of increased GPP by periphyton and submerged aquatic vegetation associated with an increase photosynthetic active radiation (PAR).
Ecosystem production estimates will be derived from water column diel dissolved oxygen (DO) measurements. Dissolved oxygen and temperature will be recorded at 15 minute intervals for 5 days using a multiparameter water quality sonde (e.g. Hydrolab Minisonde®). One sonde will be suspended from a tripod located in the center of each plot. Deployments will be made monthly for the first three months and in conjunction with the four sampling events. NEP, GPP, and R will be calculated using a modified method of Odum's diel O_2 curve method (Odum 1956) which incorporates a temperature dependent gas exchange coefficient (*k*) estimated as a function of wind speed (Cole et al. 2000). Wind speed and direction will be measured using HOBO wind speed/direction smart sensor. A total of five anemometers will be deployed (1 cattail control, 1 cattail open, 1 transition control, 1 transition open, 1 reference). Photosynthetic active radiation will be measured at each plot with a HOBO® PAR sensor. Both the PAR sensor and anemometer will be connected to a HOBO Micro Station Datalogger.

Stoichiometric analysis – We will use a stoichiometric analysis to link how abiotic and biotic factors determine the carbon and nutrient composition of the base of the food web and how

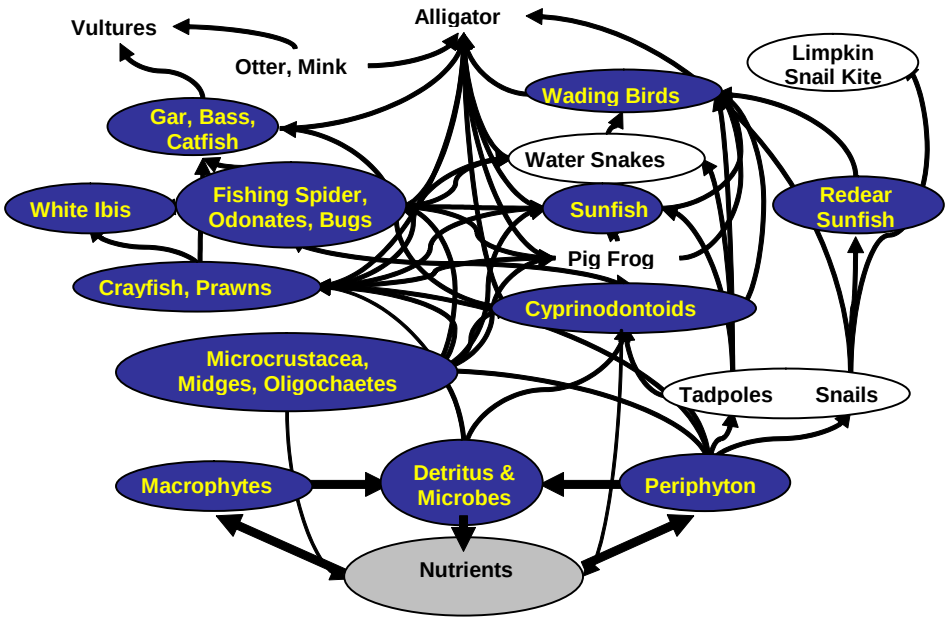

Figure 2-3. Conceptual model of the ecosystem components that will be directly measured in the Cattail Habitat Improvement Project. The grey oval indicates the nutrient pool. Blue ovals indicate the biological components in which biomass and stoichiometry will be measured. The white ovals indicate components that will be measured if encountered. Arrows indicate the potential nutrient and energy pathways. Those components that are not encircled will not be measured. Figure modified from Gunderson and Loftus (1993). Note that stoichiometry will not be determined for wading birds, snail kites, or water snakes.

Table 2-3- The frequency that each parameter will be sampled over the three year study period. White and gray columns indicate dry and wet season, respectively. B=the period immediately following the burning of the plots; RW=the 2 week period immediately following rewetting of the plots; w=weeks; m=months; Pre and Post= 1st and 2nd food web study sampling, respectively.

Sampling Year	1st Year							2nd Year					3rd Year				
Season	Dry	Wet						Dry		Wet			Dry		Wet		
Month	May	Jun	Jun	Jun	Jul	Aug	Dec	Mar	May	Jun	Jul	Sep	Mar	May	Jun	Jul	Sep
Event	B	RW	1w	2w	4w	2m	6m	Pre	Post	RW	Pre	Post	Pre	Post	RW	Pre	Post
Herbicide Application																	
Glyphosate and AMPA (buffer floc)	•						•		•			•		•			•
Glyphosate and AMPA plot interior (floc, soil, plants, periphyton, fish)									•					•			
Nutrient Flux																	
Auto Samplers Daily 2-week deployment (TP, TKN)	•	•								•					•		
Grab Samples (TP, TKN, SRP, TDP, TDKN, NH_4-N, NO_X-N, DOC, SO_4, pH, specific conductivity	•	•	•	•	•	•	•	•	•		•	•	•	•		•	•
Peepers (2 week deployment) (SRP, NH_4-N, NO_X-N, DOC)	•	•						•		•			•		•		
Periphyton																	
Taxonomy and Biomass					•		•	•	•		•	•	•	•		•	•
Nutrient Content (TC, TP, TN, TOC, TCa)					•		•	•	•		•	•	•	•		•	•
Primary Productivity					•		•	•	•		•	•	•	•		•	•
Algae Toxicity									•			•					
Heterotrophic Respiration																	
Floc (TP, TN, TC, TOC, Ash)							•	•	•		•	•	•	•		•	•
Sed 0-5 cm (TP, TN, TC, TOC, Ash)							•	•	•		•	•	•	•		•	•
Sed 5-10, 10-20, 20-30 (TP, TN, TC, TOC, Ash)												•					•
Organic P Sediment Fractionation (all depths)												•					•
Sediment P Fractionation (^{31}P-NMR)												•					•
Floc (microbial biomass C, N, P)							•	•	•		•	•	•	•			•
Floc (microbial respiration)							•	•	•		•	•	•	•			•
Floc (microbial enzyme activity)							•	•	•		•	•	•	•			•

