Assessing the potential for ecosystem enhancement in Lake Mattamuskeet

Final Progress Report to the North Carolina Wildlife Resources Commission

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Tasks

1. Assess the current status of the lake through seasonal water column sampling throughout the lake and canals
2. Determine source of light attenuation (e.g. resuspended sediment, phytoplankton, CDOM) in the lake during periods with and without resuspension
3. Conduct spatial analysis of physical and chemical factors throughout the lake
4. Conduct targeted transplant experiments by moving macrophytes from plots in the east basin to the west basin

Note:

In the period between the submission of this proposal and the initiation of the research, the east basin of Lake Mattamuskeet became dominated by algae and the rooted plants were lost. Thus all restoration experiments used transplants into areas without existing plants

Findings:

1. Light attenuation in the lake creates an environment with irradiance levels below the conventional thresholds for rooted plant growth
2. Total suspended solids are the best predictor of light attenuation in the lake
3. Algae in the lake are co-limited by both nitrogen and phosphorus
4. Denitrification does not play a major role in the lake budget as a removal term for dissolved inorganic nitrogen
5. Wild celery plantings in the east and west basins persisted for 7 months when protected from grazing, but no plantings persisted for a year

Background and rationale

The collapse of macrophyte communities and proliferation of phytoplankton in shallow lakes is well documented and a worldwide phenomenon (Berger & Vaate, 1983; Moss et al., 1986; Clugston, 1963; Engel & Nichols, 1994; Blindow, 1992, Wu et al., 2007). This shift in trophic structure is most often observed in shallow instead of deep lakes due to different impacts of abiotic factors, including light attenuation, and mixing regimes. Benthic vegetation, commonly referred to as submerged aquatic vegetation (SAV), is relegated to littoral areas in deep lakes due to light attenuation. In shallow lakes, there is relatively greater area for macrophyte growth, but actual presence is determined by interacting biotic and abiotic environmental factors (Scheffer et al., 1993; Janssen et al., 2014).

The suite of environmental variables that influence lake vegetation are those that directly or indirectly affect light attenuation. These include sediment and nutrient load, water depth, wave action, algal biomass, zooplankton and fish communities, and the presence of SAV. Once established, SAV creates self-sustaining positive feedbacks by preventing sediment resuspension, providing refuge for phytoplankton grazers (i.e. zooplankton), and reducing nutrients in the water column that would otherwise be available for algal growth (Horpilla & Nurminen, 2003, Timms & Moss, 1984; Van Donk et al., 1989; Scheffer et al., 1993).

Macrophytes die off and phytoplankton proliferate in lakes with conducive water column nutrient levels, and where light cannot penetrate to the sediment. When this happens, the positive feedbacks from benthic vegetation are lost, resulting in cascading changes that, without external forcing, prevent a shift back to a clear water state (Scheffer et al., 1993, 2001).

After transitioning to phytoplankton dominance, returning nutrient levels to pre-shift conditions may not necessarily restore a clear water state (Scheffer et al., 1993). Both vegetative states can persist under elevated nutrient conditions until a critical threshold, dependent on the environmental factors listed above, is passed (Scheffer, 1993). This complex relationship between nutrients and vegetative state has led to many studies investigating the fate of nutrients, specifically nitrogen and phosphorus, in between SAV and bare sediment shallow lakes and other aquatic ecosystems.

Previous studies have shown that SAV can use water column nutrients for growth, prevent internal loading by decreasing sediment resuspension, and may enhance denitrification (DNF) by creating oxic/anoxic interfaces at root surfaces within the sediment, as well as supplying nitrate and organic matter to denitrifying bacteria (Christensen & Sorensen, 1986; Forshay & Dodson, 2011; Caffery & Kemp, 1990; Reddy et al., 1989). There have, however, been several studies with conflicting findings; Holmroos et al. (2015) found DNF rates within a shallow Finnish lake were lower in areas with SAV compared to areas with floating plants or no vegetation, due possibly to competition for DIN between SAV and sediment bacteria. Holmroos et al. (2012) suggested that resuspension, as results from SAV loss, increases rates of sedimentary DNF by promoting conditions favorable for nitrification. While most studies have documented an overall nutrient limiting effect of SAV, these studies are generally conducted as comparisons between vegetated and unvegetated areas within the riverine, estuarine, or lake environment investigated, and therefore do not capture the shift in DNF rates and nutrient fluxes that results from a transition between SAV and phytoplankton dominance.

Study Site

A rapid regime shift, from macrophyte to phytoplankton dominance, recently occurred in the east basin of Lake Mattamuskeet, the largest natural lake in North Carolina. This lake, located in the northeastern coastal plain, has an area of ~165 km^2 and average depth of approximately one meter (Moorman et al., 2017). The watershed is comparatively small, covering just 120 km^2. The land use within the watershed is dominated by agriculture, which accounts for roughly 45 km^2. This area includes agricultural impoundments, areas of cropland that are seasonally flooded to create waterfowl habitat. The second largest land use is building development, which covers only 6 km^2. The area of undeveloped watershed is comprised mainly of impounded and natural wetlands (61 km^2), followed by forest (5.2 km^2), disconnected open water areas (2.5 km^2), and shrubs and grass (1.5 km^2) (Moorman et al., 2017). Impounded wetland and agricultural areas are present on refuge and privately-owned land within the watershed. These areas have water control structures such as pumps and flash board risers, allowing water level management by the US Fish and Wildlife Service (USFWS) or private citizens.

The lake is divided into two basins, east (~105 km^2) and west (~60 km^2), by US highway

94 (Cahoon, 1953). The basins of the lake are connected hydrologically by culverts passing beneath the highway. Despite this, the division of the east and west basins by road construction in the 1940's resulted in a divergence of vegetative states (Waters, 2010). Until recently, the east basin was clear and macrophyte dominated, and the west basin was turbid and phytoplankton dominated (Waters, 2010). Between 2013 and 2014 the vegetation in the east basin almost completely disappeared, and algal biomass increased. During this time an area of roughly 70 km^2 in the east basin that had supported dense SAV coverage transitioned to sparse or very sparse coverage (Moorman et al., 2017). Prior to this collapse, the dominant species within the vegetative community was *Vallisneria americana*. Other species present included *Najas gudalupensis*, *Potamogeton perfoliatus,* and *Chara* sp. (Sponberg & Lodge, 2005; Waters, 2010).

