# ALGAL GROWTH INHIBITION REPORT

## FOR THE

# TAYLOR CREEK WATERSHED


## JUNE 2012

## Background and Introduction

The Florida legislature passed the Lake Okeechobee Protection Act[1] in 2000 to establish the Lake Okeechobee Protection Program to restore and protect Lake Okeechobee. A primary goal is to achieve the Total Maximum Daily Load (TMDL) for total phosphorus of 105 metric tons per year from the watershed to the lake. This goal is to be achieved through the implementation of a comprehensive Lake Okeechobee Watershed Protection Plan (LOWPP), which is being implemented through the efforts of the Coordinating Agencies: the South Florida Water Management District (SFWMD), the Florida Department of Agriculture and Consumer Services (FDACS) and the Florida Department of Environmental Protection (FDEP). The LOWPP includes implementation of source control reductions through urban and agricultural Best Management Practices (BMPs) and other projects to attenuate nutrients (nitrogen and phosphorus) in the watershed before their discharge to Lake Okeechobee.

One of the projects implemented under the LOWPP was an Algal Turf Scrubber® (commonly referred to as the ATS®) technology demonstration project that HydroMentia, Inc. conducted in the Taylor Creek/Nubbin Slough (TC/NS) Sub-watershed (Figure 1), and the source water for the project was Taylor Creek. The TC/NS Sub-watershed is primarily located in Okeechobee County and flows southward into the S-191 basin, which is one of the four priority basins in the Lake Okeechobee Watershed. The primary land use in the S-191 basin is agriculture, including improved and unimproved beef pasture, dairy farms and citrus groves.

[1] (Section 373.4595, Florida Statutes – amended and expanded in 2007 to become the Northern Everglades and Estuaries Protection Program)

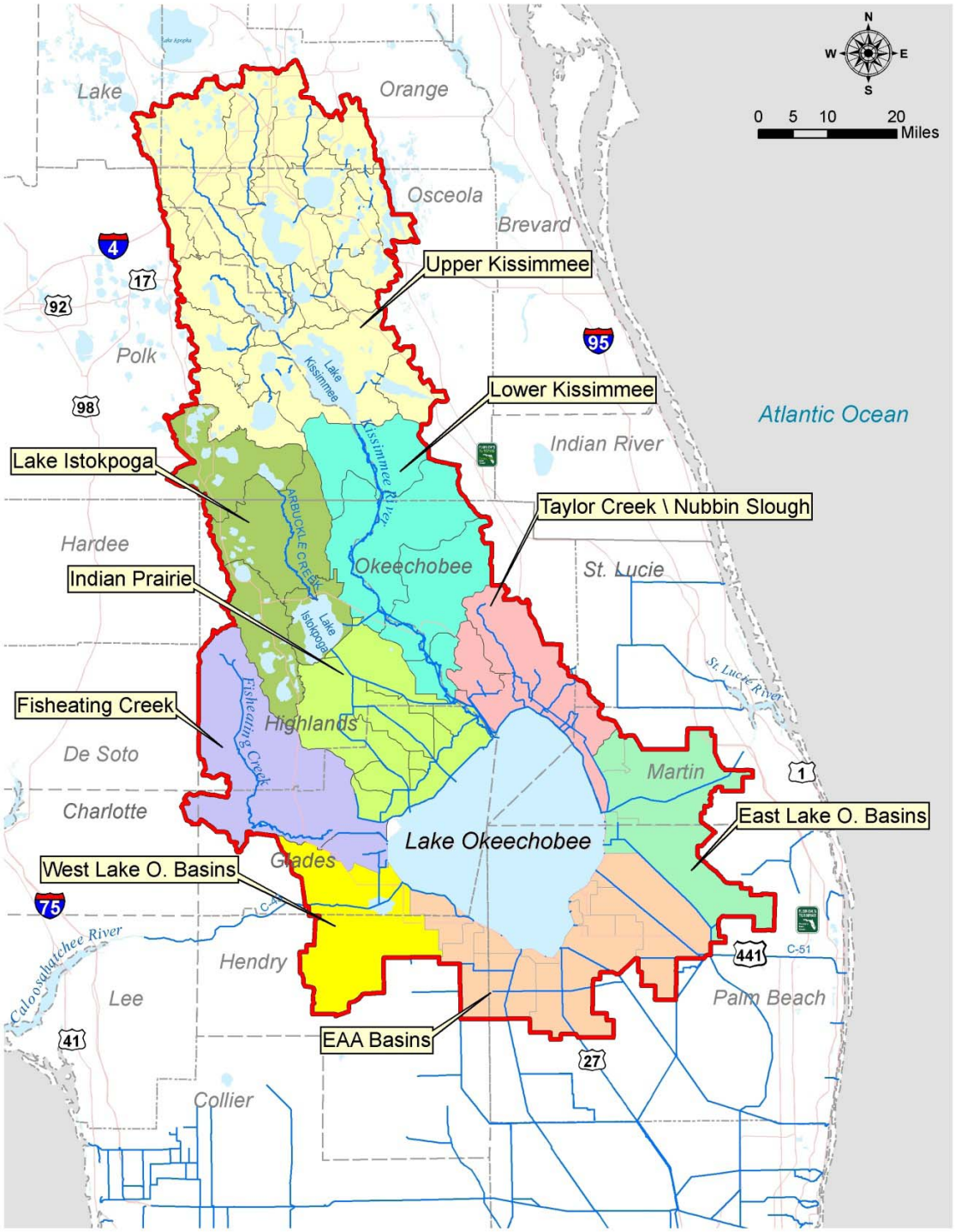



**Figure 1** – Lake Okeechobee Watersheds

The ATS™ project involved the removal of nutrients from inflow waters through the growth and harvesting of algae, i.e. periphyton. The project summary report (hereafter referred to as "HydroMentia Report"; HydroMentia, Inc., 2010) noted that: "During the three year operational period, system performance was noted to fall well below the projections included within the

Basis of Design report." The report hypothesized that some substance in the project influent water may have inhibited periphyton growth; reducing nutrient uptake and adversely affecting project performance. This finding was reported to the Florida legislature in the March 2011 LOWPP update. The HydroMentia Report noted that bioassay work conducted at the project site indicated that complex organic compounds may be responsible for the low algal production and correspondingly low system performance. The HydroMentia Report also noted that the potential complex organic compound was thought to be a surfactant that was concentrated in foam noted at the project site. Finally, the HydroMentia Report noted that increases in the inhibition of periphyton growth were more commonly found when flow within the basin increased. The report suggested that this connection could be the result of a growth inhibiting compound being "flushed"down the watershed during storm events.

