



# Taylor Creek Algal Turf Scrubber® Nutrient Recovery Facility

## Performance and Toxicity Investigation Summary Report

January 22, 2007 through January 18, 2010

Prepared for:



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## EXECUTIVE SUMMARY

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The Taylor Creek Algal Turf Scrubber® (TC-ATS) nutrient recovery facility, located within the Taylor Creek-Nubbin Slough basin tributary to and north of Lake Okeechobee, in Okeechobee County, Florida, was designed and constructed by HydroMentia, Inc. to reduce nutrients from a flow of 15 million gallons per day (MGD).

The design and layout of the TC-ATS facility, which is summarized within a Basis of Design<sup>1</sup> report submitted on March 17, 2006, was established from data collected, compiled and evaluated from nearly two years of previous operations of a full scale demonstration project (S-154 pilot study) located in the S-154 basin, to the west and contiguous to the Taylor Creek basin. The final report developed from data collected during the S-154 pilot study documented that the Algal Turf Scrubber® (ATS™) technology operated in the S-154 basin could remove phosphorus at an areal removal rate (ARR) of over 90 g/m<sup>2</sup>-year (802 lb/acre-yr) – or 20 to 40 times greater than a typical treatment wetland.<sup>2</sup>

Upon substantial completion of construction in January 2007, full operation of the TC-ATS facility was initiated on January 22, 2007. The TC-ATS was operated by HydroMentia from January 22, 2007 through January 18, 2010. During this period, data was collected and compiled by HydroMentia. The contract between HydroMentia and SFWMD for the Taylor Creek ATS™ facility ended February 1, 2010.

### PERFORMANCE RESULTS

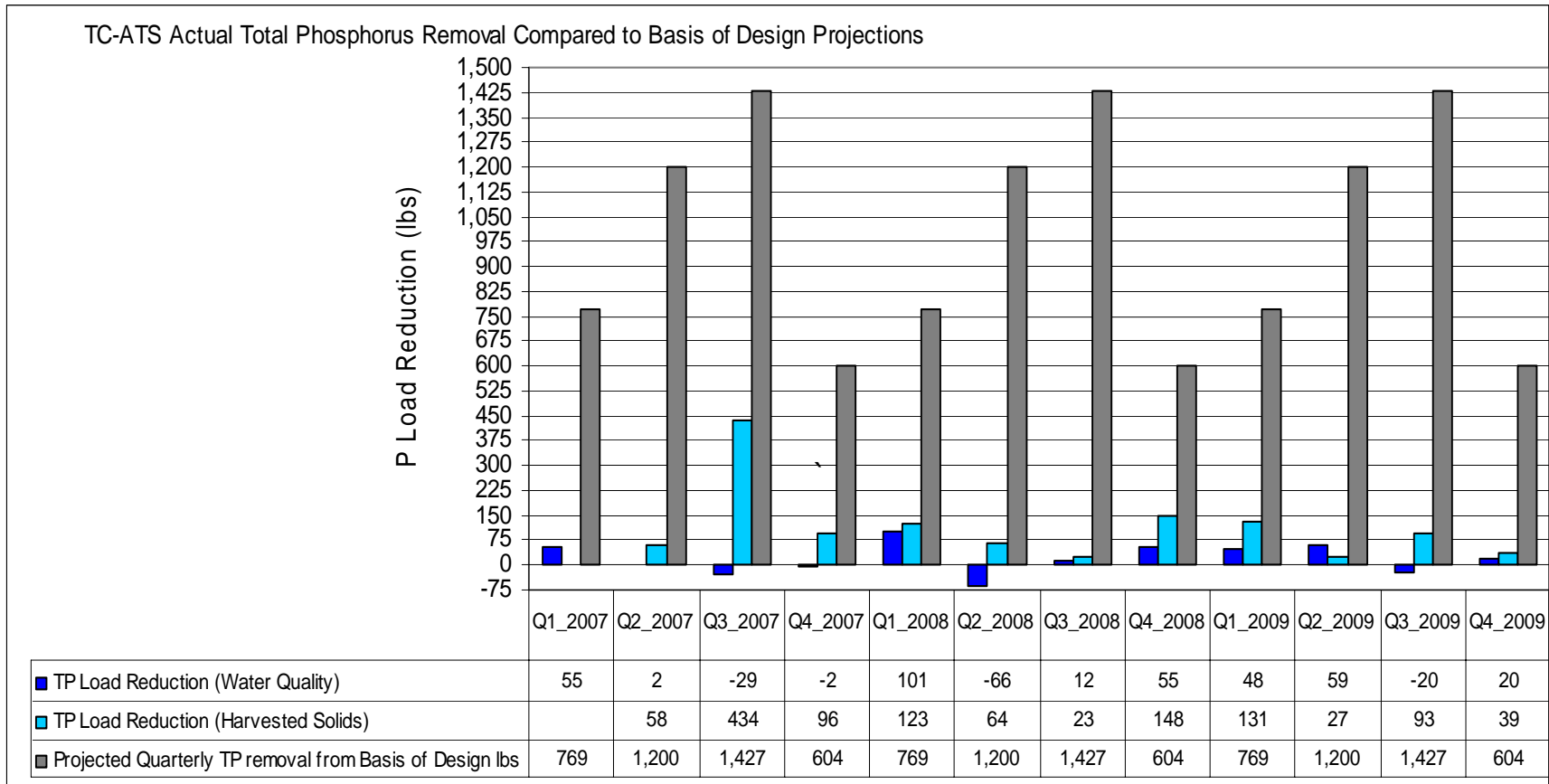
During the three-year operational period, system performance fell well below the projections included within the Basis of Design report. The system performance in terms of nutrient removal for the operational period ranged from just over 10% to less than 1% of the projected performance, depending upon the method of calculation (Table ES-1 and Figures ES-3 through ES-5).

**Table ES-1:** TC-ATS three-year system performance compared to basis of design projections.

Parameter	Basis of Design Projections	Actual Based upon Algal Harvest	Actual Based upon Water Quality and Flows
Total Phosphorus Removed (lbs)	12,000	1,236	236
Total Nitrogen Removed (lbs)	51,258	6,756	425
Algal Harvest (dry pounds)	910,000	329,422	NA

<sup>1</sup> Taylor Creek 15 MGD Algal Turf Scrubber® (ATS™) Basis of Design (2006). prepared by HydroMentia, Inc. for South Florida Water Management District and the Florida Department of Agriculture and Consumer Services

<sup>2</sup> S-154 Single Stage Algal Turf Scrubber® (ATS™) Final Report HydroMentia, Inc. March 2005. SFWMD Contract C-13933.



**Figure ES-3:** TC-ATS quarterly total phosphorus mass removal for three-year operational period compared to basis of design projections.

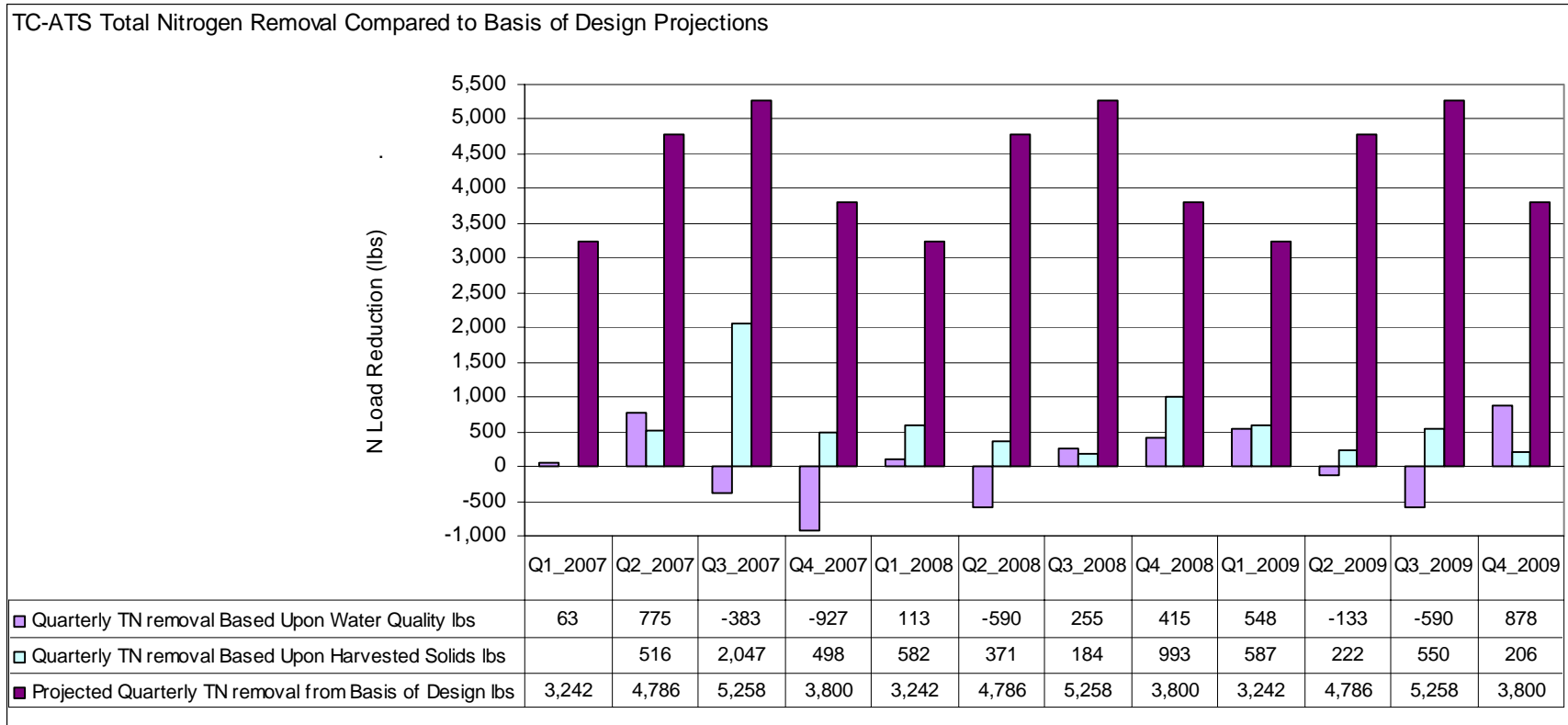
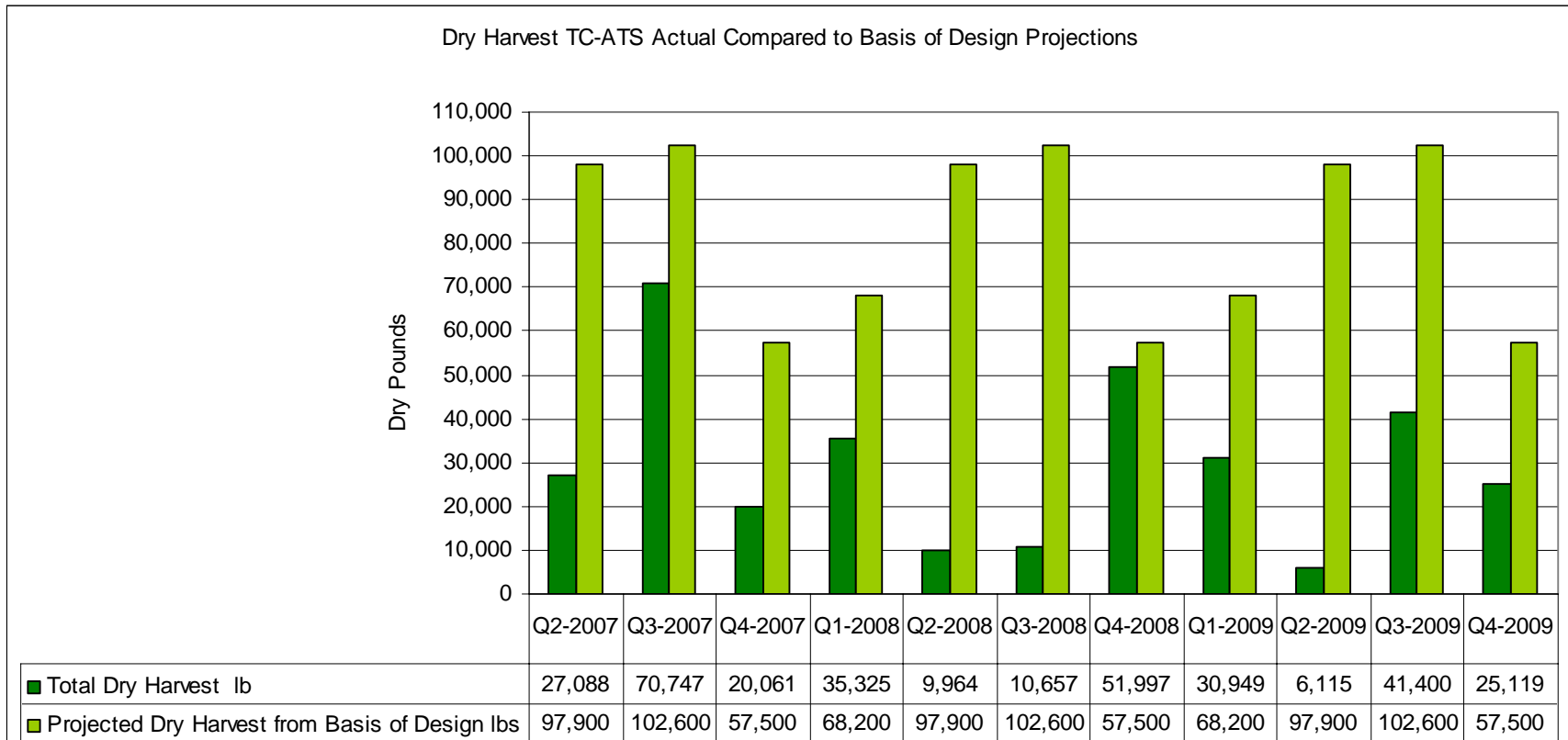


Figure ES-4: TC-ATS quarterly total nitrogen mass removal for three-year operational period compared to basis of design projections.



**Figure ES-5:** TC-ATS quarterly dry harvest mass removal for three-year operational period compared to basis of design projections.

## PERFORMANCE INVESTIGATIONS

Efforts were initiated to determine the cause(s) of the poor performance of the system. By the second quarter of the first year of operation (2007) an investigation strategy was established and implemented. The major issues considered and evaluated were

- Physical Operational and Design Issues
  - Improper Hydraulic Loading Rate
  - Improper Slope
  - Improper Surge Cycle
  - Improper Grid Design and/or Material
- Biological/Chemical Environmental Factors
  - Growth Factor Deficiencies
  - Grazing Impacts
  - Inhibitory/Toxic Materials
    - Anthropogenic
    - Naturally Occurring
  - External Nutrient Sources
  - Excessive Evapotranspiration
- Analytical/Sampling Error
  - Analytical Methods Inaccurate
  - Laboratory Error
  - Contamination during Handling of Samples

## TOXICITY INVESTIGATIONS

Initial evaluations revealed no substantial evidence related to physical operational and design issues, or analytical/sampling error. In addition there appeared to be no discernible effects from growth factor deficiencies, grazing impacts, external nutrient sources, or excessive evapotranspiration.

However, it was noted that a toxic influence was noted which was removed by activated carbon. Subsequent water analyses were then conducted to determine the extent and nature of the involved inhibitory/toxic compound(s).

- Gas Chromatography and Mass Spectrometry by the University of Florida and the Florida Department of Agriculture and Consumer Services showed comparatively large spikes which resembled signatures associated with organic solvent type compounds such as dipropylene glycol—a common surfactant/adjuvant and solvent used in agriculture.
- Formal toxicity testing by an Environmental Toxicology Laboratory in Davis, California—AQUA-Science—using EPA's Toxicity Identification Evaluations or TIE procedure applied to green algae (EPA Protocol Cf4-EPA-821-R-02-013—Chronic Algae Toxicity Evaluation), found toxicity within the Taylor Creek source water, which was concentrated within the associated foam.
- Screening tests and follow-up detailed analysis of the foam using both positive and negative electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) mass spectrometry revealed a number of surfactant type organic compounds of varying molecular weights, some of which overlapped with masses of common anionic sulfonate-alkyl chain surfactants (8-10 carbons).
- Field data showed a direct relationship between runoff rates and toxicity as manifested as productivity loss on the ATS™.

- Upstream tributaries were tested using in-situ periphytometers, and laboratory bioassays. The results showed the tributaries in the northwest reaches of the basin, roughly in the vicinity of pasture, citrus, and potato farming, tested as the most toxic.
- Analytical work completed by the USGS laboratory in Lawrenceville, Kansas revealed low levels of metabolites associated with the herbicide Metolachlor.

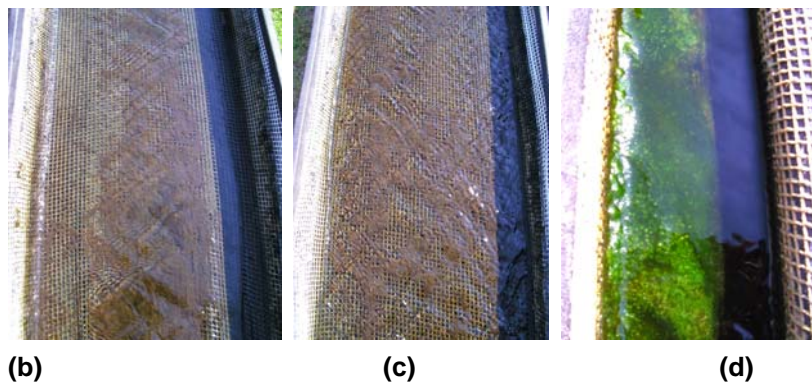
## TOXICITY ATTENUATION INVESTIGATIONS

Complementary to efforts associated with identification of the toxin(s), several investigations were completed regarding the ability to attenuate or eliminate the deleterious impacts of the toxin(s). Specifically, these evaluations revealed the following:

- **Foam Fractionation**—Selected because of TIE testing which indicated aeration removed the toxin(s), and the possibility of surfactants contributing to toxicity. Foam fractionation however was ineffective in mitigating for the toxin(s).
- **Water Hyacinth Scrubber (WHS™)**— With a two-day detention period, the WHS™ removed the deleterious impacts of the toxin. Noted in Illustration ES-1 are comparative photographs.
- **STA type wetlands**—Deleterious impacts of toxin(s) were removed from water retained for several weeks within the Taylor Creek Stormwater Treatment Area (STA).
- **Alum precipitation**—There was some indication that the deleterious impacts of toxin(s) were attenuated within Taylor Creek water pre-treated with Alum.



(a)



**Illustration ES-1:** Pre-treatment with a Water Hyacinth Scrubber (WHS™) as seen in (a). Algal Turf comparative development control (b); pretreatment with foam fractionation (c); and pretreatment with WHS™ (d).

### PRIMARY FINDINGS

Over the three-year period of operation of the Taylor Creek ATS™ facility, efforts to identify and mitigate for the compound(s) and factor(s) associated with the system's below projection performance and algal turf productivity resulted in the following primary findings.

1. Waters associated with Taylor Creek in the vicinity of the TC-ATS facility pump intake were intermittently toxic as determined by EPA's TIE methodology and as verified by in-field evidence.
2. The intermittent toxicity resulted in significantly reduced treatment performance and periphyton growth and periodic algal die-offs.
3. The TIE testing showed toxicity associated with this water was not associated with heavy metals.
4. Toxic compound(s) within this Taylor Creek water accumulated within foam generated from the agitation of this water.
5. Treatment of this water with activated carbon significantly attenuated the deleterious impacts of the toxin(s) associated with this water.
6. Treatment of this water through a water hyacinth scrubber designed for a two-day detention period could significantly attenuate the deleterious impacts of the toxin(s) associated with this water.
7. Treatment of this water through extended detention within a passive wetland system (STA) could significantly attenuate the deleterious impacts of the toxin(s) associated with this water.

## RECOMMENDATIONS

Based on the findings and evidence associated with the Taylor Creek Algal Turf Scrubber® nutrient recovery project, the following program is recommended.

### *Toxicity Identification*

1. Develop a geographic toxicity testing and sampling program to narrow the source of the toxicity.
2. Through analytical means as described herein and additional means as required, including but not necessarily limited to in-field periphytometers; static bioassays; TIE; GC-MS; NMR; ESI-MALDI; and extraction, isolation and bioassay verification, establish the specific identification and source of the compound(s) responsible for toxicity. Such analytical efforts shall include both water and sediment samples from Taylor Creek; and any water treatment systems employed within the Taylor Creek basin.
3. Once the toxin(s) is identified, assess the past and potential future impacts upon Taylor Creek and Lake Okeechobee.

### *Toxicity Treatment Evaluation*

1. Once the toxin(s) have been identified, coordinate with the applicable agencies including the Florida Department of Agriculture and Consumer Services so that efforts can be made to eliminate or reduce toxic impacts to periphyton within the Taylor Creek watershed.
2. Once the toxin(s) are identified and the source of contamination is removed from the watershed, evaluation of the ATS™ technology could be conducted if desired phosphorus load reductions have not been met within the basin.
3. Evaluate all treatment systems examined for reduction of phosphorus including their respective capabilities in eliminating over a long term, the deleterious effects of the identified toxin(s), and their cost effectiveness based upon a fifty years present worth evaluation, evaluate all systems to establish a reasonable basin wide plan for reduction of phosphorus and elimination of toxicity.
4. If identification and elimination of the toxin is not pursued, it is recommended that pilot testing a periphyton based ATS™ be employed to include pre-treatment via STA, Alum and/or WHS systems. The ATS™ pilot units will serve as long-term bioassays of the STA, Alum and WHS effluent and the Taylor Creek influent.

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## SECTION 1. INTRODUCTION

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### PROJECT BACKGROUND - S154 BASIN PILOT ALGAL TURF SCRUBBER® -

From January 27, 2003 to December 4, 2004 HydroMentia, Inc. under contract with the South Florida Water Management District (SFWMD contract C-13933), and in cooperation with the Florida Department of Environmental Protection (FDEP), and the Florida Department of Agriculture and Consumer Services (FDACS), operated a pilot facility within the S-154 basin (S-154 pilot study), located directly west of the Taylor Creek basin, just north of Lake Okeechobee and west of the City of Okeechobee in Okeechobee County, Florida. This pilot project was initiated with the intent of investigating the efficacy of two Managed Aquatic Plant Systems (MAPS) in removing and recovering nutrients from impaired surface waters—with total phosphorus as the primary target. The two MAPS technologies investigated were the Water Hyacinth Scrubber or WHS™ and the Algal Turf Scrubber® (ATS™).

Initially, for the first three quarters of the S-154 pilot study (January 27, 2003 to November 3, 2004) it was operated as a two-stage facility, with a 2.50 acre WHS™ serving as the first stage process, followed by a second stage, 2.05 acre ATS™, with flows at just under 0.50 million gallons per day (MGD). The goal for this initial period was the reduction of total phosphorus concentration to the extent practical (Concentration Reduction Optimization Mode), recognizing the Total Maximum Daily Load (TMDL) target of 40 µg/L total phosphorus concentration. During these three quarters, total phosphorus was removed at the areal removal rate (ARR) of 12.76 g/m<sup>2</sup>-yr, with the total phosphorus concentration reduced by 83.4% from 476 µg/L to 79 µg/L. Of this removal, 73% was provided by the first stage WHS™ and 27% by the second stage ATS™.

From November 3, 2003 through October 18, 2004, the S-154 pilot two-stage system was operated in a Nutrient Load Optimization Mode—the intent being to increase removal efficiency by reducing process area, while increasing hydraulic loading rates, and increasing phosphorus ARR. This would help to meet the total phosphorus load removal requirements associated with the Lake Okeechobee TMDL. During this period, the combined process area was reduced to 1.25 acres of WHS™ followed by 0.25 acres of ATS™, with a daily flow of 0.739 MGD. At this reduced area and increased flow, the system averaged a phosphorus ARR of 19.30 g/m<sup>2</sup>-yr, with 39% mass reduction of total phosphorus. The WHS™ provided a total phosphorus ARR of 15.16 g/m<sup>2</sup>-yr, while the ATS™ provided a total phosphorus ARR of 40.38 g/m<sup>2</sup>-yr.

Because the ATS™ technology required less area per unit mass removed than the WHS™ and significantly less area per unit mass removed than passive wetland treatment units such as Stormwater Treatment Areas (STAs), three independent single-stage ATS™ flowways were established so as to determine the optimal ATS™ hydraulic loading rates necessary to achieve the maximum phosphorus areal removal rate for the S-154 Basin. Each single-stage ATS™ flowway had a length of 300 feet and a width of five feet. Each was operated at different linear hydraulic loading rates (LHLR)<sup>3</sup> - South flowway 4.7 gpm/ft; North flowway 8.5 gpm/ft; and Central flowway 18.8 gpm/ft. Total phosphorus ARR was directly related to LHLR, indicating that algal uptake efficiency was enhanced by increased water velocity. The total phosphorus ARR associated with the three flowways over the operational period from May 11, 2004 to December 5, 2004 was 25 g/m<sup>2</sup>-yr; 47 g/m<sup>2</sup>-yr; and 92 g/m<sup>2</sup>-yr for the South, North and Central flowways, respectively. The percent total phosphorus removals were 23.08% for the Central flowway; 24.03% for the South flowway; and 24.85% for the North flowway. Influent total phosphorus concentration to all three flowways was 336 µg/L, with effluent total phosphorus concentrations at 250 µg/L for the South flowway; 249 µg/L for the North flowway and 258 µg/L for the Central flowway.

Based upon data collected from the single stage ATS™ study, a design model based upon first order kinetics was developed. Total phosphorus concentration and LHLR were determined to be the most influential parameters in determining community specific growth rate. This Algal Turf Scrubber® Design

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<sup>3</sup> Linear hydraulic loading rate or LHLR is defined as the flow in gallons per minute (GPM) for each foot of ATS™ width, or gpm/ft

Model, or ATSDem, was applied to water quality conditions associated with the contiguous Taylor Creek basin, a major contributor of phosphorus to Lake Okeechobee.