Methods

Monitoring

Monitoring stations included 4 sites in each basin and an additional ditch (West) and canal (East) sampled six times from April-November 2016 (Fig 1).

Figure 1. Map of monitoring stations sampled in 2016

Temperature, conductivity, salinity, dissolved oxygen (DO), and turbidity were measured *in situ* in surface and bottom water using a YSI 6600EDS-S water quality sonde. Grab samples from surface water were collected at each site and, upon return to IMS, were immediately filtered on GF/F filters.

Filters were analyzed for chl α (Welschmeyer, 1994), and filtrate was analyzed for nutrients. Nitrate/nitrite NO_x^-N (detection limit 0.05 µM), ammonium NH^{+} (0.24 µM), orthophosphate PO_4^{3-} (0.02 µM), and total dissolved nitrogen TN (0.75 µM) concentrations were measured on a Lachat Quick-Chem 8000 Nutrient Auto-Analyzer. Dissolved inorganic nitrogen species (DIN; NH_4^+, NO_x^-N) were subtracted from TN values to calculate dissolved organic nitrogen (DON). Absorbance of chromophoric dissolved organic material (CDOM) was measured from optical scans of filtered samples on a Shimadzu UVmini-1240 spectrophotometer. Absorbance values between 250 and 700 nm for each sample were corrected for background noise by subtracting the average absorbance between the 700 and 800 nm wavelengths (Green and Blough, 1994). Napierian absorption coefficients were calculated using corrected absorbance data following the equation, $a_\lambda = 2.303 \times A_\lambda / l$, where a_λ is the Napierian absorption coefficient, A_λ is the measured absorbance at wavelength λ, and l is the cuvette path length in meters (Green and Blough, 1994). The absorbance at 350 nm was used to measure the concentration of CDOM (Moran et al., 2000). Total suspended solids (TSS) in water from the east and west basins was measured as the weight difference of a clean filter and the same filter with captured TSS, dried at 105º C.

Sediment Gas and Nutrient Flux

In June of 2016 USFWS vegetation survey maps were used to separate areas within the east and west basins into three categories based on the history of SAV cover (Moorman et al., 2017).

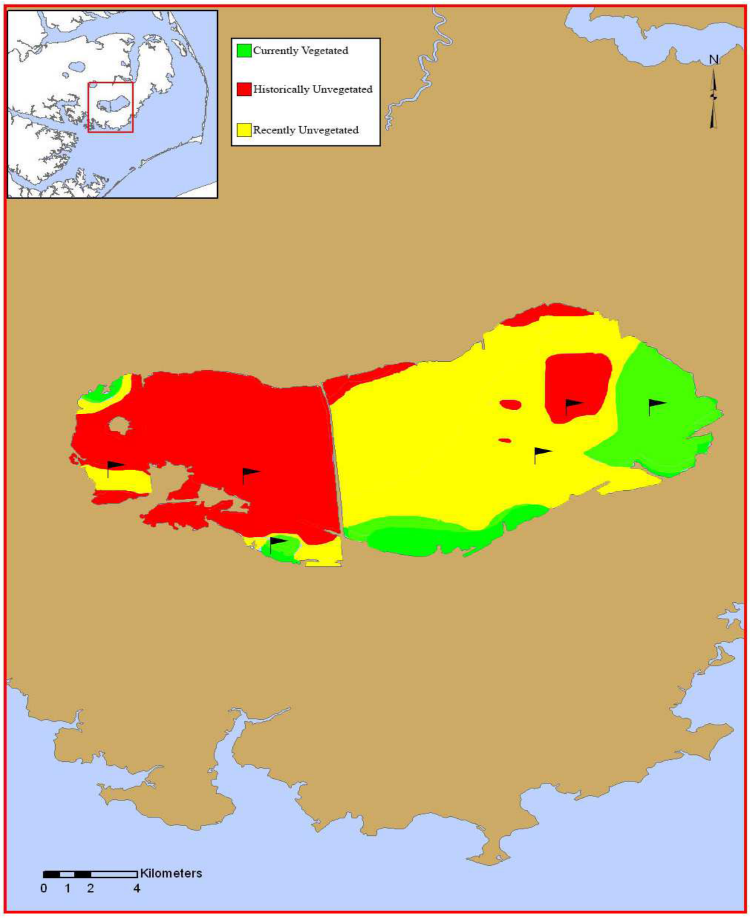


Figure 2. Map of Lake Mattamuskeet, located on the Albemarle Pamlico peninsula. Vegetated areas are shown in green, recently unvegetated areas that lost vegetation after 2015 are shown in yellow, and historically unvegetated areas that did not have vegetation prior to 2014 are shown in red. Sampling locations are denoted with black flags.

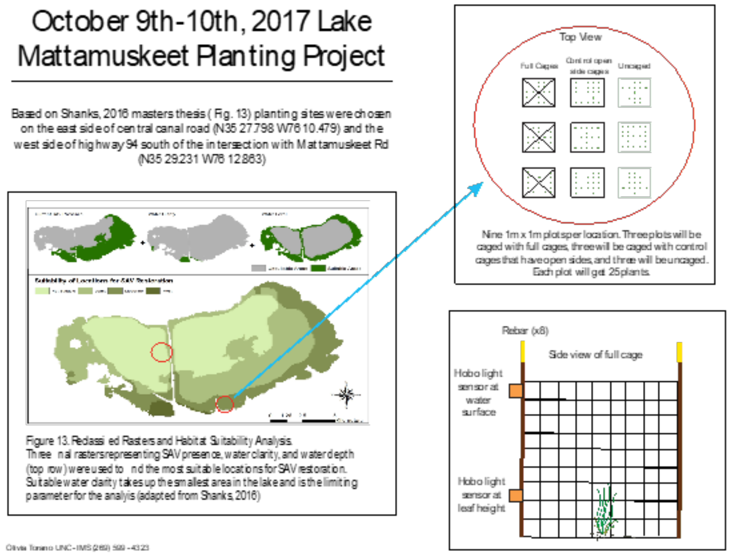
Areas with moderate to dense benthic vegetation present during the 2015 vegetation survey were categorized as currently vegetated (CV), areas that lost vegetation between 2014 and 2015 were categorized as recently unvegetated (RU), and areas with no vegetation prior to 2014 were categorized as historically unvegetated (HU) (Fig. 2). One location from each category was chosen for sampling in each basin and, when possible, in a central location within the delineated area.