The 2011 Florida budget included a specific appropriation of $100,000 for FDEP to investigate potential substances which may have inhibited algal growth in the Taylor Creek Watershed. This investigation was performed in consultation with the SFWMD and FDACS and was intended to build upon the results and recommendations of the HydroMentia Report. FDEP received the appropriation through the TMDL program funding. The appropriation included language directing FDEP to provide a written report to the SFWMD by June 30, 2012. The following report provides an overview of the results from the initial phase of this sampling effort and serves to satisfy this reporting requirement.

## Methods and Materials

### Introduction

Algal toxicity bioassays (ATBs) were used in this study to assess the biological response of samples collected in the Taylor Creek/Nubbin Slough watershed (Figure 1). The ATB is a 96-hour chronic bioassay test in which the population (growth) of the test species (*P. subcapitata* for freshwater samples) is compared to growth in a control. Samples with a biological response (cell counts, chlorophyll fluorescence, absorbance or biomass) at a defined threshold below

control suggest the presence of a biologically inhibiting constituent (note: cell count and biomass reported as mg dry wt/L were the growth responses examined in this study). Biologically inhibiting constituents can be naturally occurring in the system or anthropogenically introduced. ATBs are not used to isolate or identify constituents in the water.

**Sampling Site Locations and Descriptions**

Water samples were collected from six (6) sites in the Taylor Creek drainage basin designated as FDEP_TCATOX 1 through FDEP_TCATOX 6 (also referred to at TCATOX 1 through TACTOX 6). Site descriptions are provided in Table 1 and site locations are provided in Figure 2. Figure 2 also shows the location of the ATSTM project (TC ATSTM Site) for spatial reference. Sampling sites FDEP_TCATOX1 through FDEP_TCATOX4 are located in individual upstream tributaries of Taylor Creek, while site FDEP_TCATOX5 is located just above the weir at the convergence of these four tributaries. Site FDEP_TCATOX6 is located at the inflow to the Taylor Creek STA and is downstream of both the FDEP_TCATOX5 site and the TC ATSTM site, which is now the site of an SFWMD Hybrid Wetland Treatment Technology Facility. Land use upstream of each site is primarily improved, unimproved and woodland pasture. A few notable exceptions include: approximately 260 acres of residential area upstream of FDEP_TCATOX3; approximately 780 acres of citrus groves located northwest of FDEP_TCATOX5, but downstream of FDEP_TCATOX1 and FDEP_TCATOX2; and an 800 acre residential area on the east side of Taylor Creek between sites FDEP_TCATOX5 and FDEP_TCATOX6. All sampling locations are concurrently located with active SFWMD monitoring sites. In the interest of reducing this investigation's cost, the SFWMD agreed to leverage the collection of raw water samples for FDEP and shipped these samples to the FDEP laboratory.

Table 1. Taylor Creek sample site locations, descriptions and corresponding SFWMD site.

| Site Name | Latitude/ Longitude | Site Description | Corresponding SFWMD WQ Site |
|---|---|---|---|
| FDEP_TCATOX1 | 27° 23’ 34.94”N 80° 55’ 20.05”W | Beatty Property Wetland Drainage Ditch along CR68 | TC27353413 |
| FDEP_TCATOX2 | 27° 23’ 40.53”N 80° 53’ 41.65”W | NW Taylor Creek at HWY 68 Bridge and USGS Gauge Station | TCNS201 |
| FDEP_TCATOX3 | 27° 23’ 12.52”N 80° 51’ 58.72”W | Little Bimini at Potter Road | TCNS204 |
| FDEP_TCATOX4 | 27° 24’ 4.35”N 80° 48’ 54.52”W | Otter Creek and SR441, downstream of Mc Arthur Dairy | TCNS207 |
| FDEP_TCATOX5 | 27° 21’ 54.51”N 80° 52’ 21.38”W | Taylor Creek Headwater at S2 weir, located downstream of G-BAR E Ranch | TCNS213 |
| FDEP_TCATOX6 | 27° 18’ 50.80”N 80° 50’ 15.51”W | Located on the creek side of the Inflow Pump Station to Taylor Creek STA | S390 |

