

# Determining the Contribution of Aquatic Autotrophs to the Carbon Budgets of Restored Wetlands in the Sacramento-San Joaquin River Delta

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## Abstract

Over two decades of carbon farming experiments in the Sacramento-San Joaquin River Delta (Delta) have indicated that the restoration of freshwater marshes from highly developed farmland precipitates a net influx of carbon. Consequently, the reestablishment of historically tule- and cattail-dominated wetlands has been employed as a climate change mitigation tool, inducing the fixation and sequestration of atmospheric carbon *in-situ*. However, while much research has been conducted on the atmospheric fluxes of greenhouse gases into and out of these terrestrial systems – correlating storage with the growth and un-decayed senescence of large emergent graminoids – few studies have examined the aquatic processes that occur while these wetlands are flooded year-round. I examined four Delta wetlands that together represent a 20-year space-for-time sequence, in order to link changes in the broader productivity of the systems to changes in the aquatic primary productivity, particularly of phytoplankton, over time. I found that neither the satellites nor the on-site pheno-cameras considered could readily be accurately used to remotely sense changes in aquatic primary productivity via NDVI; that as wetlands get older their developmental trajectories likely encourage first large oxygenation then eventually slow deoxygenation; that measureable aquatic changes in carbon potentially represented between one and 20% of average yearly emergent productivity; and that differences in aquatic productivity could significantly be attributed to differences in photosynthetic biomass. This research suggests that aquatic autotrophs may contribute differentially throughout time (e.g., as reservoirs) to the fixation of carbon, and that researchers should account for these primary producers when modeling gas fluxes in newer systems and when designing additional wetland sites.



# Chapter 1

## Introduction

Wetlands are among the most valuable landscapes on the planet, covering approximately 5 to 8% of the total land surface (Mitsch and Gosselink, 2007). Conservative estimates (Costanza et al., 1997), adjusted for inflation to 2015, value them as producing approximately \$7.2 trillion in environmental services as “natural capital” per year. Beginning most expansively in the mid-1800s, unfettered industrialization and urbanization forced the wetlands of the western United States to decline in size or disappear altogether. In the Sacramento-San Joaquin Delta (“Delta” hereafter), 97% of the historical wetland area was lost in this process (Whipple et al., 2012). Byrd et al. (2014) note that consequently “The loss or degradation of coastal wetlands could increase net global atmospheric CO<sub>2</sub> inputs by 6% per year.” In fact, global greenhouse gas (GHG) emissions from such sources as the burning of fossil fuels and deforestation have already contributed to warming of the climate, associated with an average global increase in temperature by 0.85 [0.65 to 1.06] °C over the period 1880 to 2012 (Pachauri et al., 2014). These forcings are linked in a positive feedback, with peatlands alone – a subset of wetlands – representing 16-33% of the global soil carbon pool (Byrd et al., 2014). Their loss releases the carbon from this pool, mechanically and chemically (e.g., via oxidation). To mitigate future damage and adapt to imminent environmental events that follow from increasing temperatures and air and water pollution, the practice of restoring or constructing wetlands has emerged, capitalizing on the rapid and complex biogeochemical processes that take place during development. Among the structural co-benefits, for example buffering in floods, and ecological advantages, often re-establishing historical communities of co-dependent wildlife and plants, wetlands also provide

biogeochemical services like reversal of soil carbon oxidation and subsequent subsidence through peat accretion (Miller et al., 2008), filtration or chemical transformation of contaminated water (Jasper et al., 2013), and sequestration of atmospheric carbon (Miller, 2011).

However, complex nutrient cycling begets complex atmospheric fluxes of GHGs, and often this is crux of understanding wetlands in the face of climate change (Ibid.). Petrescu et al. (2015) note, for instance, that while one process (photosynthesis) may sequester carbon in a negative feedback, it may also bring about greater respiration and methane production “that may partially or completely counteract carbon uptake,” with the ratio depending on factors like water table height. For tailored maximization of carbon-based processes alone, a more dynamic ecological understanding must be sought.

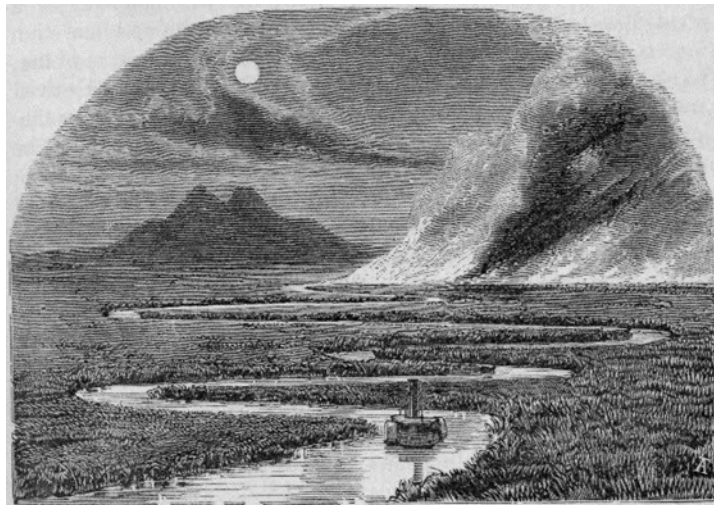
While most research has focused on large emergent plants and their direct interface with the atmosphere, it is unclear what the short-term population dynamics of aquatic primary producers are. This is particularly the case in constructed, highly regimented systems designed for mass accretion and carbon sequestration (i.e., little decomposition, low oxygen, high water saturation), but situated in a region of historical peat formation, and climatically predisposed to naturally tidal freshwater marsh. It is also unclear what the long-term aquatic contribution is in a context of controlled succession, whereby large and competitive emergents that mix, hybridize, and fill open spaces are encouraged to proliferate and dominate the landscape as self-constructing organic infill. He et al. (2015) suggest that since “there is no correlation between methanotroph abundance and methanogen or methane production, oxygen availability in the microenvironment is probably the limiting factor controlling methanotroph populations in this wetland.” If this is true, because oxygen availability is influenced in these wetlands by the presence of specific vegetation (Miller, 2011), an understanding of aquatic primary producer distribution over time is mandatory in manipulating sequestration wetlands to, for example, reduce methane production. The determination of early springtime trends in the primary production-based carbon contributions of aquatic autotrophs will contribute to a more useful baseline understanding of constructed wetland ecology and ultimately aid in the maximization of carbon sequestration and accretion efforts by the government and broader Delta community.

My testable hypotheses for this research were threefold. First, that successional changes in the wetland aquatic communities could be remotely sensed via satellite imagery (specifically, via the index 'NDVI'). Second, that

newer wetlands would exhibit higher dissolved oxygen values as well as higher aquatic primary productivity values – the latter being measured via aquatic oxygen generation per unit time. And third, that differences in productivity would be correlated with differences in aquatic autotroph biomass, measured via photosynthetic pigment concentrations. Together, these hypotheses represented my expectation that early in constructed wetland development, aquatic primary productivity makes up a larger portion of the overall carbon budget than it does later in wetland development. Previous research suggested that that this would occur because large emergent plants would eventually dominate the wetlands, dramatically reducing the contribution of aquatic carbon fixers over time.

## Chapter 2

# Background



**Figure 2.1:** Mount Diablo and Historical Tule-Dominated Tidal Marshes of the Sacramento-San Joaquin River Delta in the mid-1800s.

*“An apparently interminable sea of tules extends nearly one hundred and fifty miles, south, up the valley of the San Joaquin; and when these we on fire, as they not unfrequently are, during the fall and early winter months, the broad sheet of licking and leaping flame, and the vast volumes of smoke that rise, and eddy, and surge, hither and thither, present a scene of fearful grandeur at night, that is suggestive of some earthly pandemonium.”* (Hutchings, 1862)



**Figure 2.2:** Modern Twitchell Island (December 2015). To the right, the channelized San Joaquin River flowing from the east. To the left, subsided Twitchell Island farmland connected to the river via pump.

## 2.1 Ecological Engineering

A technical, parameterized model of wetland processes has yielded specific design strategies to bring about scalable ecosystem services. In wetlands designed for wastewater treatment, idealized fluid dynamic considerations – ranging from “completely mixed flow reactor” models to “plug flow” models – dictate the appropriate physical dimensions of the space and the introduced taxa for nutrient uptake for maximum treatment efficiency (Jasper et al., 2013; Kadlec and Wallace, 2009). Likewise, hard-to-design physical parameters like sunlight irradiance, light attenuation, input water quality, reactive chemical species concentrations, depth, and mixing are taken into consideration in predictive empirical models for photoinactivation of pathogenic contaminants like viruses (Silverman et al., 2015). To that specific end, it has been found that in terms of biotransformation, “biofilms found on roots, stalks, and detritus are more important...than planktonic microorganisms” (Jasper et al., 2013). Similarly, higher nutrient removal (nitrate, phosphate), correlated with higher microbial diversity, has been found in constructed surface-flow wetlands with 50% vegetative cover versus those with 100% (Jasper et al., 2013). This ecological heterogeneity subsequently introduces

diversity in ecosystem services. For example, if periodic construction and maintenance are considered ecological “disturbances,” then understanding the resulting food web ecology enables to link energy cycling with mechanical disturbance Wootton et al. (1996). The ecology of these constructed wetlands is thus of great importance in understanding their functional capacities.

One long-term approach is to manipulate hydrology, considered by Mitsch and Gosselink (2007) as “probably the single most important determinant of the establishment and maintenance of specific types of wetland processes.” They add that seasonal fluctuations in water level are the “rule, not the exception, in most wetlands,” impacting water renewal rates and influxes/pulses of nutrients. In the Delta, wetlands have consequently been designed for peat accretion and carbon sequestration through an artificial hydroperiod of constant inundation (Miller et al., 2008). Specifically, at Twitchell Island, following wetland construction in 1997 and continuing into 2006, it was found that accretion occurred at 4 cm/year on average under this regime, reversing subsidence due to oxidation of exposed peat and providing the basis for “carbon farming” by which farmers may be paid for reconversion of agricultural land to wetland (Ibid.). Wetlands at Twitchell and the nearby Sherman Island are the study sites considered in this study, and contain a total of four experimental constructed wetlands, developed by the California Department of Water Resources in September 1997 (East and West Ponds), 2011 (Mayberry Farms), and fall of 2013 (East End). Findings from these disparate wetlands have shown promising results in the areas of peat accretion and carbon sequestration (Miller, 2011; Miller et al., 2008). However, while whole-wetland manipulation of hydraulics and dominant taxa has been explored here and elsewhere, a community-level exploration is often sought for microenvironmental or ecological manipulation to reduce such carbon-removing processes as methanogenesis (He et al., 2015).

## 2.2 Successional Community Ecology

Changes in community-level composition result from a host of factors, including limited availability of nutrients over time, changes in the diversity of dominant – usually “emergent” – plant communities present, and the gradient of chemical fluxes which inevitably exists from inflow (here, from an urbanized, impacted river) to outflow (here, an adjacent agricultural ditch)

(Miller, 2011). The four subject site systems are characterized by perennially inundated conditions. In such stagnant hydroperiods, primary productivity is often “depressed” and the number and type of rooted plants is significantly limited. This stems from the effects of waterlogged soils, including changes in oxygen content that lead to soil anoxia, as well as changes in concentrations of soluble sulfide and salts (Mitsch, 2009). In spite of such stresses, winter-time root biomass retention in clonal dominants is integral to the perennial return of emergents, “in contrast to nearly complete winter die-off of above-ground plant biomass” (Garver et al., 1988) and Gill and Jackson (2000) via (Miller and Fujii, 2010). Mitsch and Gosselink mention that even in vascular plants that are specifically adapted to anaerobiosis, best growth still occurs in aerobic soils. Fine-tuning the physical dimensions of the site often falls short of maximizing engineered ecological processes.

## 2.3 Food Web Interactions

Aquatic primary productivity in wetlands is often significant, and elucidating aquatic contribution is necessary in determining the cycling of raw materials in the “construction” of other life forms (Keddy, 2000). Cronk and Mitsch (1994*b*) found that aquatic net primary productivity could often be as high as that of emergent macrophytes, noting that “its measurement was essential to an overall picture of the influence of hydrology on productivity.” They found that water column primary producers (mainly periphyton, phytoplankton, and submerged macrophytes) contributed between 17 and 67% of net aboveground carbon production, and was significant regardless of hydraulic regime studied. Similarly, Mitsch and Gosselink (2015) note that in tidal salt marshes and freshwater marshes, epiphytic algae is a source of high-quality food energy for meio- and macroinvertebrates in spite of their small biomass. They highlight Zedler (1980) in questioning the “paradigm that salt marshes have ‘detritus-based food webs’” (Odum, 1980) considering that the majority of secondary production by metazoans could be associated with primary production by microphytobenthos rather than through either direct (herbivory) or indirect (detritivory) links to primary production by vascular plants. While important though, primary productivity in freshwater wetland systems is complicated by differences in storage and end-use of photosynthate (Mitsch and Gosselink, 2015). Dominant primary producers in the aquatic regions of wetlands include phytoplankton like algae (including true algae,

and cyanobacteria), floating small aquatic plants, and differentially situated macrophytes (like emergent graminoids) (Kadlec and Wallace, 2009). Owing to differences in their physiology and size, contribution of aquatic primary producers is often linked in this functional grouping approach. Because of their high turnover rates, algae may be “important for short-term nutrient fixation and immobilization with subsequent gradual release and recycling” (Ibid.). They often represent high contribution to productivity, but lower contribution to biomass; that is, “apparent productivity” or “net primary production” (Cronk and Mitsch, 1994*a*). The contributions of aquatic autotrophs may be significant, but are visibly short-lived.

## 2.4 Plant Physiology

Alternatively, in the case of emergents, Byrd et al. (2014) note the impact of plant morphology on productivity, showing that in emergents the vertical stem structure contributes to high photosynthetic rates by increasing light penetration within canopies, but also increases light scattering and absorption between vegetation – posing serious challenges to its remote sensing, which are an otherwise invaluable tool for ecosystem-scale monitoring. Miller (2011) adds that plant-moderated influx of  $CO_2$  at Twitchell Island was significantly correlated with stem density of emergent vegetation. He further notes that the potential GHG forcing was greatest in areas of submerged vegetation in deeper wetlands. And regarding submerged vegetation, Schoenberg and Oliver (1988) note that diebacks of emergents allow for a peak in phytoplankton, and that aquatic productivity is thereby inversely related to water level. Disparate functional groups impart disparate productivities in the freshwater wetland system. Therefore, it is necessary to identify this differential productivity separately among the three primary sources of organic carbon: vascular marsh vegetation (macrophytes), organic material brought from upstream, and phytoplankton, often considered at an unknown quantity Mitsch and Gosselink (2015).