Sediment cores were collected in triplicate from each location by pushing plastic tubing (6.4 cm diameter x 31 cm) into the sediment 18 cm, and capping both ends. All samples and 75 L of overlying water were collected from each basin and transported to the Institute of Marine Sciences, Morehead City, NC (IMS). Upon arrival, top stoppers were removed and sediment cores were submerged in aerated basin specific water. Cores were equilibrated overnight in a dark environmental chamber (Bally Inc) set to *in situ* temperature. Submerged cores were capped excluding air bubbles as described by Gold et al. (2017). Cores were connected to a flow-through system pumping 1 mL/minute of aerated basin specific water into the top and out of the bottom of the water column for each core via peristaltic pump (Eyre et al., 2002). Capped cores were pre-incubated for 22 hours, for turnover of overlying water roughly 3 times to allow gas fluxes to come to steady state.

Samples of feed and core outlet water were collected four times over two days at intervals of at least 6 hours (1 full turnover) in gas-tight vials. Dissolved gases were measured with a membrane inlet mass spectrometer (MIMS) for masses of N_2:Ar and O_2:Ar ratios (Kana, 1994). During the midpoint of the two-day incubation (~hour 30), additional 40 mL water samples were collected from inflow water and core outlets and filtered for later analysis of nutrients as described in monitoring section above. Values from inlet and core outlet water nutrient analyses and MIMS measurements were used to calculate fluxes based on core size and flow rate. After incubation, surface (3 cm depth) sediments from cores were collected and analyzed for percent organic matter (%OM) via loss on ignition (Byers, 1978).

SAV restoration

The SAV restoration experiment was carried out in October, 2017. A total of 450 *Vallisneria Americana* plants obtained from Wetland Plants Inc. (Edenton, NC) were planted. Eighteen plots, 9 in the east and 9 in the west basins, were constructed, and 25 plants were transplanted into each restoration plot. The location of transplants and experimental design are shown in figure 3. Fully caged plots, open caged plots, and uncaged plots were used to determine the influence of light attenuation and grazing on plant survival.



October 9th-10th, 2017 Lake Mattamuskeet Planting Project

Based on Shanks, 2016 masters thesis (Fig. 13) planting sites were chosen on the east side of central canal road (N35 27.798 W76 10.479) and the west side of highway 94 south of the intersection with Mattamuskeet Rd (N35 29.231 W76 12.863)

Figure 13. Reclassified Rasters and Habitat Suitability Analysis. Three final rasters representing SAV presence, water clarity, and water depth (top row) were used to find the most suitable locations for SAV restoration. Suitable water clarity takes up the smallest area in the lake and is the limiting parameter for the analyis (adapted from Shanks, 2016)

Nine 1m x 1m plots per location. Three plots will be caged with full cages, three will be caged with control cages that have open sides, and three will be uncaged. Each plot will get 25 plants.

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Figure 3 Planting locations and experimental design.

Results and discussion

Monitoring

Water quality monitoring in East and West lake basins from April through November 2016 showed little variability among stations within basins despite wide geographical extent (Fig 1) for most parameters measured as indicated by the low standard error bars for TSS, CDOM and Chlorophyll a (Fig 4). Some distinct differences were apparent between basins with pooled station data for each basin, with significantly higher TSS in the East basin and higher CDOM in the West Basin. Chl-*a* concentration was slightly higher in the East basin, but this difference was not significant. Elevated concentration of CDOM in the West Ditch could indicate source material from watershed runoff from high organic matter farmland. This ditch was the only site with appreciable levels of nutrients (particularly ammonium) while both basins and the East Canal had consistently low nutrient concentrations throughout the sampling period (Fig.5).

Temporal variability in light attenuation was evident for the East basin but remained fairly constant in the West basin (Fig.7b). K_d showed a strong correlation with TSS on the East (Fig.8a) but this relationship was not significant for the West basin although TSS was strongly correlated between basins (Fig.8b). The duration of elevated wind speed appeared to drive the highest TSS measured in April in both basins while lower TSS was measured in the East basin on dates when wind direction was from the north or northwest. Chl-*a* did not correlate with K_d in either basin nor was TSS correlated with chl-*a* on the West side and showed a weak, negative correlation on the East side (Fig. 9). These data and observations indicate that sediment resuspension driven by wind events is the principal component responsible for light attenuation in the geographically larger and shallower East basin which has presumably been exacerbated by the loss of vegetation in the recent years preceding this monitoring in 2016. While a similar temporal pattern of TSS was evident in the West basin, concentrations were consistently lower on this side of the lake. Levels of CDOM were higher in the West basin which likely contributed the most to the stable light attenuation measured here throughout the study period (Figs.6b, 8b). Although light attenuation was high and generally similar in both basins, it is apparent that the causes are different suggesting that efforts to reestablish submerged vegetation will require basin specific strategies to alleviate light limitation.

Chlorophyll *a* tracked similarly on both sides of the lake with high concentrations maintained throughout the three seasons encompassed by the monitoring period, exceeding state water quality standards of 40 ug/L for all months except June (Fig 8a). The highest concentrations of chl-*a* (July, October and November) corresponded to lower average wind speed particularly in the precedent 6 hour interval (Fig 6a). Highest concentrations of TSS were negatively correlated with chl-*a* on the East side which could indicate light limitation for phytoplankton from resuspension of sediment under higher wind conditions in this basin (Fig 9). The constant hypereutrophic state observed on both sides of the lake resulted in very low levels of water column nutrients (Fig.5) indicative of efficient cycling of available nutrients by phytoplankton.

Algal nutrient bioassays

Nutrient bioassays were conducted during summers 2016 and 2017 to determine nutrient limitation to phytoplankton in both East and West basins and these assays showed similar ambient levels of chlorophyll *a* in both basins (Fig 10). In 2016, the West side showed elevated chl-*a* in response to combined N and P while added nutrients to the East side showed no response over control. In 2017 a

Both East and West basins showed higher response in chl-*a* than previously measured and N and P appeared to be co-limiting. Phytoplankton in the West canal appeared to be N limited and the East canal showed elevated response to either and both N and P.