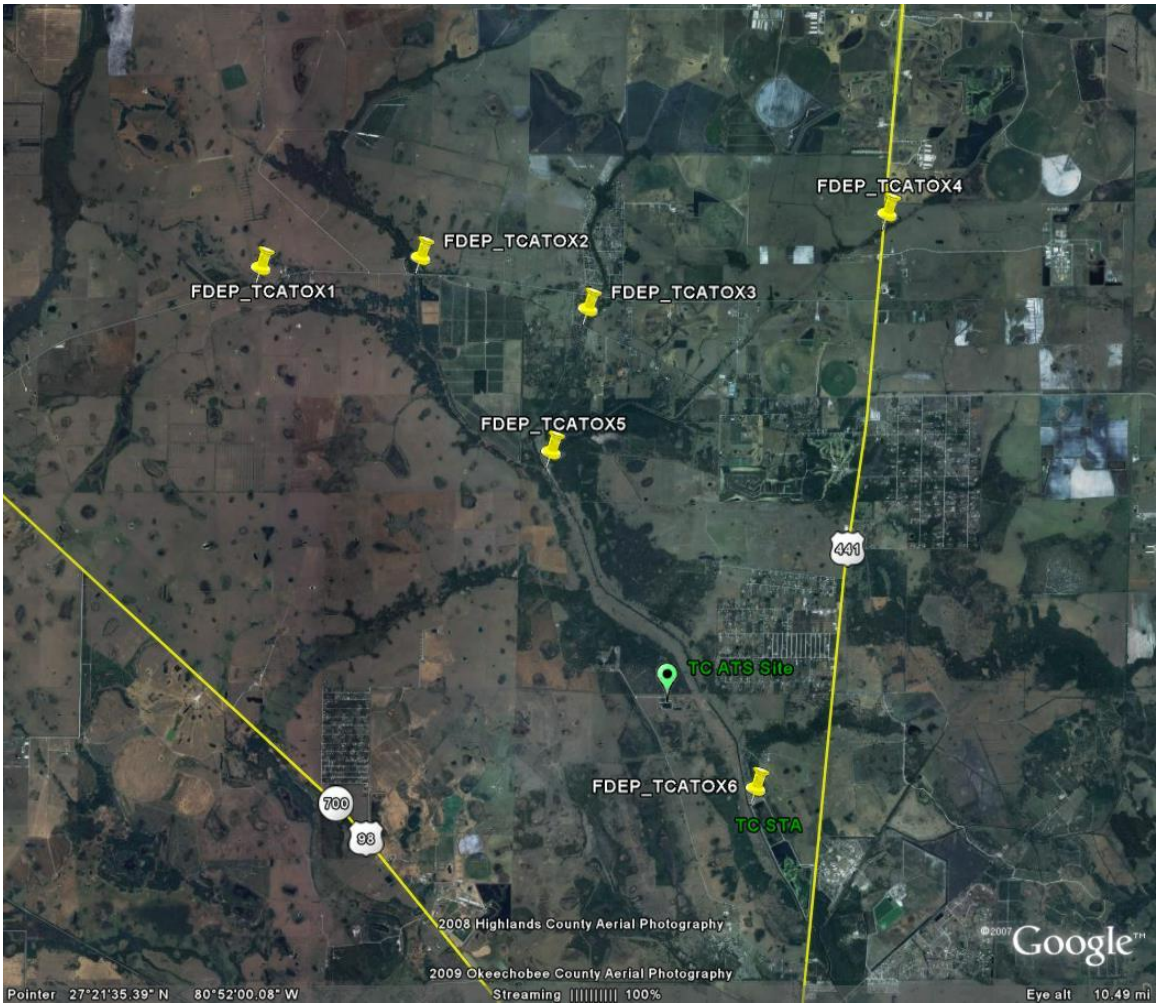



**Figure 2.** Aerial map of Taylor Creek sampling sites.

**Sample Collection**

SFWMD personnel collected surface water samples from the six stations between July 2011 and February 2012 (Table 2)**.** Sample collection only occurred when observed flow was present at the study sampling locations. Sample collection followed the procedures and requirements found in the Field Sample Collection Procedures Section of the District's FSQM (SFWMD, 2011a). Following completion of sample collection for each day, the samples were shipped overnight in coolers with wet ice at 4$^{o}$ C to the FDEP Central Laboratory in Tallahassee, Florida. Upon delivery to the Central Lab, Receiving Department staff logged samples into the FDEP Laboratory Information Management System (LIMS). After the samples were logged in, they were transferred to Biology Laboratory Staff for processing.

**Table 2**. Inventory of sampling dates at each sampling location.

| Sample Date | Sample Site | | | | | |
|---|---|---|---|---|---|---|
| | TCATOX1 | TCATOX2 | TCATOX3 | TCATOX4 | TCATOX5 | TCATOX6 |
| 7/26/2011 | yes | no | yes | no | yes | yes |
| 8/9/2011 | yes | yes | no | no | yes | yes |
| 8/23/2011 | yes | yes | yes | no | yes | yes |
| 9/6/2011 | no | no | yes | no | yes | yes |
| 9/20/2011 | yes | no | yes | no | yes | yes |
| 10/4/2011 | yes | no | yes | no | yes | yes |
| 10/18/2011 | yes | yes | yes | yes | yes | yes |
| 11/1/2011 | yes | yes | yes | no | yes | yes |
| 11/15/2011 | no | no | yes | no | yes | yes |
| 11/29/2011 | no | yes | no | no | yes | yes |
| 2/7/2012 | no | no | no | no | yes | yes |

Sample Processing

With the exceptions noted below, the ATBs were performed in accordance with EPA Test Method *Green Alga, Selenastrum Capricornutum, Growth Test Method 1003.0* found in EPA 821-R-02-013 *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* and/or FDEP SOPs TA 07.10-2.6 and TA 07-1.14 (http://www.floridadep.org/labs/cgi-bin/sop/biosop.asp) The sample holding time of 36 hours specified in the EPA method was modified to allow ATB test initiation to occur on the second day (Thursdays) after sample collection (Tuesdays). This variance was allowed for logistic reasons so that test termination(s) would occur on Monday(s) rather than Sunday(s). All ATBs were initiated within 55.5 hours of sample collection. The tests were initiated using 1500 hours as the start time. The earliest sample collection time was 0736 hours, the latest, 1133 hours. The EPA method manual (EPA 821-R-02-013) allows procedural modification especially for non-compliance enforcement testing.

Receiving water (ambient water) tests commonly employ two treatments (a control and 100% sample but may consist of receiving water dilutions). Concentrations used in these tests were the control (0% sample), 100% sample, 75% sample, 50% sample and 25% sample for the

first three events (July 26, August 9 and August 23, 2011) then control (0% sample), 100% sample, 50% sample, 25% sample and 12.5% sample in subsequent events. The 12.5% concentration was used to further refine the IC25. It was felt these concentrations were adequate to quantify the results.