**Figure 2.3:** *Mougeotia* spp., a filamentous green alga found in an East End water sample.

## 2.5 Physical Habitat

Finally, biophysical parameters inherent to local conditions in the wetland lead to varying ecological processes. He et al. (2015) note the existence of physicochemical gradients of pH, sulfate, nitrate, and dissolved oxygen correlated with differences in microbial composition and activity at the subject sites on Twitchell Island. Cronk and Mitsch (1994a) similarly highlight that in their wetlands, aquatic productivity was highest near the inlet. In spite of recorded (but unanalyzed) observations on obvious changes in composition of aquatic taxa over time, no conclusion has been reached as to why this compositional change is so dramatic on a local scale, over the course of wetland development – owing to heterogeneity of physical covariates at the subject sites. Mitsch and Gosselink (2015) note that in freshwater

marshes, most primary productivity is “routed through detrital pathways,” owing to high pH substrates, high available soil calcium, medium or high nutrient loading, and high soil microbial activity (in regions experiencing atmospheric exposure). Dissolved nitrogen and phosphorus can often reflect the degree of plant dormancy, with high concentrations in winter reflecting mineralization. Miller and Fujii (2010), from destructive harvests conducted between 1998 and 2000, determined that ash-free dry weight of standing live aboveground emergent marsh vegetation “was an order of magnitude greater than that of standing crop biomass measurements of submerged and floating vegetation.” This differential biomass is corroborated by sources that claim (contradictory to other sources like Cronk and Mitsch (1994*a*)) that aquatic macrophytes, phytoplankton, and attached microalgae “all exhibit similar low production figures relative to terrestrial plants” (Keddy, 2000). Significant atmospheric gas emission and physical parameter differences exist between regions of emergent and regions of submerged or floating vegetation, with the latter exhibiting lower rates of  $CO_2$  influx (Miller, 2011). This difference is exacerbated by wetland-scale differences of hydraulic regimes, with shorter residence times in shallow, 25-cm Old West Pond vs. 50-cm Old East Pond subject sites (Ibid).

# Chapter 3

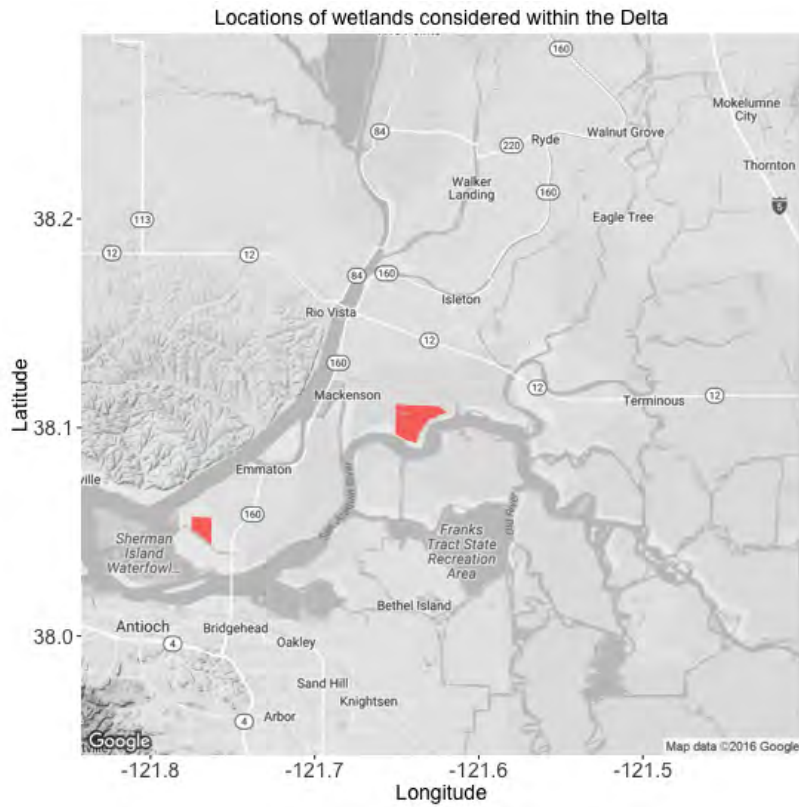
## Methods

### 3.1 Locations

The subject sites considered here are four constructed freshwater palustrine (i.e., non-tidal) marshes that are located in the Sacramento-San Joaquin River Delta (Delta). Each wetland represents a disparate degree or type of vegetative re-establishment, with each having been restored from an intensely agricultural use at a different point in time over the course of approximately two decades. The hydroperiod of all sites is permanently flooded, with intermittent inflow from the adjacent San Joaquin River, at the discretion of subject site managers (CA DWR P.E.s). Inflow to the sites contains minimal amounts of sediment (Byrd et al., 2014).

TABLE I: *Wetland Background*

<i>Wetland Designation</i>	<i>Construction Complete</i>	<i>Maintained Water Table Height</i>	<i>Tower Location</i>	<i>Area</i>
Old West Pond (Twitchell Island) <sup>1</sup>	1997	25 cm	(38.10743333, -121.6469)	12.6 acres
Old East Pond (Twitchell Island) <sup>1</sup>	1997, 2013 <sup>2</sup>	55 cm	–	11.7 acres
Mayberry Farms (Sherman Island) <sup>3</sup>	2010	UNK	(38.0498, -121.7651)	441.4 acres
East End (Twitchell Island) <sup>1</sup>	2013	Varies	(38.1027, -121.6413)	1288 acres



**Figure 3.1:** Locations of wetlands considered here (in WGS 1984/EPSS 4326). North is directly up. Maps of individual wetland plots are shown below.

Site lithology is characterized predominantly by peat underlain by fluvial sand, silt, and clay deposits (Gamble et al., 2003), meaning that the establishment of plant communities and the leaching of organic matter likely do not follow typical patterns of freshwater marshes that experience tidal flooding and fluxes in fertility and aeration (Mitsch and Gosselink, 2015). Additionally, given that subject site vegetation is perennially submerged by

<sup>1</sup>Was previously: Rice (*Oryza sativa*) cropland.

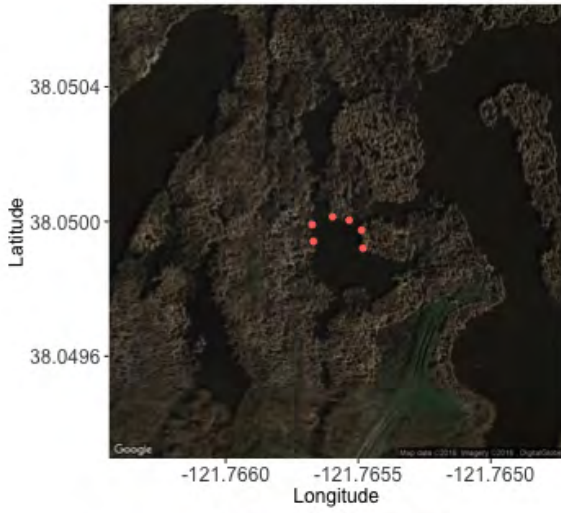
<sup>2</sup>Old East Pond experienced mechanical disturbance again in 2013, likely confounding its representativeness for the year 1997. Therefore, I consider it to be more representative of somewhere between 20-year and 15 year restoration, falling between Old West Pond and Mayberry Farms in the space-for-time sequence.

<sup>3</sup>Was previously: Pepperweed and annual grassland pasture.

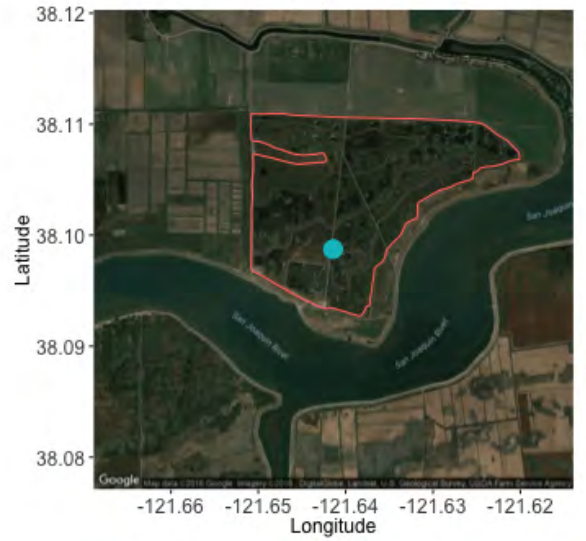
water from the adjacent San Joaquin and Sacramento Rivers, and is situated adjacent to densely maintained agricultural parcels, aquatic taxa are possibly impacted by runoff and aerial dispersion of air pollutants like pyrethroid pesticides. Similarly, the inflow of water is possibly impacted by upstream urbanization.

At any rate, initial examination of specific conductivity between two sites (East End, Old West Pond) indicated lower-than-expected ions/salts, suggesting somewhat better water quality than in the river channel. Soil temperatures appear to be higher at sites located closest to inlets, and are cooler with increasing distance (Miller, 2011).

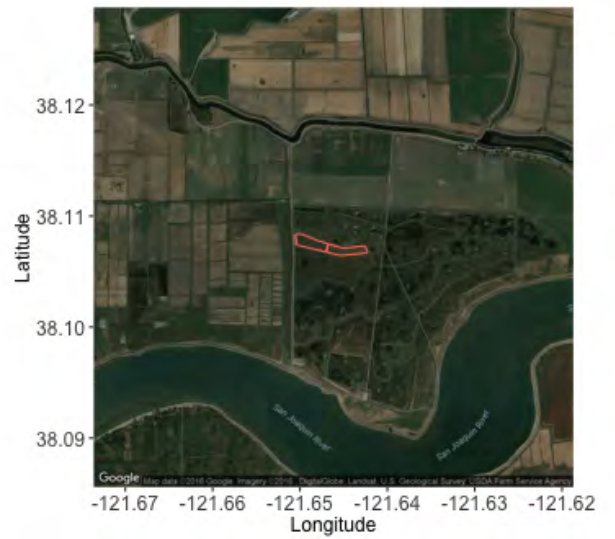
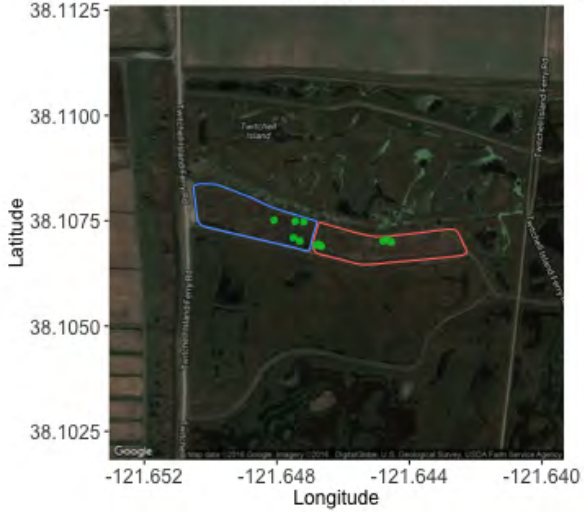
Mayberry Farms Sampling Points



East End Sampling Points



West (Blue) + East Pond (Red) Sampling Points (Green)



## 3.2 Measurements

The ecosystem approach that I employ here takes into consideration the relationship that aquatic primary producers are both affected by, and affect, their surrounding environment. Constant inundation, for instance, means that annual plant seeds do not have the opportunity to annually germinate for lack of exposure (Mitsch and Gosselink, 2015), so subsequently the systems are naturally converging to nearly monospecific perennial stands (Miller and Fujii, 2010). I employ two types of pre-existing datasets (remotely sensed imagery, and remotely recorded oxygen and carbon dioxide measurements), as well as field-collected data (light/dark bottle experiment data). The purpose of using these data was to establish baseline conditions and trends from which more specific ecological hypotheses could be made.

### 3.2.1 Remotely sensing changes in aquatic primary productivity using high resolution satellite imagery.

I employed remotely sensed imagery gathered and processed by NASA and retrieved through the Google Earth Engine API to fill-in long-term *in-situ* measurements gaps that existed among wetland sites. Although I found this method was not directly helpful, it informed my understanding of on-site biological processes. For the purposes of wetland-to-wetland comparison, I isolated Landsat 7 imagery and took a normalized difference vegetation index (NDVI) of bands 3 and 4 (red and near-infrared). NDVI data were derived from February 2000 to March 2016 imagery. To compare high-temporal resolution trends (within-year comparisons), I supplemented Landsat data with Moderate Resolution Imaging Spectroradiometer (MODIS) imagery, also from NASA. While Landsat data had a higher spatial resolution than MODIS composites, MODIS spatial resolution incredibly limits precision of spatially explicit trends. I used Landsat imagery to quantitatively compare wetlands.

TABLE II: *Satellites Used for Remote Sensing*

<i>Satellite Source</i>	<i>Grounding Track Repeat Cycle</i>	<i>Spatial Resolution</i>	<i>Launch Year</i>
Landsat 7 ETM+	16 days	30 meters	1999
MODIS/MCD43A4 (NDVI)	1-2 days (with 16 day composites)	500 meters	2000

The spatial resolution of Landsat data is approximately 30-meters, but

band values were averaged out for the entirety of the wetland footprint, which was digitized by hand and converted to the Arc Shapefile data structure. NDVI is a calculation of the portion of infrared radiation out of infrared and red bands; the more positive the number, the higher the productivity (Herring, 2000). While Byrd et al. (2014) note the limitations of using NDVI in these freshwater marshes (e.g., 100% cover biomass measurements are often incorrect due to asymptotic limits), I hypothesized that these data are useful in distinguishing differential trends between wetlands.

NDVI is calculated as follows:

$$NDVI = \frac{(NearInfraredBand - VisibleLightBand)}{(NearInfraredBand + VisibleLightBand)}$$

### 3.2.2 Remotely sensing changes in aquatic primary productivity using continuous recording of changes in dissolved oxygen and carbon dioxide.