These results show some distinct differences from seasonal bioassays conducted a decade earlier during which the ambient concentrations of chl-*a* were about 2 times greater in the West basin compared to the East during fall 2005 and summer 2006 (Fig. 10). Seasonal response to nutrients during this period were similar in both basins with N and P co-limitation for phytoplankton apparent in the summer while N, either alone or with P, stimulated higher biomass in the fall. Assays conducted in the winter and spring of 2006 showed lower ambient chl-*a* that was similar between basins with no response over controls indicating nutrients were not limiting phytoplankton growth during these seasons. In the West basin ambient chl-*a* was much higher in summer 2006 compared to 2016 while the East basin showed higher ambient chl-*a* in 2016 similar to West basin levels during this year.

Overall, the recent assays and monitoring data confirm a shift in trophic state on the East basin from macrophyte to phytoplankton dominance, matching conditions that were present but limited to the West basin a decade earlier. Phytoplankton communities in both basins of the lake are currently sustained in a hypereutrophic state and poised to efficiently utilize available nutrients.

Denitrification

Several sediment N_2 flux experiments conducted in 2016, 2017 and 2018 investigated the role of denitrification in lake N cycling. Because this microbial process converts biologically available N to inert N_2 gas, denitrification could alleviate lake eutrophication. Conversely, N_2 gas can be fixed into biomass by select organisms including cyanobacteria. It was hypothesized that submerged vegetation would show elevated rates of denitrification over historically or recently unvegetated sediments as has been observed for seagrasses and marshes in marine systems. In summer 2016 gas fluxes (N_2 and O_2) were compared in sediment cores collected from vegetated, recently unvegetated and historically unvegetated sites (Fig 11). Similar rates of negative N_2 flux were measured in cores from vegetated sites on both sides of the lake indicating net N-fixation (Fig 11). Both recent and historically unvegetated sites showed net N-fixation on the East side and low net denitrification on the West side. However, in subsequent years, unvegetated sediments collected from both East and West basins showed only net N-fixation with rates highest in 2017 (Fig. 11). N_2 fluxes measured a decade earlier, in 2006 showed variable results with net N-fixation apparent on both sides in July and low net denitrification measured on the East side in August. These results of both recent and previous sediment N_2 flux measurements show that lake conditions in either basin are rarely favorable for denitrification and conversely, support N-fixation. It appears that the sustained high demand for nutrients by phytoplankton results in very low ambient water column nutrients available to sediment processing by denitrifers and furthermore, microbes in lake sediments may add bioavailable N to the system.

Further investigation into N_2 gas fluxes, in 2017, focused on canals and ditches on both sides of the lake where higher nutrient concentrations were observed in 2016 monitoring. However, mean N_2 fluxes measured in these sites were not significantly different from zero with individual core replicates varying from negative to positive (data not shown). Low DIN concentration in site collected feed water for these incubations indicated that denitrification was limited by available nitrate in these sites that periodically experience pulses of DIN. In 2018 N_2 fluxes were measured in fringing wetlands (*Phragmites australis*) on East and West sides and in wet and dry impoundments bordering the lake (Fig 12). Negative fluxes were measured in sediments collected from fringing wetlands from both sides and from the flooded impoundment. Cores collected from the dry impoundment showed positive N_2 flux and could represent conditions of a recently flooded impoundment as incubations required overlying water.

Nutrient fluxes, also measured during the gas flux experiments reported here, showed negligible exchange of DIN between the sediment and water column with nitrate exchanges often below detection and ammonium fluxes low and variable, exhibiting both influx and efflux with the sediment interface (data not shown).

In summary, the net balance of N_2 gas flux measured in sediments was almost always negative through a period spanning 10 years, demonstrating net N-fixation from both basins of the lake. This was evident even in habitats that were predicted to favor denitrification, including SAV and fringing wetlands. Sediments from ditches and canals that experience elevated DIN levels also showed net N-fixation although N levels were low during the measurements. Wildfowl impoundments that boarder the lake could be sources for nutrients to the lake but show potential for N removal via denitrification when recently flooded although wet impoundments favored N-fixation. These findings reiterate the lake wide eutrophic state that favors phytoplankton that appear to successfully compete for nutrients at the expense of sediment bacteria.

SAV restoration experiments

While originally proposed for earlier in the project, various setbacks in obtaining plants in a biodegradable medium delayed planting to October, 2017. HOBO light meters were placed just beneath the surface of the water and above the sediment at leaf height in a fully caged and uncaged plot. This was repeated in both basins to assess the light attenuation caused by the cage material. Figure

13 shows the percent of light, measured as lux, reaching leaf height in the caged and uncaged plots. Light attenuation was high in both caged and uncaged plots, indicating that the presence of cages will likely not impact growth despite any small amount of additional light attenuation that may be occurring.

Plant survival was assessed in May, 2018. Figure 14 shows plant survival in the east and west basins. Transplanted SAV survived in a total of four plots all of which were fully caged. Two of the fully caged plots could not be assessed for plant survival due to lack of equipment to allow visibility in the murky water. No open cage or uncaged plots supported SAV survival. Cages were resampled in October of 2018 and the results for vegetation presence were inconclusive due to near zero visibility and structures on the sediment surface that could have been plants. Sampling in Spring of 2019 using lights and underwater scopes determined there were no plants remaining.

Due to the survival of SAV in fully caged plots over the first winter, grazing is likely inhibiting plant growth. Full cages excluded fish, bird, and turtle grazers, so we are unable to speak to the type of grazers responsible for eliminating SAV in the open and uncaged plots. The persistence of SAV, despite high light attenuation and an ice over event during the winter of 2017, suggests larger scale caged plantings could be successful re-establishing vegetated areas in both basins. However, none of the experimental plantings persisted beyond 1 year.