### **FULL SCALE ALGAL TURF SCRUBBER® - TAYLOR CREEK BASIN**

As both the Taylor Creek and S-154 basins are largely in agricultural land use, and the water quality conditions appear very similar in terms of nutrients and organic and mineral content, it was assumed that the performance dynamics of a proposed Taylor Creek ATS™ would mimic those of the S-154 pilot study, and that additional pilot work on Taylor Creek was not necessary. Consequently, HydroMentia proceeded to develop a Basis of Design Report<sup>4</sup> applicable to a proposed full scale ATS™ facility located within the Taylor Creek basin. Based upon the Taylor Creek water quality conditions, it was calculated that the average annual total phosphorus ARR would exceed 90 g/m<sup>2</sup>-yr, and the annual average productivity in terms of dry biomass generated per unit time would exceed 40 g/m<sup>2</sup>-day.

Following submittal of the Basis of Design Report to the District, and revision and re-submittal of the Report in response to comments by the District, HydroMentia designed and constructed a 15 MGD, 3.54 acre ATS™ facility on Taylor Creek in fulfillment of contractual obligations to the South Florida Water Management District (SFWMD), as delineated within Contract # OT051045, executed and dated on August 15, 2005. Upon substantial completion of the Taylor Creek Algal Turf Scrubber® Nutrient Recovery Facility (TC-ATS) in January 2007, HydroMentia assumed operational responsibilities for the facility through contractual provisions included as Addendum #3600000952-A01 to Contract #OT051045 executed October 27, 2006. Full operation of the facility was initiated on January 22, 2007.

From January 22, 2007 through January 18, 2010 data were collected and compiled by HydroMentia. The contract between HydroMentia and SFWMD for the Taylor Creek ATS™ facility ended February 1, 2010. During the three-year operational period, system performance fell below projections. Ensuing investigations related to the cause(s) of this poor performance resulted in the following items:

- The discovery that Taylor Creek source water contains one or more compounds which render the water toxic to periphytic and epiphytic algae.
- An assessment of the general region within the upstream watershed from which these compound(s) are captured within the stormwater runoff which is released into Taylor Creek.
- Discovery through specialized chemical and biological analytical techniques of the general classification of compound(s) which may be associated with these toxic influences.
- Evaluation of reasonable pretreatment processes for the reduction of toxicity prior to release to the ATS™ process.
- Field testing and detailed analysis of an effective pretreatment program, including recommendations for further pilot level investigations in which an effective pretreatment would be applied.
- Discussion and recommendations related to the implications associated with the existing level of toxicity within the Taylor Creek water, and additional measures necessary to isolate and identify the involved compound(s), and to locate the source(s) of this compound(s).

This Final Report is arranged into two primary sections, Section 2 titled Data Review and Evaluation is an assessment of performance data related to the three years of operation of the Taylor Creek Algal Turf Scrubber® nutrient recovery facility (TC-ATS); and Section 3 titled Investigation into Inhibitory and Toxic Influences Impacting System Performance is a review of efforts associated with investigations related to

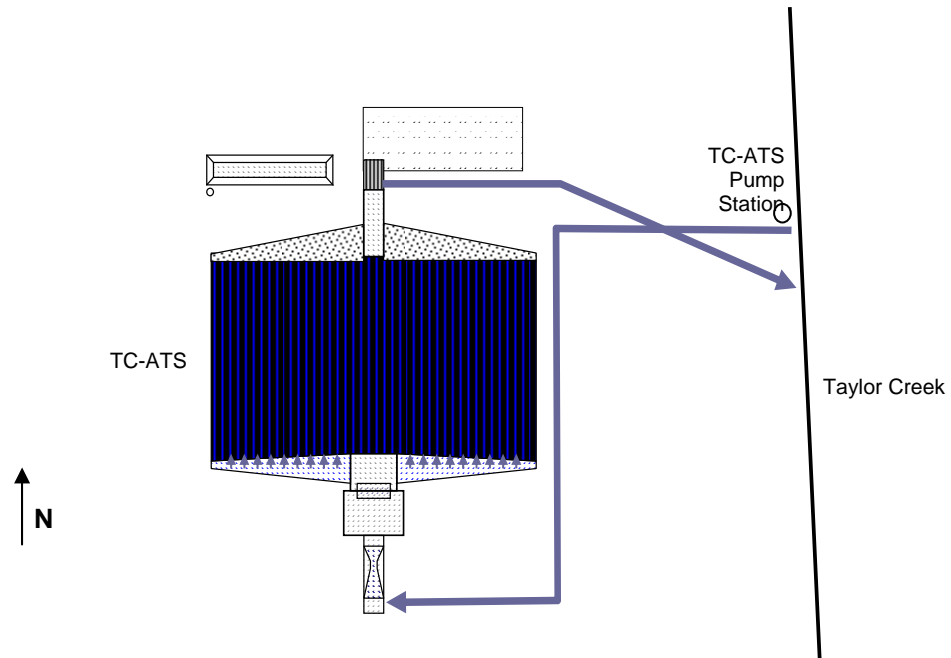
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<sup>4</sup> Taylor Creek 15 MGD Algal Turf Scrubber® (ATS™) Basis of Design, March 17, 2006. Prepared by HydroMentia, Inc. for SFWMD.

the low level of system performance over the operational period, which includes evaluations related to the discovery of toxicity within the Taylor Creek water.

### TAYLOR CREEK ATS™ FACILITY LAYOUT

The TC-ATS facility includes 3.6 acres of effective Algal Turf Scrubber® treatment area ( Figure 1, Illustration 1 to 8).



**Figure 1.** Illustration of TC-ATS (not to scale).

Water enters the TC-ATS via a pump station located on Taylor Creek about 2 miles north of the US 441 bridge in Okeechobee County, Florida. Inflow is discharged in pulsed flows equitably along the 521 foot width of the Algal Turf Scrubber® flowway. Water then gravity flows in a shallow laminar manner down the 300-foot treatment flowway. At the bottom of the flowway, treated water is collected and routed to a centralized biomass recovery station. Outflow from the TC-ATS returns to Taylor Creek via a gravity return line that discharges 400 feet south of the pump intake.



**Illustration 1:** Areal image of the TC-ATS facility looking northwest.



**Illustration 2:** Image of the 15-MGD mixed-flow pump station at the TC-ATS.



**Illustration 3:** Image of the TC-ATS inflow surge, distribution manifold and laterals and monitoring station.



**Illustration 4:** Image of the TC-ATS outflow and first stage solids recovery unit.



**Illustration 5:** Image of the TC-ATS Duperon FlexRake.



**Illustration 6:** Image of the TC-ATS outflow discharge to Taylor Creek.



**Illustration 7:** Image of harvested algae in April 2007 during initial start-up and period of extended drought.



**Illustration 8:** Image of TC-ATS facility composting operation.

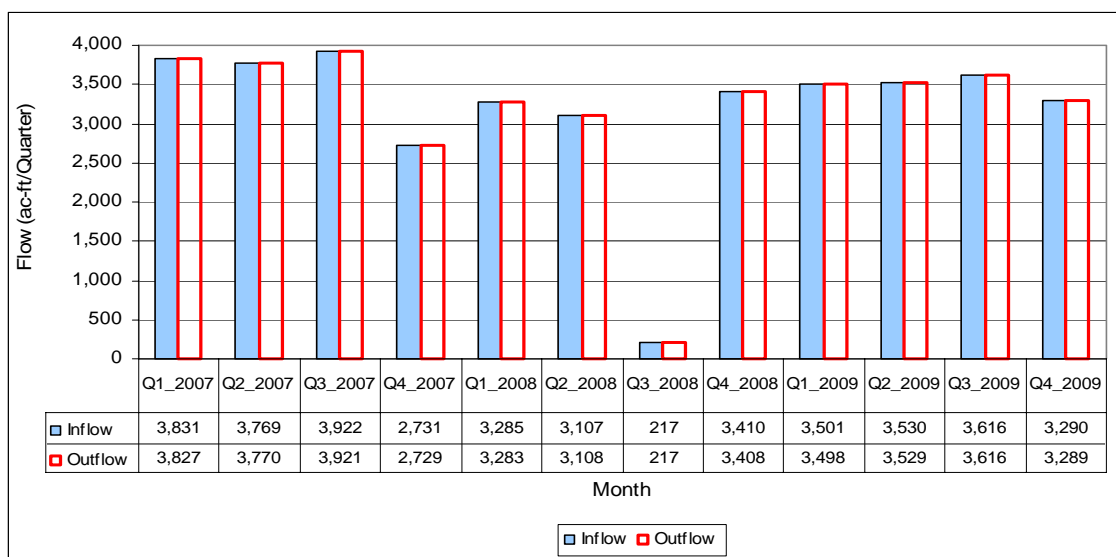
## SECTION 2. DATA REVIEW AND EVALUATION

This final data review covers the entire operational period from the week beginning January 22, 2007 to the week ending January 18, 2010. Included in this review is a summary of operational and performance data generated in conjunction with the approved Monitoring Plan to include analysis of nutrient load removal; biomass production, recovery, and quality; and compost volume and quality.

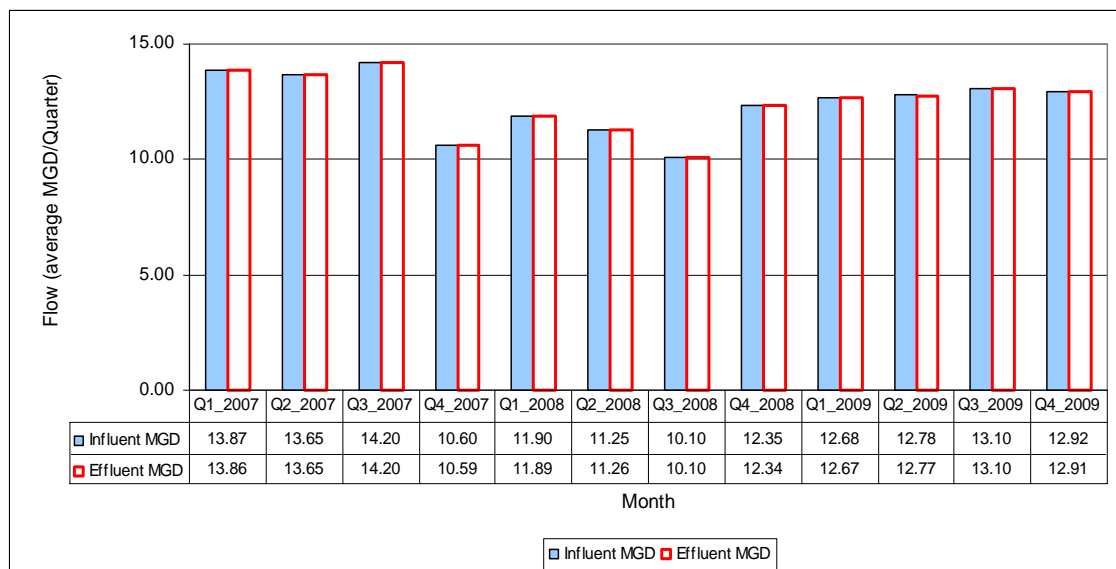
### OPERATIONAL DATA REVIEW

#### Flow

The system, originally designed for about 15 MGD, was adjusted during 2008 to about 12-13 MGD in an effort to enhance system performance (Figure 2). During Q3-2008, the pumps were removed for repair and the system was out of operation for over two months.



(a)

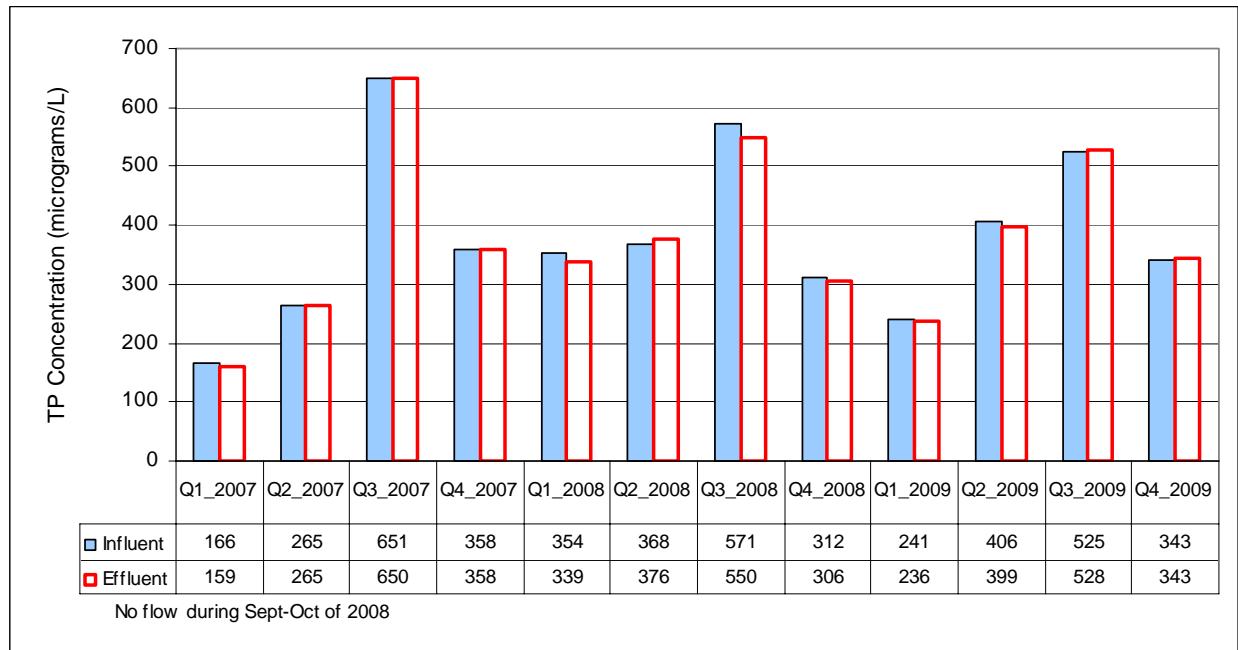


(b)

**Figure 2.** Summary of quarterly flows (a) total volume in acre-ft (b) average flow rate MGD.

## Phosphorus

Mean quarterly inflow and outflow total phosphorus (TP) concentrations for the operational period (as a seven-day composite) ranged from a low of 166 µg/L for first quarter of 2007 (Q1-2007), to a high of 651 µg/L for the third quarter of 2007 (Q3-2007), with an average influent for the operational period of 367 µg/L (Figure 3). Ortho phosphorus, as measured from grab samples, consistently accounted for more than 50% of the total phosphorus.



**Figure 3:** Quarterly mean inflow and outflow total phosphorus concentrations for operational period.

The total phosphorus mass removals for each quarter, based upon water quality and flow data (water quality based) and upon harvest data (harvested solids based) show considerable variability, and when evaluated using the t-test at 95% confidence levels, the means of the differences were found to be statistically different, with a two-tailed t-critical at 2.23, and the t-value determined at 2.32 (Figure 4). Divergence of these two mass removal calculations is clearly evident when shown as cumulative values (Figure 5). This trend is also reflected in the total phosphorus areal removal rates (ARR) for each quarter (Figures 6 and 7).

Mass removal based upon harvested solids is calculated as:

$$P_{mh} = (sH_w)p$$

Where  $P_{mh}$  = mass of phosphorus removed through harvesting  
 $s$  = solids content as fraction of wet harvest  
 $H_w$  = mass of wet harvest  
 $(sH_w)$  = mass of dry harvest  
 $p$  = tissue phosphorus content as fraction of dry harvest

Mass removal based upon water quality is calculated as:

$$P_{mw} = I_p Q_I - E_p Q_E$$

Where  $P_{mw}$  = mass of phosphorus removed based upon water quality

$I_p$  = influent total phosphorus concentration

$E_p$  = effluent total phosphorus concentration

$Q_I$  = influent totalized flow

$Q_E$  = effluent totalized flow

Phosphorus Areal Removal Rates are calculated using harvest data as:

$$R_{AP} = 365 * (P_{mh}/A t)$$

Where  $R_{AP}$  = harvest based areal removal rate as mass removed per unit process area over a year

$P_{mh}$  = mass of phosphorus removed through harvesting

$A$  = process area

$t$  = days since last harvest

Phosphorus areal removal rates therefore are calculated using water quality data as:

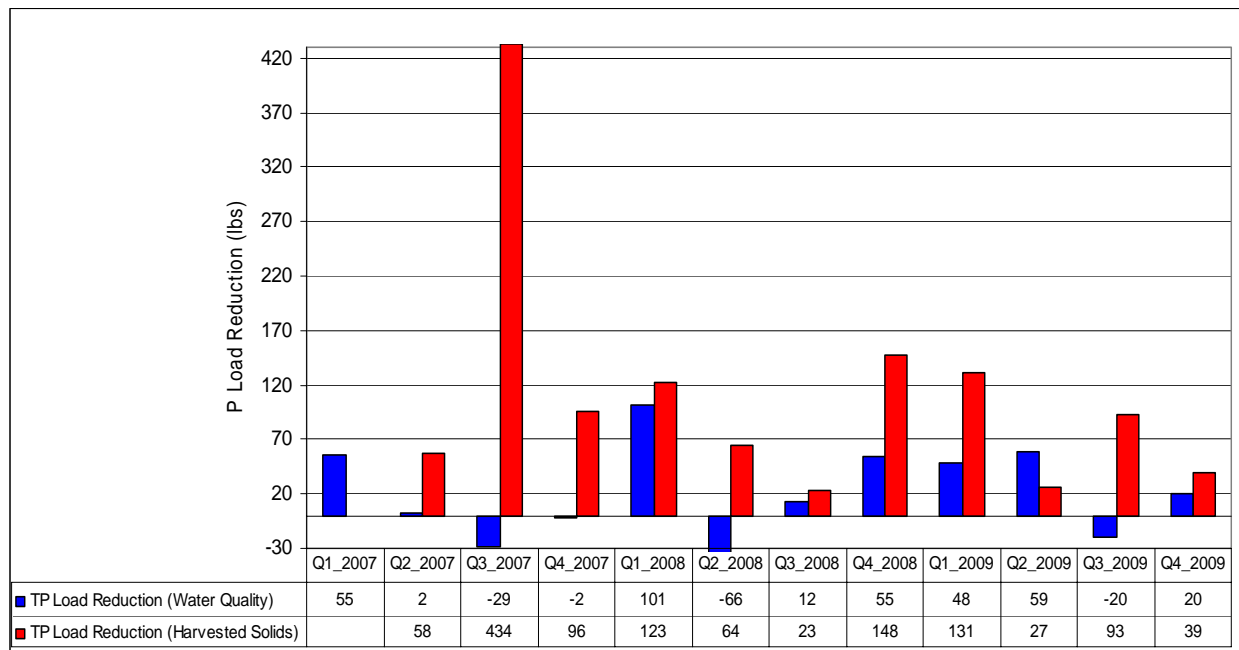
$$R_{AW} = 365 * (P_{mw}/A t)$$

Where  $R_{AW}$  = water quality based areal removal rate as mass removed per unit process area over a year

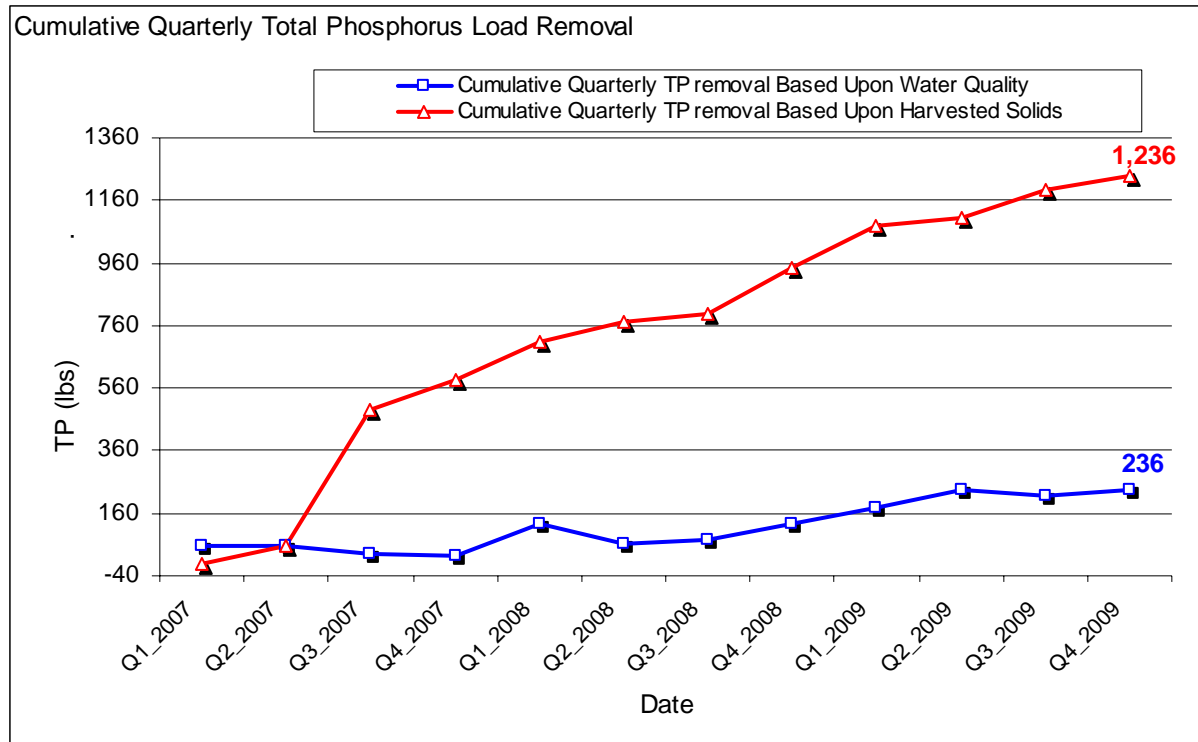
$P_{mw}$  = mass of phosphorus removed based upon water quality

$A$  = process area

$t$  = days since last sampling



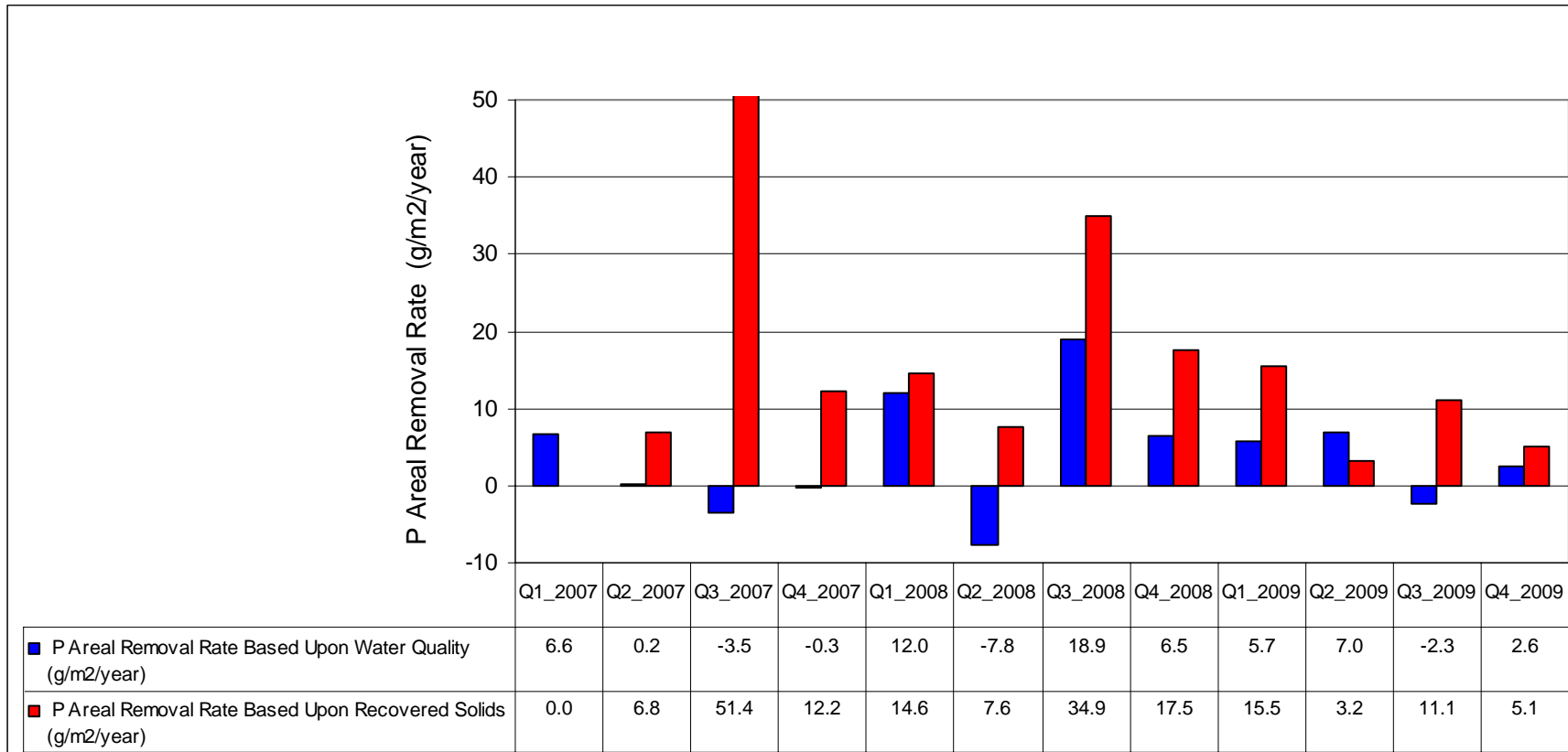
**Figure 4:** Quarterly mass removal total phosphorus based upon water quality calculations and harvest solids recovered for operational period.