Next, I retrieved “eddy covariance tower” (a micrometeorological instrument) atmospheric flux and physical parameter data directly from the Dennis Baldocchi Lab (UC Berkeley), unprocessed. Note that only three of the four wetlands had associated data collection from the Baldocchi Lab. These data are retrievable online, and relevant equipment used in data collection are referenced below.

TABLE III: *A list of the parameters and associated devices for pre-existing (i.e., continuous) data collection.*

	<i>Twitchell Island: Old West Pond</i>	<i>Twitchell Island: East End</i>
Ecosystem Flux: CO <sub>2</sub>	Licor LI-7500A	Licor LI-7500A
Dissolved Oxygen (DO) and Water Temperature	Sensorex DO6400/T	Sensorex DO6400/T
Dissolved CO <sub>2</sub>	Forerunner/Ecosense GP-001	Forerunner/Ecosense GP-001
Specific Conductivity	Campbell Sci CS547A	N/A
Phenocam (Greenness = GCC)	UNK	UNK
Water Level	Campbell Sci CS450	N/A

The greenness index (GCC), is the “green chromatic coordinate,” and is calculated as follows:

$$GCC = \frac{(GreenDN)}{(RedDN + GreenDN + BlueDN)}$$





**Figure 3.2:** Servicing YSI meters for long-term aquatic data collection at Mayberry Farms, atop an eddy covariance tower.

Where DN is digital number. Adopted from Klosterman et al. (2014).

Collection from the Baldocchi sources occurs automatically at varying intervals, with some data collected several times per second (10 hz). The outputs that I use here spans averaged 30-minute intervals. These data are inspected for accuracy and their corresponding equipment maintained by Baldocchi Lab field technicians, who collect the data from on-site data loggers approximately every two weeks.

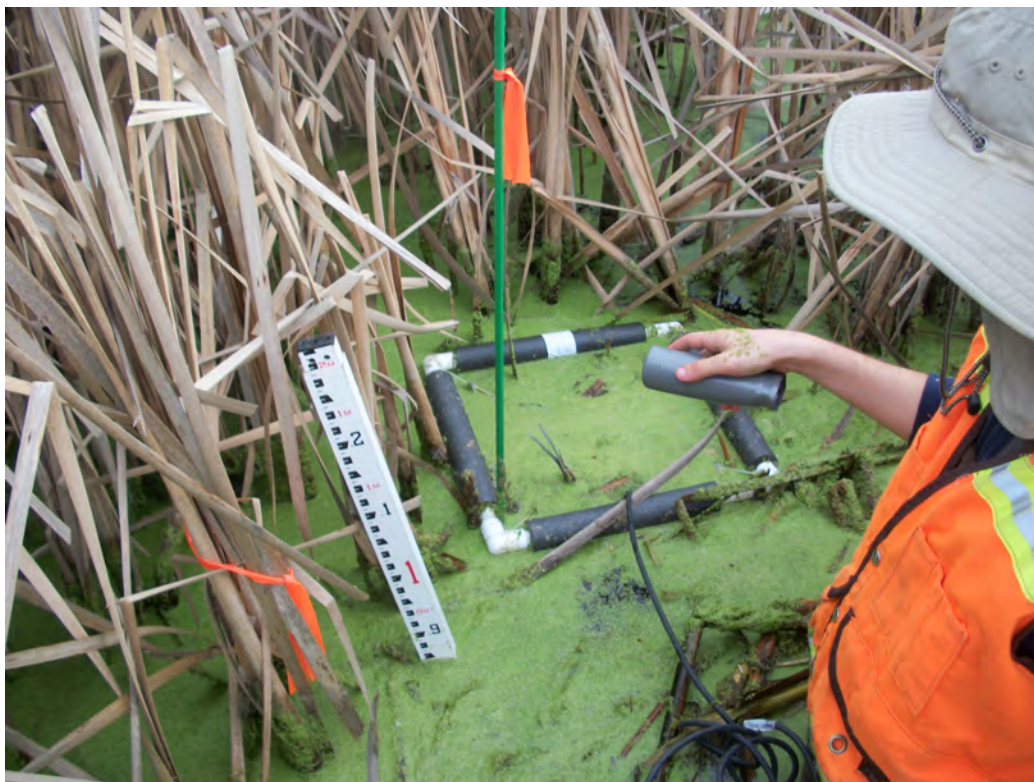
### **3.2.3 Measuring physical habitat covariates to characterize environmental baselines.**

Next, I measured physical habitat parameters in the field with a YSI probe, with sampling points co-located at water sampling points for light/dark bottles. Collection of these covariates allowed for comparisons to the findings of other studies regarding physical gradients on site (e.g., those of He et al.

(2015) and Cronk and Mitsch (1994a)), and allowed for associations with the growth and productivity of measured flora. Measurement occurred with a sonde/probe handheld device, calibrated prior to use in the field.

TABLE IV: *Physical Covariates*

<i>Measurement</i>	<i>Unit(s)</i>
pH	–
Temperature	°C
Dissolved Oxygen	mg/L and %
Specific Conductivity	$\mu S/cm$
Pressure	mbars and mmHg
Depth	meters

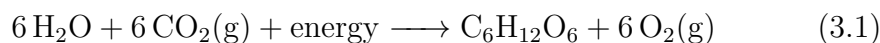


**Figure 3.3:** Sampling physical covariates in Old East Pond, meter stick emerging from duckweed next to square sampling locations.

### 3.2.4 Conducting field experiments and biomass estimation to attribute changes in dissolved oxygen to differences in the presence and intensity of aquatic primary production.

I estimated gross primary productivity per unit time from net primary productivity measurements in the field (light/dark bottle experiments), employing the general stoichiometric relationship of sugar production during photosynthesis to estimate grams of carbon produced per unit volume of water per unit time:

**Photosynthesis**  $\sim$



**$\Delta$  Dissolved Oxygen** =

$$\begin{aligned} & \text{Final bottle concentration (after)} \\ & - \text{Initial bottle concentration (before)} \end{aligned} \quad (3.2)$$

**Conversion of carbon (mgC/h)<sup>4</sup>** =

$$\frac{(\text{Average } \Delta \text{ in } mg \text{ O}_2) * (1/PQ^5) * (12/32)]}{\text{Average time (approximately 1h)}} \quad (3.3)$$

**Gross Primary Production<sup>6</sup>** =

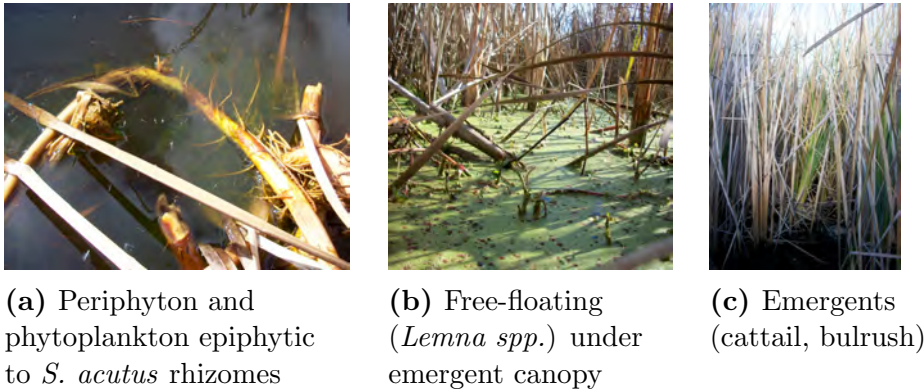
$$\begin{aligned} & \text{Light bottle } \Delta \text{ carbon} - \text{Dark bottle } \Delta \text{ carbon} \\ & \approx \text{photosynthesis} - \text{dark respiration} \end{aligned} \quad (3.4)$$

For productivity estimation, I isolated and performed light/dark bottle incubations on 300 mL of water from 10 cm below the surface at each of six sampling locations (five for West Pond) located approximately five meters apart at similar depths (see attached maps). At each sampling point, I concurrently sealed shut two clear glass biochemical oxygen demand (BOD) bottles (see Figure 3.6) and directly measured initial and final dissolved oxygen

<sup>4</sup>Adapted from (Dodds and Whiles, 2010).

<sup>5</sup>Where PQ is the photosynthetic quotient (moles of O<sub>2</sub> released during photosynthesis per moles CO<sub>2</sub> incorporated = 1.2 for algae at moderate light levels) (Ibid.).

<sup>6</sup>Other sources refer to this as “photoassimilation,” as it fails to consider the loss of carbon due to photorespiration, the respiration that occurs during light reactions.



**Figure 3.4:** Primary Producer Functional Groups Present in Subject Wetlands (*Photos : 12/28/15*)

concentrations with a YSI probe submerged directly into bottle water. Dark bottles were completely covered with aluminum foil to exclude all incoming solar radiation that would encourage light reactions in photosynthesis. I made sure to exclude bubbles as often and reasonably as possible, sealing caps so that no fluids could be exchanged between bottle interior and exterior regions.

In order to estimate algal biomass (or “apparent” as opposed to “gross” primary productivity), I performed fluorometric analysis of 10 mL subsamples of wetland water, originally collected in 50 mL centrifuge tubes in the field, at each light/dark bottle experiment location within the wetlands. I carefully removed a full falcon tube of H<sub>2</sub>O from each sampling point at approximately 10 cm below the surface, attempting to not disturb the local sediment or suspended organics or include bubbles. Following an adaptation of Sartory and Grobbelaar (1984) by Keith Bouma-Gregson (KBG) (of Mary Power Lab, UC Berkeley), I kept these samples in a cooler during transport, then within 5 hours filtered through glass fiber filters and stored in sanitized film cannisters, in a freezer for 24 hours. I then added 10 mL of 90% acetone to each film cannister (in the dark), then refrigerated (in the dark) at 4 °C for 24 hours. To test the samples fluorometrically on a Turner Design TD-700, I removed approximately 5 mL from each sample and analyzed in a 13 by 100 mm culture tube. A calibration curve had already been developed by KBG for the fluorometer, but prior to each set of analytes I confirmed accuracy via a solid standard. The calculation for chlorophyll-*a* concentration is detailed

below:

With  $[\text{chlFL}]$  = concentration of chl-*a* (g/L) measured in acetone extract by the fluorometer:

$$\begin{aligned} V_{\text{extract}} &= \text{volume of acetone extract } (L) \\ V_{\text{sample}} &= \text{volume of water sampled filtered } (L) \\ [\text{chl-}a] &= \text{concentration of chl-}a \text{ in water sample } (g/L) \quad (3.5) \\ [\text{chl-}a] &= [\text{chlFL}] * \frac{V_{\text{extract}}}{V_{\text{sample}}} \end{aligned}$$



(a) Falcon tubes

(b) Filter

**Figure 3.5:** Extracting chlorophyll-*a* from water samples.

### 3.3 Treatments

The design of this experiment follows what Hurlbert (1984) calls a “comparative mensurative” experiment and Gotelli and Ellison (2013) call a natural experiment, whereby manipulations are pre-existing differences between systems, with measurements being taken in the natural state of those isolated (i.e., not interspersed) systems. Of importance to this design is “Assuring that the replicate samples or measurements are dispersed in space (or time) in a manner appropriate to the specific hypothesis being tested.”

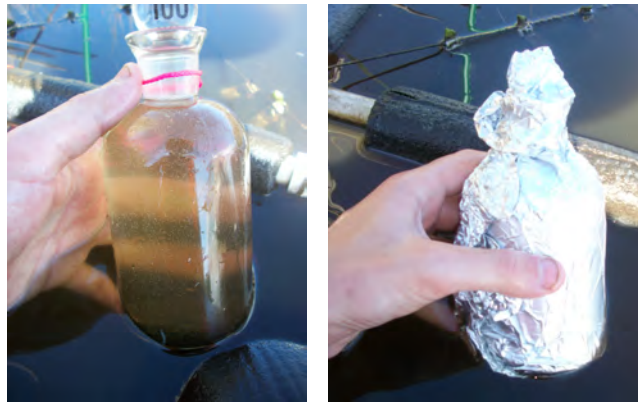


Specifically, I employ here a “space-for-time substitution” in which the selection of study sites represents an intentional temporal gradient (“chronosequence”) that enables a shorter-term (single-season, early spring 2016) examination of successional difference. A total of 23 sampling points were implemented for in-water data (6 per wetland, with exception of West Pond which had 5 for physical covariate measurements and 3 for productivity bottle experiments), in an attempt to survey four sites at disparate temporal gradients, I replicated samples among those sites three times (i.e., on three separate days) to control for, or at least determine, variation within wetlands.

I took two gradients into consideration in my sampling, whereby I attempted two categorical distances (as evenly spaced as possible) from inlet and one categorical distance from plants, in this case directly against emergents. This approach ensured that spatial trends noted in literature (e.g., He et al. (2015), Miller and Fujii (2010)) were controlled for (for distance from emergents) or recorded along with sampling data (for distance from inlet), to allow for spatial trends in data analysis. With six sampling points per wetland, I could approach statistical significance with data (if appropriate) in a parametric analysis that assumed normal distribution, as feasibly close to Gotelli and Ellison’s “Rule of 10” as possible.

Sampling frequency occurred at approximately three-week intervals, with approximately three to four hours spent at each wetland per day, and all wetlands sampled in the same order on the same day. This repeated from February (02/28) to mid-April (04/10/2016) totaling approximately three sampling points, presumably representing an average of the first third of the year, as productivity begins to increase from dormant emergents to active emergents across wetlands.

My rejection criteria for selecting sampling locations were associated with available space, known biogeochemical gradients, and accessibility. The area sampled had to be physically accessible by foot (in waders), and be able to contain a 2-ft. by 2-ft. periphyton sampler. Sampling had to also occur at the very least 5 meters away from the edge of the wetland, to prevent “edge effects.”



(a) Light

(b) Dark

**Figure 3.6:** Light and Dark Bottles for *in-situ* Productivity Measurement.



**Figure 3.7:** Experimental Field Setup for Light/Dark Incubations (Mayberry Farms). PVC frames that delineated sampling regions were kept in place by attachment to stakes. I tied BOD bottles to the PVC samplers and hung them  $\sim 10$  cm below the water surface for the approximately one hour duration of productivity experiments.