**ATB Results and Discussion**

Cell counts and biomass[2] (expressed as mg dry wt/L) were measured in the ATB tests. "Inhibited growth" or "inhibited biological response" is defined in this document as the reduction in cell count or biomass of ≥25% in a sample when compared to the cell count or biomass in the control.

Thirty-five (35) of the 43 samples analyzed were valid tests (Table 3). Seven (7) analyses resulted in invalid tests in which the control cell counts were less than the minimum required count ($1 \times 10^6$ cells/mL). The problem of test invalidation due to low control counts was resolved by using four (4) day old cultures rather than seven (7) day old cultures for the algal inoculum (the allowable range for ATBs is 4 to 7 days).

**Table 3.** TCATOX samples received by the laboratory and valid ATB tests by site.

| TCA Site | Algal Toxicity Samples | Algal Toxicity Valid Tests |
|---|---|---|
| TCATOX 1 | 7 | 5 |
| TCATOX 2 | 5 | 4 |
| TCATOX 3 | 8 | 8 |
| TCATOX 4 | 1 | 1 |
| TCATOX 5 | 11 | 8 |
| TCATOX 6 | 11 | 9 |
| TOTAL | 43 | 35 |

The results of the ATBs are presented as 25% Inhibition Concentrations ($IC25^2$) for cell counts (Table 4) and for biomass (Table 5). In samples when IC25 values are below 100, algae growth is considered inhibited. As an example of how to interpret IC25, a hypothetical sample

with a cell count IC25 of 40% would indicate that when the sample was diluted to 40% of its original concentration it exhibited a 25% reduction in cell count compared to the control. Stronger concentrations of this sample (higher IC25 values) would have exhibited a greater reduction (>25%) in cell counts compared to control. Thus the lower the IC25 value the greater the potential biological inhibition of the source water.

Cell counts were substantially lower in the 100% samples than in the controls in 32 of the 35 valid tests, producing IC25 values < 100% sample and suggestive of a growth inhibiting constituent (Table 4). It was noted during the analyses of the first few sets of TCATOX ATBs that mean cell volume was usually greater in the 100% sample than in the controls, resulting in greater biomass per cell in the sample test concentrations; hence, calculated IC25 values for biomass (Table 5) were greater [a higher concentration (calculated) of sample showing a 25% reduction in the endpoint compared to the control] than IC25 values for cell counts. A comparison of IC25 values for biomass and cell count (within each valid test) shows that 32 of the 35 samples had higher IC25 values (however slight) for biomass than for cell count. The biomass endpoint showed no algal growth inhibition in 12 samples; whereas no algal growth inhibition was noted in only 3 samples based on cell count. No algal growth inhibition in this report means that calculated IC25 values for the measured endpoints (cell count and biomass) were greater than 100% sample. By inference, cell density and biomass may have been less in the 100% sample than in the control but was/were not greater than 24.9% less.

Heavy metals, pesticides and complex industrial wastes are some of the potential causes for an increase in mean cell volume in *P. subcapitata* bioassays (Miller et al, 1978). The cause of the increase in mean cell volume in the TCATOX samples was not determined. As noted, there can be multiple factors involved in “increased cell volume”. Without specific chemical analyses, the determination of increased cell volume is not feasible. Algal cells from 100% sample test concentrations were examined (at test termination) by the Biology Laboratory’s chief algal taxonomist (Dr. Maosen Hua) who determined that the cells showing an increase in mean cell volume were healthy, rather than simply enlarged due to increased membrane permeability.

Increased cell volume may be due to inhibition of the cell division mechanism; thus, biomass may fail to detect the occurrence of a significant effect (Chao and Chen, 1999). The physiological processes and/or factors involved in the increase in algal cell volume are beyond the scope of this report.

Cell count IC25s suggest that TCATOX 1, TCATOX 2 and TCATOX 6 had the most reduced biological response followed by TCATOX 5, while TCATOX 3 had the least affected biological response with three samples showing no significant difference (IC25 >100) in biological response compared to control (Table 4). This pattern is consistent with the finding of the HydroMentia Report Based on cell counts, 91% (32 out of 35) of the samples yielding valid results had cell count IC25 values below 100 suggesting the presence of an algal growth inhibiting constituent(s)

**Table 4.** Cell Count 25% Inhibition Concentration (IC25 expressed as % sample) for samples collected from TCATOX sites from July 26, 2011 through February 7, 2012

| DATE | TCATOX 1 | TCATOX 2 | TCATOX 3 | TCATOX 4 | TCATOX 5 | TCATOX 6 |
|---|---|---|---|---|---|---|
| 7/26/2011 | 18 | - | > 100 | - | No Result | No Result |
| 8/9/2011 | No Result | No Result | - | - | No Result | No Result |
| 8/23/2011 | 17.6 | 21.9 | 37.2 | - | 20.7 | 13.5 |
| 9/6/2011 | - | - | 34.1 | - | 74.7 | 56.6 |
| 9/20/2011 | No Result | - | > 100 | - | No Result | 73.7 |
| 10/4/2011 | 22.4 | - | > 100 | - | 74 | 55.5 |
| 10/13/2011 | 9.75 | 13.8 | 11.3 | 12.4 | 9.7 | 16.5 |
| 11/1/2011 | 16.5 | 13.1 | 25.9 | - | 27.1 | 11.2 |
| 11/15/2011 | - | - | 18.9 | - | 10.9 | 9.0 |
| 11/29/2011 | - | 9.6 | - | - | 31.7 | 21.6 |
| 2/7/2012 | - | - | - | - | 39.7 | 23.4 |

No Result - invalid test
Not Sampled (no flow) = -

= no or low (<25%) inhibition
= moderate inhibition (IC25 50% -100%)
= high inhibition (IC25 < 50%)

Biomass IC25 (Table 5) appears to follow the same pattern as cell count IC25. TCATOX 1, TCATOX 2 and TCATOX 6 showed the most reduced biological response followed by TCATOX 5, while TCATOX 3 had the least affected biological response with six samples showing no significant difference (IC25 >100%) in biological response from control. Based on biomass, 66 percent (23 out of 35) of the valid tests had biomass IC25 values below 100 suggesting the presence of an algal growth inhibiting constituent(s) in the source water.