Data figures

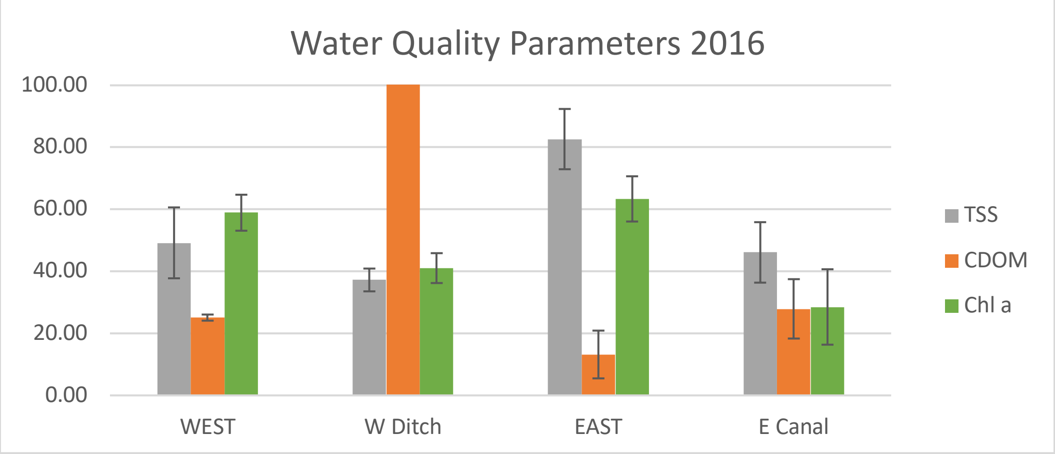


Figure 4. Mean and standard error for parameters measured in 6 monitoring trips in basins (4 sites combined) and Nixon's Ditch and Waupoppin Canal.

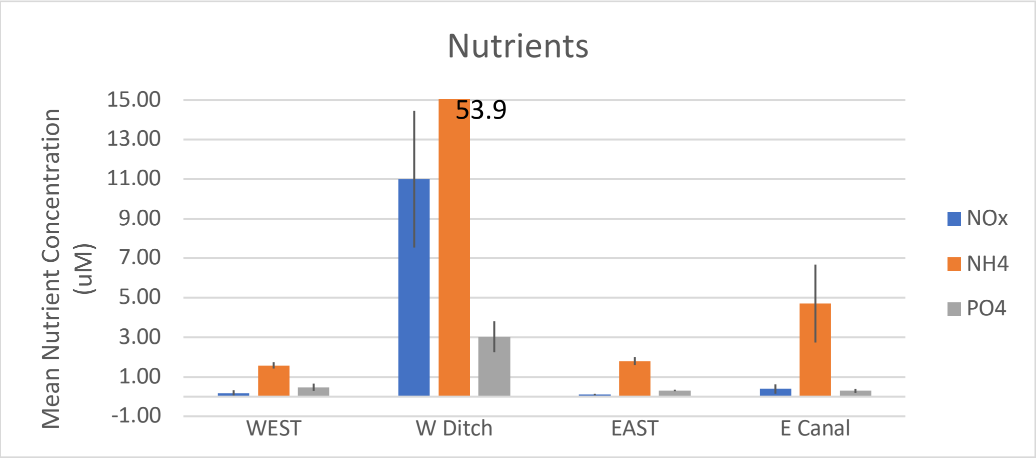
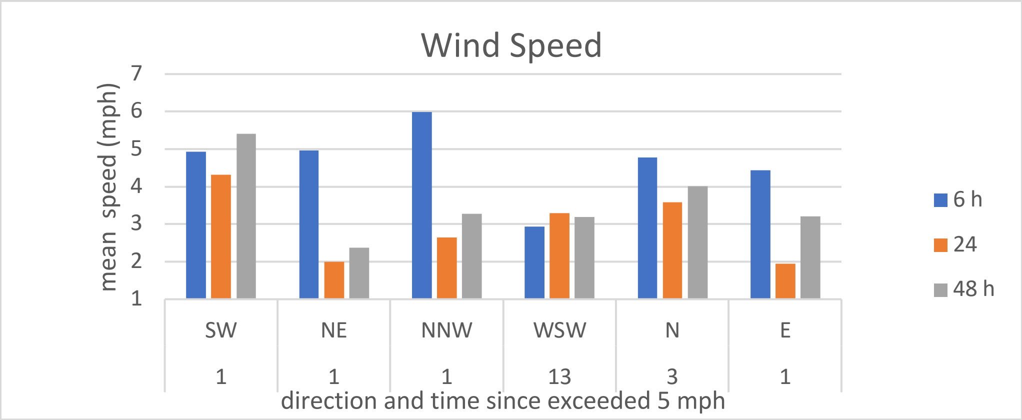
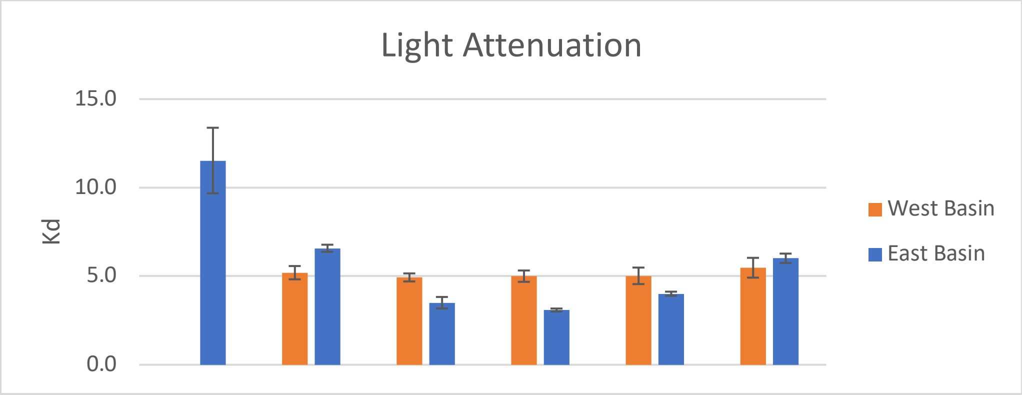


Figure 5. Mean and standard error for nutrients measured in 6 monitoring trips in basins (4 sites combined) and Nixon's Ditch and Waupoppin Canal