Table 2-3. Continued

Sampling Year	1st Year							2nd Year					3rd Year				
Season	Dry	Wet						Dry		Wet			Dry		Wet		
Month	May	Jun	Jun	Jun	Jul	Aug	Dec	Mar	May	Jun	Jul	Sep	Mar	May	Jun	Jul	Sep
Event	B	RW	1w	2w	4w	2m	6m	Pre	Post	RW	Pre	Post	Pre	Post	RW	Pre	Post
Decomposition																	
Decomp Bags (TP, TN, TC, TOC)									•			•		•			•
Decomp Bags (microbial biomass C,N,P)									•			•		•			•
Decomp Bags (microbial respiration)									•			•		•			•
Decomp Bags (microbial enzyme activity)									•			•		•			•
Macrophytes																	
Percent Cover (aerial digital photography)												•					•
Species Composition												•					•
Above Ground Biomass												•					•
Above-ground Tissue Nutrient Content (TC, TP, TN, TOC, TCa, Ash)							•	•	•		•	•	•	•		•	•
Below Ground Biomass												•					•
Below Ground Tissue Nutrient Content (TC, TP, TN, TOC, TCa, Ash)												•					•
Secondary Production (Inverts)																	
Species Composition									•		•	•	•	•		•	•
Abundance/Biomass									•		•	•	•	•		•	•
Nutrient Content (TC, TN, TP)									•		•	•	•	•		•	•
Secondary Production (Fish)																	
Species Composition & Biomass (Drift net array, fyke net, gill net)								•	•		•	•	•	•		•	•
Nutrient Content (Drift net array, fyke net, gill net)								•	•		•	•	•	•		•	•
Wading Birds																	
Species composition, abundance & behavior								•	•				•	•			
Secretive Birds																	
Point count							•	•					•				•
Ecosystem Production																	
Hydrolab sonde (diel DO, pH, temperature, specific conductivity)	•	•			•	•	•	•	•		•	•	•	•		•	•

the quantity and quality of primary production impact higher trophic levels (Figure 2-3; Elser et al. 1998, Sterner et al. 1998, Elser et al. 2000, Cross et al. 2003). Essential to comprehensive stoichiometric analysis are the simultaneous measurements of biomass and nutrient content in the different ecosystem components. We have outlined above the methods we will use to characterize several ecosystem components, all of which contain both a measure of biomass and nutrient content. For autotrophs and detritus (floc), C:N:P ratios will be used to compare nutrient limitation among plots. These values will be compared to biomass specific primary production and respiration rates to establish patterns of biomass production with productivity for each treatment. In addition, nutrient use efficiency (NUE), defined as biomass C divided by biomass N or P (C:N and C:P) will be calculated.

The C:N:P ratios for individual species of invertebrates and vertebrates (primarily fish) will be used to determine the nutritional requirements of each species. In general, because invertebrates and vertebrates are homeostatic (i.e. their C:N:P remains constant regardless of the elemental composition of its food (Sterner and Elser 2002), the C:N:P ratio can be used to infer its nutritional requirements. The C:N:P of individual consumers will be compared to the C:N:P of their food to determine the degree of imbalance calculated as the arithmetic difference between the two (Cross et al. 2003). The stoichiometric imbalance will be used to determine if consumer-resource relationships differed between treatments.

The carbon use efficiency (CUE), calculated as secondary production divided by primary production, will be used to assess how efficiently carbon is transferred. These values will be compared among treatments. To determine if nutrient recycling by invertebrates and fish varies between treatments, we will assess interspecific variation by measuring the TN and TP excreted from fish and invertebrates following the methods of Vanni et al. (2002). The ultimate goal is to determine the stoichiometric balance of each plot, where stoichiometric balance is defined as the similarity in nutrient content between available nutrients and autotrophs or consumers and food resources (Sterner and Elser 2002).

2.5 SUMMARY

The Cattail Habitat Improvement Project aims to test: 1) whether creating openings within densely vegetated areas will sufficiently alter trophic dynamics such that wildlife diversity and abundance is increased; and, 2) to what extent does the structure and function of these created open areas compare to those of the natural Everglades using and ecosystems approach. Measures of ecosystem structure, ecosystem function, and ecosystem stoichiometry will be simultaneously made to discern food web dynamics and compare among treatments (Figure 2-3). Our predictions regarding how created openings will differ between control plots (objective 1) and from the natural Everglades (objective 2) are outlined in Tables 2-1 and 2-2, respectively. This experiment is anticipated to run for 3 years. At the end of this period, combining the results of objectives 1 and 2 will allow us to provide a preliminary assessment of the role of active management in accelerating the improvement of cattail habitat. This experiment will not provide recommendations on methods of cattail management, nor the size and locations of openings, should they be determined to be a desirable option. Assuming these open-water areas are sustainable after the first three years, further studies, such as the interaction of vegetation mosaics, can be explored in subsequent years.

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