TCATOX 6, located approximately 6.8 kilometers downstream of TCATOX5, often had lower IC25 values than TCATOX5 suggesting an increase or an input of an inhibiting constituent between sites TCATOX 5 and TCATOX 6.

**Table 5.** Biomass 25% Inhibition Concentration (IC25 expressed as % sample) for samples collected from TCATOX sites from July 26, 2011 through February 7, 2012

| DATE | TCATOX 1 | TCATOX 2 | TCATOX 3 | TCATOX 4 | TCATOX 5 | TCATOX 6 |
|---|---|---|---|---|---|---|
| 7/26/2011 | 29.4 | - | > 100 | - | No Result | No Result |
| 8/9/2011 | No Result | No Result | - | - | No Result | No Result |
| 8/23/2011 | 36.4 | 36.8 | > 100 | - | 37.7 | 25.2 |
| 9/6/2011 | - | - | > 100 | - | > 100 | > 100 |
| 9/20/2011 | No Result | - | > 100 | - | No Result | 94.2 |
| 10/4/2011 | 35.7 | - | > 100 | - | > 100 | 86 |
| 10/13/2011 | 14.5 | 20.1 | 74.5 | 53.8 | 31.3 | 32.2 |
| 11/1/2011 | 19.5 | 27.1 | 92.4 | - | 42.3 | 17.3 |
| 11/15/2011 | - | - | > 100 | - | 79.5 | 47.6 |
| 11/29/2011 | - | 62.5 | - | - | > 100 | 83.9 |
| 2/7/2012 | - | - | - | - | > 100 | > 100 |

No Result - invalid test
Not Sampled = -

= no or low (<25%) inhibition
= moderate inhibition (IC25 50% -100%)
= high inhibition (IC25 < 50%)

To determine if there was an actual difference in response versus a difference in the rate of response, cell counts and biomass at varying endpoints (96-hours, 120 hours and 168 hours) were compared (Figures 3 and 4). In these figures the error bars represent the value at or above which the 100% sample would not be considered to have a growth response significantly different from the control and therefore the presence of a limiting constituent is not expected. Values below the error bar displayed inhibited algal growth. The 96-hour cell density endpoint appeared to be a more conservative[3] endpoint than 96-hour biomass endpoint in the Taylor Creek ATBs based on the number of ATBs falling below the error bar. Regardless of the endpoint used, the portion of the Taylor Creek drainage area sampled during this study appeared to have a constituent or constituents that consistently reduced biological response (cell count and biomass) in the ATBs (Tables 4 and 5). Data from the ATBs performed on samples (TCATOX 2, TCATOX 5 and TCATOX 6) collected on November 29, 2011, suggests that the inhibiting constituent measured at 96-hours might be transient (for this event) (Figures 3 and 4). The ATBs duration was extended to 168-hours, with measurements of cell density and

[3] Conservative as used here means more samples showing potential growth inhibition to algae.

biomass taken at 120 hours and 168 hours in addition to the standard 96 hour measurements. At 96-hours, cell density data suggests inhibited growth in each of the three samples and biomass data suggests inhibited growth in samples TCATOX 2 and TCATOX 6 but not TCATOX 5. At 120 hours, the cell density IC25 test at TCATOX 6 is the only sample suggesting inhibited growth by either endpoint. At 168 hours, TCATOX 5 again shows reduced biological response based on cell density but not biomass and is the only sample suggesting inhibited growth by either endpoint. This (168 hour TCATOX 5 cell density -28.7% less than control) might be an artifact of the test; however, the release of substances from dead, dying or lysed cells causing inhibited algal growth cannot be ruled out. Chemical analyses have not been performed to identify potential inhibiting constituents. Possible reasons for the reduced biological response shown at 96-hours and not shown at 120-hours or 168-hours include recovery of initially susceptible cells, metabolizing of an inhibiting constituent by the algae and photolysis (Adams et. al., 1985).

Figure 3. Comparison of cell count data from extended duration ATBs performed on samples collected on November 29, 2011. The error bar (25% of control) represents the value at or above which 100% sample would not be considered to have a biological response different from control.