# Chapter 4

## Results

### 4.1 Physical covariate measurements

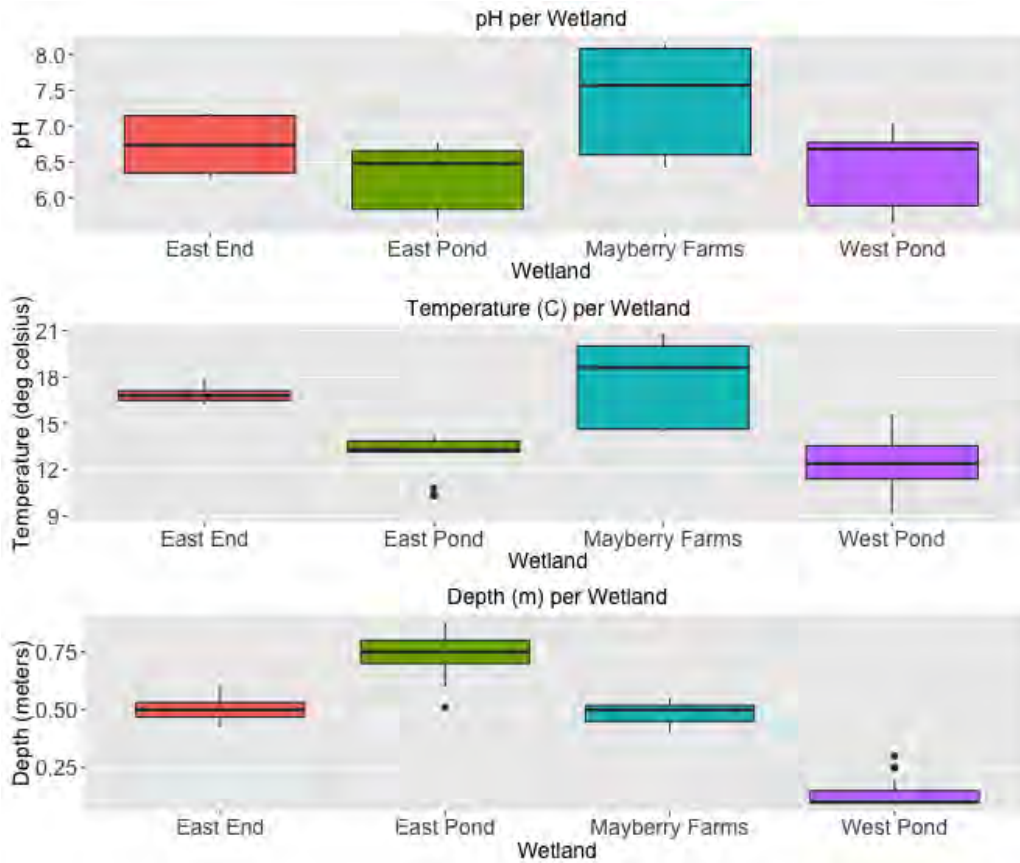
I took physical habitat measurements concurrent, and co-located, to productivity light/dark bottle experiments at each of the six sample locations (three within West Pond), for each of the two to three field visits. These measurements were recorded on a YSI probe after a 30-second to one minute stabilization period, approximately 10–15 cm under the surface of the water, or in the case of depth, approximated on a meter-stick to the nearest centimeter.

TABLE I: Average physical habitat measurement ( $n = 6$  (for West Pond) to 18 measurements/cell on majority).

<i>Wetland</i>	<i>Depth (m)</i>	<i>Temperature (°C)</i>	<i>Pressure (mmHg)</i>	<i>Dissolved Oxygen (%)</i>	<i>Conductivity (μS/cm)</i>	<i>pH</i>
West Pond	0.1	12.6	764.4	6.1	1140.7	6.5
East Pond	0.7	13.0	765.1	5.9	408.5	6.3
Mayberry Farms	0.5	17.8	765.0	97.3	6965.2	7.4
East End	0.5	16.8	764.8	21.2	974.9	6.7

Between West Pond and East Pond, East Pond was much more inundated with water, likely inducing more chemically reducing conditions that produced a more acidic pH value. However, both older wetlands were more acidic than the newer wetlands, Mayberry Farms and East End. According to HOBO meter measurements (two per site, data not presented here), temperatures were also consistently different between sites, even outside of sampling events, closely following values taken at nearby eddy covariance

towers. In other words, within-wetlands measurements were much closer than those between wetlands. The two newer wetlands had, on average, 4.5 degrees higher average temperatures, attributable to the presence of greater open water regions. Baseline pressure did not significantly differ between sites. The range of these differences are shown below, in Figure 4.1.



**Figure 4.1:** Boxplots of pH, water temperature, and depth data taken across all field visits and sample points per wetland.

Neither depth nor temperature significantly differed within wetlands during the study period, with the exception of Mayberry Farms, where pH ranged from sub-pH 7 conditions to over pH 8 conditions – indicating over an order of magnitude change in the concentration of hydrogen ions at that site. Similarly, water temperature greatly varied at Mayberry Farms with over 5 degrees celsius changes between sampling periods.

The most apparent difference in physical habitat measurements between wetlands, aside from pH, was specific conductivity values, which ranged from approximately 400 to almost 7,000 microSiemens/cm. These data suggest higher salt or other ion concentrations in Mayberry or West Pond. Higher values should be expected at Mayberry, which is geographically situated at the interface of salt and freshwater mixing of the Sacramento and San Joaquin Rivers and the saline San Francisco Bay.

## 4.2 Remotely sensing changes in aquatic primary productivity using high resolution satellite imagery.

Using Google Earth Engine API, I programmed in an adaptation of JavaScript the extraction, clipping, and Normalized Difference Vegetation Index calculation of six years of Landsat 7 imagery for each wetland. I performed this manipulation on four disparate polygon regions that I digitized based on each wetlands engineering management plan footprints, and approximate delineations between roads and wetland vegetation. I then exported these data and analyzed daily, weekly, monthly, and yearly average patterns for each wetland as a proxy for overall photosynthetic primary productivity. Table II below lists some summary statistics for the approximately six years of NDVI data, for each wetland.

TABLE II: Mean, median, minimum, and maximum average wetland NDVI values derived from Landsat 7 imagery (January of 2010 to April of 2016). Wetlands are arranged below from left to right with decreasing time since restoration.

	<i>West Pond</i>	<i>East Pond</i>	<i>Mayberry Farms</i>	<i>East End</i>
<b>Mean</b>	0.04996	0.06158	-0.02004	0.02901
<b>Median</b>	0.03363	0.05179	-0.04663	0.01484
<b>Minimum</b>	-0.23979	-0.23489	-0.26694	-0.23049
<b>Maximum</b>	0.44284	0.45333	0.28064	0.40795

NDVI magnitude for mean, median and extrema did not appear consistently associated with time since restoration; however patterns in NDVI

peaks during a given year did change. Figure 4.2 below is a line graph of all NDVI data available since 2010 for each wetland, with an approximate return interval/temporal resolution of one month.



**Figure 4.2:** All average wetland NDVI data, derived from Landsat 7 imagery. Line opacity corresponds to the magnitude of NDVI, with darker regions being larger NDVI values.

Data in Figure 4.2 indicate the tendency for NDVI peaks to stay in (in older wetlands) or transition to (in newer wetlands) a unimodal, late year peak from what was once bimodal. This trend is apparent in isolating Mayberry Farms NDVI by day-of-year, across years in Figure 4.3 below. Values suddenly dropped in latter half of 2010, slowly extending toward a unimodal summer to fall peak in following years. This shift corresponds temporally to the dates of wetland construction, and conversion of the area considered from farmland to emergent communities. These data suggest the movement

from bimodal annuals to unimodal perennial plants (e.g., cattails, bulrush).



**Figure 4.3:** Line graph of day-of-year Mayberry Farms NDVI values derived from MODIS imagery, which exists at a much higher temporal resolution (i.e., return interval) but much lower spatial resolution than Landsat imagery. Pinker values indicate values for years closer to the present.

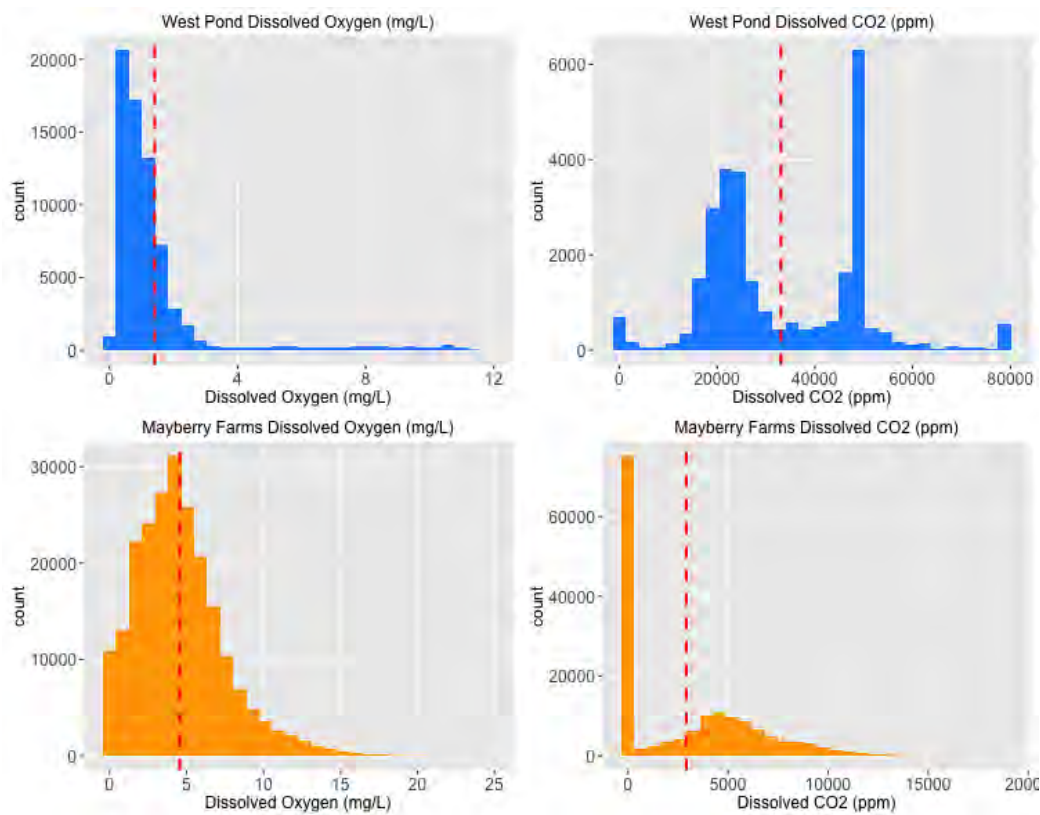
### 4.3 Remotely sensing changes in aquatic primary productivity using continuous recording of changes in dissolved oxygen, dissolved carbon dioxide, and phenocam greenness data.

I analyzed several years of dissolved gas and in-situ camera data to indirectly detect long-term, high-temporal-resolution trends in changes of aquatic productivity. These data were collected by YSI devices co-located at the eddy covariance towers used in atmospheric gas measurements, at intervals of 15 or 30 seconds. Only West Pond and Mayberry Farms had complete data for these measurements; East Pond had no data because it had no corresponding eddy covariance tower, and East End had only phenocam greenness data. Therefore, I was only able to compare West Pond and East End data for long term dissolved oxygen and dissolved carbon dioxide trends. Averages of the three utilized variables are shown below in Table III.

TABLE III: Average remotely sensed (via YSI probe) dissolved oxygen, dissolved carbon dioxide, and phenocam greenness data across all recording time. For Mayberry Farms, data was collected from 10/15/2010 to 2/12/2016. For West Pond, data was collected from 7/14/12 to 2/12/2016.

	<i>West Pond</i>	<i>East Pond</i>	<i>Mayberry Farms</i>	<i>East End</i>
Mean Dissolved CO <sub>2</sub> (ppm)	33,051.7	N/A	2,914.6	N/A
Mean Dissolved Oxygen (mg/L)	1.40	N/A	4.6	N/A
GCC (1st quartile - 3rd quartile)	0.33 - 0.39	N/A	0.34 - 0.37	0.335 - 0.392

Not only were these data limited geographically to Mayberry Farms and West Pond, but the length of recording time was different; five years for Mayberry Farms, and three years for West Pond. Additionally, while the spread of these data were both fairly normal for dissolved oxygen, the spread of these data exhibited somewhat different general trends for dissolved carbon dioxide. In Mayberry Farms, the most frequent CO<sub>2</sub> concentration value recorded was a “zero,” whereas the most frequent CO<sub>2</sub> ppm value in West Pond was closer to 5,000. This may point to differences in the recording device or in environmental conditions surrounding the device. These spreads are shown in Figure 4.4 below.



**Figure 4.4:** Histograms of long-term dissolved oxygen and carbon dioxide data collected at Mayberry and West Pond. Means are shown with a dotted red line. The West Pond CO<sub>2</sub> data is likely indicating an instrumental upper recording limit at around 50,000 ppm (seen in its second bimodal peak). Inversely, the Mayberry Farms CO<sub>2</sub> data likely exhibits a lower instrumental limit (and/or false zeros). I did not exclude these data when averaging across time frames (e.g., days, weeks, months, years).

Mayberry Farms dissolved oxygen data exhibited much larger spread in dissolved oxygen and dissolved carbon dioxide values compared to West Pond, suggesting larger fluctuations at an unknown time scale.

## 4.4 Conducting field experiments and biomass sampling to attribute changes in dissolved oxygen to differences in the presence and intensity of aquatic primary production.

Finally, I completed two to three field visits per each of all four wetlands to collect *in-situ* aquatic primary productivity and biomass data in the form of light/dark bottle experiments combined with lab-based fluorometric analysis of samples collected at every sampling location and processed within 48 hours. I tested for productivity by isolating 300 mL of water with suspended phytoplankton from each sampling site, measuring the difference in dissolved oxygen after an hour of enclosure in environmentally normal conditions (i.e., 10–15 cm below the surface of the water, attached to floating sampling units that delineated sample locations).

### 4.4.1 Light/Dark bottle experiments to measure changes in primary productivity *in-situ*.

I visited all four wetlands within 48 hours of one another, at least twice. For Mayberry Farms, I visited three times. The average results of the light/dark bottle experiments performed at each wetland are presented below to the appropriate significant digit, in Figure 4.5. Each value in the table corresponds to the average change from the beginning of the measurement hour to the end of the measurement hour, with negative values highlighted in red and positive values highlighted in green. Positive values would indicate a net increase in the measured value over the one hour bottle experiment study duration. Negative values indicate a net decrease.