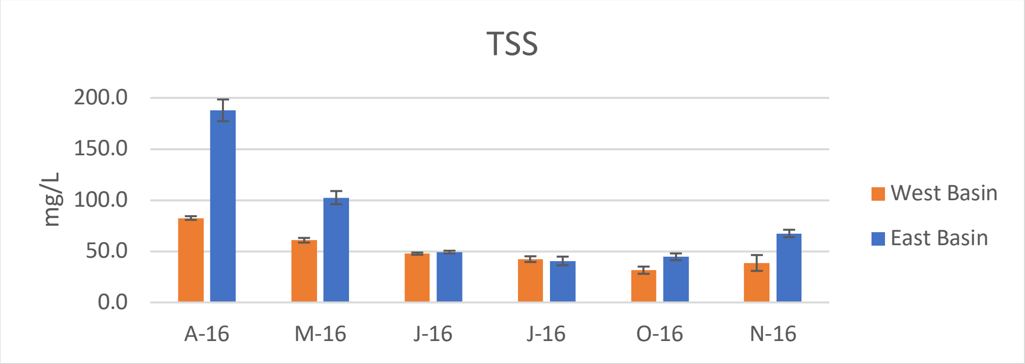
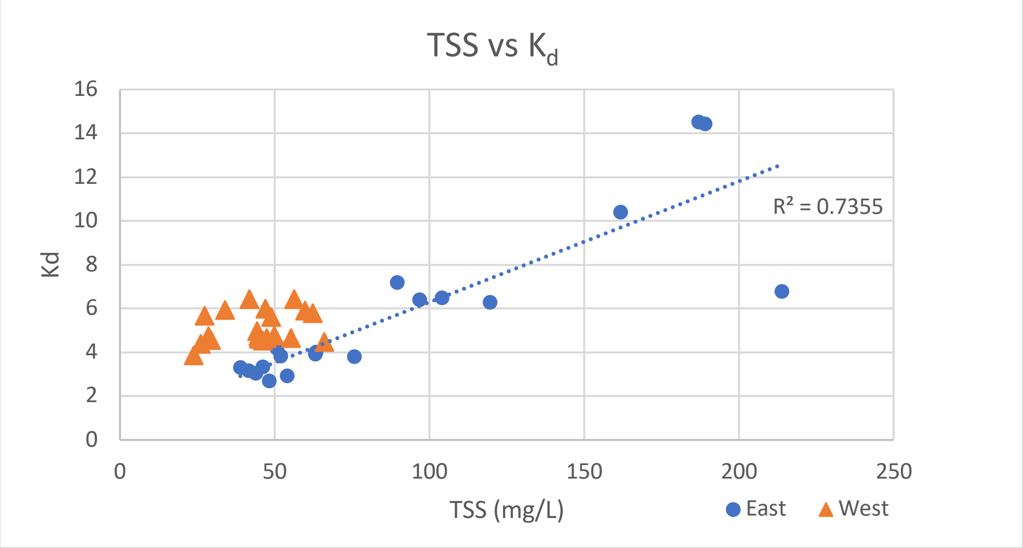


Figure 6. a) Mean wind speed over indicated periods preceding sampling trip b) Light attenuation coefficient and c) TSS as means and standard error of 4 sampling stations in each basin.



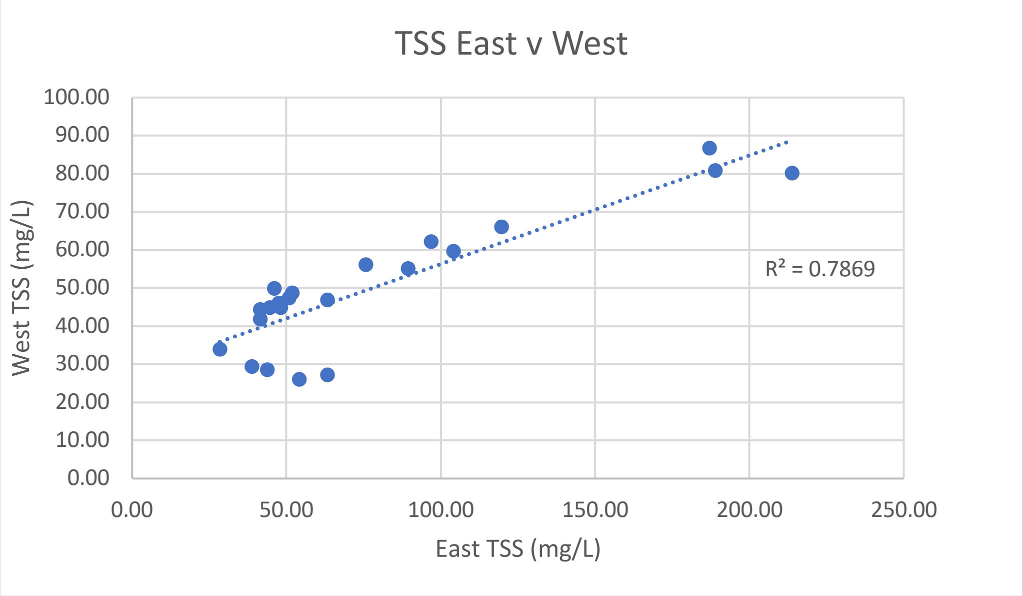


Figure 7 Regression of a) TSS vs K_d and b) TSS of East vs. West for all stations and monitoring trips