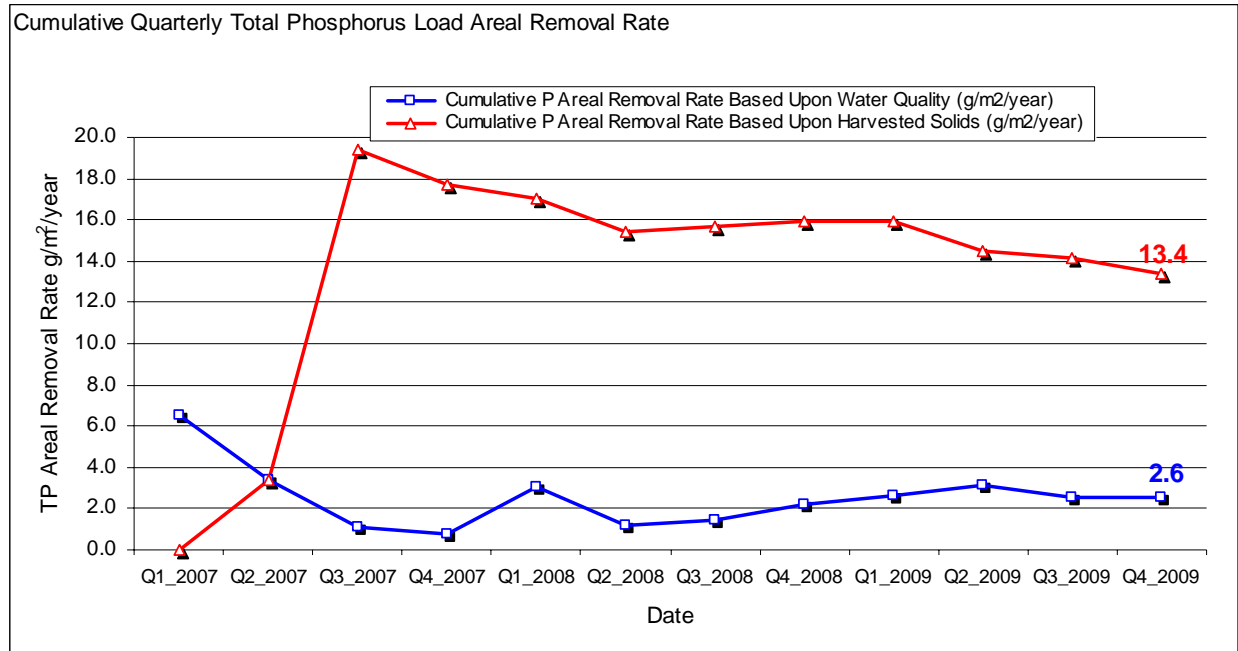
**Figure 5:** Cumulative quarterly total phosphorus mass removal rates based upon water quality and harvested solids recovery over operational period.

There is considerable disparity between the harvest based and water quality based calculations for total phosphorus mass and rate of removal (Figure 5). Typically, one would expect these numbers to more closely track one another. The fact that the harvest based calculations are much higher than the water quality based calculations generated some concern early in the operational period, and statistical review showed that these indeed were statistically different value sets. As discussed in Section 4, while efforts were made to try to explain this disparity, no satisfactory explanation was found, and the question remains whether this disparity is related to sampling error or a weakness in laboratory methodology—e.g. the digestion step required for total phosphorus determination.

Regardless of whether the water quality based calculation of 236 pounds total phosphorus removed over the operational period, or the harvest solids based calculation of 1,236 pounds total phosphorus removed were used, the total mass removal was well below the projection of approximately 12,000 pounds. Similarly the total phosphorus ARR for the operational period was 2.6 g/m<sup>2</sup>/year based upon water quality calculations and 13.4 g/m<sup>2</sup>/year—both well below the projection of over 90 g/m<sup>2</sup>/year. As previously mentioned in Section 1, and as discussed in detail within the upcoming section, this below expected performance relates to the presence of toxic factor(s) within the Taylor Creek influent.



**Figure 6:** Total phosphorus average quarterly areal removal rates as calculated from water quality and harvested solids data.



**Figure 7:** Total phosphorus cumulative quarterly areal removal rates as calculated from water quality and harvested solids data.

## Nitrogen

Mean quarterly inflow and outflow total nitrogen (TN) concentrations for the operational period (as a seven-day composite) ranged from a low of 1.17 mg/L for the first quarter of 2009 (Q1-2009), to a high of 2.41 mg/L for the third quarter of 2007 (Q3-2007), with an average influent for the operational period of 1.75 mg/L (Figure 8). The majority of the nitrogen was as Total Kjeldahl Nitrogen (TKN), with most of the TKN as organic nitrogen. Nitrate and ammonia levels typically represented less than 10% of the total nitrogen.

The TN mass removals for each quarter based upon water quality and flow data (water quality based) and upon harvest data (harvested solids based) show considerable variability, although less than noted for total phosphorus. When evaluated using the t-test at greater than 90% but less than 95% confidence levels, the means of the differences were found to be statistically different, with a two-tailed t-critical at 0.01 at 1.81, and the t-value determined at 2.11 (Figure 9). Divergence of these two mass removal calculations is clearly evident when shown as cumulative values (Figure 10). This trend is also reflected in the TN areal ARR for each quarter (Figures 10 and 11). Note that these values are presented as calculations based upon water quality and recovered harvested solids. The method of calculation is the same as that presented for total phosphorus.

As with phosphorus there was considerable disparity between the harvest based and water quality based calculations. Typically, one would expect these numbers to more closely track one another, although the fact that nitrogen can interact with the atmosphere through nitrogen fixation, denitrification, and ammonia volatilization, which can explain to a certain extent why the tracking is not as close as typically seen with phosphorus. As with phosphorus, the nitrogen removals based upon harvested solids were significantly higher than those based upon water quality calculations. In such cases, nitrogen fixation may be providing nitrogen to the algal turf, although the same concerns regarding possible sampling or analytical error as applied to phosphorus also pertain to nitrogen.

Regardless of whether the water quality based calculation of 425 pounds total nitrogen removed over the operational period, or the harvested solids based calculation of 6,756 pounds total nitrogen removed were used, the total mass removal was well below the projection of approximately 51,000 pounds. Similarly the TN ARR for the operational period was 4.6 g/m<sup>2</sup>/year based upon water quality calculations and 73.2 g/m<sup>2</sup>/year based upon the harvest solids—both well below the projection of about 418 g/m<sup>2</sup>/year. As previously mentioned in Section 1, and as discussed in detail within the upcoming section, this below expected performance relates to the presence of toxic factor(s) within the Taylor Creek influent.

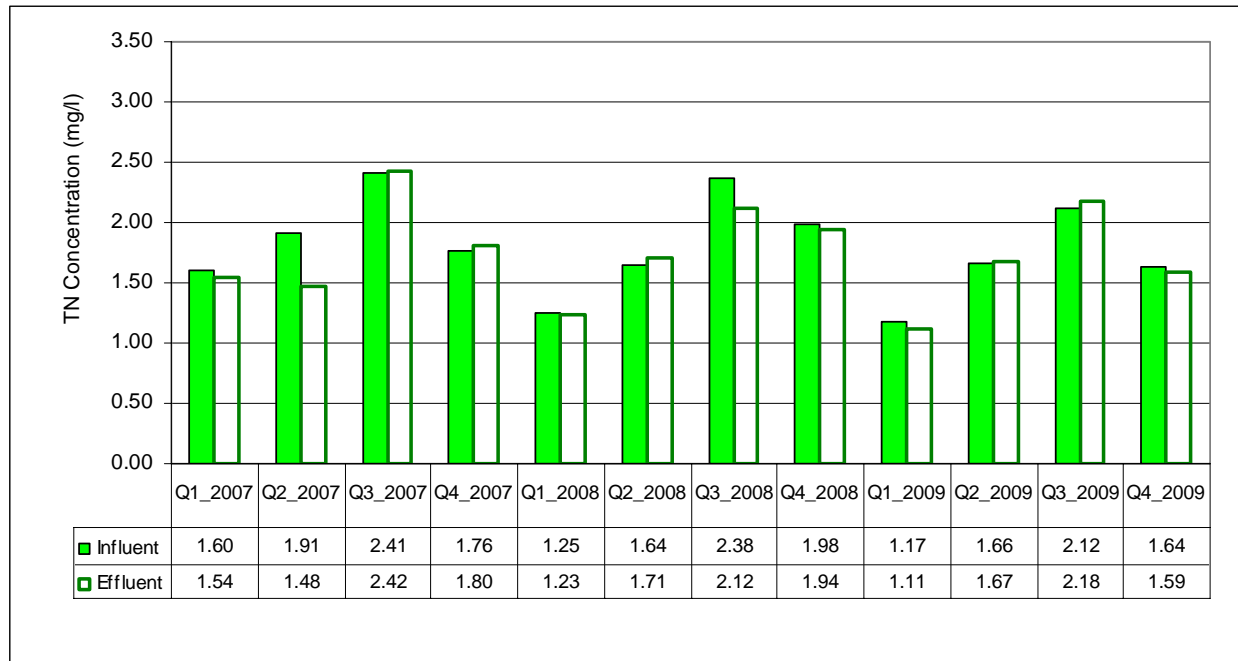
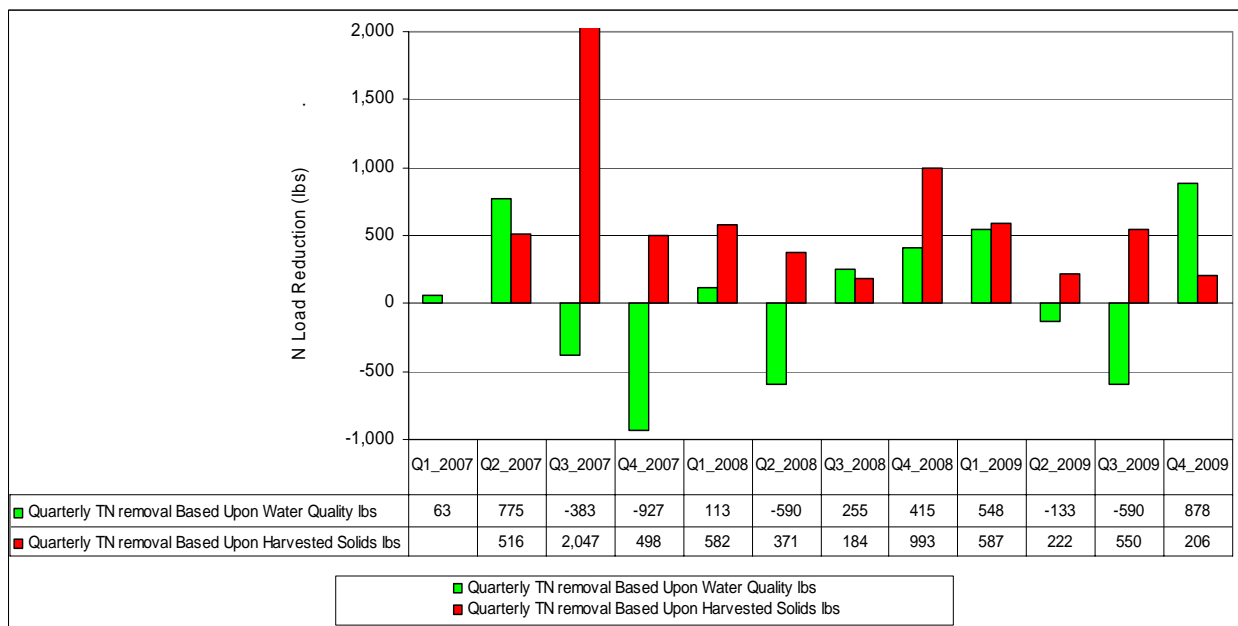
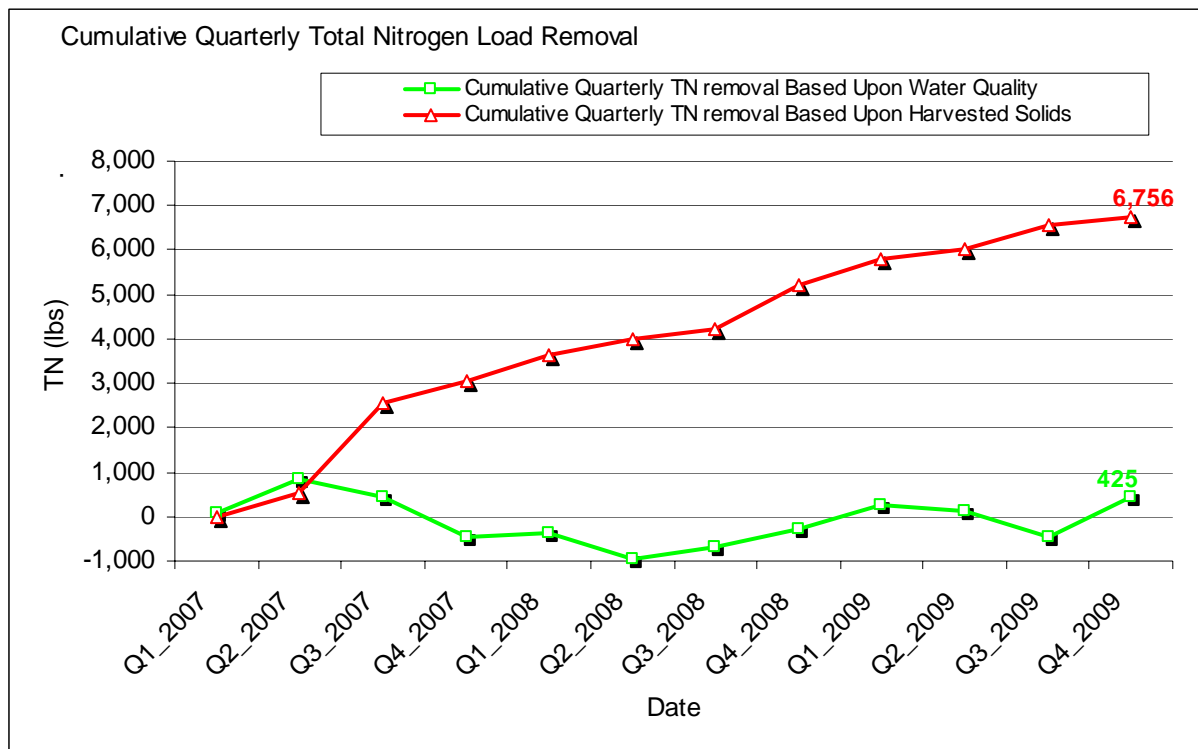


Figure 8: Quarterly mean inflow and outflow total nitrogen concentrations for operational period.



**Figure 9:** Quarterly mass removal total nitrogen based upon water quality calculations and harvest solids recovered for operational period.



**Figure 10:** Cumulative quarterly total nitrogen mass removal rates based upon water quality and harvested solids recovery over operational period.

### Other Water Quality Parameters

During the three years of operation, general water quality was monitored for the ATSTM influent and effluent (Table 1). The influent water from Taylor Creek is comparatively low in minerals, with total hardness of about 100 mg/L as CaCO<sub>3</sub>. The water is highly colored, and color was noted to increase with increase rainfall and nutrient levels, indicating color was likely associated with tannins and other complex, stable organic molecules which was contributed by both vegetation associated with native wetland areas and with runoff from grazed pastures and other agricultural activities. The color was noted to remain unchanged or to increase slightly through the ATSTM. Heavy metals were notably absent within the influent and effluent flows, although small concentrations of copper, zinc and selenium were documented. The observed concentrations were below any regulatory limits.

Taylor Creek water was typically low in biodegradable organics and total organic carbon, while typically being above 5.00 mg/L dissolved oxygen (DO). Conductivity was comparatively low, typically being somewhat higher during drought periods, and decreasing during periods of heavy runoff. Total alkalinity was also comparatively low, typically being below 100 mg/L as CaCO<sub>3</sub>. Influent pH levels were usually at or just above neutral.

A battery of organochlorine pesticides and herbicides showed undetectable levels, except on three occasions, when low levels of alpha and/or gamma chlordane were noted, and one event when low levels

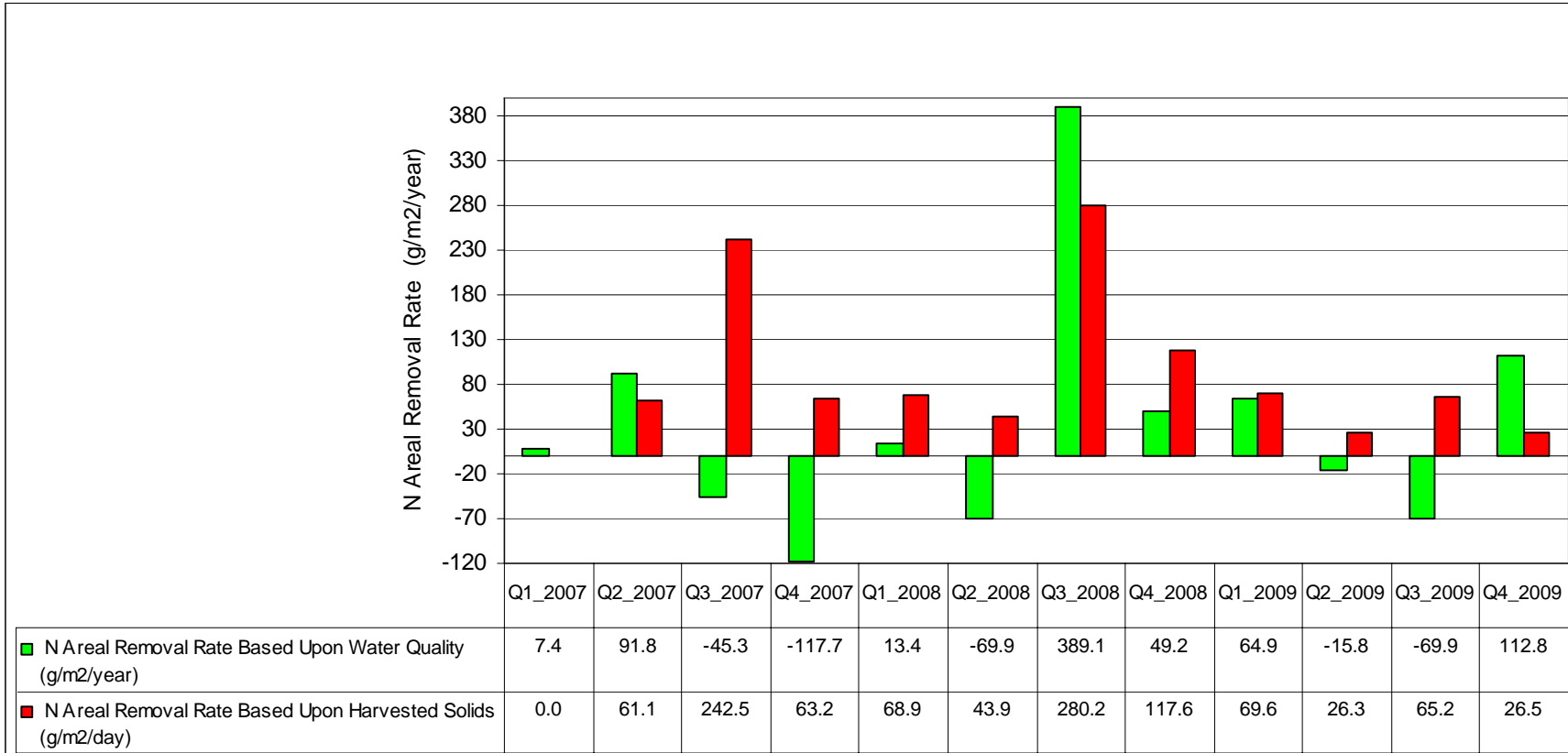
of 2,4-D was detected in the effluent. No organophosphorus pesticides were noted. Additionally, more detailed herbicide/pesticide analyses done as part of the toxicity investigation are presented in Section 3.

For the first two years of operation pH, DO, water temperature and conductivity were determined in the field using a handheld YSI unit (Table 1). The typical trend for daytime DO, is an increase from influent to effluent, as algal productivity generates oxygen, causing a DO increase within the effluent. The pH values also typically increase within the effluent as algal production consumes carbon dioxide while converting bi-carbonate and carbonate alkalinity to hydroxyl alkalinity.

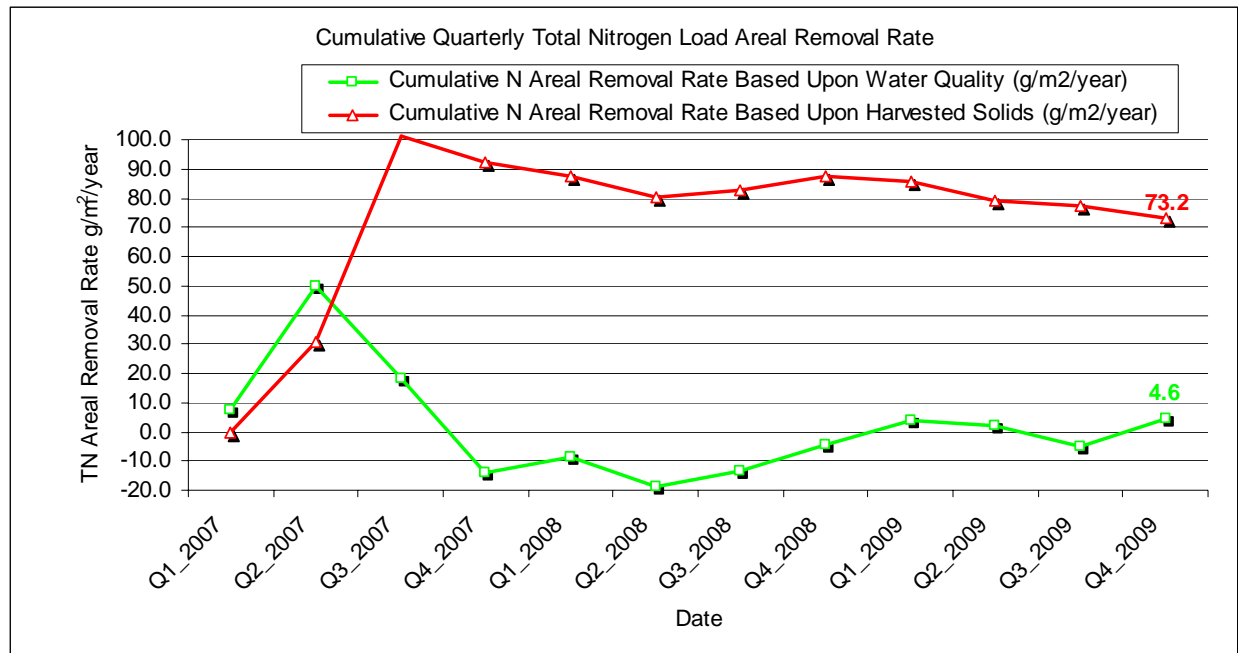
In addition, for portions of 2007 and 2008, a Sonde was placed in both the influent and effluent to facilitate time active in-situ monitoring of pH, DO, water temperature, and conductivity (Figures 13 through 16). The Sonde data revealed the typical fluctuations for pH, DO, and water temperature, with pH and DO increasing during the daytime during photosynthesis and dropping at night. The fact that the influent pH tracks the effluent pH to some extent (Figure 14), indicated there may be some recycling of effluent to the pump intake, and this is most noticeable during drought conditions, when there is no flow within this segment of Taylor Creek [Figure 14(a)]. It is also possible that increased influent pH during the day is attributable to photosynthesis in the creek. This is however, unlikely, as similar studies done in S-154 (L-62), in which plant growth was much more abundant, revealed little diurnal pH fluctuation in the canal.

Water temperature (Figure 15) showed typical increases during the warm months, and decrease during the winter months, which is as would be expected. As with pH, the influent diurnal pattern tracked the effluent pattern to some extent, unlike the S-154 pattern, in which canal (influent) water temperature remained rather constant through the diurnal cycle. The tracking of influent and effluent trends indicates there was some effluent recycling to the pumping station.

Conductivity patterns (Figure 16) are not consistent with S-154 patterns, as the effluent levels are consistently lower than the influent patterns from the Sonde data. The handheld data does not reflect this pattern, indicating a possible inconsistency between the two instruments. There is no reasonable explanation for the lower effluent conductivity levels, as increased temperature and ET losses would both tend to cause a conductivity increase in the effluent.



**Figure 11:** Total nitrogen average quarterly areal removal rates as calculated from water quality and harvested solids data.



**Figure 12:** Total nitrogen cumulative quarterly areal removal rates as calculated from water quality and harvested solids data.

### Biomass Management

During the operational period, 437,050 pounds of wet biomass were removed by the FlexRake. This harvested material was composed largely of filamentous green algae and diatoms, and had an average solids content of 9.9%, or a total dry solids harvest from the FlexRake of 43,300 pounds (Figure 17).

Those solids not captured by the FlexRake were diverted into a two-stage settling pond. The settled solids were periodically removed and composted. The diverted solids were estimated as total suspended solids (TSS) within the diverted flow minus any solids associated with recycled flows (clarified water from the settling ponds returned to the floway). This allowed an estimate to be made of the diverted harvest solids on a dry weight basis as the product of the TSS concentration and the diverted flow volume minus any solids associated with the recycled clarified flow. The diverted harvest typically was significantly greater than the rake harvest, on a dry solids basis<sup>5</sup>. For the full operational period, diverted dry solids harvest amounted to 286,122 lbs of a total dry solids harvest of 329,422 pounds, or nearly 87% of the entire harvest (Figure 18).

The tissue nutrient quality for rake solids showed higher tissue nutrient levels than diverted solids (average 0.52% TP and 2.77% TN for the rake solids versus average 0.35% TP and 2.17% TN for the diverted solids, Figures 19 and 20). This suggests there is considerable necrotic debris in the diverted solids, verified by microscopic examination. In addition, the tissue nutrients from both the rake and diverted solids were well below projections of 0.79% TP and 3.20 % TN. This indicates that the algal turf was stressed.