Average West Pond dissolved oxygen values were positive, indicating a net increase in the dissolved oxygen of the 300 mL study mesocosms. This net positive average existed for both light and dark bottles, contrary to what would be expected in an isolated photosynthetic system whereby respiration decreases oxygen concentrations (in dark bottles) and net primary production increases oxygen concentrations (in light bottles). Nevertheless, by using the stoichiometric relationship listed in Methods, I converted these changes in dissolved oxygen to changes in carbon, indicating an approximately 0.03 mg production of carbon compounds per liter of water per hour.



	Change in Dissolved Oxygen (mg/L/h)	Change in Temperature (C/h)	Change in Pressure (mbars/h)	Change in Carbon (mg/L/h)
<b>West Pond</b>	0.1	0.0	0.2	0.03
Dark bottles only	0.1	0.2	0.2	0.03
Light bottles only	0.1	-0.2	0.2	0.03
<b>East Pond</b>	-0.1	-0.5	-38.8	-0.02
Dark bottles only	0.0	-0.5	0.5	-0.01
Light bottles only	-0.1	-0.5	-78.6	-0.04
<b>Mayberry Farms</b>	0.1	-0.2	-0.4	0.03
Dark bottles only	0.3	-0.2	-0.3	0.09
Light bottles only	-0.1	-0.1	-0.4	-0.03
<b>East End</b>	-0.4	-0.8	0.2	-0.12
Dark bottles only	-0.3	-0.8	0.3	-0.10
Light bottles only	-0.5	-0.8	0.2	-0.15

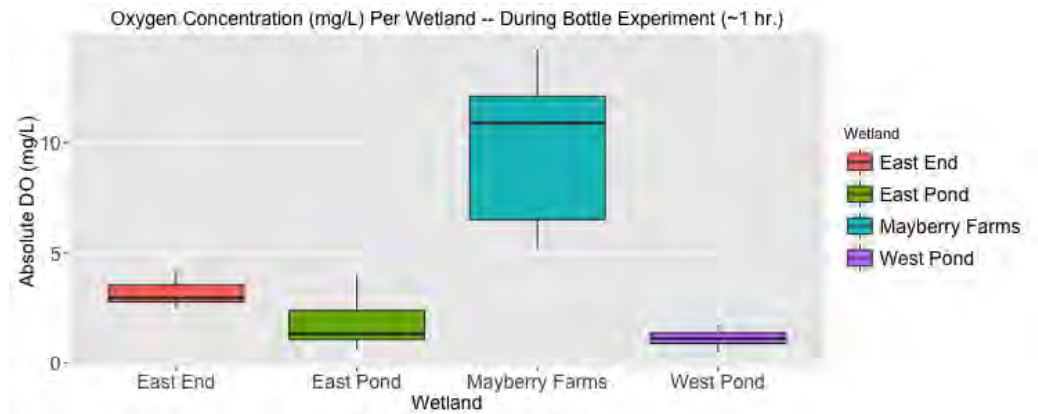
**Figure 4.5:** Averages for all field days shown, across all sample points per wetlands (3 for West Pond, 6 at remaining three wetlands). The line corresponding with the wetland designation is an average of both light and dark bottles.

Average East Pond and East End changes in dissolved oxygen were negative for all bottles, indicating either faulty experimental execution/equipment, or a biochemical process that uses up more oxygen than was created. The magnitude of differences between light and dark bottles was about the same. Because of these negative values, stoichiometric conversion also resulted in a net negative average value for carbon production.

Mayberry Farms exhibited a net increase in dissolved oxygen in dark bottles, and a net decrease in dissolved oxygen in light-exposed bottles – the exact opposite trend as would be expected in an isolated photosynthetic system.

The boxplots in Figures 4.6 and 4.7 below indicate the extent to which dissolved oxygen values varied over the course of the bottle experiments. The

former combines all collected dissolved oxygen values (not differences over time) between wetlands, and the latter compares bottles within wetlands, and among field experiment days. Values above 0 mg/L change in dissolved oxygen indicate net increases in the corresponding bottles; sub-zero represented net decreases.



**Figure 4.6:** Absolute values of dissolved oxygen concentration over the course of all bottle experiments and field days.

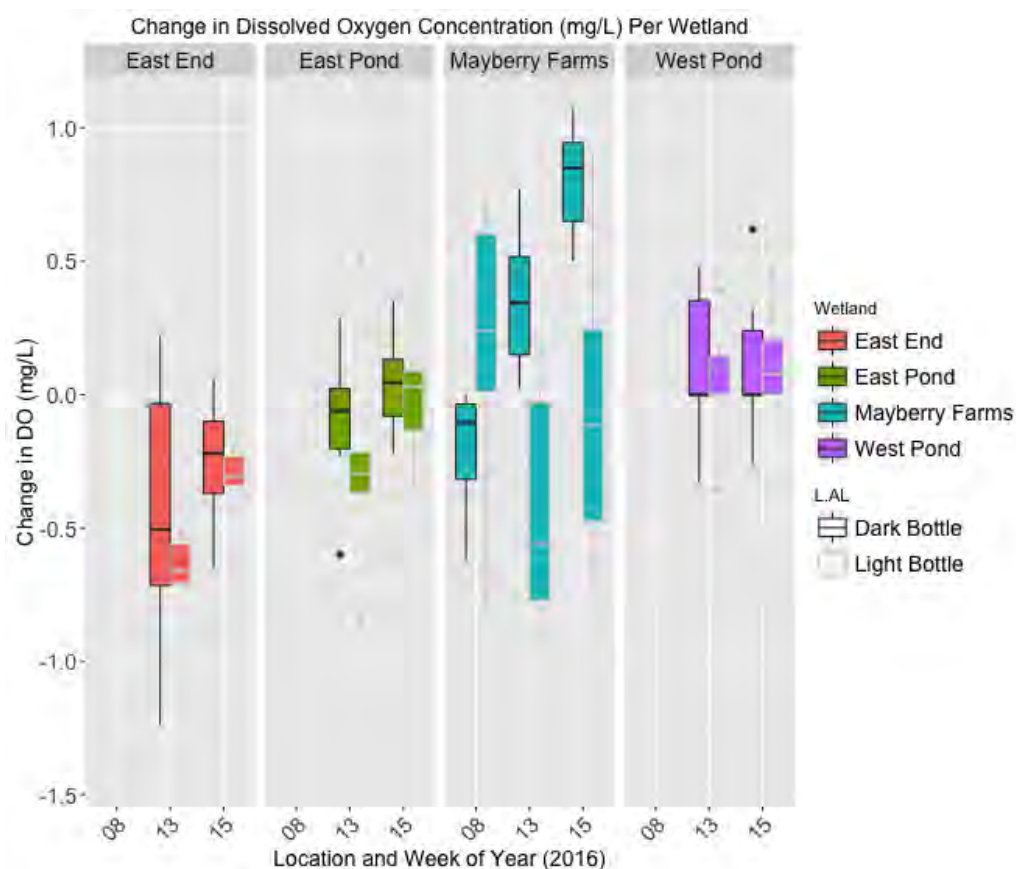
After determining that the individual light vs. dark bottle differences were not biologically distinct (whereby dark bottles had larger respiration than oxygen production, light bottles had larger oxygen production than respiration), I decided to consider only their averages at each wetland for further analysis. To answer my original research question on time having an impact on the amount of aquatic primary productivity that can occur in wetlands, I subsequently considered the bottles and their beginning- and end-of-hour differences together. Given that bottle experiments yielded values of the wrong sign (+/-) for conventional productivity conversions, it would have been arbitrary to claim that dark bottles truly represented respiration and light bottles represented photosynthesis. In many cases, the data suggested otherwise.

Table IV below shows the primary location-specific geographic and productivity-oriented data used in the estimation of primary production rates.

<sup>1</sup>Across light and dark bottles.

<sup>2</sup>Approximating half of day was of the same productivity as bottles' measured hours.

<sup>3</sup>Assuming Miller (2011) range of 1.3 to 3.2 kgC/m<sup>2</sup>/year.



**Figure 4.7:** Boxplot of changes in dissolved oxygen concentration per bottle set, per wetland, per day. Each set of adjacent black-bordered and grey-bordered boxes indicates a single sampling day, with black border representing all sets of dark bottles, and grey border representing all corresponding sets of light bottles for that day. Note that values above zero represent net increases in oxygen in bottles for the sampling hour, and values below zero represent net losses in oxygen per bottle.

Based on the bottle experiments, Table IV demonstrates that, by magnitude, the older the wetland, the smaller the aquatic contribution was to changes in primary productivity. This assumes that the aquatic autotrophs like photosynthetic phytoplankton and suspended periphyton were the only origins of changes in dissolved oxygen in the isolated bottles, which may not be correct according to the reversed trends between light and dark bottles.

TABLE IV: A table detailing the values used in the determination of (1) wetland aquatic primary productivity, and (2) the proportion of average wetland primary productivity that could be attributed to aquatic primary production or other biochemical processes, based on these field experiments.

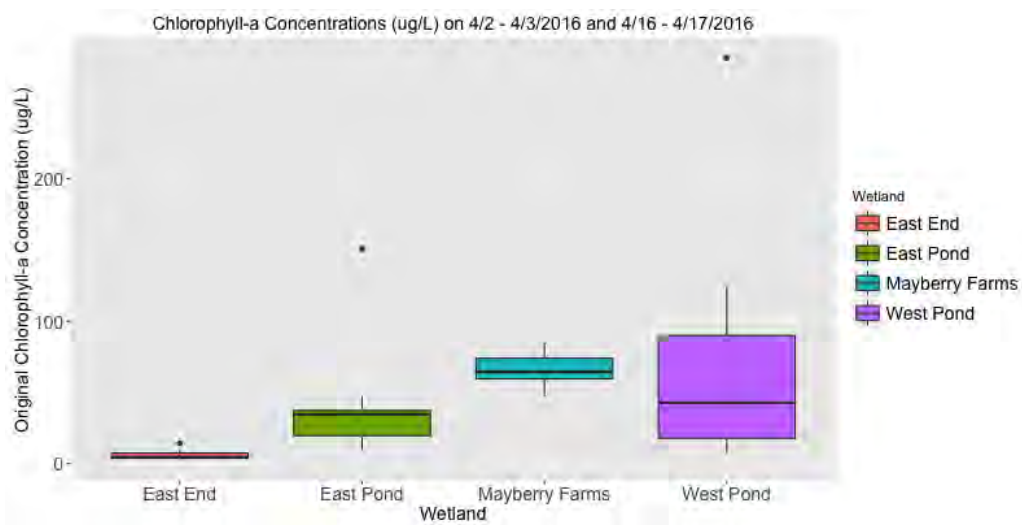
	<i>West Pond</i>	<i>East Pond</i>	<i>Mayberry Farms</i>	<i>East End</i>
Area ( $m^2$ )	50,990.4	47,348.3	1,786,284.0	5,212,355.7
Depth (m)	0.1	0.7	0.5	0.5
Approx. Volume ( $m^3$ )	5,099.0	33,143.8	893,142.0	2,606,177.8
Average Productivity <sup>1</sup> (mgC/L/h)	0.03	-0.02	0.03	-0.12
Productivity (mgC/ $m^3$ /h)	30.2	-22.5	31.2	-123.7
Hourly Production per Wetland (gC/h)	154.2	-744.4	27,842.4	-322,466.4
Yearly Production (kg C/yr) <sup>2</sup>	675.9	-3,262.9	122,033.4	-1,413,370.3
Proportion of emergent productivity <sup>3</sup>	<b>0.4 to 1%</b>	<b>-5.3 to -2.2%</b>	<b>2.1 to 5.3%</b>	<b>-20.9 to -8.5%</b>

Nevertheless, changes in aquatic carbon – whether or not they are biologically driven – appear to represent between one and 20 percent of the wetland carbon transformation rates. The newest wetland (East End) appears to have approximately one order of magnitude greater aquatic carbon transformation rates that the oldest wetland (West Pond).

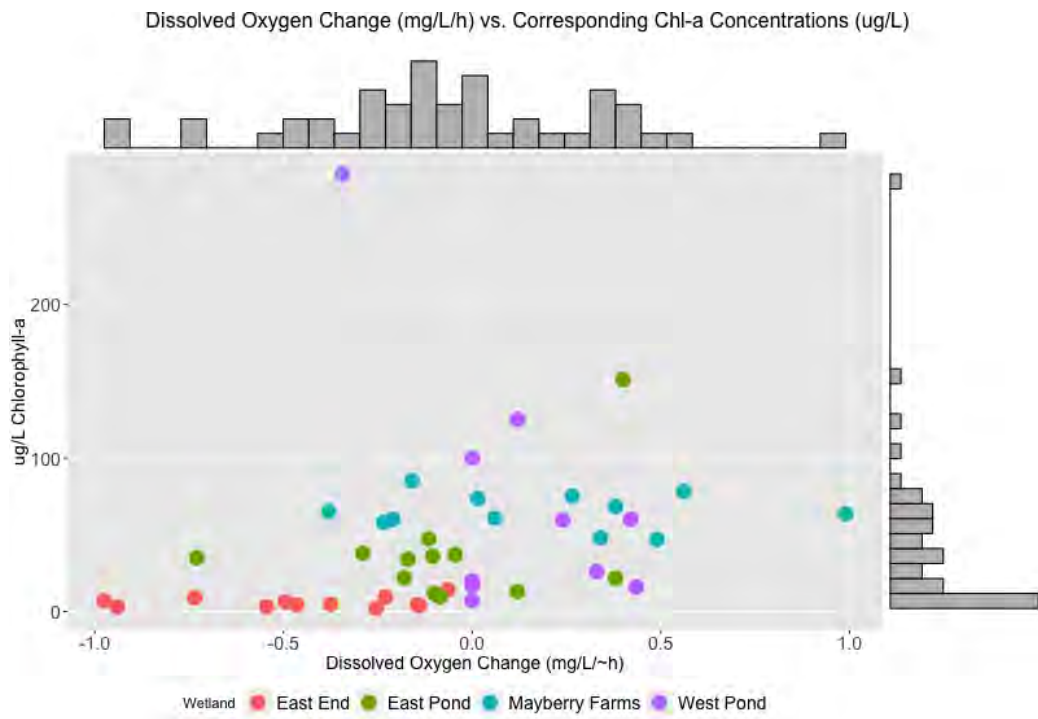
#### 4.4.2 Chlorophyll-*a* fluorometric analysis to estimate phytoplanktonic biomass.