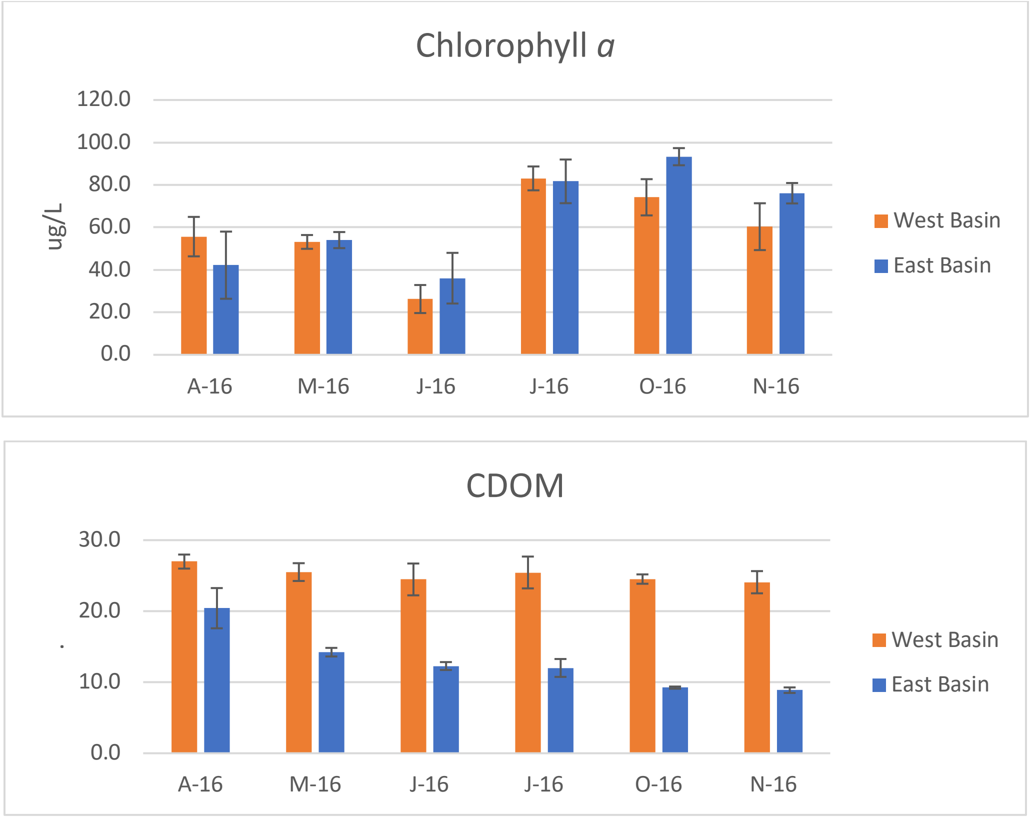


Figure 8. a) Chlorophyll *a* concentrations and b) CDOM as means and standard error of 4 sampling stations in each basin.

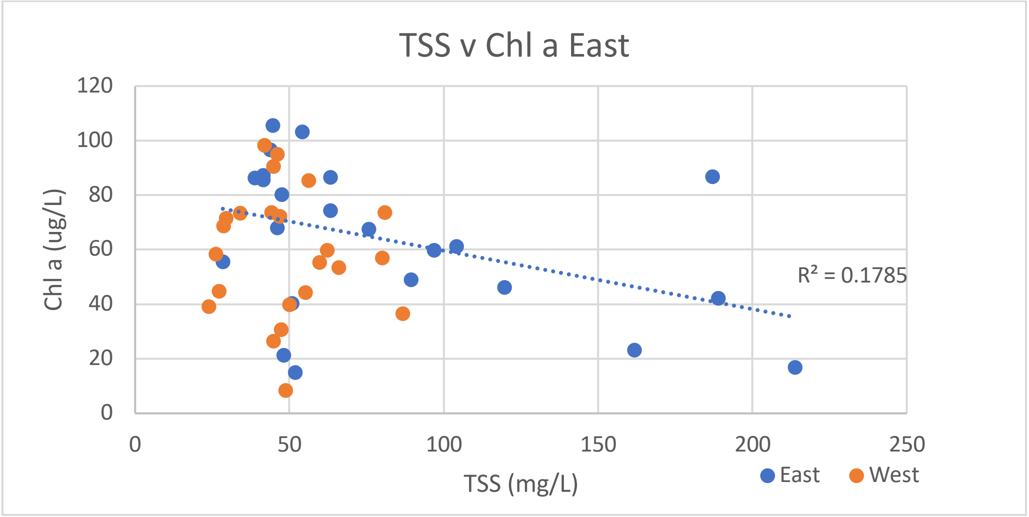


Figure 9. TSS vs Chl-*a* for all stations and monitoring trips

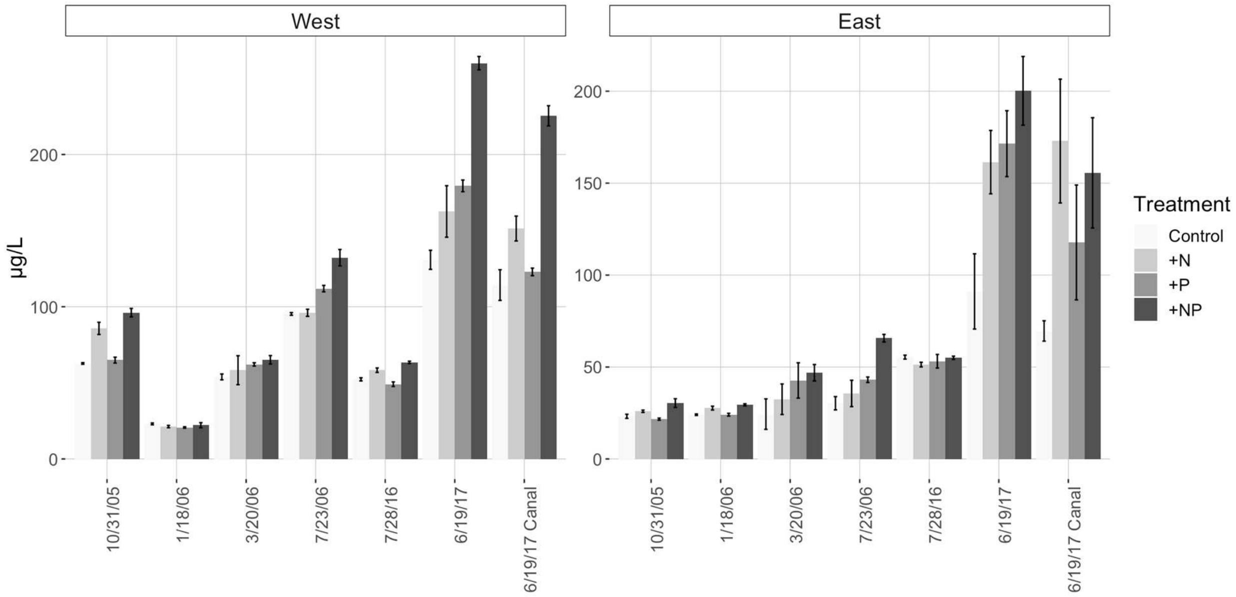


Figure 10. 2005 – 2017 bioassay chlorophyll *a* (µg/L) response to nutrient additions (note difference in scale between East and West sides). The 2017 bioassays were amended with 160 uM N (NO3- and NH4+) while previous assays were amended with 20 uM combined N species. The 2017 bioassay included canal water collected from each basin.