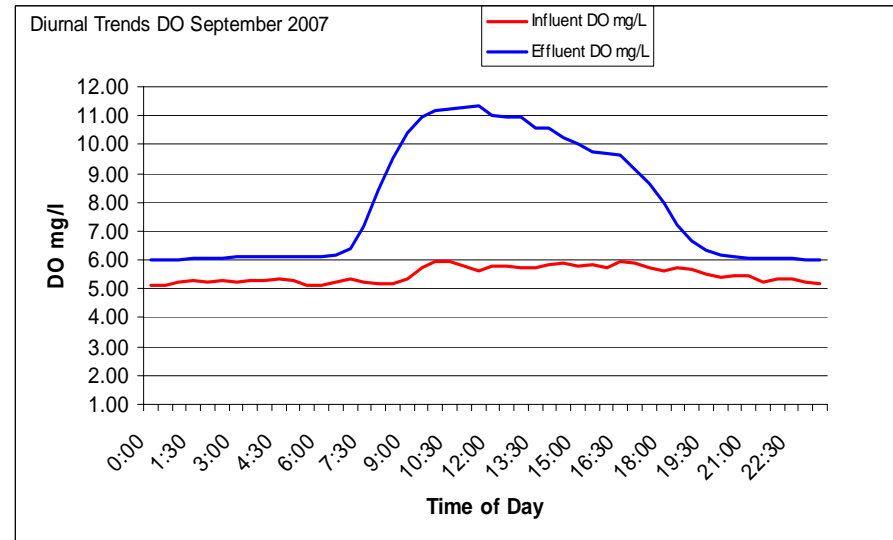
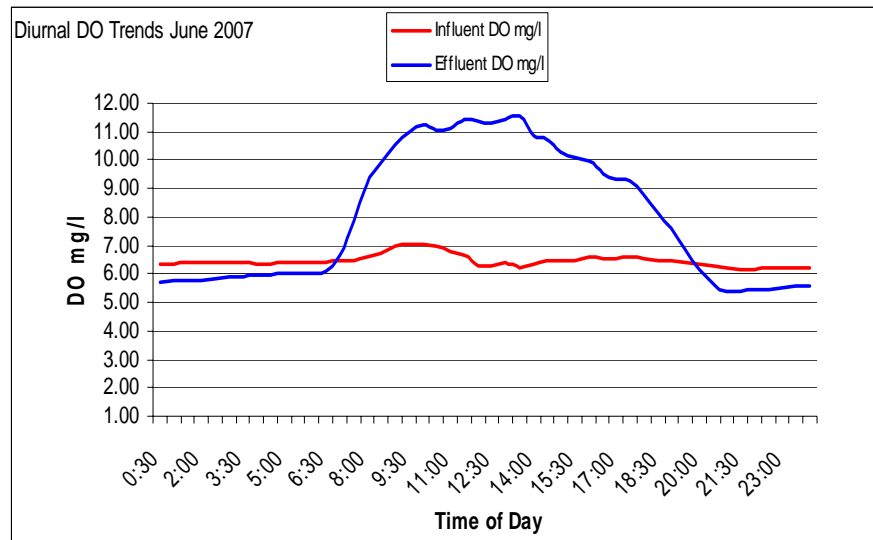
Stressed conditions are also implied by the lower than expected levels of net productivity. For the operational period, the average net productivity as measured by dry biomass was 10.7 g/m<sup>2</sup>-day, as compared to the projected average net productivity as measured by dry biomass of 44.3 g/m<sup>2</sup>-day. Quarterly net productivity over the operational period is shown in Figure 21.

<sup>5</sup> T-test reveals means different at 0.01 (99%) confidence level with  $t = 10.49$  and critical two tailed  $t = 1.99$ .

**Table 1: Influent and Effluent Water Quality, Exclusive of Nitrogen and Phosphorus**

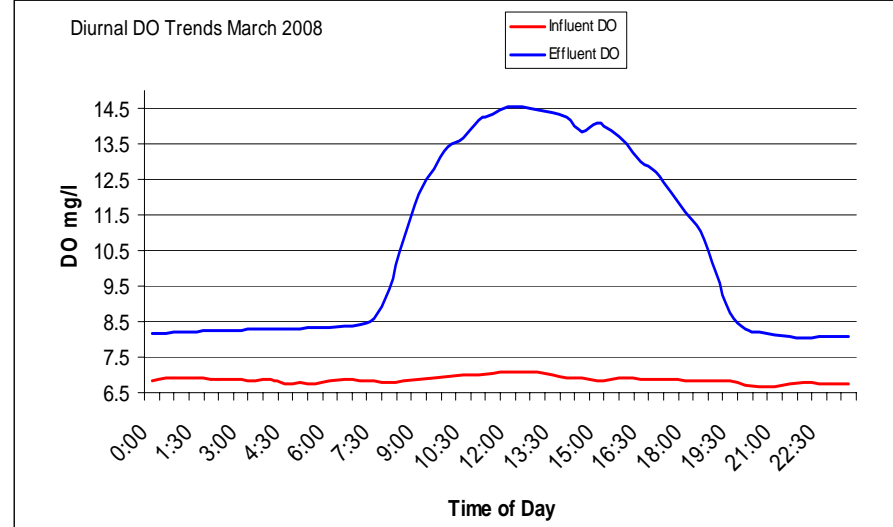
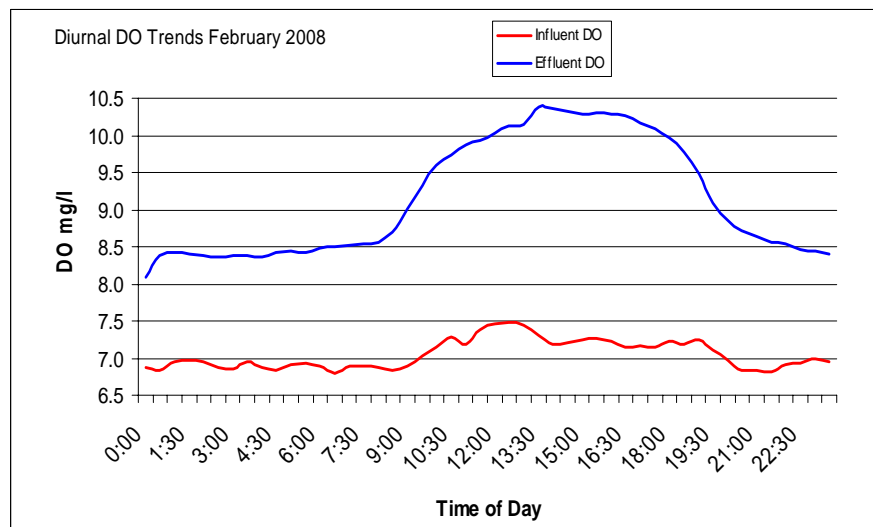
	Start-Up 2007		Quarter 1 2007		Quarter 2 2007		Quarter 3 2007		Quarter 4 2007		Quarter 1 2008		Quarter 2 2008		Quarter 3 2008		Quarter 4 2008		Quarter 1 2009		Quarter 2 2009		Quarter 3 2009		Quarter 4 2009			
	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl		
Daytime pH (grab)			8.51	8.69	8.36	8.81	7.57	8.13	8.21	9.03	8.08	9.14	7.57	8.60														
Daytime DO mg/L (grab)			8.26	8.89	6.45	8.48	4.25	10.00	6.89	12.24	7.05	9.48	4.56	9.04														
Daytime Water T °C (grab)			22.50	23.00	27.70	29.40	28.50	30.30	22.10	23.70	21.36	21.41	28.14	28.15														
Daytime Conductivity microS/cm (grab)			529	530	390	391	364	351	540	533	590	555	457	481														
Alkalinity mg/L as CaCO <sub>3</sub>	100	100			65	65	68	69	117	115	75	75	64	63			116	119	107	112					67	62	40	40
BOD <sub>5</sub> mg/L	<11	<4.5	U	U	2.0	2.0	4.3	3.4	2.0	2.3	2.0	2.4	2.3	2.3			2.0	2.0	8.2	3.9					2.8	3.5	2.8	2.8
Color pcu	80	80	50	50	150	150	300	350	90	90	200	200	400	450			100	100	80	85					250	250	150	150
TOC mg/L	23	23	17	17	28	28	31	331	22	19	30	31	41	43			21	20	19	21					27	32	32	32
TSS mg/L	2.7	2.3	3.3	4.4	11.0	U	10.3	9.8	5.0	5.0	5.0	5.0	8.7	9.8			7.8	13.0	5.0	5.0					17.0	8.5	5.0	5.5
TVSS mg/L	U	U					5.0	5.0	5.0	5.0	5.0	5.0	8.0	7.0			5.0	7.0	5.0	5.0					14.0	10.0	6.0	6.0
Calcium Hardness as CaCO <sub>3</sub> mg/L			126	123																								
Magnesium Hardness as CaCO <sub>3</sub> mg/L			45	45	45	45																						
Total Hardness as CaCO <sub>3</sub> mg/L			171	165	115	115	111	110	162	163	120	120	100	100					150	160					110	99	157	155
Ca mg/L	38	38	51	49	28	28	29	29	43	43	28	28	28	29			40	39	44	46					28	26	40	40
K mg/L	26.0	26.0	20.0	19.0			17.0	17.0	26.0	27.0	23.0	23.0	15.0	15.0			26.9	26.4	21.0	23.0					15.0	15.0	32.1	31.7
Mg mg/L	11.0	11.0	11.0	10.0	11.0	11.0	9.0	9.0	13.0	13.0	11.0	10.0	7.7	7.9			11.7	11.4	9.2	9.7					8.7	8.2		
Fe mg/L	0.08	0.08	U	U			0.93	0.87	0.18	0.18	0.39	0.40	0.88	0.94			0.31	0.29	0.19	0.20					0.74	0.67	0.35	0.30
As µg/L	U	U							U	U			U	U			U	U										
Cd µg/L	U	U							U	U			U	U			U	U							U	U		
Cr µg/L	1.6	1.4							U	U			U	U			U	U							U	U		
Pb µg/L	U	U			U	U			U	U			U	U			U	U	U	U					U	U		
Se µg/L	U	U			U	U			U	U			U	U			U	8	U	U					U	U		
Zn µg/L	U	U			U	U			U	U			16	17			U	U							U	U		
Hg µg/L	U	U			U	U			U	U			U	U			U	U	U	U					U	U		
Cu µg/L	U	U			U	U			U	U			U	20	U	U	U	U							2.1	2.0		
OrganoChlorine Pesticide Series	U	U			U	U			U	U			U	U			U	U	U**	U					U***	U		
OrganoChlorine Herbicide Series	U	U			U	U			U	U			U	U			U	U	U	U					U	U^		
OrganoPhosphorus Pesticide Series	U	U			U	U			U	U			U	U			U	U	U	U					U	U		

\* Alpha Chlordane 0.003 µg/L  
 \*\*Gamma Chlordane 0.9 µg/L  
 \*\*\*Gamma Chlordane .007 Alpha Chlordane .006 µg/L  
 ^ 2,4, D 0.654 µg/L



(a)

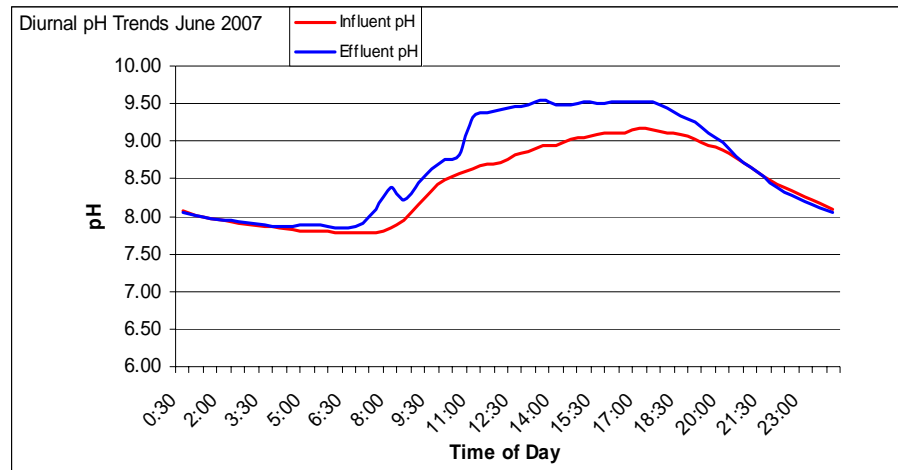
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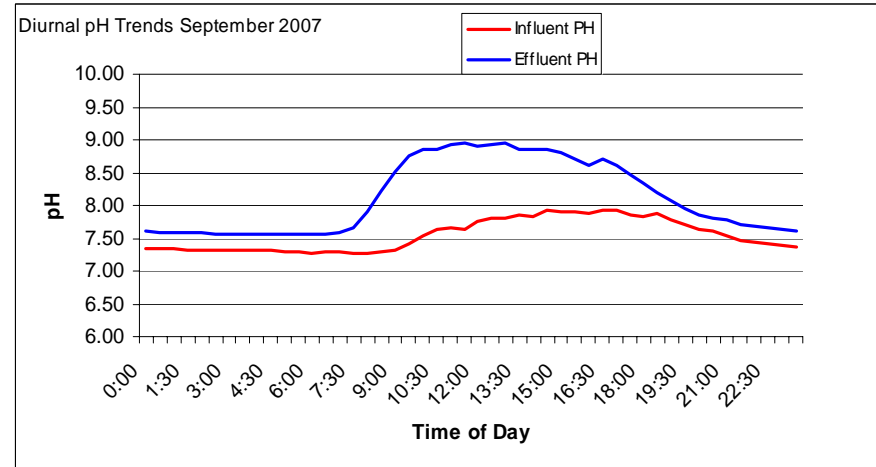
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(d)

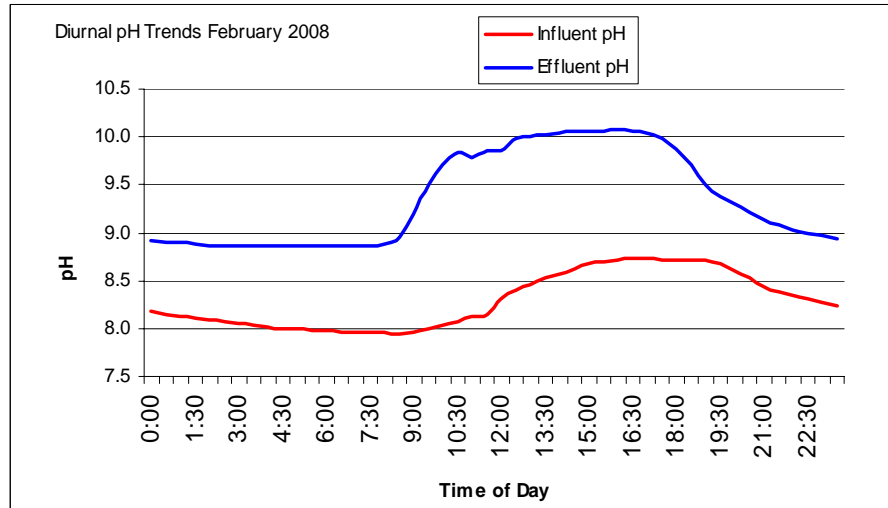
**Figure 13:** Typical diurnal DO trends ATST™ Influent and Effluent (a) June 2007; (b) Sept 2007; (c) Feb 2008; March 2008



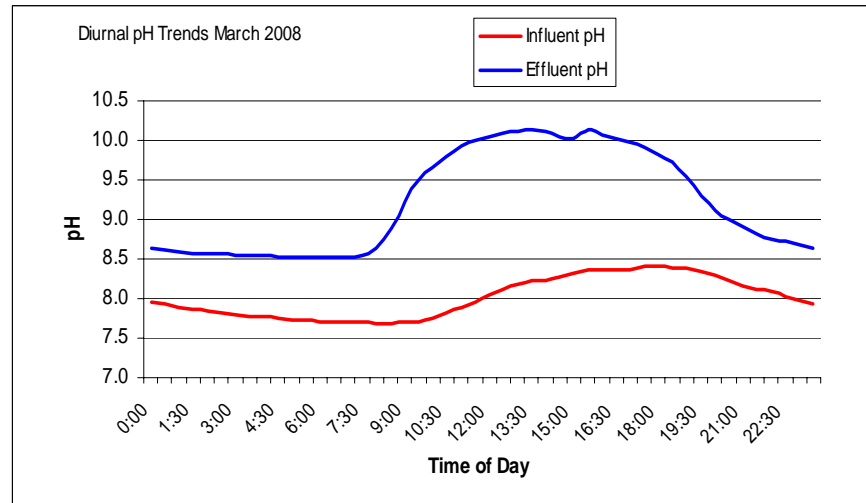
(a)



(b)

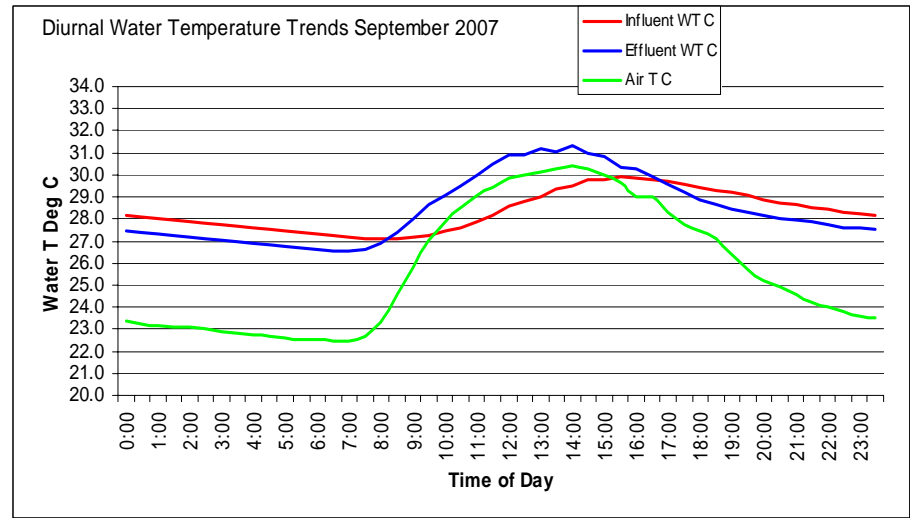
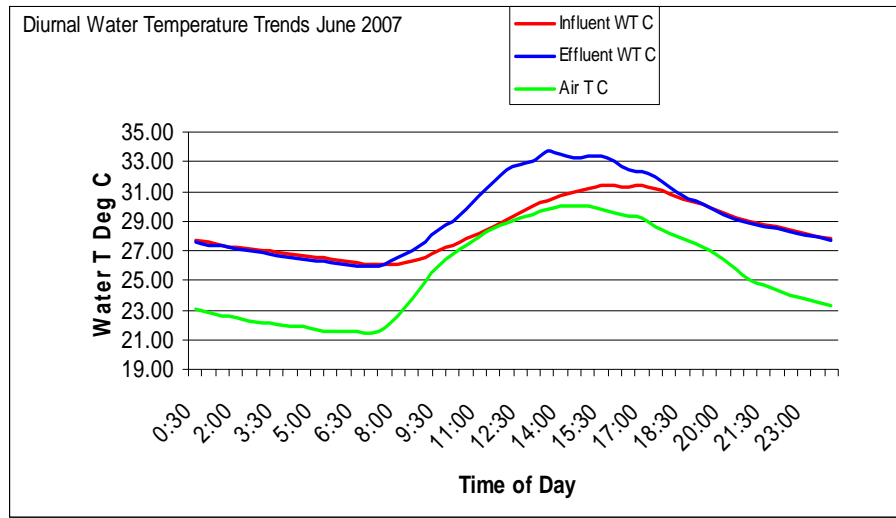


(c)



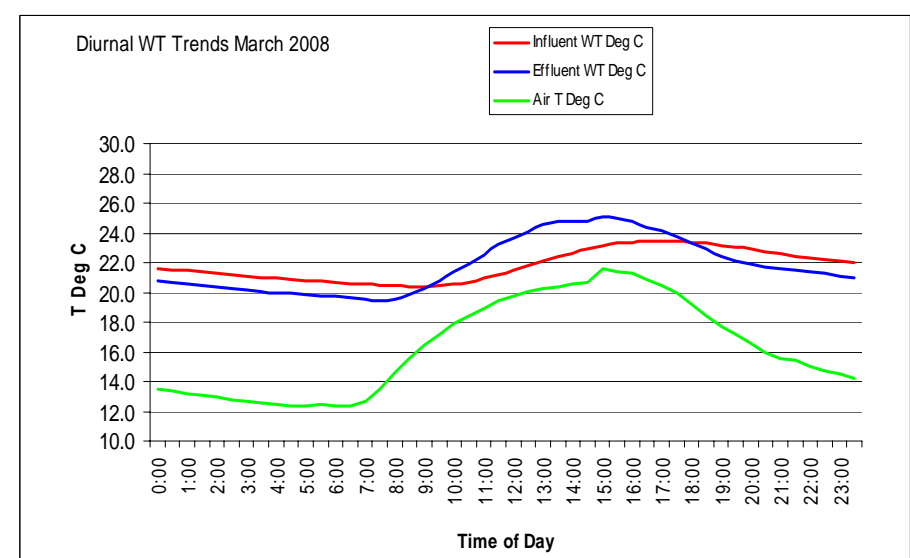
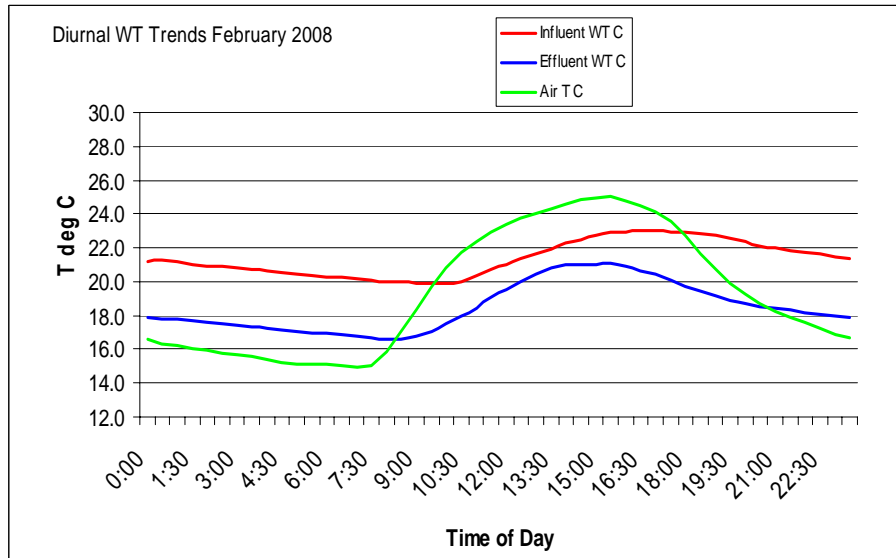
(d)

Figure 14: Typical diurnal pH trends ATSTM influent and effluent (a) June 2007; (b) Sept 2007; (c) Feb 2008; March 2008



(a)

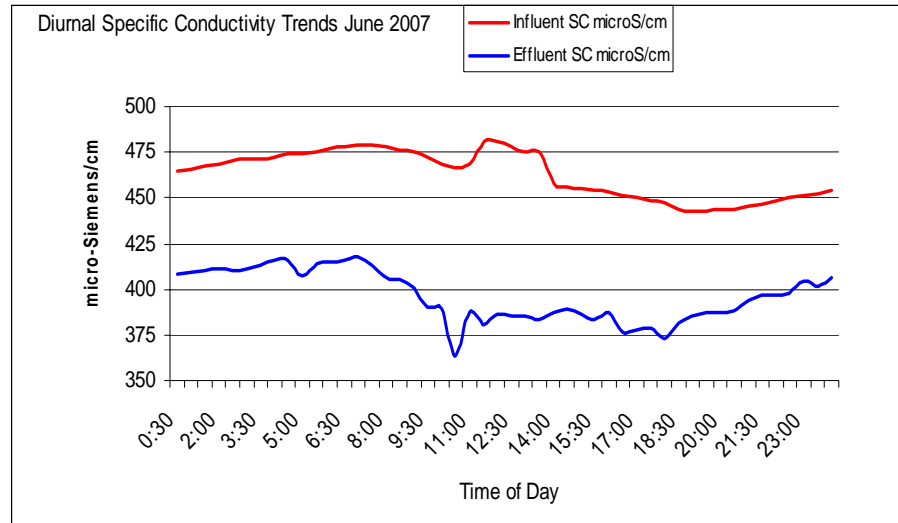
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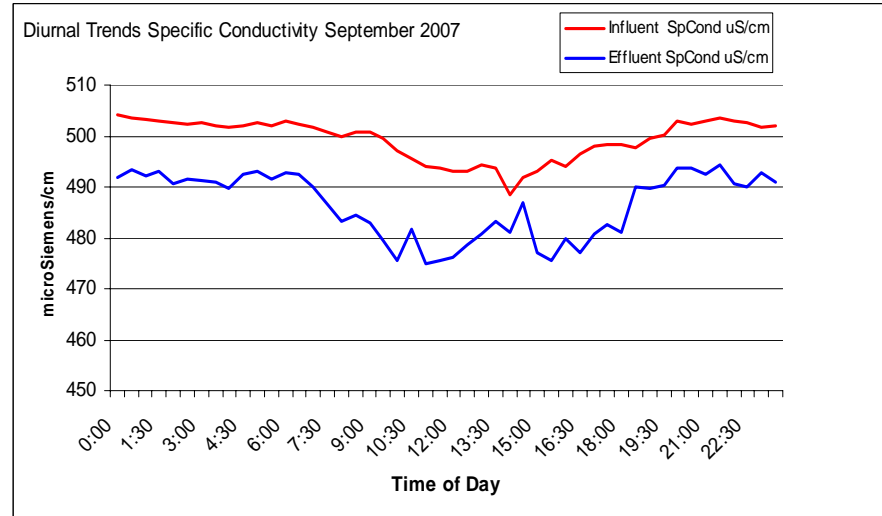
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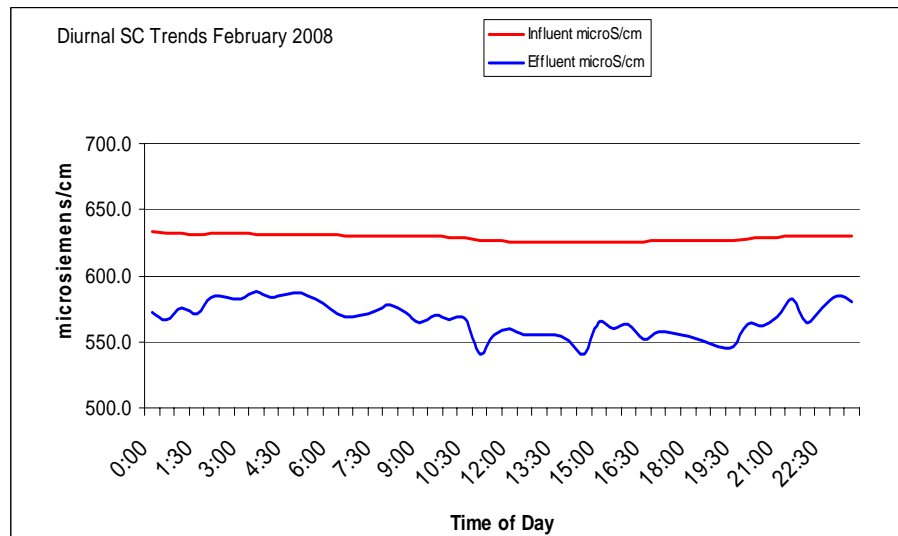
Figure 15: Typical diurnal water and air temperature trends ATST<sup>TM</sup> influent and effluent (a) June 2007; (b) Sept 2007; (c) Feb 2008; March 2008



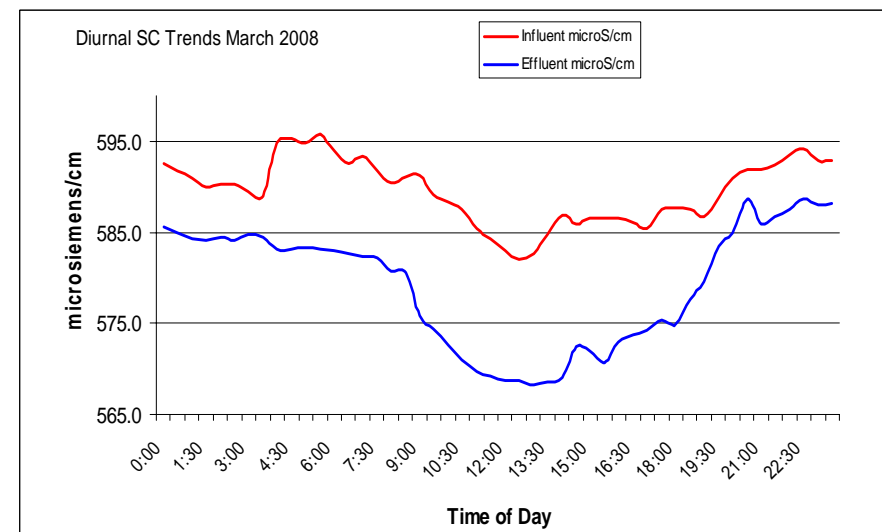
(a)



(b)



(c)



(d)

**Figure 16:** Typical diurnal conductivity trends ATST™ influent and effluent (a) June 2007; (b) Sept 2007; (c) Feb 2008; March 2008

During the operational period, harvested solids were windrow composted (Illustration 9). During the three years of operation approximately 34 tons of compost was produced (Table 2).