In order to associate changes in dissolved oxygen with biological processes (rather than through the failed light/dark dichotomy), I fluorometrically estimated chlorophyll-*a* in the water by processing six water samples from each wetland, from each field visit. West Pond was the exception, for which I only retrieved five water samples per visit due to a loss in a sampling region. Because this experiment did not isolate functional group differences in primary producers (i.e., productivity in periphyton vs. phytoplankton vs. other photosynthetic organisms in the wetland water), I was limited to optical analysis of water samples for overall chlorophyll-*a* concentration. Because samples were filtered, however, it is likely that chlorophyll concentrations represent those primarily of suspended algae. Figure 4.9 graphs the range of resulting concentrations for each wetland, across all sampling dates.

Based on these data, West Pond had a larger range of chlorophyll-*a* concentrations, but Mayberry Farms had a higher average. Alternately, East End and East Pond had lower averages for chlorophyll-*a*, with not much variation between sampling periods.



**Figure 4.8:** Boxplots of chlorophyll-*a* concentration variation between wetlands across the two sampling events when samples were taken and processed within 48 hours from each wetland.



**Figure 4.9:** Scatter plot of chlorophyll-*a* concentration versus change in dissolved oxygen concentration at same locations. The graph exhibits positive association, suggesting a link between photosynthetic activity and change in dissolved oxygen concentration.

# Chapter 5

## Discussion

By comparing indices of productivity between each of the four wetlands, the data suggest a statistically significant impact of time since restoration on the aquatic productivity of constructed and perennially inundated freshwater marshes. Further, these differences in productivity appear associated with differences in aquatic autotrophic biomass, suggesting that differences in productivity over time may be attributed to changes in the presence of aquatic photosynthesizers like phytoplanktonic algae. While this association would imply that successional development of the wetlands at a landscape-scale influence differences in aquatic productivity, this trend does not appear associated proportionally or sequentially to time-since-restoration. Overall, these findings suggest that while time and successional changes may play a role in the changing contributions of aquatic primary producers to the carbon budgets of constructed wetlands, time is not the only primary driver.

### 5.1 Findings

#### 5.1.1 Physical covariates do not clearly influence changes in dissolved oxygen and carbon dioxide in the system; however, other photosynthesizing organisms like emergent graminoids might.

While past studies have emphasized the importance of hydraulics or water table height on the presence of macrophytes (Miller and Fujii, 2010) and others have emphasized the biogeochemical gradients as a function of dis-

tance from inlet to outlet (He et al., 2015), differences in physical covariate averages in my work do not appear to be first-order drivers for differences in aquatic primary productivity. Additionally, historical constraints challenge assumptions that must be made about the subject site. For instance, previous studies on hydraulic conductivity and gradients noted that water was fed into the wetlands via gravity siphon, and that “Current Delta management practices change the environment in the peat through the course of a year, promoting or inhibiting microbial oxidation through the cyclical wetting and draining of the upper few feet of the peat” (Gamble et al., 2003). Managing site engineers claim that in recent history, there have been no fluctuations in water table height that would expose the peat to the atmosphere (Bryan Brock, in person, 2015). While the composition of microbial communities differs along the inlet-outlet gradient (He et al., 2015; Miller, 2011) the differences in dissolved oxygen change did not appear dependent on location within the wetlands. The low temperature, conductivity, pressure, and baseline dissolved oxygen conditions on site suggest that the sites are very well mixed (perhaps in completely mixed flow reactor conditions). The only gradient not considered in the sampling regime here was the likely microenvironmental gradient to exist as a function of distance from emergent plant communities (Capers and Les, 2005), which often coincides with differences in water depth (Miller and Fujii, 2010). I controlled for distance from emergents in my sample locations for this study, and therefore this gradient should not impact my results.

Miller (2011) noted that between Old West and Old East Ponds, which are shallow ( $\sim 25$  cm) and deep ( $\sim 55$  cm) respectively, higher water surface losses of methane and lower losses of  $CO_2$  occurred in the deeper wetland between 2000 and 2003, following initial wetland development. He attributed the differences in  $CO_2$  efflux to the plant community differences – with the deeper wetland producing less  $CO_2$  because it contained fewer emergents – since in both wetlands it was found that emergent vegetation emitted both  $CH_4$  and  $CO_2$  more than areas of submerged and/or floating vegetation, including algae. At both wetlands, initially significant increases in gas production occurred following construction from 1998 to 1999, with interannual variability occurring thereafter. These findings suggested that indirectly, hydroperiod impacted the emissions of  $CO_2$  and  $CH_4$  via plant communities present, submerged and floating vegetation contributed less to GHG emissions on site than emergents, and that aquatic primary production may co-occur at locations of high methane production. They also suggested



that initially high emissions following construction were short term, and that seasonal plant and microbial activity dictated emissions thereafter.

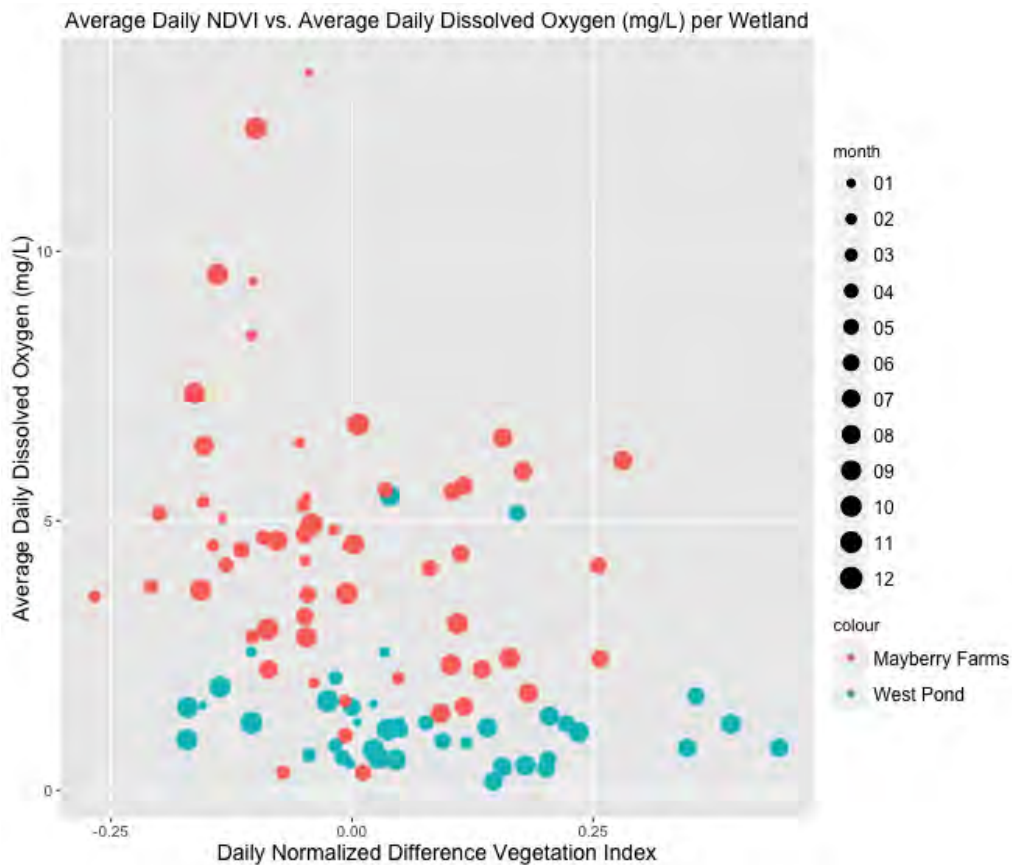
Additionally, while Miller (2011) noted  $CO_2$  uptake into the water column following initial inundation (at Old West and East Ponds), with “plant uptake exceeding respiration,” he also noted that following the first growing season, no direct influxes of  $CO_2$  occurred thereafter (following emergent establishment). Influx via plant moderation did continue, however, and was greatest in emergent stands. Together, with the seasonal peaks shown in the monthly average aquatic  $CO_2$ , these data suggested that the  $CO_2$  inputs in these wetlands were largely from respiration or transport through vegetation, not direct interfacial mass transfer influxes. With East End containing more aquatic vegetation and fewer emergents than West Pond, and the differences between wetlands largely disappearing during times of emergent dormancy, it is likely that respiration from and transport through emergents is the dominant driver of aquatic  $CO_2$  in these systems as well.

Because this study was limited only to the very beginning of the emergent growing season, normally obvious differences along the vegetational gradients may not have been possible. Miller (2011) noted on this subject that areas of emergent vegetation had lower dissolved oxygen, pH, and water and soil temperatures than areas of submerged vegetation, with spatial variability in gas exchange strongly related to plant community. Water dissolved oxygen and pH were significantly correlated to plant-moderated influxes in Old East Pond (only), “reflecting activity of submerged and floating vegetation.” These data suggested that submerged and floating vegetation plays a critical role in the influx of  $CO_2$  into the system, but I was not able to capture these differences in my controlled study.

### **5.1.2 Changes in aquatic primary productivity could not be adequately sensed via highresolution satellite imagery.**

Structural changes within broader wetland plant communities, or land use, could be sensed with MODIS imagery at its highest spatial resolution, but remotely sensed data via Landsat 7 could not fill the data gaps that existed in directly measured dissolved oxygen or dissolved carbon dioxide data between wetlands.

While the magnitude of changes in the water columns of Mayberry Farms

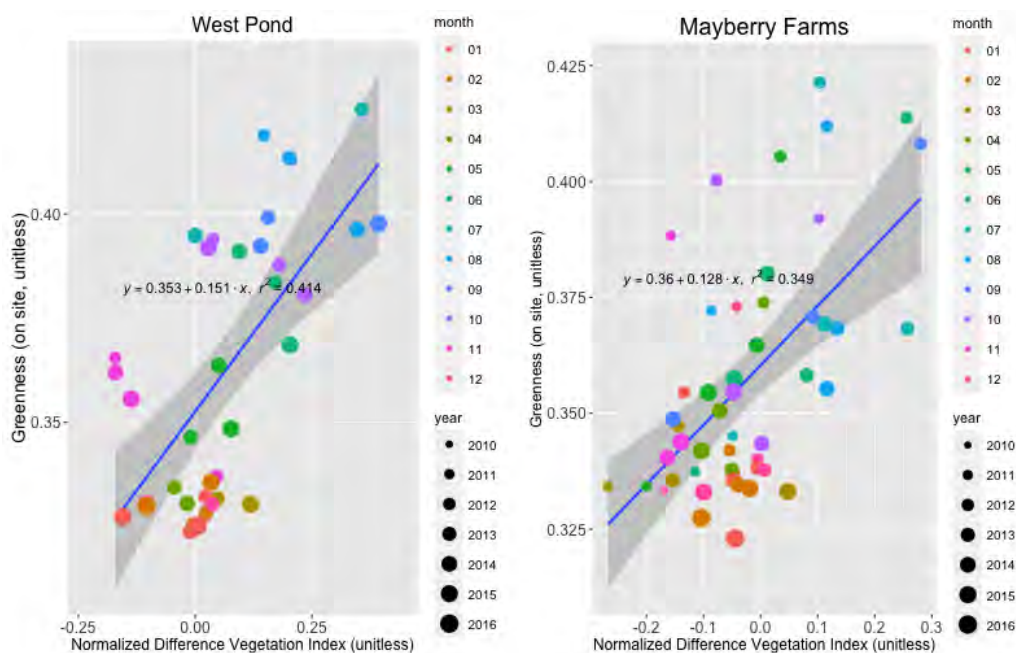


**Figure 5.1:** A scatter plot of average daily dissolved oxygen (mg/L) and corresponding NDVI values for that day. An adequate regression could not be performed on average daily NDVI data and their corresponding average changes in dissolved oxygen. Red dots are associated with values at Mayberry Farms; blue with West Pond.

and West Pond could not be correlated with changes in NDVI (See Figure 5.1), these data do suggest that emergents are not the only driver of inputs of oxygen into the aquatic system. Schile et al. (2013) used higher resolution and better-classified imagery to successfully sense areal changes in emergents, which would be helpful in substantiating this disjunction between emergent season growth and differences in aquatic oxygen and carbon dioxide.

Furthermore, these changes in the water column could not even be well-correlated (see Figure 5.2) with the on-the-ground greenness index (GCC),

indicating either that the greenness index is a poor predictor for the entire wetland, or (more likely), that the resolution of the satellite imagery (500 m) considered here was too low or the emergent plant communities honestly make small contributions to the greater aquatic system outside their immediate periphery.



**Figure 5.2:** Attempting to correlate average daily greenness index (GCC) with corresponding NDVI values for Mayberry Farms and West Pond. A linear regression correlation coefficient of 0.349 and 0.414, respectively, indicate an adequate but imperfect fit. This suggests NDVI from Landsat should not ultimately be used as a universal proxy for GCC or sensing emergent growth. Standard error is shown. Dot color corresponds with month in the year.

The majority of the subject site wetland surface area cover (post-construction year, which differs per wetland) is emergents (Miller, 2011), so remotely sensing measures of productivity can be considered as average productivity of the systems, albeit constrained by the realities of light transmission in dense vegetation, leaf litter, and inundation (Schile et al., 2013). About 50% of total solar energy available (300-3,000 nm) is absorbed by leaves (Taiz, 2002), and only approximately 4-7% of this photosynthetically active radiation is converted by the subject sites dominant emergents Typha and Phragmites

to photosynthate (Mitsch and Gosselink, 2015). However, among species, amount of cumulative biomass varies (Byrd et al., 2014).