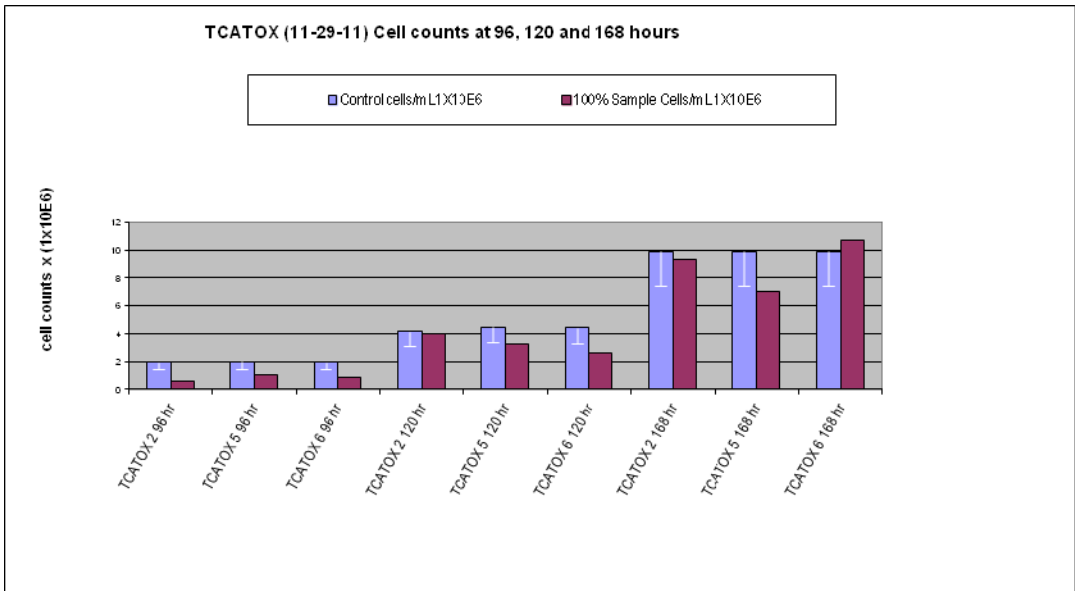
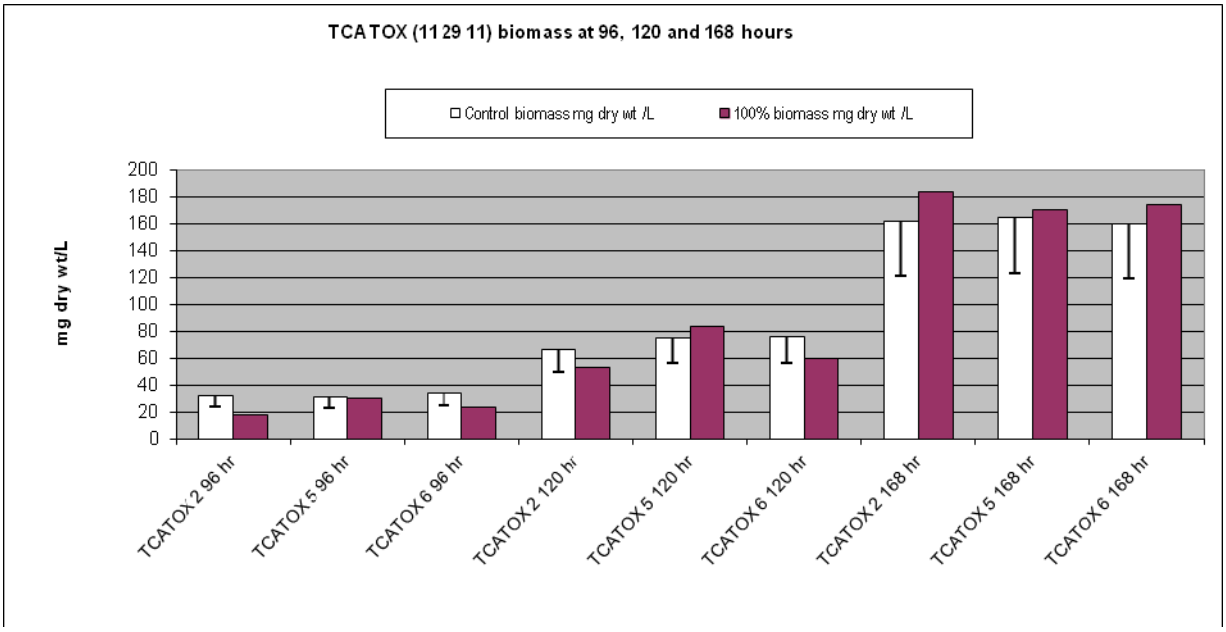


Figure 4. Comparison of biomass data from extended duration ATBs performed on samples collected on November 29, 2011. The error bar (25% of control) represents the value at or above which 100% sample would not be considered to have a biological response different from control .




The HydroMentia Report (HydroMentia, 2010) reported die-offs in early summer (first flush) and die-offs in the fall (September-October). It also associated observed algal die-offs with runoff patterns and suggested that this might be associated with agricultural practices within the basin. At the time of this report samples were only collected over a seven month period and did not capture early wet season “first flushes”. In addition, the results following some storm events that did occur during the sampling period did not show a consistent pattern. All IC25s were depressed following the August 2011 flow event, but did not evidence such consistent depression following a similar flow event in September 2011. The IC25s remained consistently depressed in samples from all of the sample sites following large spikes in flow in early to mid-October 2011 (Figure 5). Finally, the IC25s for the two samples obtained in February 2012 were both significantly depressed, despite the absence of any significant flow events during the several weeks preceding the sampling date. Therefore, there are insufficient data and not

consistent enough results to make a definitive statement concerning temporal variation in observed biological response in the ATBs.

**Nutrient Analysis**

Samples for total phosphorus (TP) analysis were also collected during each sampling event (Table 6 below). The TP data were collected and analyzed by SFWMD as part of their existing watershed monitoring program. All data are available in the DBHydro database under the corresponding site names given in Table 1.

Figure 5. Cell Count IC25 compared to Mean Flow. Notes: Mean flow was obtained from the US Geological Survey database for USGS site 02274325.