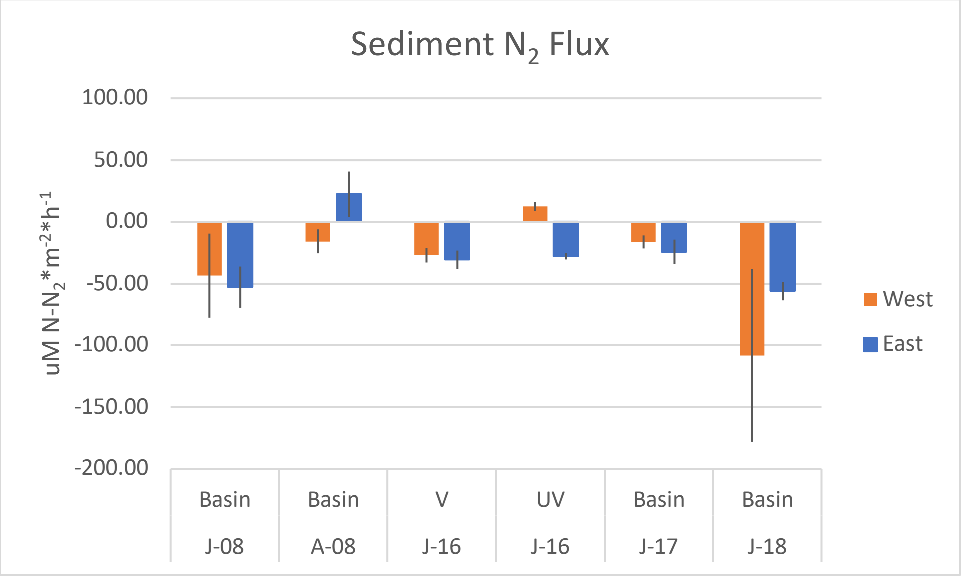


Figure 11. Sediment fluxes of N_2in East and West basins including locations with (V) and without (UV) measured in 2016. Net fluxes above 0 represent denitrification and below 0 indicate N-fixation.

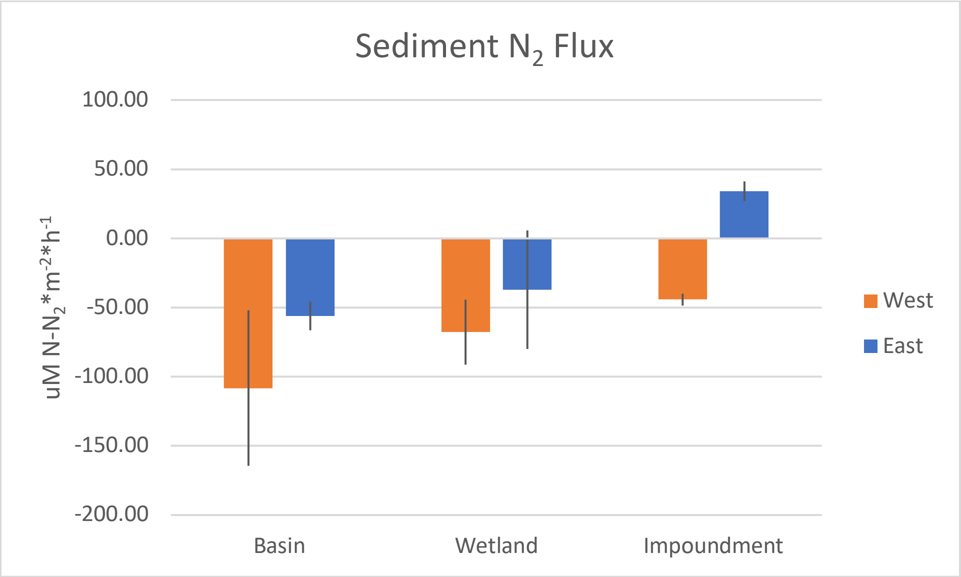


Figure 12. June 2018 flux measures in sediments of open basins, fringing wetlands and wet (West) impoundment and dry (East) impoundment.

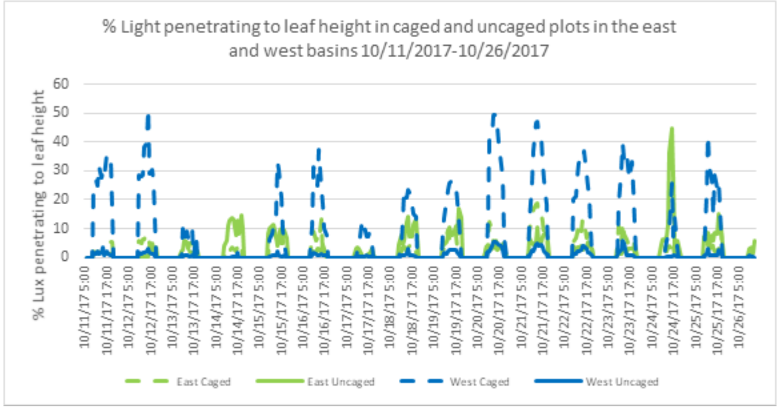


Figure 13 Percent of light measured in lux reaching leaf height for caged (dotted) and uncaged (solid) plots over a two-week period after planting.

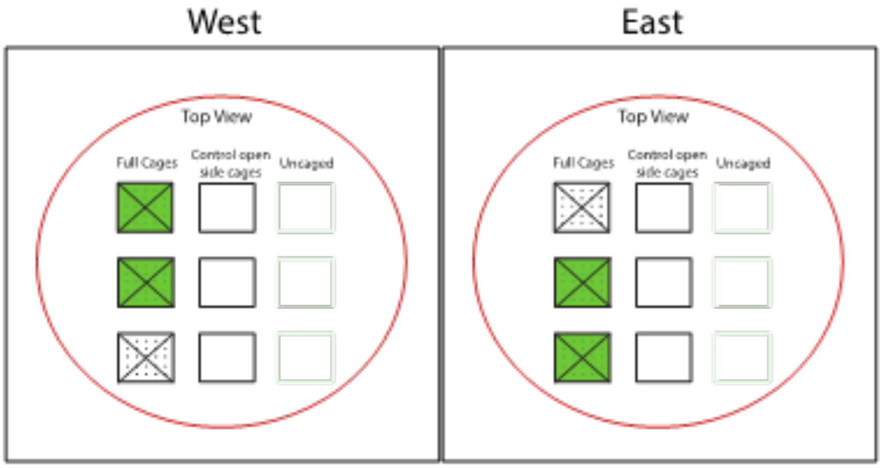


Figure 14. Plants present shown in green, undetermined survival in black, no survival blank. May 2018 sampling.

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