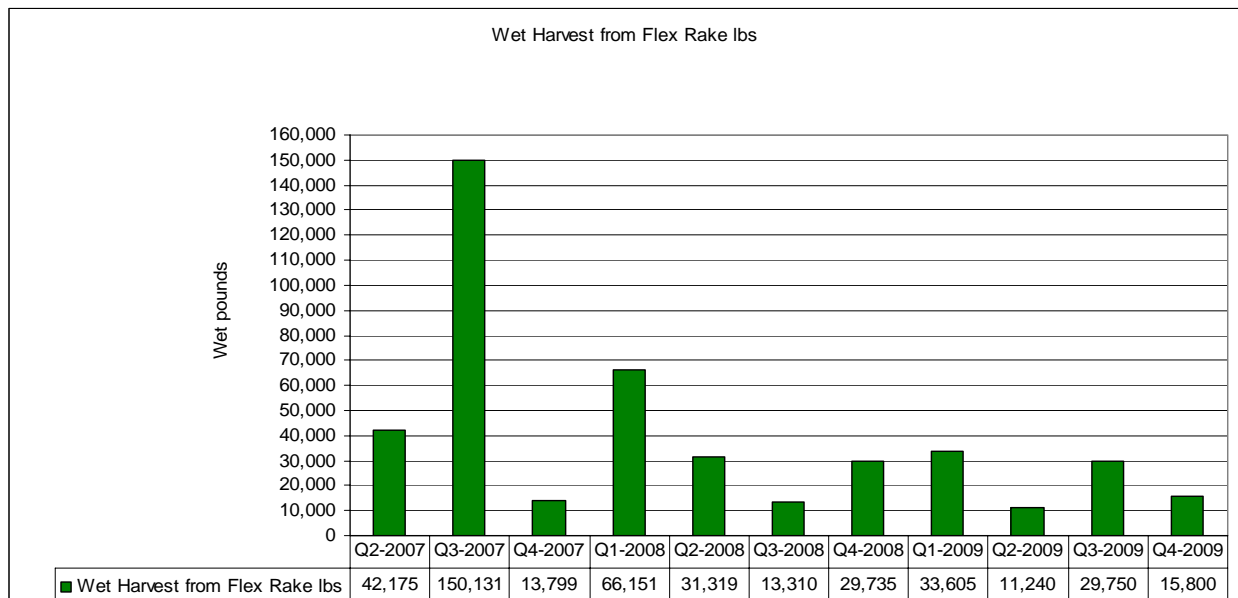


Figure 17: Wet pounds of harvest via FlexRake over operational period.

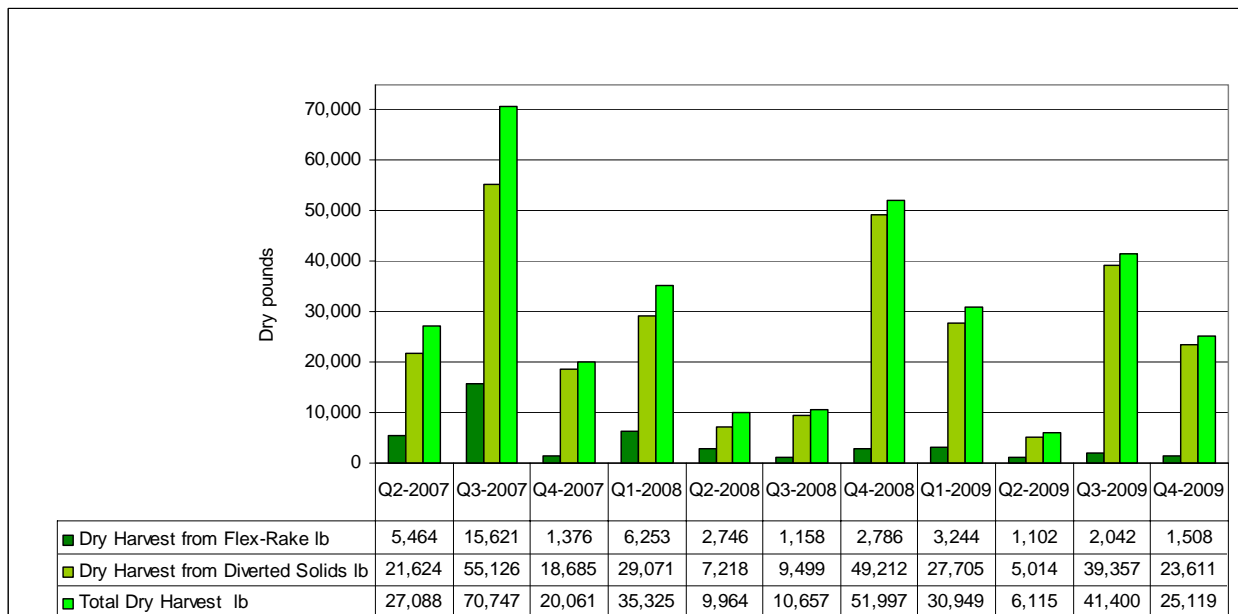


Figure 18: Harvest over operational period in dry pounds.

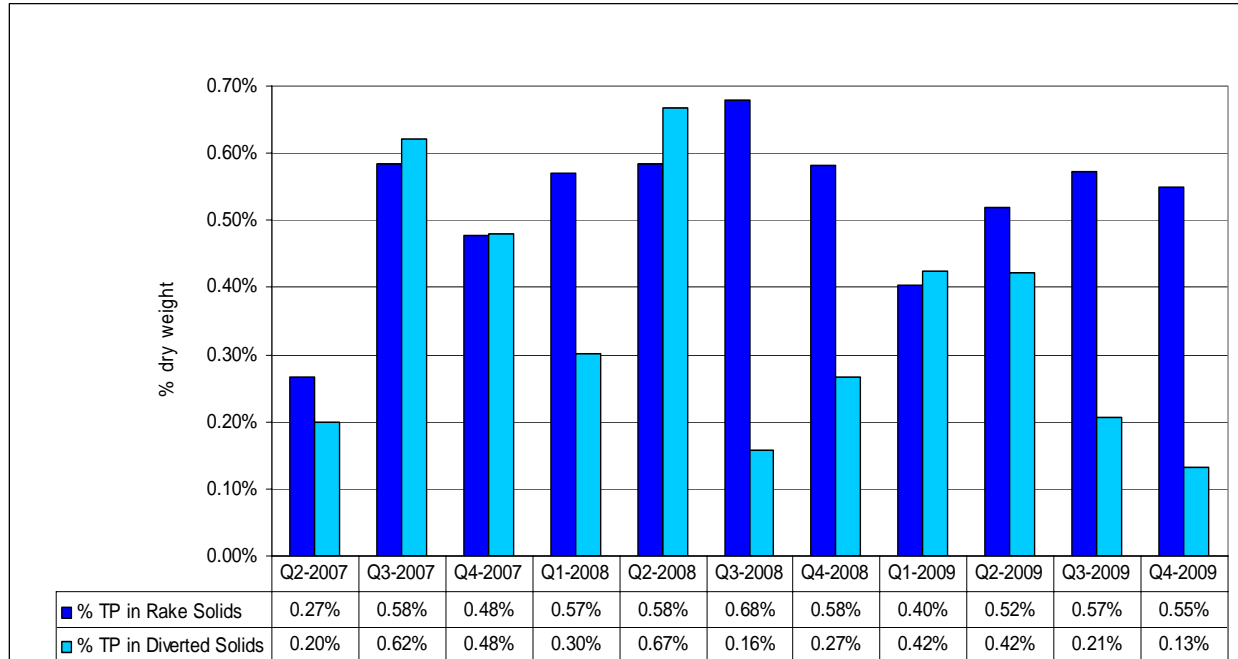


Figure 19: Harvest solids total phosphorus tissue levels over operational period.

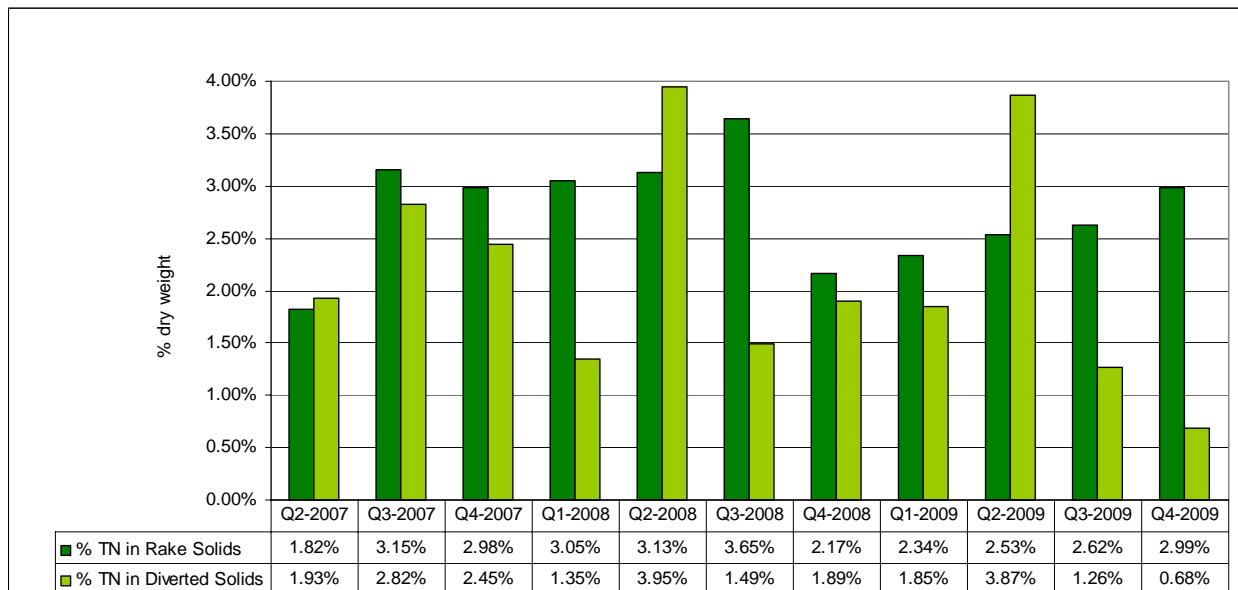
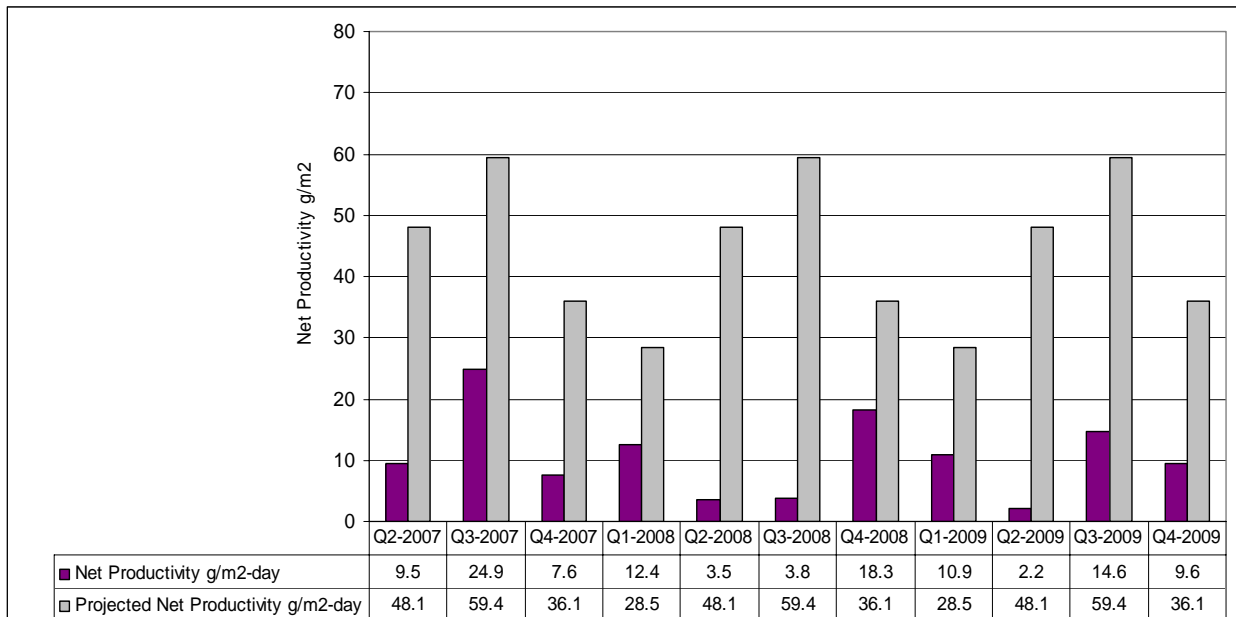


Figure 20: Harvest solids total nitrogen tissue levels over operational period.



**Figure 21:** Comparative quarterly net productivity actual vs. projected over operational period.



**Illustration 9:** Composting operation at the TC-ATS facility.

**Table 2:** TC-ATS facility compost analysis.

Parameter	Unit	Batch #6	Batch #10	Batch #18	Batch #0	Batch #19	Batch # 17	Batch #21	Batch #26	Batch #30
pH	-	7.0	7.0	8.1	7.6	7.5	7.8	7.6	7.5	6.8
Moisture	%	33.9	47.0	26.0	42.1	70.3	54.2	42.2	37.6	54.7
Ammonia-N	%	0.001	0.001	ND	ND	ND	ND	ND	ND	ND
Total N	%	0.91	0.53	0.52	0.35	0.47	0.53	0.66	1.34	0.67
P <sub>2</sub> O <sub>5</sub>	%	0.48	0.37	1.00	0.39	0.37	0.72	0.41	0.59	0.72
K <sub>2</sub> O	%	1.04	0.73	1.18	0.70	0.57	1.07	0.54	0.95	0.50
S	%	0.50	0.33	0.60	0.32	0.20	0.37	0.26	0.30	0.26
Ca	%	2.57	3.43	1.13	3.41	0.36	0.67	2.05	1.10	1.32
Mg	%	0.17	0.15	0.31	0.16	0.08	0.15	0.12	0.13	0.14
Na	%	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03
Fe	mg/Kg	1,729	1,940	5,912	2,070	2,149	2,615	2,285	2,458	4,525
Mn	mg/Kg	180	303	2,378	329	920	6,18	412	623	1,103
Cu	mg/Kg	ND	ND	ND	ND	ND	ND	ND	ND	ND
Zn	mg/Kg	29	30	51	28	ND	33	31	40	49
TOC	%	9.5	6.5	21.9	6.8	6.5	10.0	7.7	12.2	6.1
Cl	%	0.16	0.13	0.23	0.12	0.14	0.26	0.09	0.22	0.06

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## SECTION 3. INVESTIGATION INTO INHIBITORY AND TOXIC INFLUENCES IMPACTING SYSTEM PERFORMANCE

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### BACKGROUND - PERIPHYTON AS AN INDICATOR OF AQUATIC STRESSORS

Periphyton is a complex assemblage of algae, fungi, cyanobacteria, microinvertebrates and organic material.

In addition to periphyton's important function of providing food for invertebrates, and thus fish, in local and downstream ecosystems, the U.S. Environmental Protection Agency (EPA) identifies periphyton as an ecological indicator of aquatic stressors in aquatic environments which include excess nutrients and toxic chemicals, as well as habitat alteration and suspended sediments.

EPA defines the term "Ecological indicator" as a characteristic of an ecosystem that is related to, or derived from, a measure of biotic or abiotic attributes that can provide quantitative information on ecological condition, structure and function. An indicator can contribute to a measure of integrity and sustainability.

Periphyton are useful indicators of environmental condition because they respond rapidly to anthropogenic disturbances including contamination by excess nutrients, metals, herbicides, and hydrocarbons. As an example, a shift in community composition from large filamentous green algae to smaller diatom species and blue-green species has been documented as a possible sign of herbicide effects on a wetland.<sup>6 7 8 9</sup>

The Algal Turf Scrubber® technology has been developed, and as is based on periphyton's high rate of biomass production and diverse biological community. Based on research by HydroMentia Inc, and Dr. Water Adey, the Algal Turf Scrubber® technology has demonstrated the capacity to adapt to a broad range of source waters while providing removal of pollutants including nutrients, metals, and organic compounds from contaminated source waters from urban and agricultural stormwater; and municipal, industrial and agricultural wastewater.

Since ATS™ research began in the early 1980's, to HydroMentia's knowledge, no ATS™ system has ever documented sustained inhibition of periphyton growth prior to application of the technology in the Taylor Creek basin

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<sup>6</sup> Goldsborough, L.G., and G.G.C. Robinson. 1983. The effect of two triazine herbicides on the productivity of freshwater marsh periphyton. *Aquatic Toxicology* 4:95-112.

<sup>7</sup> Gurney, S.E., and G.G.C. Robinson. 1989a. The influence of two triazine herbicides on the productivity, biomass, and community composition of freshwater marsh periphyton. *Aquatic Botany* 36:1-22

<sup>8</sup> Hamilton, P.B., G.S. Jackson, N.K. Kaushik, and K.R. Solomon. 1987. The impact of atrazine on lake periphyton communities, including carbon uptake dynamics using track autoradiography. *Environmental Pollution* 46:83103

<sup>9</sup> Herman, D., N.K. Kaushik, and K.R. Solomon. 1986. Impact of atrazine on periphyton in freshwater enclosures and some ecological consequences. *Canadian Journal of Fisheries and Aquatic Sciences* 43:19171925

## CHRONOLOGY AND DISCUSSION

### Investigations into Identification and Source of Inhibitory/Toxic Agents

During the first quarter of start-up operation of the Taylor Creek ATS™ facility (TC-ATS)—January 22, 2007 to April 30, 2007—the region was experiencing an extended drought that resulted in reduced flows from Taylor Creek. With Taylor Creek functioning virtually as an impoundment, operation of the ATS™ combined with reduced pollutant loads contributed to lower than expected nutrient levels within Taylor Creek. For example, the February, March and April 2007 total phosphorus concentrations within the Taylor Creek influent were, 154, 123 and 108 µg/L respectively, as compared to historical total phosphorus concentrations for these months of 410, 450 and 400 µg/L, respectively. During this first quarter of start-up operation, the Taylor Creek ATS™ provided a modest level of nutrient reduction, with total phosphorus areal removal rate (ARR) averaging about 6.0 g/m<sup>2</sup>-yr. At the end of the quarter, the total phosphorus ARR had increased to 14.1 g/m<sup>2</sup>-yr. It was presumed that these somewhat lower than projected total phosphorus ARR were related to these lower total phosphorus concentrations and start-up operations. The upward trend of ARR towards the quarter's end appeared to indicate the system was moving out of a start-up phase, and that the algal turf was reaching some level of maturity and stability.

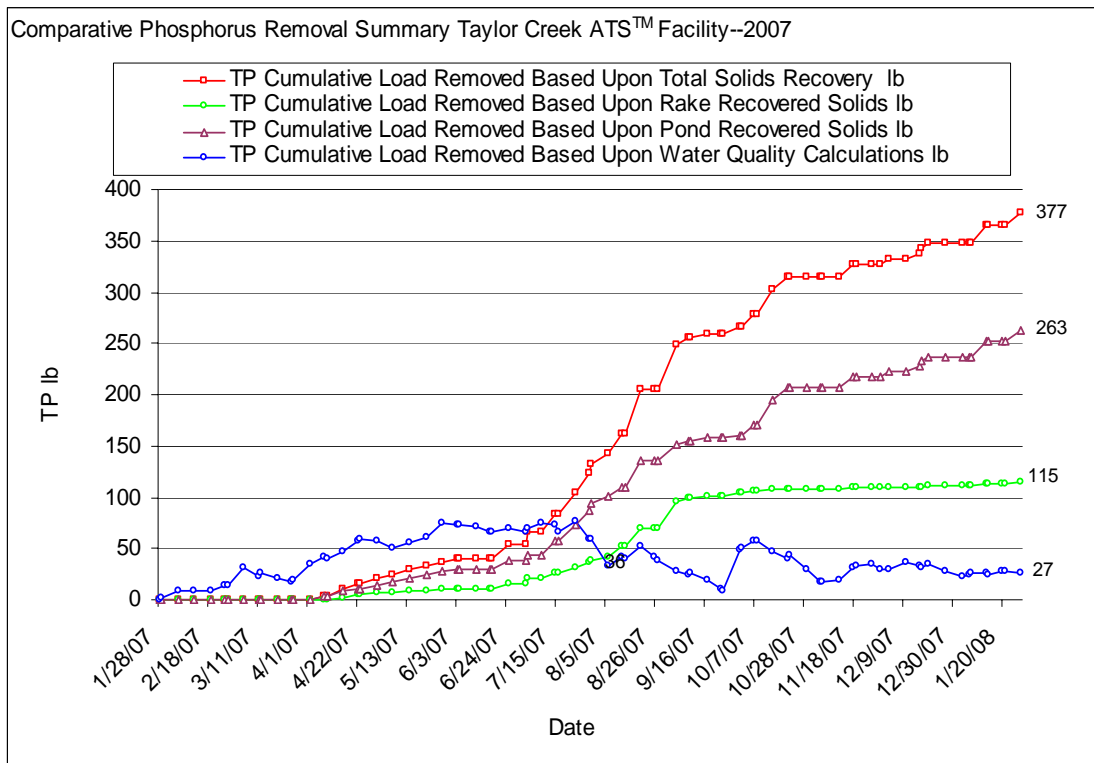
Harvesting of the algal turf began in early April, 2007, and over the month of April, 2007, the system was harvested five times, with a total of 14,953 dry pounds of solids removed. The calculated productivity as dry biomass per unit time over the quarter was calculated as 4.7 g/m<sup>2</sup>-day. Of the total harvest, 24.5% was removed by the harvest rake as filamentous algal turf, with the remainder as solids recovered in a settling pond after passing through the harvest rake. The solids recovered were noticeably low in phosphorus and nitrogen content - 0.21% and 1.72% phosphorus and nitrogen, respectively for rake recovered solids and 0.14% and 1.05% phosphorus and nitrogen, respectively for pond recovered solids. The low tissue nutrient levels were well below values noted during the S-154 pilot study, and offered an initial indication that perhaps algal turf health may be less than optimal.

During the second quarter (April 30, 2007 to July 30, 2007), for the months of May and June, 2007, phosphorus levels within Taylor Creek continued to decline, reaching an average of only 41 µg/L by June. However, by July 2007, summer rainfalls began and total phosphorus concentration increased to 679 µg/L as a monthly average. Total phosphorus loads increased substantially during July 2007, however the system performance did not respond as expected. Based upon water quality calculations, there was no net removal of total phosphorus in the second quarter. When the total phosphorus removed was calculated from algal turf harvesting, the total phosphorus ARR was estimated at 31.2 g/m<sup>2</sup>-yr. During July 2007, an estimated 19,022 pounds of solids were removed through harvesting, with 29.6% removed as rake recovered solids, and 70.4% removed as pond recovered solids. Estimated productivity for July 2007 was 19.4 g/m<sup>2</sup>-day. Tissue nutrient content also increased somewhat in July 2007, to 0.55% and 3.00% phosphorus and nitrogen, respectively for rake recovered solids; and 0.29% and 1.67% phosphorus and nitrogen, respectively for pond recovered solids or an average of all solids recovered of 0.41% phosphorus and 1.89% nitrogen. However, these levels were also well below the tissue concentrations of 0.90% total phosphorus and 3.24% total nitrogen projected for July, based upon S-154 findings.

### Identified Trends - Algal Productivity and Treatment Performance

By the end of quarter two and extending into the third and fourth quarters of 2007, two trends became evident.

The first trend related to the sizable difference noted between total phosphorus mass removal calculated through water quality data and the total phosphorus mass removal calculated through harvest data<sup>10</sup> (see Figure 22). This difference was of sufficient magnitude to suggest there may be significant error in either sampling or sample analysis for both water and/or algal biomass.