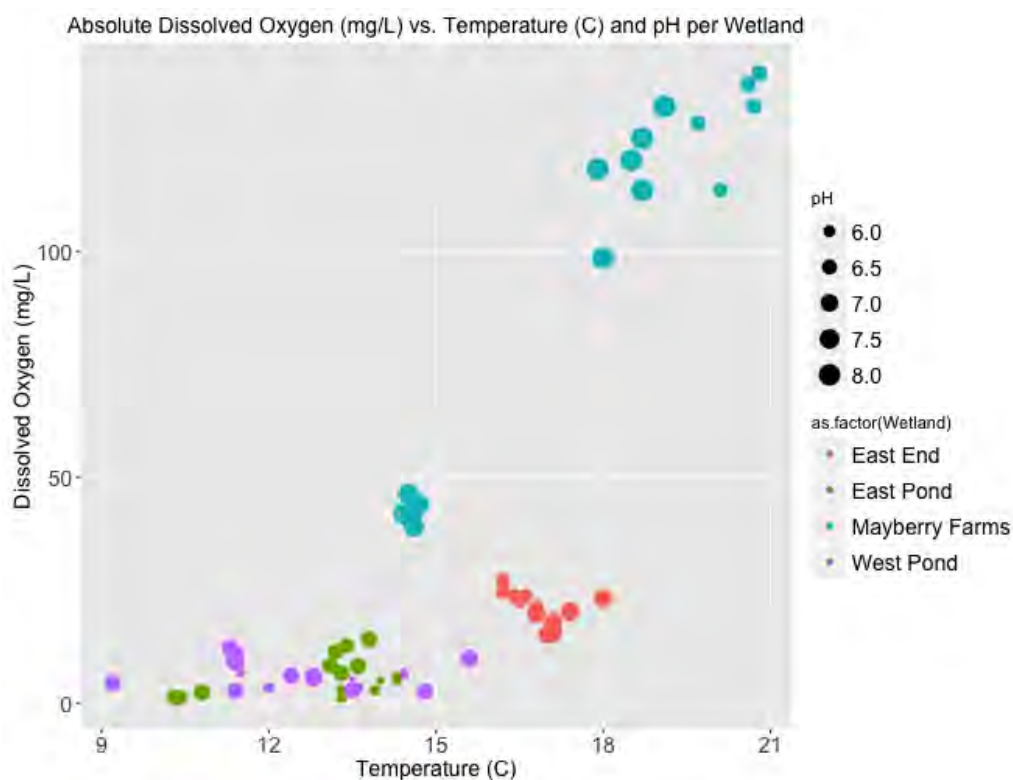
The previously bimodal nature of each MODIS-based NDVI graph indicates that the data analyzed here may extrapolate from data also collected from adjacent agricultural land – which is often known to have two annual peaks in remotely sensed productivity (Miller, 2011). Nevertheless, year of shift from bimodal to unimodal appears to coincide with year of conversion to wetland for each of the identified wetland sites. These data suggest that, following conversion from agriculture to crop, overall productivity of the wetland system shifts from bimodal to unimodal yearly pattern, with greater productivity happening later in the year. If so, this late-summer productivity would not have been sensed in this study in my field work.

Similarly, observations made by (Byrd et al., 2014) suggest that aquatic vegetation occurs early in wetland development, and in cumulative patches rather than evenly throughout the site. This granularity would not have been sensed in my project, either. They found that West Pond and East Ponds were characterized by plots with lower amounts of biomass compared to Mayberry (at Sherman Island), and of emergent patches of a variety of sizes (ranging from 10  $m^2$  to 1000  $m^2$ ). They further found that most of their plots (65%) did not contain any floating aquatic vegetation, with the exception of the 7% that had floating aquatic vegetation comprise over 50% of the plot area. This coincided with hydrologic differences between wetlands, with the Old West Pond containing virtually no open water areas, and the deeper Old East Pond – at the time of survey – having three ponds over 1000  $m^2$  in size, and additional smaller areas of open water, “typically covered by floating aquatic vegetation” such as *Lemna spp.* and *Azolla spp.* These findings suggest that dominant factors in the persistence of open water areas appropriate for aquatic photosynthesis are age and depth, whereby older and shallower wetlands have few if not any open water areas when perennially inundated. Since I generalized a point-water measurement to a remotely sensed image of the entire site, resolution loss may have therefore confounded and lowered correlation coefficients.

### 5.1.3 Aquatic primary productivities differ between wetlands, likely due to differences in aquatic autotrophic biomass.

There were significant differences in oxygen production between wetlands during my field site visits and subsequent light/dark bottle experiments, suggesting that time since restoration plays a role, albeit an uneven one, in the contribution of aquatic primary producers to freshwater marsh carbon budgets.

In order to link the differences dissolved oxygen (both absolute, and production-based) to biological dynamics, I first examined absolute (i.e., non-differenced) dissolved oxygen values versus temperature, and by pH. Figure 5.3 shows these relationships below.



**Figure 5.3:** Dissolved oxygen and corresponding temperatures, wetlands, and pH values at time of measure during field visits.

These data suggested that with increasing temperature is increasing dissolved oxygen concentration. Because lower temperatures allow for greater storage of gas, the positive correlation between temperature and dissolved oxygen here indicates a possible relationship between increasing biological activity (with increasing temperature) due to increased metabolic reactions and subsequent increases in oxygen. What these data do not show, however, are these values parsed by proximity to different plant functional groups.

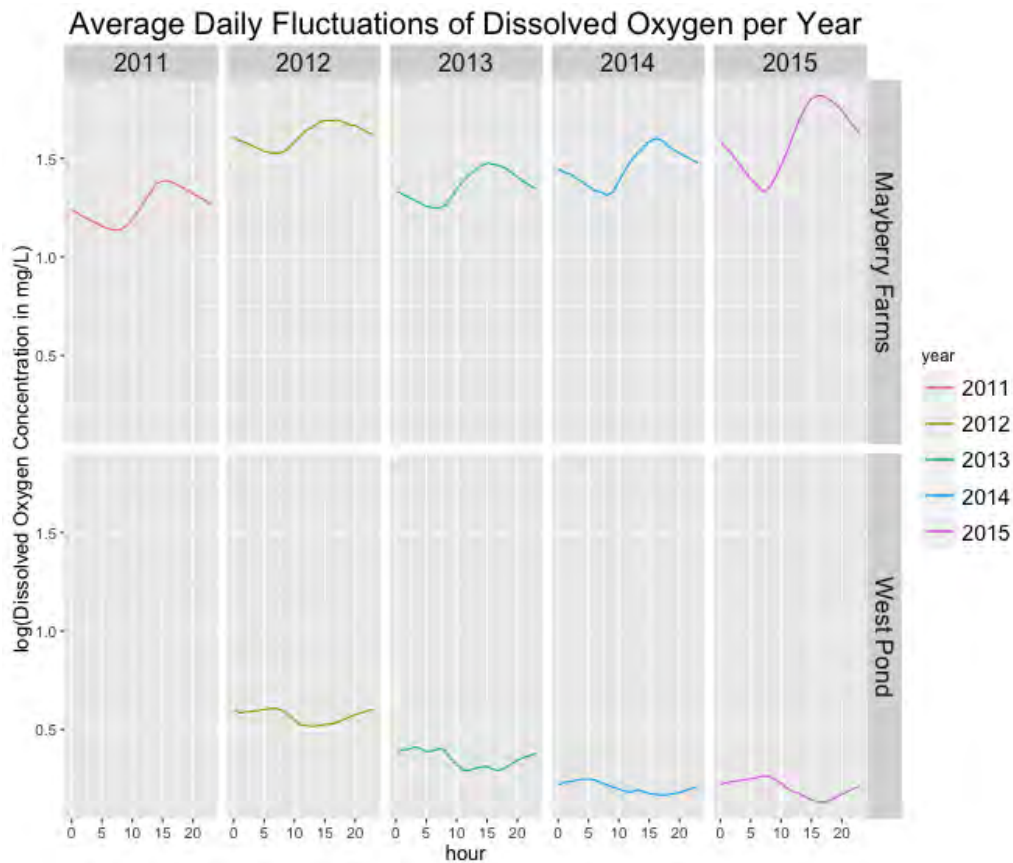
Furthermore, these data show that newer wetlands – Mayberry and East End – are both higher in temperature and in corresponding dissolved oxygen. This suggests that newer wetlands are characterized by the same possible biological activity that leads to higher aquatic dissolved oxygen values.

Next, I examined average daily fluctuations of dissolved oxygen for each hour of the day, differentiated by year.

The daily fluctuations of dissolved oxygen in Mayberry follow typical photosynthetic patterns, with lows in early morning and peaks at late afternoon. West Pond instead has higher peaks earlier in the day for all years considered here, suggesting an alternative controller for changes in dissolved oxygen that that of Mayberry.

The overall trend in Figure 5.4 is the stagnating or lowering daily fluctuations of dissolved oxygen in the oldest wetland (West Pond) and increasing daily fluctuations and daily averages in Mayberry Farms, the newer wetland. This is substantiated by hourly regressions in Figure 5.5, which show high hysteric movement between low and high concentrations of dissolved oxygen throughout the day, with larger fluctuations with each following year in Mayberry.

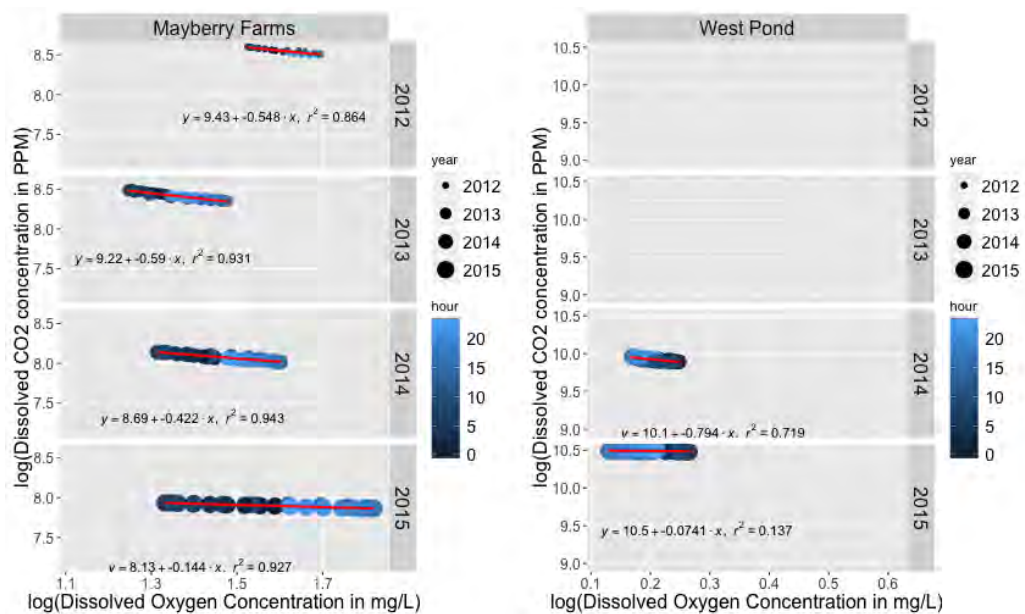
These data suggest that yearly average dissolved oxygen is staying stagnant in the older wetland, and increasing in the newer wetland for a given  $CO_2$  input. Correlation coefficients for the fit linear regressions range from 0.864 to 0.943 in Mayberry Farms, and between 0.137 and 0.719 in West Pond, indicating a very good fit more often than not. The linear regressions do not necessarily indicate linear trends, but rather sinusoidal returns to lows or highs at the same times of day across days (i.e., a flattened version of Figure 5.5).



**Figure 5.4:** Log(Average Hourly Dissolved Oxygen Concentration), by Year. Data from 2016 are preliminary, and do not represent the same 365-day average of the previous four to five years, so are not presented here.

#### 5.1.4 Productivity and chlorophyll-*a* concentration appear linked.

Finally, in order to determine if the proportional differences in productivity noted in Table IV could be attributed to differences in the presence of aquatic autotrophs, rather than other biogeochemical processes or mechanical mixing, I regressed all average chlorophyll-*a* values on average dissolved oxygen values for corresponding wetlands. I then completed two one-way Analyses of Variance (ANOVAs) to determine if the differences between dissolved oxygen means were real, and based separately on (1) the treatment



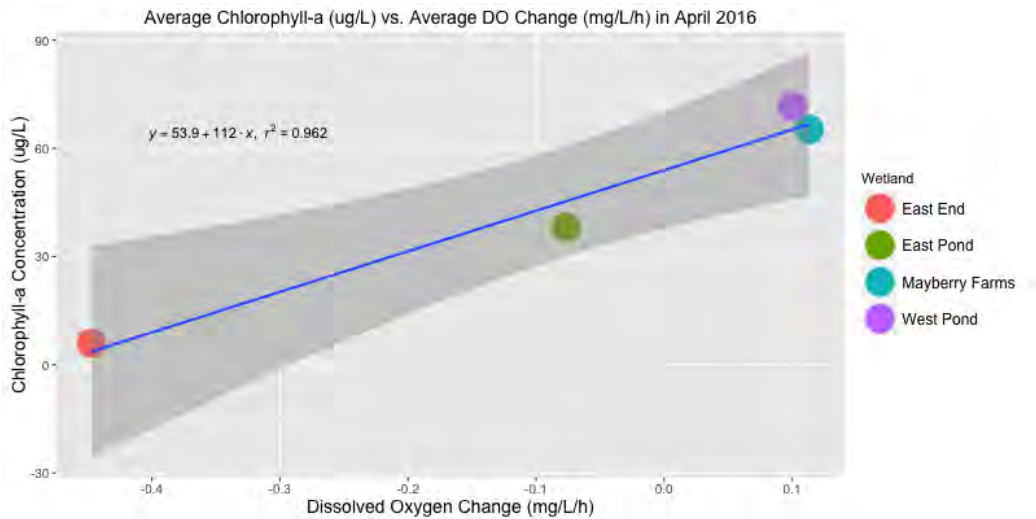
**Figure 5.5:** Average Hourly log(Dissolved Oxygen) by Year and Wetland. Note that lighter blue indicates later times in the day. Longer periods represent larger daily fluctuations. 2016 values are higher on average than other years because the averages only represent wintertime fluctuations; for this reason, they are excluded.

of time via wetland designation, and (2) differences between light and dark bottles. Up until this point, I deemed (2) as insignificant for purposes of simplicity after achieving unconventional bottle experiment results.

The result of the chlorophyll-*a*-dissolved oxygen change regression (seen in Figure 5.6) was a correlation coefficient (R-squared) of 0.962. Chlorophyll-*a* averages encompassed two field visits, and productivity averages encompassed two visits for all wetlands but Mayberry, for which it encompassed three visits. This result suggests a biological driver for dissolved oxygen change. However, this does not necessarily correlate with time since restoration, as the oldest wetland (Mayberry) had high positive chlorophyll-*a* and dissolved oxygen change values, contrary to my original hypothesis that older wetlands would have much less of both compared to their younger counterparts. Additionally, the strength of this correlation is reduced by considering only four wetlands.