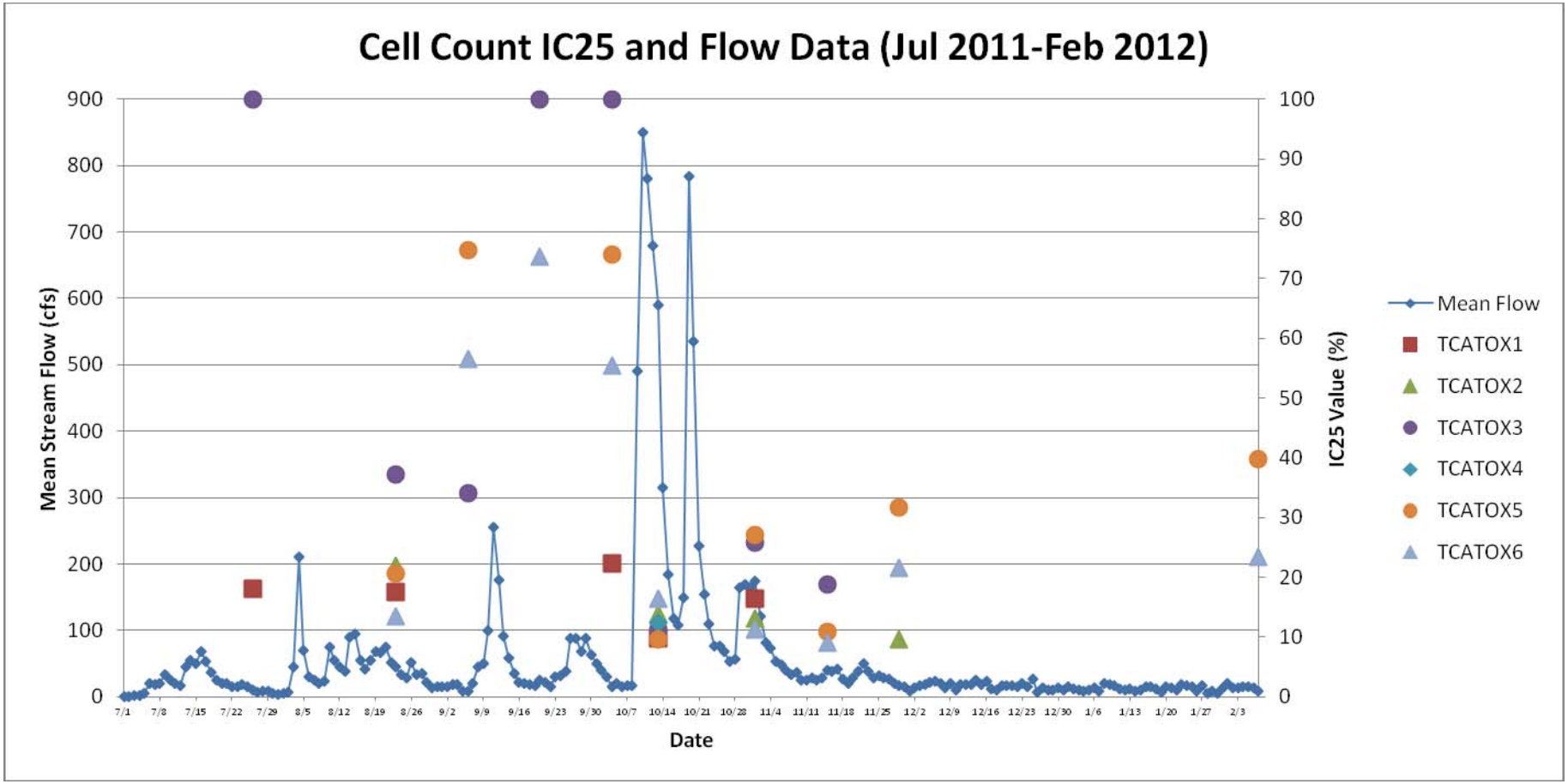


**Table 6**. Corresponding Total Phosphorus (mg/l) data analyzed by the SFWMD Analytical Laboratory for corresponding sampling locations.

| | Sample Site | | | | | |
|---|---|---|---|---|---|---|
| Sample Date | TCATOX1 | TCATOX2 | TCATOX3 | TCATOX4 | TCATOX5 | TCATOX6 |
| 7/26/2011 | 0.744 | - | 0.871 | - | 0.661 | 0.583 |
| 8/9/2011 | 0.487 | 0.476 | - | - | 0.599 | 0.436 |
| 8/23/2011 | 0.382 | 0.621 | 1.169 | - | 0.549 | 0.54 |
| 9/6/2011 | 0.313 | - | 0.74 | - | 0.435 | 0.255 |
| 9/20/2011 | 0.231 | - | 0.656 | - | 0.451 | 0.321 |
| 10/4/2011 | 0.273 | - | 0.941 | - | 0.54 | 0.472 |
| 10/18/2011 | 0.435 | 0.843 | 1.683 | 0.387 | 0.786 | 0.814 |
| 11/1/2011 | 0.579 | 0.48 | 1.133 | 0.41 | 0.953 | 0.725 |
| 11/15/2011 | - | - | 0.695 | - | 0.485 | 0.355 |
| 11/29/2011 | - | 0.45 | - | 0.247 | 0.484 | 0.323 |
| 2/7/2012 | - | - | - | 0.827 | 0.354 | 0.161 |

## Conclusions

As stated earlier, the purpose of this report is to provide an overview of the results from the initial phase of this sampling effort. Initial results of ATBs suggest the presence of a constituent(s) in the source water that inhibits the growth of the algae *P. subcapitata*. This is consistent with the finding of the HydroMentia Report that algal growth inhibition was occurring, which may have reduced nutrient uptake & adversely affected project performance. The HydroMentia Report hypothesized that inhibited periphyton growth was correlated to runoff patterns. Although this study has insufficient data to make correlations between flow and IC25 values, an increase in algal growth inhibition occurred at all sites following a spike in flows from regional wide heavy rainfall in October 2011.

At this time, a cause of the reduced biological response of *P. subcapitata* in the source water has not been identified nor linked to any activity in the basin. Drought conditions in the watershed hindered the consistent collection of additional samples which would be necessary to further define the spatial and temporal extent of the affected source water and identify a potential cause. It should also be noted that samples collected over the last several years for pesticides and other toxicants in surface water and in large and small bodied fish, as a requirement of the Taylor Creek STA Operational Permit (0194485-001-GL),

showed no exceedances of state water quality standards (SFWMD 2012) or of critical tissue benchmarks for pesticides (SFWMD 2011b). Surface water samples, sediments, large and small fish were collected upstream and downstream of the STA and within both cells of the STA. Tests within the STA were also performed to determine if algal growth was being inhibited once HydroMentia reported the performance problems at the ATS located upstream of the STA. The tests resulted in successful growth of algae in specific locations of the STA. Therefore, the coordinating agencies will continue to assess the performance of the STAs and other nutrient reduction projects in the watershed which utilize algal uptake as a nutrient reduction method and will periodically monitor during storm events.