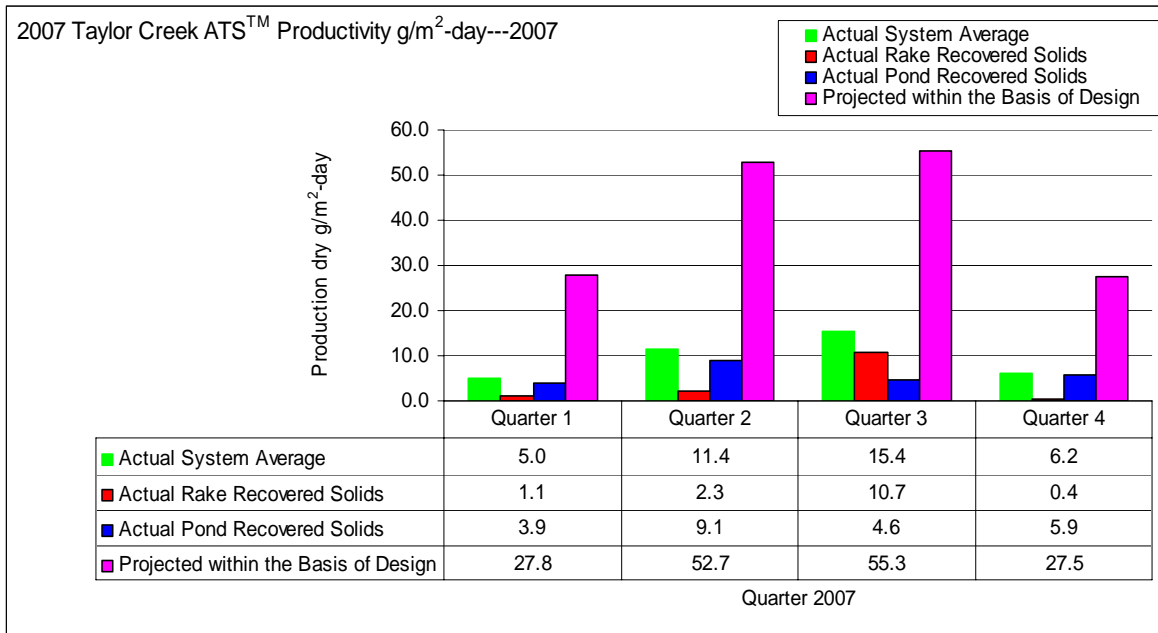


**Figure 22:** Total phosphorus removal TC-ATS through the first year's operation---2007.

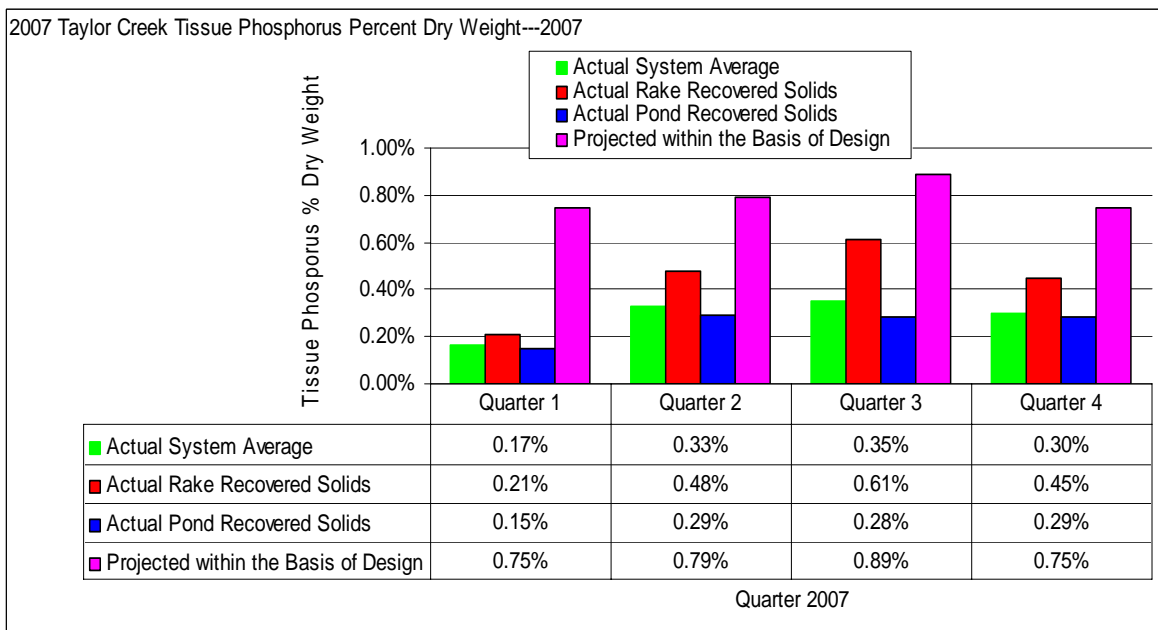
The second trend was associated with the lower levels of both algal turf productivity and phosphorus content of the turf biomass when compared to projections developed within the Basis of Design report<sup>11</sup> (see Figures 23 and 24) and shifts in the algal community type (see Illustration 10 and 11).

<sup>10</sup> Water Quality Based Performance (WQBP) for phosphorus removal is the difference between the incoming phosphorus load as calculated by influent total phosphorus concentration times total flow volume and effluent load as calculated by effluent total phosphorus concentration times total flow volume adjusted for rainfall and evapotranspiration. Recovered Solids Based Performance (RSBP) for phosphorus removal is calculated as the sum of the rake recovered solids (dry weight) times the tissue phosphorus levels and the pond recovered solids which are calculated as the harvest diverted flow volume times the total phosphorus concentration in the diverted flow. With proper sampling and analysis the two calculations should reconcile.

<sup>11</sup> Taylor Creek 15 MGD Algal Turf Scrubber® (ATS™) Basis of Design, March 17, 2006. Prepared by HydroMentia, Inc. for SFWMD.



**Figure 23:** Actual field algal turf productivity compared to projections–TC-ATS through the first year’s operation---2007.



**Figure 24:** Actual field algal turf nutrient content compared to projections–TC-ATS through the first year’s operation---2007.



**Illustration 10.** Filamentous green algae on Taylor Creek Algal Turf Scrubber® flowway on July 31, 2007.

During the first two weeks of September of 2007 a significant shift occurred in the algal turf community. The filamentous green algae died, resulting in a diatom dominated community (Illustration 11). For over 4 months (September 2007 through January 2008) green filamentous algae remained virtually absent from the Taylor Creek ATS™, with the exception of a limited number of elevated areas on the flowway.



**Illustration 11.** Diatom dominated algal mat illustrating absence of green algae.

Typically the Water Quality Based Performance (WQBP) for phosphorus removal load calculation method is considered more reliable than the Recovered Solids Based Performance (RSBP) for phosphorus removal load calculation method, as the sampling matrix (water) with WQBP is homogenous, meaning a random sample is more likely to be representative of the entire volume. However, it is quite reasonable to

deduce that if material is actually being removed and documented via harvest from the ATS™ floway, and this material contains phosphorus, then there should be reflected a parallel reduction noticed within the water source from which this phosphorus would be extracted. In other words, the harvest mass stands as indisputable evidence of phosphorus removal and recovery, and when the phosphorus within this mass cannot be reasonably accounted for from water quality data, then it is necessary to examine more closely the phosphorus dynamics of the system and the reliability of sampling and analytical methods. It was presumed the reason(s) for this incongruity would likely be one or more of the following, applicable to both water and algal biomass samples:

- There is an external phosphorus source such as atmospheric fallout or wildlife contributions.
- The analytical methods used were not providing accurate results.
- There is significant sampling error, e.g. the removal is below the detection from the difference between inflow and outflow water quality samples (e.g. censoring effect)
- The water loss through evaporation was significant enough to result in nullifying concentration reduction, i.e. water vapor loss tracked phosphorus loss.

There were factors within the Taylor Creek flows that appeared to be interfering with algal turf productivity and system performance, and that these factors could be related to the physical design and operation of the system; chemical influences; biological influences; or a combination of these. By mid-summer 2007, it became clear that extensive investigative efforts were needed to identify the cause(s) giving rise to these issues of concern associated with these two trends, and to mitigate their impacts. Consequently, an investigation strategy was developed (Figure 25). By March, 2008 considerable work had been completed regarding this investigation strategy. These findings were summarized in a report dated March 2008, entitled **Evaluation of Phosphorus Removal by the Taylor Creek Algal Turf Scrubber® Nutrient Recovery Facility in Okeechobee County, Florida for the Period July 2007 through December 2007.** This report is included as Appendix A. A series of tables developed from investigations related to the flowchart noted in Figure 25 during this time provide a summary of the findings during the various investigations (Tables 3 through 10).

Through this first series of investigations, the following was documented.

- Design and physical operational features such as floway slope, hydraulic loading rate, grid material, and surge cycling did not contribute to the issue of system's poor performance.
- There did appear to be a statistically significant difference between phosphorus removals based upon Water Quality Based Performance (WQBP) and Recovered Solids Based Performance (RSBP).
- There did not appear to be any significant external contributions of phosphorus which would confuse the sampling and analytical data.
- There was no indication that the evapotranspiration losses down the floway were of a magnitude sufficient to significantly concentrate the total phosphorus levels in the effluent.
- There was no supportive evidence that macro or micro nutrient limitations were responsible in any significant manner for below projected algal turf productivity or system performance.
- In a static bioassay, Taylor Creek water treated with activated carbon rendered the water amenable to healthy algal growth, while a non treated control of Taylor Creek water showed extensive growth inhibition, providing indication that one or more organic toxins may be involved, and responsible for below projected performance and productivity.

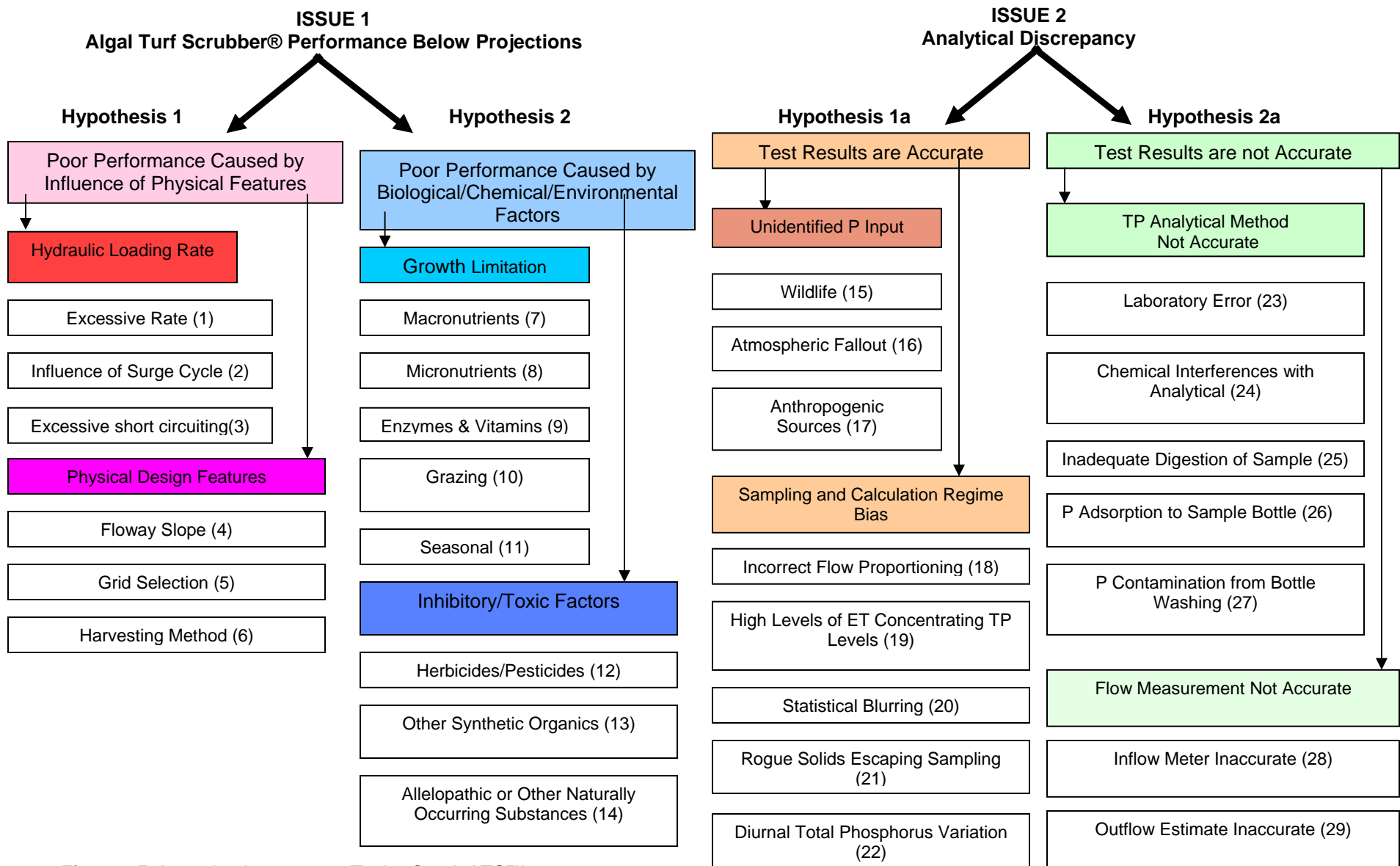


Figure 25: Investigation strategy Taylor Creek ATS™--2007.

**Table 3:** Summary of Investigations—Poor Performance Caused by Influence of Physical Feature—Hydraulic Loading Rate

ISSUE 1. ALGAL TURF SCRUBBER® PERFORMANCE BELOW PROJECTONS						
Hypothesis 1: Poor Performance Caused by Influence of Physical Feature						
Id #	Importance #	Status	Issue	Testing Strategy	Summary of Results	Comments
Hydraulic Loading Rate						
1	7	Reduced to 13.4 gpm/lf late November 2007	Excessive Hydraulic Loading Rate	Run field tests to see if lower hydraulic loading rate results in improved performance	Initial tests on an isolated 5 foot floway showed some improvements when Linear Hydraulic Loading Rate (LHLR) was reduced from 20 gpm/lf to 14.4 gpm/lf. West side of the floway adjusted accordingly also showed some improvements in performance. The entire floway was then adjusted to 13.3 gpm/lf. To date, no documented improvements noted.	LHLR relates to velocity down the floway (along with slope). Noted at S-154 18-20 gpm/lf yielded best performances. Indication this may not be the case at Taylor Creek, although nothing conclusive to date.
2	17	Completed Surge cycle impacts tested during October, 2007 on pilot unit	Influence of Surge Cycles	Surge cycling relates to the benefits to algae production associated with oscillatory waves which increase surface area exposure and reduce diffusion barriers.	Surge cycles tested at various intervals on a small 1 foot wide, 100 foot long pilot unit. At optimal cycle of 30 seconds or less, no improvement in algal productivity was noted after several weeks of continuous operation.	Surge cycling may be adjusted to optimize production, but is not considered responsible for extensive periods of negligible productivity. Full scale floway has been adjusted to a 30 second cycle without any noticeable improvements in performance.
3	25	Completed Isolated floway set up in August, 2007	Excessive short-circuiting	High areas on the floway forces flow rate (velocity) variations on portions of the floway, which may result in decreased or increased detention time. Five foot isolated floway was set on the main floway to eliminate any short-circuiting to see if any changes in algal productivity or performance occurred.	Eliminating any short circuiting did not result in improved algal productivity or performance.	There is no evidence that short circuiting is a significant contributor to poor algal production or reduced system performance.

**Table 4:** Summary of investigations—poor performance caused by influence of physical feature—physical design.

ISSUE 1. ALGAL TURF SCRUBBER® PERFORMANCE BELOW PROJECTONS						
Hypothesis 1: Poor Performance Caused by Influence of Physical Features						
Id #	Importance #	Status	Issue	Testing Strategy	Summary of Results	Comments
			Physical Features	Design		
4	26	Completed  Slope tested at 2% during October 2007 on pilot unit	Floway Slope	Concern that the floway slope of 0.5% may not be sufficient to adequately support algal productivity. A 100 foot long, 1 foot wide pilot unit was established at a 2% slope at the same LHLR as the main floway to see if algal productivity improved.	For a period of 2 months the pilot unit showed no notable algal productivity, and no growth at levels higher than the main floway set at 0.5% slope.	Slope of the floway relates to establishing velocity on the floway per Manning's equation. The design velocity, based upon literature and field experience was set at >0.66 fps. The main floway at 0.5% slope provides a velocity of about 0.75fps as an average. Increasing slope increased velocity above this optimum value, but did not improve productivity.
5	23	Various grid materials placed on main floway in September 2007.	Grid Material	Grid material is provided to support algal attachment. Different grid materials were tested on the floway.	No algal productivity enhancement noted with any of the grid materials tested.	Grid material needs to provide adequate area for attachment of algae. Non-floating grid is preferred.
6	24	Future	Harvesting Method	It was suggested that more complete harvesting, such as would be provided by a vacuum type harvester would improve productivity.	A section of the floway near the central lateral was harvested and cleaned completely to emulate vacuum harvesting. There was no indication of enhanced productivity	Vacuum harvesting is being investigated as a next level design feature. Harvesting must leave sufficient standing crop to permit biomass recovery.

**Table 5:** Summary of investigations—poor performance caused by biological/chemical/environmental factors—growth limitation.

<b>ISSUE 1. ALGAL TURF SCRUBBER® PERFORMANCE BELOW PROJECTONS</b>						
<b>Hypothesis 2: Poor Performance Caused by Biological/Chemical/Environmental Factors</b>						
<b>Id #</b>	<b>Importance #</b>	<b>Status</b>	<b>Issues</b>	<b>Testing Strategy</b>	<b>Summary of Results</b>	<b>Comments</b>
<b>Growth Limitation</b>						
7	6	Initial efforts on isolated floway in September 2007. May conduct more formal test again on pilot unit	Macronutrients	Supplement isolated 5 foot floway on main floway with dolomite (Ca, Mg, alkalinity) and potassium nitrate to see if the macronutrients might be deficient.	After several weeks of supplementation no enhanced algal productivity noted.	The levels of nitrogen, calcium, magnesium and alkalinity (relates to available carbon) at Taylor Creek are very similar to S-154, which showed no signs of growth limitation factors.
8	8	Completed  Initial efforts on isolated floway in September 2007. May conduct more formal test again on pilot unit	Micronutrients	Boron, the one micronutrient which appeared from water quality to be potentially limiting, was supplemented on the isolated 5 foot floway	After several weeks of supplementation no enhanced algal productivity noted.	Micronutrient deficiencies were not documented at S-154, where water quality related to micronutrients was similar to Taylor Creek. Usually systems with growth limiting factors involved show good growth at the front end of the process, diminishing towards the effluent end. Just the opposite is noted at the Taylor Creek site, indicating a potential growth inhibitor.
9	27	None Planned	Enzymes Vitamins	No testing strategy planned	None	Considered to be highly unlikely factor, because of the typical diversity of algal community, which would allow a non-impacted group to prevail through selective advantage. From September 2007 to end of the operating period, the first 150 feet (+/-) of the floway has shown almost no algal growth.
10	28	Completed  Examinations and Observations conducted July through November 2007	Grazing	Excessive grazing by invertebrates, such as Chironomid larvae, could retard biomass development	Microscopic and macroscopic examinations revealed no indication of excessively abundant grazers.	The presence of a diverse invertebrate community generally is considered indicative of a healthy algal turf community. While formal counts were not conducted, daily observation indicated there was a paucity of invertebrates at Taylor Creek, as compared to S-154 .
11	29	None Planned	Seasonal	Shifts in temperature and photoperiod can be expected to influence algal turf community structure	Loss of productivity was noted through the late summer into winter	Seasonal shifts in ecology are expected, but the temperature drop in Florida during winter is not sufficient to eliminate algal productivity. A viable, diverse algal community was sustained during the winters at S-154.

**Table 6:** Summary of investigations—poor performance caused by biological/chemical/environmental factors—inhibitory/toxic factors.

ISSUE 1. ALGAL TURF SCRUBBER® PERFORMANCE BELOW PROJECTONS							
Hypothesis 2: Poor Performance Caused by Biological/Chemical/Environmental Factors							
Id #	Importance #	Status	Issues	Testing Strategy	Summary of Results	Comments	
			Inhibitory/Toxic Factors				
12	2	Screening since January 2007. Bioassay January, 2008	Herbicides/Pesticides	General Screening is part of original monitoring plan. Specific testing for select compounds used in watershed needs to be pursued. Bioassay testing continues.	To date the screening has not shown any herbicides or pesticides. A list of herbicides used in the watershed which were not included in the screening has been developed, and testing for these compounds is planned. Bioassay work with control and activated carbon treatment (see Appendix B), indicates complex organic compounds may be responsible for low algae production. Such compounds could include herbicides.	Herbicides and perhaps to a lesser extent, pesticides, have been noted to impact algae productivity. The patterns of inhibition noted on the flowway indicate a compound that is more or less omnipresent, at least during the wet season. Herbicides are typically used intermittently, and because of this may not be expected to be associated with the flow at all times. Herbicides could augment influence of other agents.	
13	1	Bioassay January 2008	Other organics	synthetic	Antibiotics and anti-parasitic compounds added to feed or used with crops could be present in flows. The bioassay work was conducted initially during January 2008, and follow-up assays are continuing. Future testing for specific compounds is being considered	Bioassay work with control and activated carbon treatment, indicates complex organic compounds may be responsible for low algae production. Such compounds could include antibiotics/anti-parasitic compounds as well as hormones and other endocrine disruptors.	Antibiotics, such as sulfonamides, tetracyclines, and ionophores are used regularly within the dairy industry, and could be omnipresent in the associated flows in Taylor Creek. Literature review includes implication that such compounds could be algacidal.
14	3	Bioassay January 2008	Allelopathic naturally occurring substances	or other naturally occurring	Lignin, phenols, fulvic acid and other naturally occurring organic compounds have been indicated as potentially algacidal.	Bioassay work with control and activated carbon treatment (see Appendix A), indicates complex organic compounds may be responsible for low algae production. Such compounds could include allelopathic and other naturally occurring organic compounds	Lignins and other naturally occurring compounds could be associated with cow manure, and concentrations could be at an algacidal level. No such compounds were noted at S-154.

**Table 7:** Summary of Investigations Analytical Discrepancy—Unidentified Phosphorus Inputs

ISSUE 2. ANALYTICAL DISCREPANCY HYPOTHESIS 1a: TEST RESULTS ARE ACCURATE						
Id #	Importance #	Status	Issue	Testing Strategy	Summary of Results	Comments
			Unidentified phosphorus inputs			
15	13	General Observation	Wildlife	Observation of bird and wildlife visitation	No indication of extensive import from birds or wildlife	Bird visitation not greater than that observed at S-154.
16	15	Completed July 2007	Atmospheric Fallout	Set open bottles of deionized bottles inside and outside to determine if atmospheric contamination occurs	No phosphorus found in samples	Testing of open DI water containers inside and outside
17	16	Completed July 2007	Anthropogenic Sources	Set open bottles of deionized bottles inside and outside to determine if Anthropogenic contamination occurs	No phosphorus found in samples	Testing of open DI water containers inside and outside

**Table 8:** Summary of Investigations—Analytical Discrepancy—Sampling and Calculation Regime Bias

<b>ISSUE 2. ANALYTICAL DISCREPANCY</b>							
<b>HYPOTHESIS 1a:</b>							
<b>TEST RESULTS ARE ACCURATE</b>							
Id #	Importance #	Status	Issue	Testing Strategy	Summary of Results	Comments	
<b>Sampling and calculation regime bias</b>							
18	21	Completed  Calculation review July 2007	Incorrect Flow Proportioning	Review flow data and calculated influence on sampling sequencing between influent and effluent	Influence assessed as inconsequential		
19	12	Completed  Chloride Balance September through December 2007	High levels of ET concentrating P, thereby increasing detected levels.	Reviewed conductivity changes from influent to effluent to determine if evapotranspiration (ET) was significantly reducing flow volume, and thereby concentrating phosphorus within the effluent. Proceeded to test influent and effluent for chlorides to test same concern.	Based upon chloride studies, the ET loss rate was determined to be only slightly higher than expected pan evaporation, and not significantly contributory to changes in effluent phosphorus concentration.	Similar findings were documented at the S-154 study	
20	5	Statistical evaluation of data	Statistical Blurring	Conduct initial statistical review to determine if the difference between Water Quality Based Performance (WQBP) and Recovered Solids Based Performance (RSBP) are statistically distinguishable, and if treatment provided is obscured by statistically allowed range. Proceed with more sophisticated statistical reviews.	The water quality results for phosphorus appear statistically improbable when compared to analytical method accuracy. As additional data collected, need more complete statistical analysis.	There is a statistical difference between WQBP and RSBP, indicating a question regarding reliability of analytical procedure. However, even RSBP is below projected performance.	
21	11	Completed  September 2007	Rogue Solids Escaping Sampling	The sampling intake device will capture only solids 1/8 " or smaller. Net of 1/8" mesh was placed for specified time at discharge laterals to facilitate capture and quantification of larger solids	Capture of larger solids indicated negligible impact in terms of phosphorus inputs		
22	14	Completed  August 2007	Diurnal total phosphorus variation	Collect 2 hour composite samples over a continuous 24 hour period to see if there are any diurnal extremes regarding the difference between influent and effluent. Also collect daily composite samples for a week to see if there was any variation in difference between influent and effluent.	No such patterns noted		

**Table 9:** Summary of Investigations—Analytical Discrepancy—TP Analytical Method not Accurate

ISSUE 2. ANALYTICAL DISCREPANCY						
HYPOTHESIS 2a:						
TEST RESULTS ARE NOT ACCURATE						
Id #	Importance #	Status	Issues	Testing Strategy	Summary of Results	Comments
			TP Analytical Method not Accurate			
23	18	Completed August 2007	Laboratory Error	Split samples with certified laboratories and District Laboratory	Results within %RPD allowable of 20%	Method provides reproducible results
24	4	Initial Testing September 2007	Chemical interference with analytical method	Conduct split analysis of a series of samples in October using methods EPA365.1 (colorimetric with persulfate digestion); EPA 200.7 (ICP-photo spectrometry) and EPA 200.8 (ICP-mass spectrometry). Evaluate aluminum and arsenic as possible interference with EPA 365.1	The EPA 365. 1 and EPA 200.7 yielded results within 20% RPD. EPA 200.8 yield results below 20% RPD when compared to EPA 365.1. Aluminum and arsenic values not high enough to be interfering agents.	The deviation disclosed by EPA 200.8 may indicate interferences with photometric procedures, but data is inconclusive
25	20	Completed Testing August 2007	Inadequate Digestion	The University of Florida split samples, with one analyzed per EPA 365.1, the other evaporated, ashed, and evaluated for orthophosphate (all organic phosphate being converted during ashing)	The pre-ash samples significantly lower than digested samples (EPA 365.1), indicating digestion may be adequate, but interferences could be an issue, as with the evaluation using EPA 200.8	Differences are notable but inconclusive
26	9	Completed Testing September 2007	P adsorption to sample bottles	Evaluate composite samples taken from sample bottles with and without acid rinsing of bottles—the acid intended to dislodge adsorbed P.	For samples with high TP, some increase in concentration related to acid washing. The procedure of sample collection was adjusted to include acid rinsing (sulfuric) of sample bottles.	Influence not significant
27	22	Completed Testing January 2008	P contamination from bottle washing	There was a concern that bottle washing was leaving residual P, causing contamination. Washing was done in conformance with District guidelines. Bottles with deionized water were set in sampler for one week to see if there was any TP gain	No TP detected in test bottles	Field blanks are a normal part of the QA/QC protocols, and none have shown any sign of contamination with P.