To then test the significance of differences between wetlands, I employed



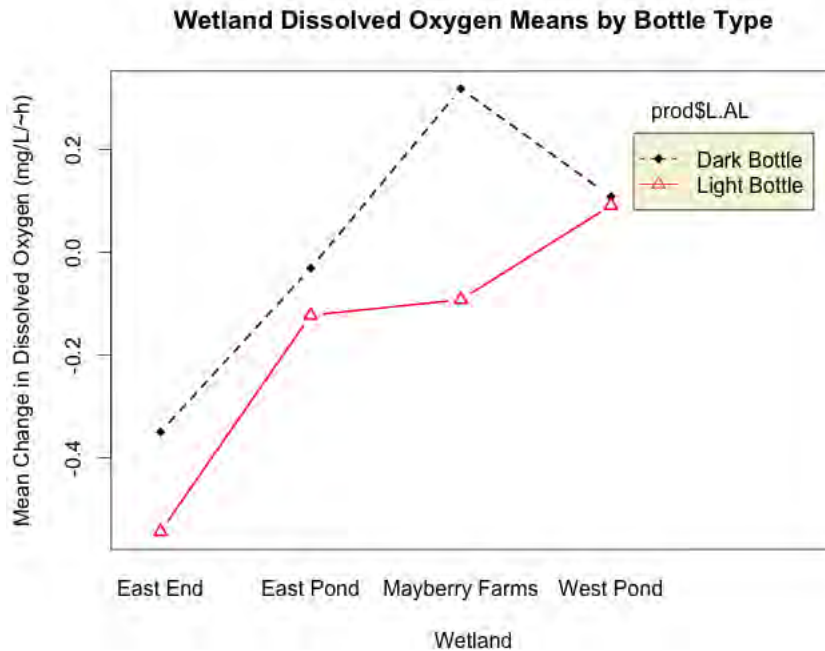


**Figure 5.6:** Average chlorophyll-*a* concentrations ( $\mu\text{g/L}$ ) vs. their corresponding average dissolved oxygen changes ( $\text{mg/L/hr}$ ) at each wetland. Note that each point represents the average of approximately twelve to eighteen water samples and six to twelve productivity measurements (with West Pond having the lower number for each).

one-way ANOVA tests (completing one for bottle-type as well, to confirm or deny their similarity). The ANOVA tests indicated treatment interactions with statistically significantly different means at the  $p < 0.01$  level for both wetlands [ $F(3, 104) = 10.003$ ,  $p = 7.535\text{e-}06$ ] and bottles [ $F(1,106) = 5.3$ ,  $p = 0.02328$ ]. In other words, both the wetland identity and bottle-type had a statistically significant effect on average change in dissolved oxygen in bottles. Dissolved oxygen change means are plotted below in Figure 5.7.

Because only two visits were conducted for all but one wetland, I determined it was not necessary to treat individual sampling events (days) as repeated measures in a “repeated measures ANOVA,” whereby the conditions in each preceding sampling period are dependent on the conditions in the period prior. Sampling periods were discrete but not far apart seasonally. Therefore, I considered sampling periods together as a single measurement in a one-way ANOVA.

Outside of ANOVA tests, I ran a series of diagnostic statistical operations on my light/dark bottle experiment data using functions provided by R or by open source packages available to R users through Comprehensive

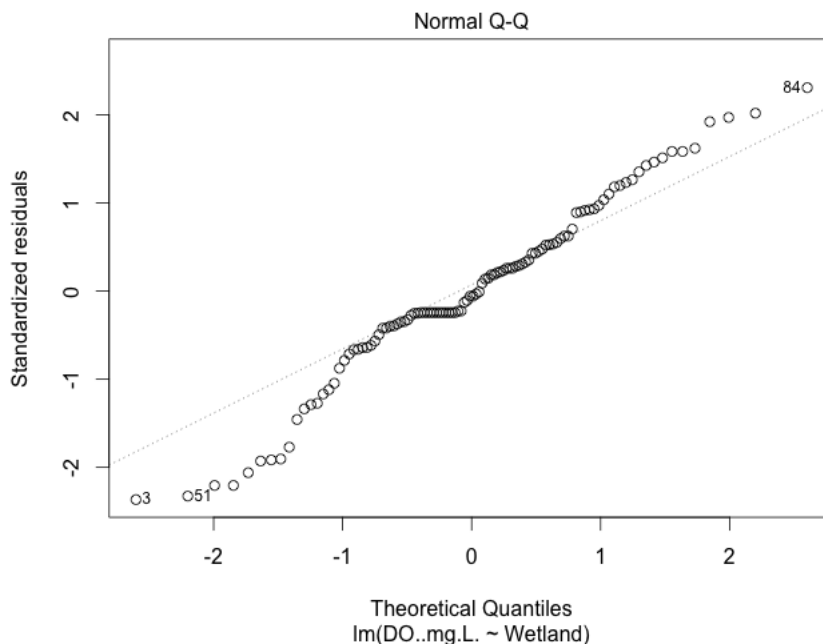


**Figure 5.7:** An interaction plot that shows the means for change in dissolved oxygen concentration, by bottle type and wetland. I tested the significance of these differences using one-way ANOVAs.

R Archive Network (CRAN). I focused on the individual light/dark bottle results for these diagnostic tests, not distinguishing between light and dark bottles. I confirmed these basic statistical assumptions, adding validity to my ANOVAs:

The ANOVA assumption for this test that dissolved oxygen change data are distributed normally was confirmed by the Shapiro-Wilk Test, which yielded a p-value of 0.6017, indicating a high probability versus a 0.05 lower bound that the sample was extracted from a population of data that were normally distributed. I also modeled Q-Q Plots of real versus theoretical residual quantiles for fitted linear models to confirm normality visually (see Figure 5.8 below).

I also tested homoscedasticity (i.e., homogeneity of variance) via the Levene test. This test indicated that the variances were statistically equal ( $F = 6.8106$ ,  $p = 0.0003$ ). All normality tests indicated that the assumptions

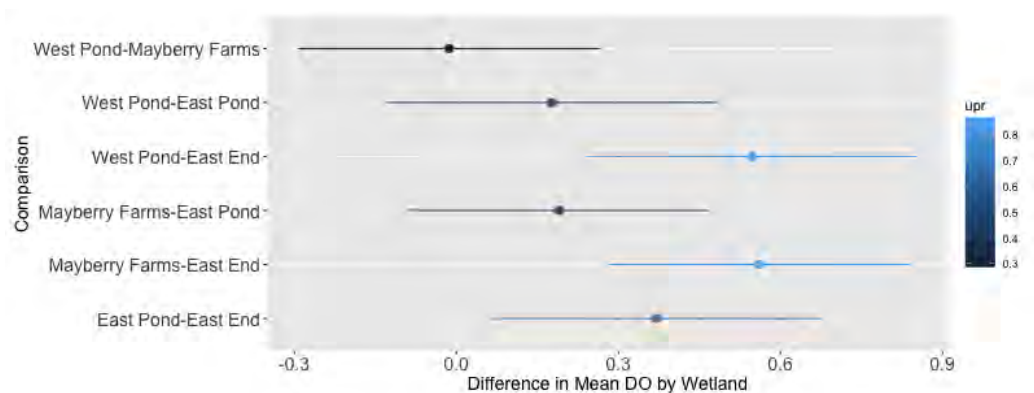


**Figure 5.8:** Quantile-Quantile (Q-Q) Plot indicating fairly "normal" distribution of residuals for a fitted linear model of dissolved oxygen change regressed on wetland identity. This residual quantile distribution closely matches what would be expected in residuals of a normal distribution.

of normality and homoscedasticity, e.g., for ANOVA tests, were reasonable ones.

Finally, I completed a Tukey's Honestly Significant Difference (HSD) test to determine the individual magnitudes of difference between wetland means, testing my hypothesis that older wetlands would on average have lower productivities than newer wetlands (see Figure 5.9 for results).

Post-hoc comparisons using Tukey's HSD test indicated that the mean dissolved oxygen change values between West Pond and East End (adjusted  $p < 0.0001$ ), Mayberry Farms and East End (adj.  $p < 0.0001$ ), and East Pond and East End (adj.  $p < 0.01$ ) were significantly different from one another. The other wetland combinations did not yield significantly different mean DO change values (adj.  $p \gg 0.01$ ). These results indicate significant differences between the older and newer wetlands means, substantiating my



**Figure 5.9:** Tukey’s Honestly Significant Difference (HSD) Test Output

primary hypothesis on the effect of time on aquatic primary productivity.

Note that while the assumption of equal sample sizes is technically incorrect – Mayberry Farms had more samples due to one extra day of sampling – the Tukey HSD test source noted its applicability in testing. It said that the “function incorporates an adjustment for sample size that produces sensible intervals for mildly unbalanced designs.”

I tested the effect size of each independent variable (wetland identity and bottle-type) using an Omega-squared test. The result of this test indicated a 3.8% strength of association with bottles, and 20% strength of association with wetland identity. In other words, wetland age (via its identity in the chronosequence) appeared to have a strong association with change in dissolved oxygen in bottle experiments.

## 5.2 Limitations

I could only compare long-term dissolved oxygen and dissolved carbon dioxide measurements between two wetlands: Mayberry Farms and Old West Pond. This meant that while I could make assumptions about time being the primary treatment, I could not say with confidence that statistical differences could be attributed to differences in time-since-restoration. It is also likely that the time range (~ 1 hr.) of measurement does not produce a significant productivity signal.

In addition, there are greater biological characteristics of the study system to take into consideration in determining primary drivers of productivity,

namely: herbivory by invertebrates and fish; the chemical effects to the water: of senescence, anoxia, and peat accretion; and competitive strategies among photosynthesizers. Others include differential flood tolerance that leads to zonation, and differential germination patterns that impact competitive strategies and recruitment (Mitsch and Gosselink, 2015).

### 5.3 Future Directions

This research suggests that biological changes in carbon in constructed wetland water represent a measureable and a perhaps significant portion of expected wetland primary productivity with respects to the reported productivity of emergents. However, it does not delineate the functional or trophic groups, or more specifically, the taxa that contribute to these changes. Future research should involve productivity measurements that are capable of parsing these differences in order to elucidate food web interactions and specific flows of energy directly. Identifying carbon sources and sinks would contribute to a higher-resolution understanding of the impact of physical configuration (e.g., shallow versus deep pools, spatial contiguity of open water regions) on the creation and liberation of organic compounds like methane and sugar. Furthermore, the presence or absence of certain taxa would indicate the level of ecological complexity of the systems through time, and subsequently the manifested ecosystem services.

Additionally, an alternative method of primary productivity measurement should be employed in future research. The light/dark bottle experiments, while inexpensive and rapid, introduce measurement error. The method ignores the degree to which photorespiration occurs (reducing the amount of oxygen measured and therefore carbon accounted for). It also assumes that single-point measurements via optical dissolved oxygen probe are representative for the region being sampled, and that oxygen change can solely be attributed to biological chemistry. In fact, the failure of average light bottles to express positive oxygen change, and average dark bottles to express negative oxygen change, suggests that alternate chemistry is taking place but is not elucidated here.

## 5.4 Broader Implications/Conclusions

Basic visual observation indicated that aquatic communities are vibrant in the newer wetlands (East End, Mayberry Farms). Invertebrates in biofilms, like ephemeroptera and trichoptera, appeared active and abundant. Smelt juveniles were identified during sunny days. Otters were observed several times in Mayberry Farms and East End. These observations suggested that open water regions allowed for more diverse communities to proliferate.

With regard to primary production, oxygen baselines and change appears strongly linked to the photosynthetic pigment, chlorophyll-*a*. This suggests that a large process should be aquatic-autotrophic, and surprisingly, not necessarily a function of percent emergent canopy cover (as West Pond had the highest concentrations of chlorophyll-*a*). Additionally, emergent plants, while influential, do not appear to be the only driver of changes of oxygen or carbon dioxide in the system. This was suggested by the changes in dissolved oxygen that occurred in the isolated mesocosms of glass BOD bottles for light/dark bottle manipulations. Together, time and emergent development do not appear to be the only/primary drivers of aquatic primary productivity.

While direct productivity does not appear sequentially associated with wetland development, daily fluctuations indicate that for a given  $CO_2$  concentration and by itself, the older wetland has less dissolved oxygen, and the newer one has more dissolved oxygen over time. Furthermore, fluctuations appear to grow in magnitude in the newer wetland (Mayberry). Based on fluctuations alone, newer wetlands appear more productive in the water, and older wetlands appear less productive.

Given that in other marsh types (e.g., tidal salt), much of algal production occurs when overstory plants are dormant (Mitsch and Gosselink, 2015), and above-ground biomass (mainly emergents, at the subject site) experience nearly complete winter die-off (Miller and Fujii, 2010), a wintertime/early springtime study was ideal for examining the contribution of aquatic photosynthesis to wetland productivity. This seasonal dependency – although apparent in atmospheric fluxes of carbon dioxide and methane and mirrored by photosynthetically active radiation (Personal communication: Baldocchi), does not appear at first examination to be inversely mirrored by aquatic productivity. While one would expect an artificially high trough in  $CO_2$  influx to the system at eddy covariance towers, due to the potential/anticipated buffering of productivity in-water, this does not appear to be a significant difference (Personal communication: Dr. Patty Oikawa). This seemingly in-

significant contribution is derived from either limitations of instrumentation – with towers measuring fluxes from several meters off the water surface or emergents insulating atmospheric gases above aquatic regions – or from truly insignificant contributions from in-water. This distinction must be resolved through transect-based field work.

This work will provide an enhanced understanding of the cycling of energy and carbon in these disparate systems, thereby elucidating a basis for understanding food web interactions and temporal trends given specific environmental changes. Knowing how energy (via carbon storage) is cycled in tandem with constrained emergent growth will allow for greater maximization of carbon sequestration in these systems, and perhaps the concomitant and active exploration of greater ecosystem services like habitat for native taxa and water treatment. Food web ecology necessitates an understanding of food web interactions, and this necessitates understanding the abundances and productivities of functional groups present. As submerged and floating aquatic vegetation distribution and productivity has not been yet explicitly studied in the context of this system, all productivity-oriented measurements in the water were novel.

# Chapter 6

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