- ***Literature Cited***


Adams, N., S., Goulding, K.H., and A. J. Dobbs. 1985. Toxicity of Eight Water-Soluable Organic Chemicals to Selenastrum capricornutum: A Study of Methods for Calculating Toxic Values Using Different Growth Parameters. *Archives of Environmental Contamination and Toxicology* 14: 333-345.

Chao M. R. and C. Y. Chen, 1999. No-Observed Effect Concentrations in Batch and Continuous Algal Toxicity Tests. *Environmental Toxicology* and Chemistry Vol. 19, No. 6, pp: 1589-1596, 2000.

Hydromentia, Inc., 2010. Taylor Creek Algal Turf Scrubber® Nutrient Recovery Facility Performance and Toxicity Investigation Summary Report. 82 p.

Miller, W. E., T. E. Maloney, and J. C. Greene. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA-600/9-78-018. U. S. EPA, Cincinnati, Ohio. 126 p.

SFWMD, 2011a. Field Sampling Quality Manual. SFWMD-FIELD-QM-001-07. South FloridaWater Management District, West Palm Beach, FL.

SFWMD, 2011b. Justification for Terminating Pesticide Monitoring for the Taylor Creek Stormwater Treatment Area. April 2011. FDEP Permit #0194485-008-GL.

SFWMD, 2012. South Florida Environmental Report Volume III Appendix 4-2 Available on-line at: SFWMD.gov.

## Appendix A. Culture Medium Nutrients

TABLE 1. NUTRIENT STOCK SOLUTIONS FOR MAINTAINING ALGAL STOCK CULTURES AND TEST CONTROL CULTURES

| STOCK SOLUTION | COMPOUND | AMOUNT DISSOLVED IN 500 mL MILLI-Q® WATER | |
|---|---|---|---|
| 1. MACRONUTRIENTS | | | |
| A. | $MgCl_2 \cdot 6H_2O$ | 6.08 | g |
| | $CaCl_2 \cdot 2H_2O$ | 2.20 | g |
| | $NaNO_3$ | 12.75 | g |
| B. | $MgSO_4 \cdot 7H_2O$ | 7.35 | g |
| C | $K_2HPO_4$ | 0.522 | g |
| D. | $NaHCO_3$ | 7.50 | g |
| 2. MICRONUTRIENTS | | | |
| | $H_3BO_3$ | 92.8 | mg |
| | $MnCl_2 \cdot 4H_2O$ | 208.0 | mg |
| | $ZnCl_2$ | 1.64 | mg[1] |
| | $FeCl_3 \cdot 6H_2O$ | 79.9 | mg |
| | $CoCl_2 \cdot 6H_2O$ | 0.714 | mg[2] |
| | $Na_2MoO_4 \cdot 2H_2O$ | 3.63 | mg[3] |
| | $CuCl_2 \cdot 2H_2O$ | 0.006 | mg[4] |
| | $Na_2EDTA \cdot 2H_2O$ | 150.0 | mg |
| | $Na_2SeO_4$ | 1.196 | mg[5] |

1 $ZnCl_2$ - Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to Stock 2, micronutrients.
2 $CoCl_2 \cdot 6H_2O$ - Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to Stock 2, micronutrients.
3 $Na_2MoO_4 \cdot 2H_2O$ - Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to Stock 2, micronutrients.
4 $CuCl_2 \cdot 2H_2O$ - Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to Stock 2, micronutrients.
5 $Na_2SeO_4$ - Weigh out 119.6 mg and dilute to 100 mL. Add 1 mL of this solution to Stock 2, micronutrients.

**Appendix A. Continued**

TABLE 2. FINAL CONCENTRATION OF MACRONUTRIENTS AND MICRONUTRIENTS IN THE CULTURE MEDIUM

| MACRONUTRIENT | CONCENTRATION (mg/L) | ELEMENT | CONCENTRATION (mg/L) |
|---|---|---|---|
| $NaNO_3$ | 25.5 | N | 4.20 |
| $MgCl_2 \cdot 6H_2O$ | 12.2 | Mg | 2.90 |
| $CaCl_2 \cdot 2H_2O$ | 4.41 | Ca | 1.20 |
| $MgSO_4 \cdot 7H_2O$ | 14.7 | S | 1.91 |
| $K_2HPO_4$ | 1.04 | P | 0.186 |
| $NaHCO_3$ | 15.0 | Na | 11.0 |
| | | K | 0.469 |
| | | C | 2.14 |

| MICRONUTRIENT | CONCENTRATION (µg/L) | ELEMENT | CONCENTRATION (µg/L) |
|---|---|---|---|
| $H_3BO_3$ | 185.0 | B | 32.5 |
| $MnCl_2 \cdot 4H_2O$ | 416.0 | Mn | 115.0 |
| $ZnCl_2$ | 3.27 | Zn | 1.57 |
| $CoCl_2 \cdot 6H_2O$ | 1.43 | Co | 0.354 |
| $CuCl_2 \cdot 2H_2O$ | 0.012 | Cu | 0.004 |
| $Na_2MoO_4 \cdot 2H_2O$ | 7.26 | Mo | 2.88 |
| $FeCl_3 \cdot 6H_2O$ | 160.0 | Fe | 33.1 |
| $Na_2EDTA \cdot 2H_2O$ | 300.0 | -- | ---- |
| $Na_2SeO_4$ | 2.39 | Se | 0.91 |