**Table 10:** Summary of Investigations—Analytical Discrepancy—Flow Measurement not Accurate

ISSUE 2. ANALYTICAL DISCREPANCY							
HYPOTHESIS 2a:							
TEST RESULTS ARE NOT ACCURATE							
Id #	Importance #	Status	Issues	Testing Strategy	Summary of Results	Comments	
			Flow measurement not accurate				
28	19	Calibration	Inflow Inaccurate	Meter	Parshall flume calibrated periodically by checking zero distance, and distance to water in stilling well.	Parshall flume measurements appear accurate (+/- 5%).	Influent measurements coincide with pump curves, parshall flume accepted open channel flow method, Flume is always in non-submerged state
29	10	Completed	Outflow Inaccurate	Estimate	Effluent flow estimated by adding rainfall and deducting ET loss based on historical records.	The ET issue was addressed as part of Hypothesis 1a review.	The weir at the effluent is not designed to provide comparable accuracy to parshall flume. If comparatively accurate effluent flows are desired, a more reliable flow measurement device must be installed.

*Activated Carbon Investigations*

Of all of the testing conducted during this period, that which provided the most insight into the type of factor(s) or agent(s) impacting system performance was the static bioassay study in which (1) containers of untreated Taylor Creek water (control) and (2) activated carbon treated water from Taylor Creek were seeded with filamentous green algae taken from an unrelated source. Water from (3) a nearby stormwater pond was also included in the test.

Water quality for the three conditions indicate the treatment by activated carbon significantly reduced color (75%), total organic carbon (78%) and total nitrogen (62%), with more modest reduction of total phosphorus (14%, Table 11). Alkalinity was noted to increase by 59%. The increase in arsenic level in the treated water was also noted from levels below detection to 0.058 mg/L. Phosphorus levels in the stormwater were significantly lower than the Taylor Creek water, as expected.

**Table 11:** Water quality associated with bioassay test water.

Parameter	Units	Taylor Creek Not Treated (Control)	Taylor Creek Activated Carbon Treated	Stormwater
Aluminum	mg/L	0.193	0.174	-
Arsenic	mg/L	BDL (0.005)	0.0582	-
Boron	mg/L	0.0749	0.0606	-
Calcium	mg/L	41.5	36.2	-
Copper	mg/L	BDL(0.0025)	BDL(0.0025)	-
Iron	mg/L	0.193	0.204	-
Magnesium	mg/L	13.1	12.2	-
Manganese	mg/L	0.0129	0.00424	-
Zinc	mg/L	BDL (0.0100)	BDL (0.0100)	-
Alkalinity	mg/L	115	183	29.9
TOC	mg/L	22.4	5.0	-
Color	mg/L	100	25	220
Nitrate-Nitrite-N	mg/L	0.242	0.024	0.031
Total Kjeldahl Nitrogen	mg/L	1.17	0.52	1.26
Total Nitrogen	mg/L	1.41	0.54	1.33
Total Phosphorus	µg/L	278	240	59
Total Dissolved Solids	mg/L	362	305	83
Total Suspended Solids	mg/L	BDL (5.0)	BDL (5.0)	BDL (5.0)
Total Volatile Suspended Solids	mg/L	BDL (5.0)	BDL (5.0)	-

Values in red indicate an increase with activated carbon treatment  
 Values in blue indicate a decrease with activated carbon treatment

Each test unit was seeded with the same amount of algae (0.6 gm estimated dry weight) from the same source. This algae was noted to be dominated by green algae, with the periphytic genus *Bumillaria sp.*, *Rhizoclonium sp.*, and the epiphytic species *Scenedesmus sp.* and *Cosmarium sp.* predominant. After thirteen days (1/10/08 to 1/24/08) the algae was recovered, and a dry weight determined.

The change in weight over time within the activated carbon treated units was significantly higher than the two controls—3.3 grams, 0.7 grams and 0.5 grams, respectively for the activated carbon treated Taylor Creek water, untreated Taylor Creek water (control) and stormwater. This rate of change was used to calculate specific growth rate as:

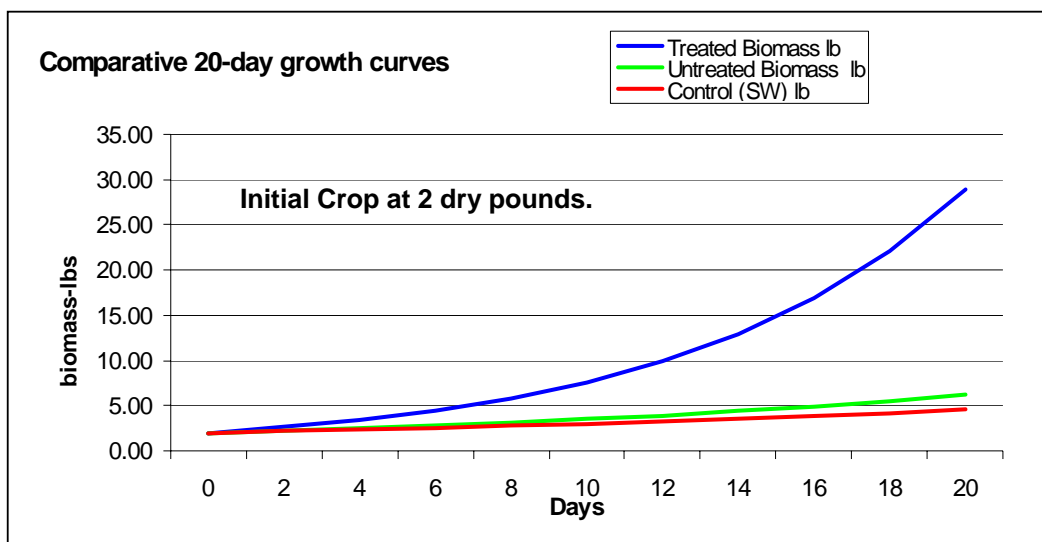
$$[\ln(W_t / W_0)] / t = \mu$$

Where  $W_t$  = dry weight of algae in grams after test period  
 $W_0$  = dry weight of algae in grams at the beginning of the test period  
 $t$  = test period in hours  
 $\mu$  = specific growth rate 1/hour

Specific growth rates calculated for each set-up were as follows:

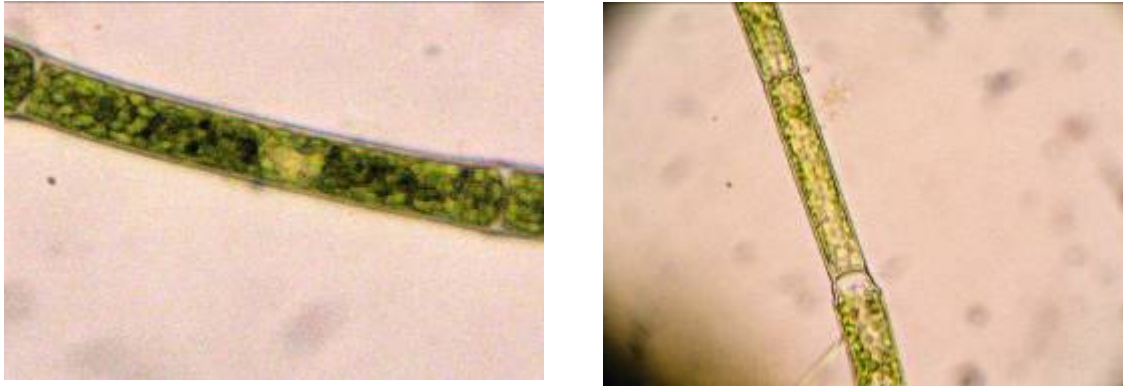
Taylor Creek Treated with Activated Carbon	0.0056/hr
Taylor Creek Untreated Control	0.0023/hr
Stormwater	0.0017/hr

Because these values are applied to logarithmic growth through the equation  $W_t = W_0 e^{\mu t}$  the significance of these findings is clear. As an example, suppose a system begins with a standing crop of two dry pounds of biomass. The extent of divergence, when these three specific growth rates are applied, is substantial, with the higher specific growth rate resulting in a 20-day biomass increase of 27.4 dry pounds, while the growth rates associated with the control and stormwater sample yield a 20-day biomass increase of only 4.0 and 2.5 dry pounds, respectively (Figure 26).



**Figure 26:** Comparison of projected growth rate influences over twenty day period from activated treated and non-treated bioassays.

In addition to yielding a higher growth rate, the water associated with the activated carbon treatment supported the development of what appeared to be a healthier algae stock. This was evident both in general appearance and from microscopic comparison. For example, two algae strands associated with the filamentous green algae *Rhizoclonium sp.* (Illustration 12). The strand on the left was taken after the test period from the activated carbon treated unit. It shows an abundance of chloroplasts, indicating active photosynthetic capabilities. The strand on the right is from the non-treated test cell (control) taken after the test period. A clear paucity of chloroplasts is evident, indicating toxic influences, which appear to repress photosynthetic activity.



**Illustration 12:** Comparative photomicrograph of a strand of *Rhizoclonium* sp. from Taylor Creek activated carbon treated test unit (a) and Taylor Creek non-treated test unit (b).

This initial bioassay was repeated again on 2/5/08 for 21 days, with the activated carbon treatment unit again showing a significantly higher specific growth rate when compared to the untreated unit. These results provided the following insights into the issue(s) related to poor productivity and system performance:

- Significantly improved algal turf productivity was solicited by treatment of Taylor Creek water with activated carbon, clearly indicating that one or more organic compounds are likely responsible for the poor productivity and system performance.
- Even though treatment with activated carbon reduced total nitrogen levels significantly, there was no evidence of nitrogen limitation. In fact even with the lower nitrogen levels, algae growth rate increased. This indicates toxic influences, not nutrient limitation are likely responsible for poor productivity and system performance.
- Treatment of Taylor Creek water with activated carbon improved the number of chloroplasts within the sampled algal cells, indicating improved vigor of the algal turf.

Following these two bioassays, investigations continued into the possibility of micro and macronutrient limitations contributing to poor productivity. Initially, it appeared that nitrogen supplementation could enhance productivity, based upon batch type static bioassays. However, when nitrogen was supplemented (as potassium nitrate) on a five foot wide, 300 foot long isolated section of the existing floway, no improvement in productivity was observed. The same lack of response was noted with supplementation of boron, bicarbonate alkalinity, calcium, magnesium or iron.

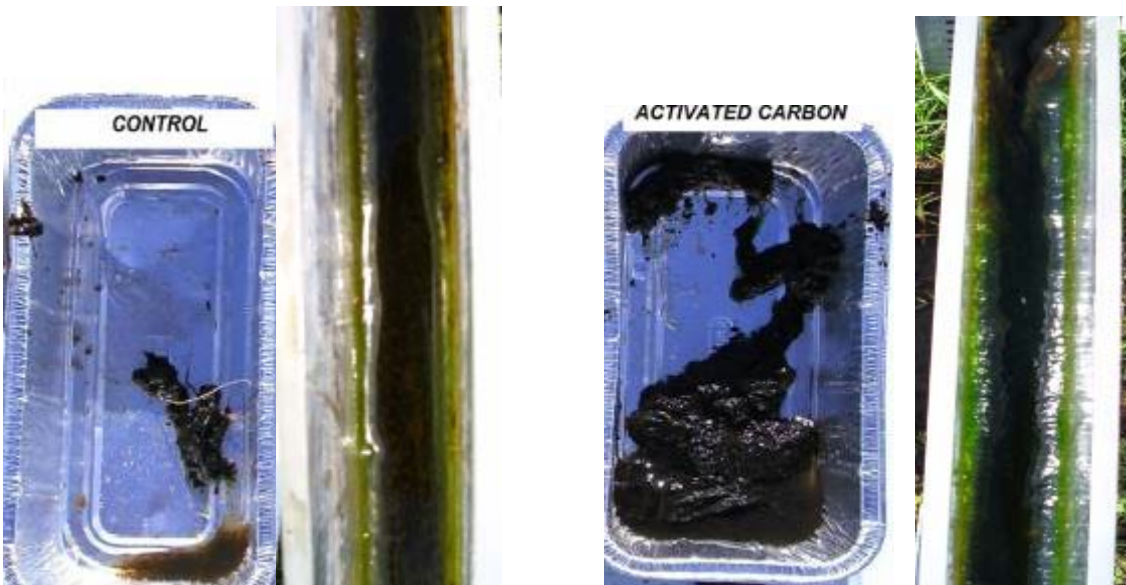
#### *Micro-ATS™ Investigations*

During the course of investigating the influence of activated carbon treatment, static bioassays were not sufficiently emulative of ATS™ dynamics, as the hydraulic detention associated with the static bioassays was measured in days, while the hydraulic detention time was measured in minutes (7-10 minutes over 300 feet) over the ATS™ floway. In order to better represent floway conditions, while avoiding the high costs of treating and supplementing the high flows (circa 100 gpm) associated with a five foot wide floway, small plexiglass systems were developed which operated at 0.5-1.0 gpm. These systems, referenced as “Micro-ATS™ Units” or MAU, provided reasonable qualitative and to some extent, quantitative testing of various water quality scenarios under ATS™ type conditions. An early set-up of the MAU in operation was recorded on YouTube as <http://www.youtube.com/watcg?v=mvb02GcSCqY> . During April, 2009 the MAU was used to repeat the activated carbon treated Taylor Creek water and non-treated Taylor Creek water bioassay (Illustration 13 and Figure 27). The algal turf productivity and vigor,

as with the static bioassay, was significantly enhanced by treatment with activated carbon. This result eliminated concerns that the organic toxin was labile, and attenuated by a prolonged detention time within the static bioassay units. The response within the MAU provided clear indication that enhanced production and vigor was related to a substance(s) removed by activated carbon, and this substance most likely was an organic compound(s).



(a)



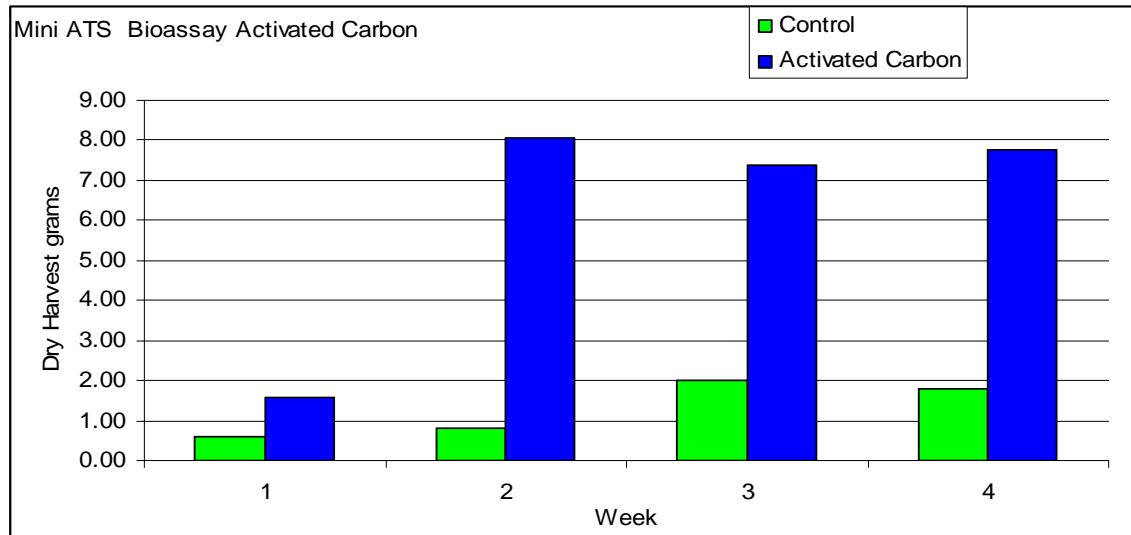
(b)

(c)

(d)

(e)

**Illustration 13:** Bioassay – Micro ATSTM Unit (MAU) April 7 to April 30,2009 Taylor Creek water with activated carbon treatment compared to Taylor Creek water with no treatment (a) Typical MAU set-up (b) Wet harvest non-treated unit (control) (c) Algal turf non-treated unit (control) (d) wet harvest activated carbon treated unit (e) Algal turf activated carbon treated unit.



**Figure 27:** Bioassay productivity results Micro ATSTM Unit (MAU) April 7 to April 30, 2009 Taylor Creek water with activated carbon treatment compared to Taylor Creek water with no treatment (Control).

Following the completion of most of the items included in the initial investigation strategy (see Figure 25, Tables 3-10, and Appendix A) and the disclosure of the benefits rendered by activated carbon treatment, efforts were initiated in early 2008 to examine more closely (1) the nature, identity and source of the toxic compound(s), (2) the seasonal frequency and amplitude of impacts of the toxic compound(s); and (3) the effectiveness of viable mitigation processes beyond the already established activated carbon treatment<sup>12</sup>.

#### *Herbicide Testing*

As part of the initial assessment and monitoring plan of the Taylor Creek water quality, a general screen for a wide variety of pesticides and herbicides (Methods 8151, 8051, and 8041) was conducted on the Taylor Creek influent. These screens, which include a large number of commercially available compounds, showed no measurable levels of any of these agents. This testing was considered during initial design efforts to determine if the water was contaminated by herbicide or pesticide residuals.

However, with the revelation that a toxic compound(s) was associated with the Taylor Creek water, an expanded list of anthropogenic organic compounds was developed based on FDACS listed compounds used with the crops associated with the crops cultivated in the Taylor Creek basin - primarily citrus, pasture, dairy, and row crops (potatoes).

Methods 8081, 8151 and 8141 only include analysis of 3 of the 21 or (14.3%) herbicides that FDACS reports as being used on crops in Florida that are produced in the Taylor Creek basin (See Attachment A)

Select agriculturally applied antibiotics also were included within this updated list of compounds. When practical, samples were submitted for testing for these additional compounds. In addition to private laboratories, several agencies assisted in completing these analyses. Specifically, these were the United States Geological Survey (USGS) laboratory in Lawrence, Kansas; the Florida Department of Agriculture and Consumer Services Bureau of Pesticides (FDACS); and the University of Florida, Institute of Food and Agricultural Sciences (IFAS).

<sup>12</sup> While activated carbon proved effective in removing the toxic compound(s), it is not a viable full scale treatment approach because of the magnitude of the costs involved in treating large volumes of water.

On April 2, 2008 a set of water samples was sent to the USGS Organic Geochemistry Group in Lawrence, Kansas for analysis of a number of herbicides and associated metabolites, and antibiotics. Among those compounds tested included the herbicides Acetochlor, Metolachlor, Alachlor, Atrazine, Bromacil, Deisopropylatrazin, Didealkylatrazin, and the antibiotics Azithromycin, Chloramphenicol, and Oxytetracycline. The recorded results are included in Appendix C.

Of eleven antibiotics tested, none were detected. Of the herbicides and their metabolites, most were undetected, with the following exceptions:

Dairy Canal <sup>13</sup>	Metolachlor ESA	0.28 µg/L
	Metolachlor OXA	0.05 µg/L
Citrus Canal	Metolachlor ESA	0.23 µg/L
	Metolachlor OXA	0.05 µg/L
Pump Intake	Metolachlor ESA	1.42 µg/L
	Metolachlor OXA	0.22 µg/L
Settling Pond	Metolachlor ESA	1.48 µg/L
	Metolachlor OXA	0.22 µg/L
	Deisopropylatrazin	0.03 µg/L
	Didealkylatrazin	0.06 µg/L

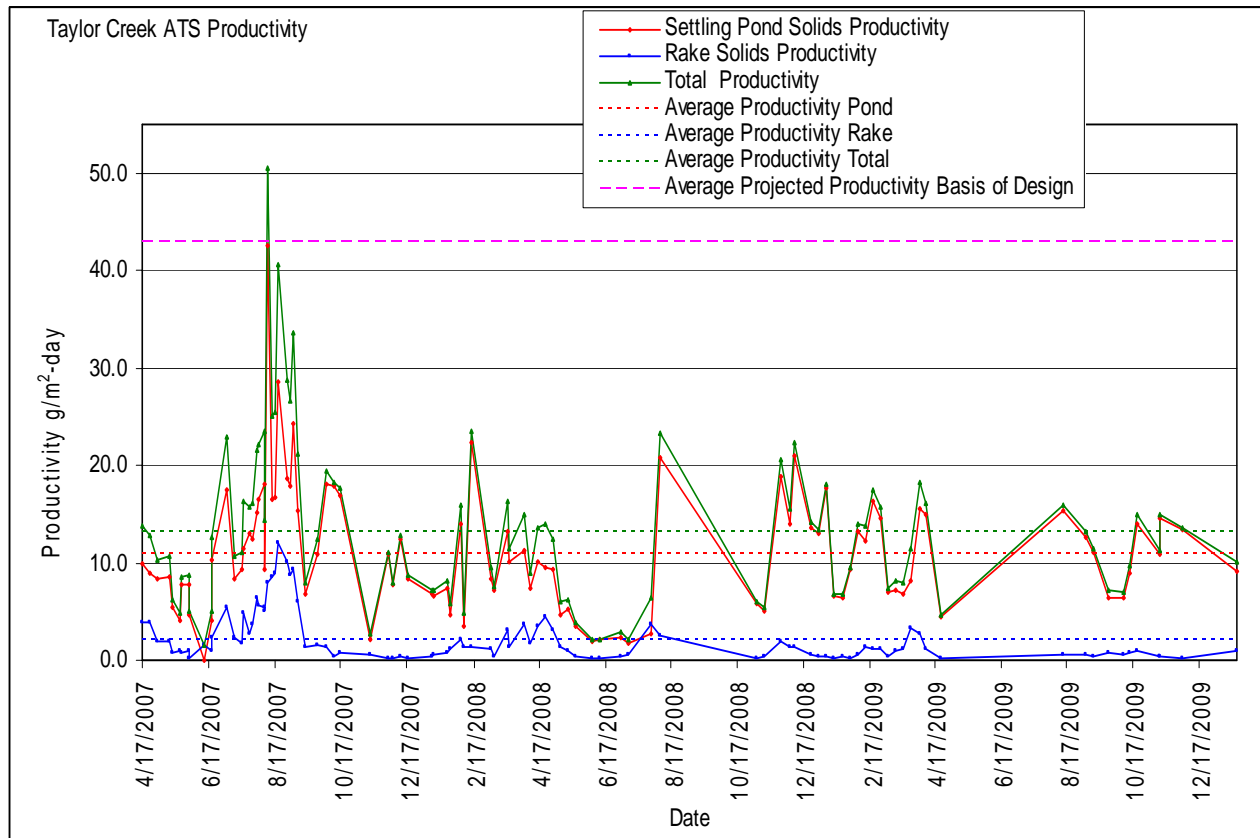
It is not surprising that the higher levels were found in the settling pond, as the pond is used to store harvested algae and detrital solids which pass through the harvest rake. There is apparently a tendency for bioaccumulation within this pond. Of all of these findings, the presence of Metolachlor and its metabolites arouse the most suspicion. This herbicide is widely used and is a common groundwater contaminant. As with many of these compounds the nature and extent of its toxicity to ecological communities is not well established, particularly regarding impacts upon attached algae communities.

In reviewing the available information, it appears a Metolachlor concentration of 7.8 µg/L is generally the limit that has been accepted regarding potential deleterious impacts upon aquatic organisms. The concentrations found in this sample of course are well below this level. However, it is not clear if the metabolites are less or more toxic. While the Metolachlor findings are suggestive of possible involvement, they are far from conclusive.

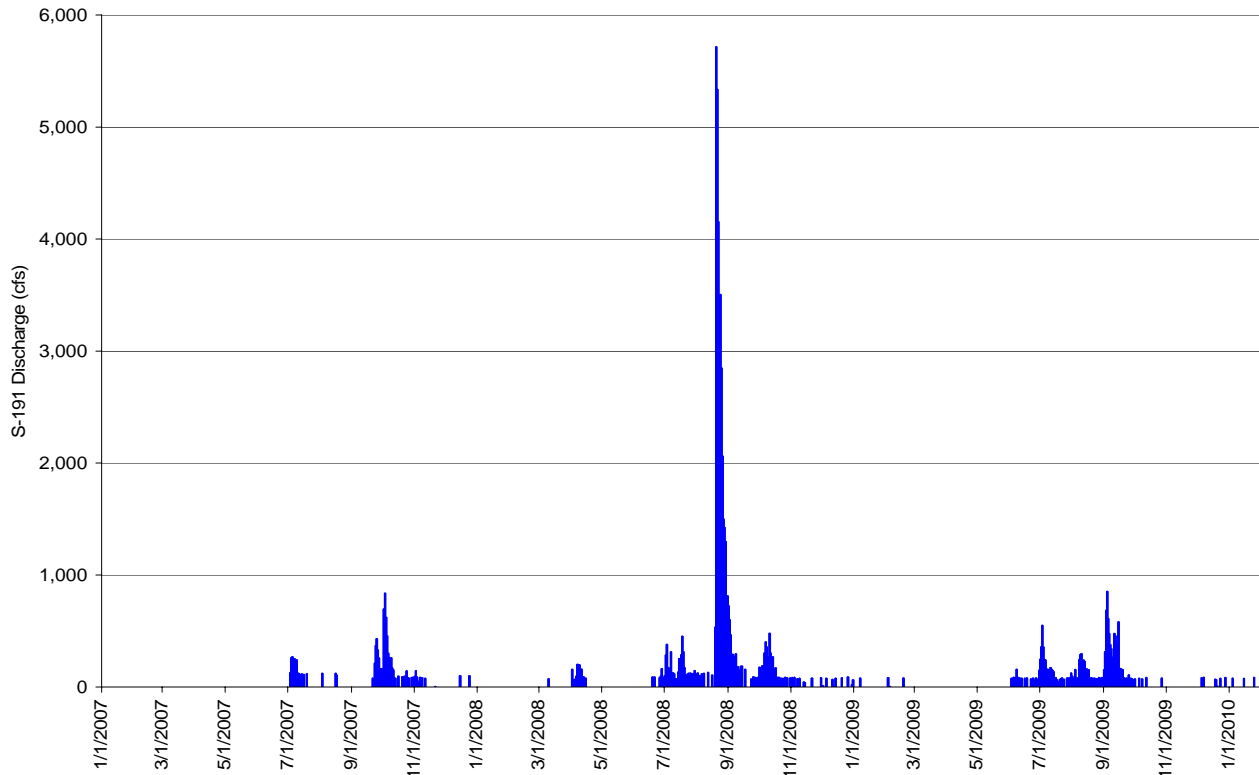
#### *Algal Productivity and Stormwater Runoff*

In addition to the paucity of algae specific toxicity data related to many of these herbicides, the Taylor Creek investigation into the presence and extent of specific herbicides and pesticides were impeded by the frequency of the presence of the toxic compound(s) within the water. During the three years of operation at the TC-ATS, periods of algal turf growth were typically followed by rapid algal turf die-off (Figure 28). During the operational period, the lowest algal productivity periods occurred in early summer and fall, with highest productivity in the winter months and late summer. The early summer die-off (May-June) appears to be associated with the beginning of the rainy season, and the attendant "first flush" runoff. The relationship between toxic influence and runoff is not as obvious with the fall die-off (September-October), and it is possible that the patterns may be as dependent upon upstream application of the toxic compound(s) as they are upon runoff fluctuations. However, when algal production is temporally matched with runoff as indicated by flows within Taylor Creek (releases at Lake Okeechobee through structure S-191), there is noted a clearer relationship between production and runoff associated flows (Figure 29). The observed toxicity appears largely associated with runoff patterns. The implication is that the toxic compound(s) originate from activities associated with purposeful material distribution (e.g. spraying) within the watershed, and quite possibly this distribution is associated with one or more of the agricultural practices within the basin.

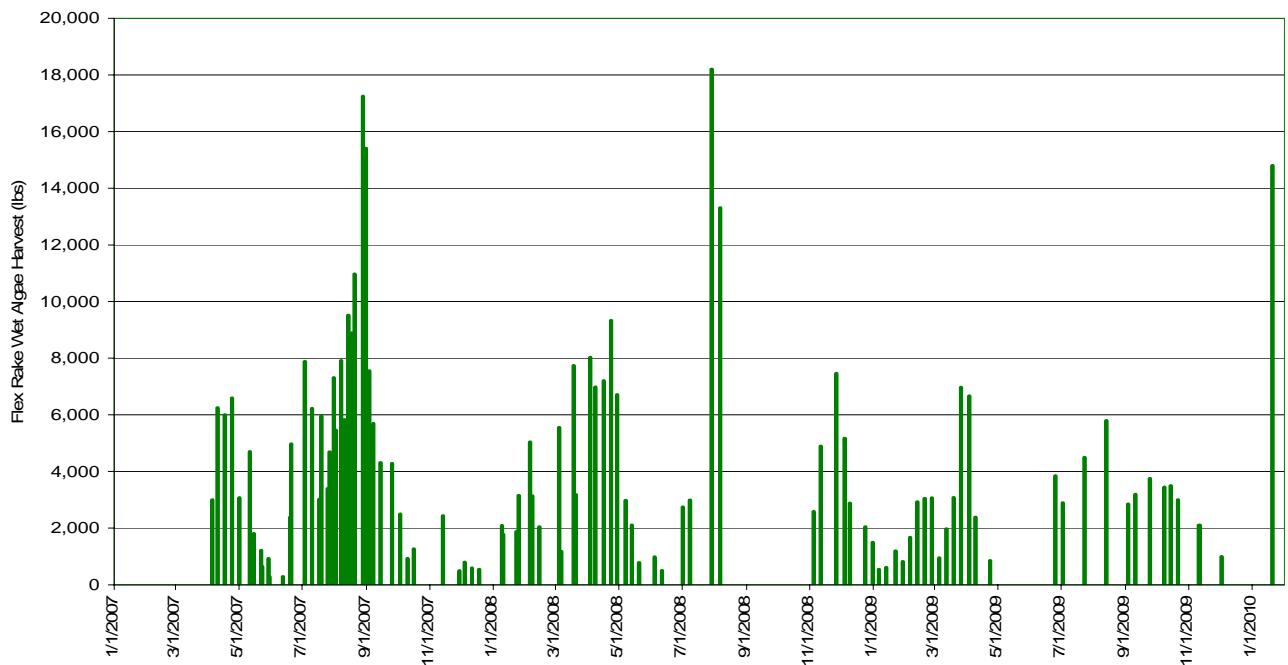
<sup>13</sup> The Dairy Canal Reference is to the upstream portion of Taylor Creek which transverses the dairy region of the watershed. The Citrus Canal Reference is that portion associated with the citrus and potato farms. The pump station is the combined flows at the pump station intake, and the settling pond is a pond where harvested solids accumulate.



**Figure 28:** Algal turf productivity over TC-ATS operational period.



(a)



(b)

**Figure 29:** (a) Taylor Creek discharge into Lake Okeechobee at S-191 from January 2007 to January 2010, compared to (b) wet harvest at FlexRake TC-ATS Facility for same time period.

*Unidentified Compound via GC-MS*

In addition to the testing provided by the USGS, analyses for specific herbicide and pesticide compounds identified as likely candidates for use in the Taylor Creek basin were conducted by FDACS and IFAS. The findings of the FDACS analyses, as noted in Appendix D, showed no detection of a long list of compounds, including Atrazine, Metolachlor, Diquat and Paraquat. Similarly, related compounds were also undetected through analyses completed by the IFAS Laboratory in Ft. Pierce, Florida.

However, Dr. Chris Wilson, who completed the Gas Chromatography-Mass Spectrometry (GC-MS) analyses for IFAS, noted a large peak of an unidentified compound in three of the composite samples and one foam sample analyzed (see Table 12 and Appendix E). When asked, FDACS lab personnel provided verbal confirmation that they also noted similar large peaks in their analyses. Analysis of field and equipment blanks with deionized water showed these peaks were not sample contaminants.

The unknown peak was associated with the composite samples, while absent in the grab samples (Table 12). It also was noted in higher concentrations in foam taken from the influent. This suggests the material(s) associated with the peak may be released intermittently. This pattern would be consistent with some type of scheduled application (such as spraying episodes or planned wash downs), although the evidence is not conclusive. Because the peak is large, implying comparatively high concentrations, and because the peak is notably absent within the grab samples, it is suspected that the spike concentrations during the release period would be even higher. The higher association of the peak with the foam than with the composite water samples indicates the material may be a surfactant.

**Table 12:** GC-MS unknown chemical detection patterns.

Sample	Date	Sample Type	Unknown Chemical Present
TCATSO-062508g	06/25/2008	Influent Grab	-
TCATSO-062508c		Influent Composite*	+
TCATSO-070908g	07/09/2008	Influent Grab	-
TCATSO-070908c		Influent Composite*	+
TCATSO-072108g	07/21/2008	Influent Grab	-
TCATSO-072108c		Influent Composite*	-
TCATSO-080408g	08/04/2008	Influent Grab	-
TCATSO-080408c		Influent Composite*	+
TCATSO-021609f	02/16/2009	Influent Foam Concentrate	+

\*Composite samples via autosampler over one week. Samples taken as 100 mL aliquots every three hours.

The identity of the unknown peak was investigated using MS and the spectra were queried against a NIST MS spectra library. The top two matches included the compounds, dipropylene glycol monomethylether and 1-[1-methyl-2-[2-propenyloxy]ethoxy]-2-propanol (see Appendix E). Dipropylene glycol, which has many commercial synonyms including Dowanol 50B and Arcosolv (<http://www.osha.gov>), is an industrial co-solvent used widely in agriculture and industry.

Interestingly, glycol ethers work as co-solvents because of their surfactant-like nature and are used to emulsify and stabilize the active ingredients in agricultural pesticide formulations (Dow Chemicals Answer Center <http://dow-gco.custhelp.com>). Although propylene glycol ethers are generally believed to have low aquatic toxicity, there is inadequate data regarding the impacts of these chemicals on the environment. As stated in a technical bulletin regarding propylene and ethylene glycol ethers toxicity: "The 'spottiness' of the data is evident, as is the lack of test results for certain (chemical) species and the fact that no single laboratory appears to have tested propylene and ethylene glycol ethers at same time with the same protocol (<http://www.lyondellbasell.com>)."

Overall, the GC-MS analysis and database query enhanced understanding of the types of compounds which are present within the Taylor Creek water. However, the data alone do not offer conclusive evidence that the materials discovered were algal toxins. More complex, specialized analysis would be necessary to precisely identify the unknown compound(s) and to identify a cause-effect relationship between the unidentified compound and algae productivity.

While the discovery by IFAS of this peak does not confirm the toxicity or even the involvement of this compound(s) in the impedance of algal production, its presence in comparatively high concentrations did demand further consideration, and gave rise to the possibility that the toxic agent(s) may be a substance other than a pesticide or herbicide, and that a surfactant type compound(s), which either serves as an adjuvant to assist in improving the efficacy of pesticides and herbicides, or perhaps a detergent used in one or more of the agricultural activities within the basin, needed to be included within the list of suspects. This suspicion was further reinforced by the presence of heavy foaming associated with the influent, and the fact that the foam was often dark, and emitted a chemical odor (Illustration 14).



**Illustration 14:** Typical foaming at influent surge box at TC-ATS facility.

#### *Toxicity Identification Evaluations (TIE)*

To confirm or reject the presence of toxicity within the Taylor Creek water and further evaluate the nature of any toxic agent(s) detected, it was decided to conduct more formal toxicity testing using EPA's Toxicity Identification Evaluations or TIE procedures.

The TIE methodologies are EPA approved bioassay methods for determining the presence, nature and virulence of toxic agents in water. There is a TIE procedure (EPA Protocol Cf4-EPA-821-R-02-013—Chronic Algae Toxicity Evaluation) applied to the green algae, *Selanastrum capricornutum*, that is designed to determine if a water is toxic to algal productivity. *Selanastrum* is common green alga that presents itself as single cells or small colonies. It would be expected to thrive within an algal turf community. The *Selanastrum* response, grown as a monoculture within the laboratory in conformance

with the testing protocols established for the TIE procedure, is accepted by EPA as an indicator of algae toxicity within the waters tested.

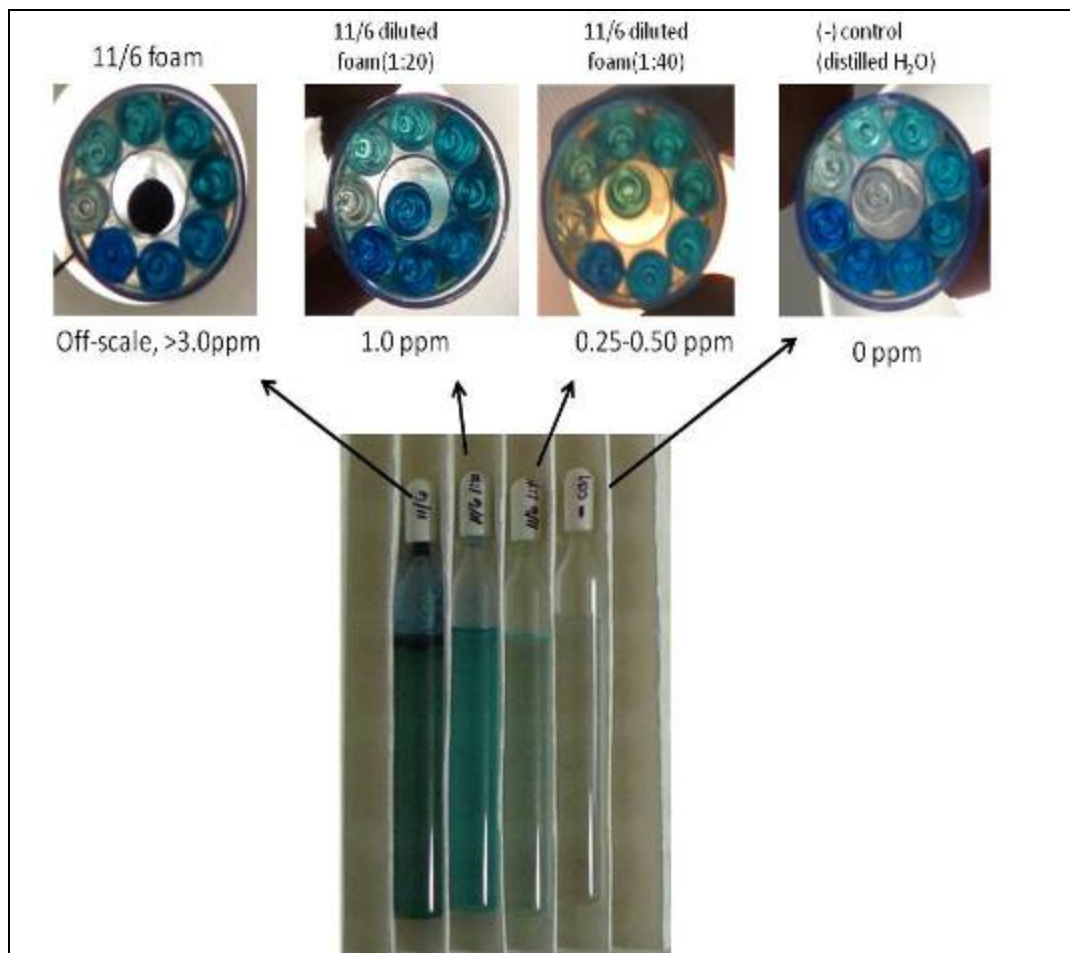
An Environmental Toxicology laboratory in Davis California—AQUA-Science—conducted TIE testing on a sample of Taylor Creek water submitted on 12/17/2008. They concluded that “statistically significant algal toxicity was detected at the highest concentrations.” *Selenastrum* grown in this sample of undiluted Taylor Creek water using the TIE protocol showed 25% reduction in growth response when compared to the controls. This was stated as an Inhibition Concentration 25% or IC<sub>25</sub>.

Recognizing that the presence of toxicity within the Taylor Creek water would likely be intermittent, and that there was some likelihood that the toxin(s) would be concentrated within the influent foam, samples of the foam diluted with deionized water were submitted on two occasions—February 16, 2009, and March 30, 2009. The first sample was composed of collected foam diluted 50% with deionized water; the second was collected foam diluted about 75% with deionized water. Both showed statistically significant algal toxicity, with the more concentrated sample showing 53% reduction of growth initially. After one week the sample was retested, showing 41% reduction in growth, indicating the toxin(s) was rather stable. Further testing with EDTA addition showed the toxin(s) was not removed by EDTA. This provided evidence that heavy metals were not responsible for the toxicity. Treatment with activated carbon, as with earlier tests by HydroMentia, showed the toxin was removed. Similarly, aerating (sublimating) the sample appeared to remove toxicity, implying the toxin(s) may have a rather high volatility; is removed by the foaming; or is readily oxidized.

#### *Methylene Blue Active Substance Testing*

Ancillary to the TIE evaluations, a Methylene Blue Active Substance (MBAS) test kit was used as a means of detecting anionic surfactants at levels ranging from 0.25 – 3.0 mg/L, within diluted Taylor Creek foam samples. The test kit employs the methylene blue extraction method in which anionic detergents react with methylene blue to form a complex that is extracted and phase separated into an organic solvent. The intensity of the blue color is directly proportional to the concentration of ‘methylene blue active substances’ (MBASs) in the sample and comparison to standards allows quantitative estimates of MBASs ranging from 0-3.0 mg/L.

Diluted foam samples (1:20 and 1:40) were collected from Taylor Creek (11/6/09) and tested according to the manufacturer’s protocol. The results indicated the presence of ‘MB active substances’ (an indirect measure of anionic surfactants) and provided an estimate of the anionic surfactant levels in the undiluted foam at about 40 mg/L (Illustration 15).



**Illustration 15.** MBAS testing of foam and diluted foam samples collected at Taylor Creek on 11/6/09. The top photos illustrate comparison to the anionic surfactant standard (from 0-3.0 mg/L; standard = linear alkylbenzene sulfonate). The bottom photo is a side-by-side comparison of the same samples. Note that foam was a 50% mixture of water/foam; therefore, the estimated level of anionic surfactants in the foam is 40 mg/L.

The MBAS test can over-estimate the amount of anionic surfactants due to environmental interferences, although the chemical extraction method reportedly minimizes these impacts. However, according to Barsh *et al.* 2008<sup>14</sup>, "While it has some cross-reactivity with other natural and anthropogenic compounds, environmental chemists generally agree that any over-estimation of LAS by this method is comparable to its under-estimation of *total* surfactants in the environment."

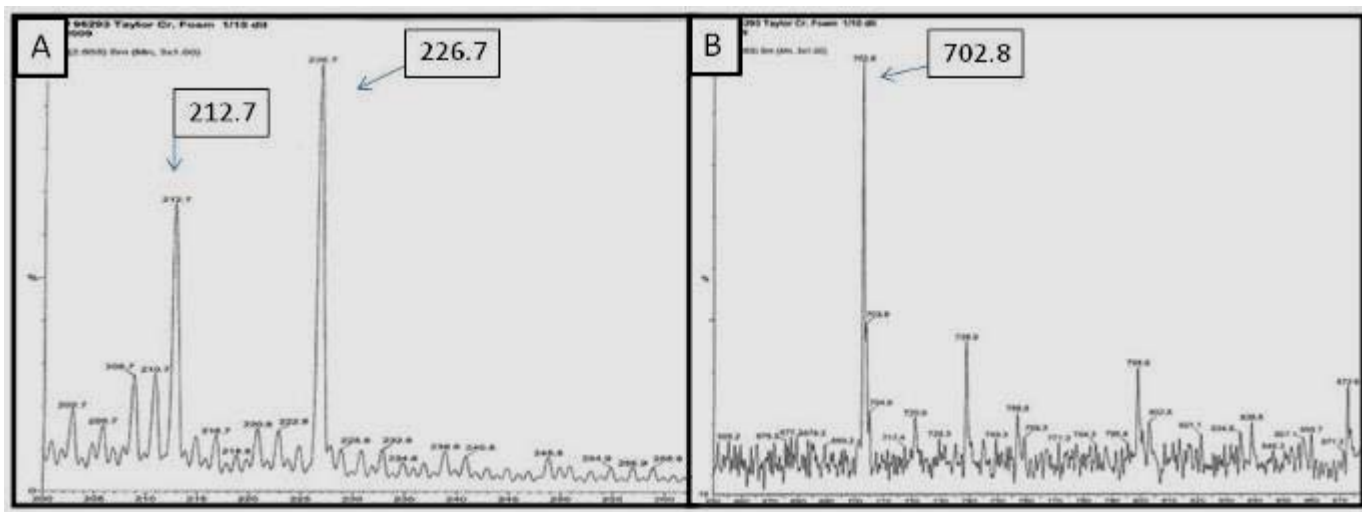
Since the MBAS assay is considered the industry standard for detecting anionic surfactants in waste water (and is the basis of EPA standards), this finding added further evidence that surfactants may be one class of anthropogenic compounds negatively impacting Taylor Creek. Although the MBAS test is non-specific and does not provide information on identity of the surfactant(s), it can function as an important screening tool to identify samples with higher loads of surfactants that can be further characterized.

<sup>14</sup> R. Barsh, J. Bell, H. Halliday, M. Clifford and G. Mottet. 2008. Preliminary Survey of Pyrethroid Pesticides and Surfactants in San Juan County Surface Waters. Report for Center for the Historical Ecology of the Salish Sea. Lopez, WA.

### *Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry*

To further examine the nature of surfactants within Taylor Creek, a screening study was conducted using both positive and negative electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) mass spectrometry to detect anionic cationic, and/or non-ionic surfactants in Taylor Creek foam samples. On 11/21/2009, foam samples were collected and shipped to M-SCAN (Westchester, PA) for analysis.

The negative ESI and MALDI (THAP spectra only) analyses resulted in the detection of unknown chemical components, including compounds with masses of 214.7 or 226 Da, 228 Da and 703.8 Da (see Figure 30).



**Figure 30.** Negative electrospray expansions illustrating unknown chemical components in diluted samples of Taylor Creek foam.

The positive ESI and MALDI analyses also detected unknown chemical components, with masses ranging from 200-910 Da (data not shown). Although both positive and negative ESI and MALDI mass spectra appeared to contain components at detectable levels, further analyses and data would be required to determine the identity of the components. This finding is perhaps not surprising considering that surfactants are among the most widely disseminated xenobiotics that enter the aquatic environment and identification is challenging due to the diversity of surfactants and the complexity of environmental matrices.

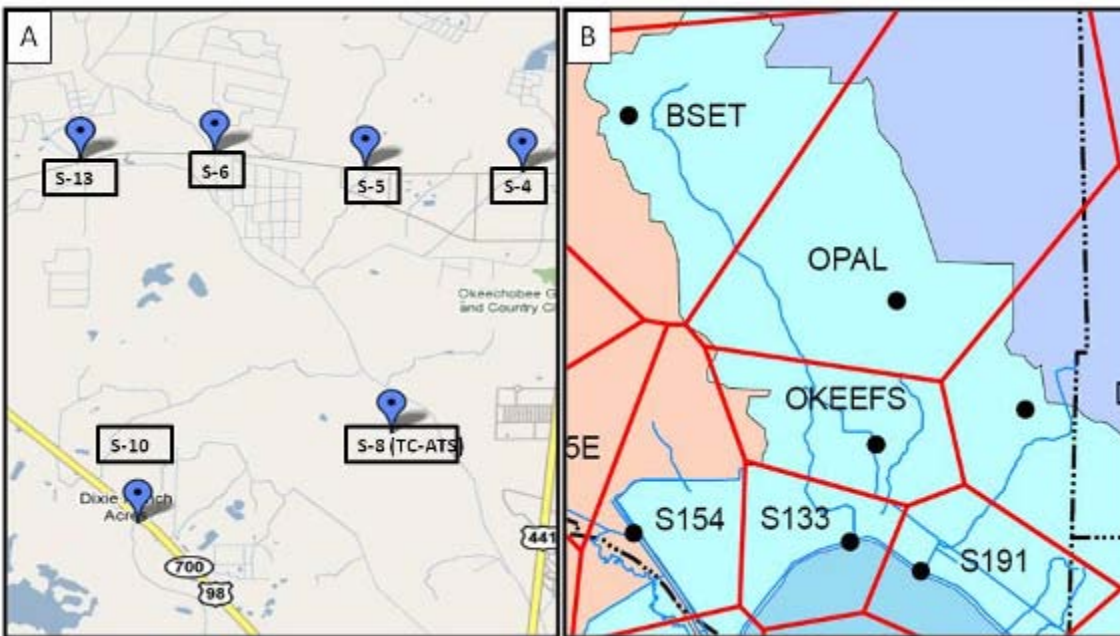
Although this testing did not facilitate identification of the components in the spectra, the analyses did provide some valuable information. The ESI and MALDI data did not show clear evidence of certain common polymeric surfactants, including polyethoxylates, which would have been represented in the spectra as a series of ions with constant repeating intervals (44 daltons). The components with masses ranging from 200-250 Da in the negative MS analyses do, however, overlap with masses of common anionic sulfonate-alkyl chain surfactants (8-10 carbons). Structural and identity assignments would require additional data since thousands of chemicals have masses in this range. A full report on these MS analyses is presented in Appendix F.

It is important to emphasize that in the weeks following the 11/21/2009 foam sample, the ATSTM floway exhibited a rebound in periphyton growth, which may suggest that the sampling period was not optimal for detecting the toxic compound (s). In addition, the 11/21/2009 foam samples were prescreened using the

MBAS test, which yielded inconclusive results due to interferences from 'colored' substances in the foam that caused the reaction to appear green, not blue (negative controls = clear). Analysis of 11/21/2009 TC influent indicated ~ 0.25 mg/L of MBAS's (reaction was a 'blue' color); however, since the limit of detection of the kit is 0.25 mg/L, this represents very low level.

While the static and dynamic bioassays; the TIE analyses; the GC-MS evaluations, MBAS screening; and ESI-MALDI testing either verified or indicated likelihood of the presence of a toxic agent(s), and enhanced understanding of the nature of the toxin(s) involved, they did not offer any insight related to the source(s) responsible for release of this toxin(s). Review of harvest and productivity data provide a rather clear indication that these releases are associated with runoff, and that delivery and subsequent deleterious impacts are, accordingly, intermittent. In a parallel effort to identification evaluations, sampling and testing protocols were established and implemented to track the relative abundance and scheduling of the toxin(s) upstream of the intake of the Taylor Creek pump station, which delivered water to the TC-ATS floway. Sites selected for sampling are shown in Figure 31, -site S-8 is the intake to the TC-ATS facility, and site S-10 is within the upstream reaches of the S-154 basin.

### Geographic Toxicity Sampling



**Figure 31:** Geographic toxicity sampling sites and rainfall reporting stations.

Static bioassays from each site was conducted from August 30<sup>th</sup> to October 25<sup>th</sup>, 2009. Five independent bioassays were completed during this time, with the intent of measuring site-specific toxicity within the Taylor Creek basin. Two-liter water samples were recovered from these sites and three 500 mL aliquots were decanted into 600 mL beakers. Replicate beaker samples were spiked with F/2 growth media and a highly homogenized periphyton inoculum. After about 1 week of growth, the filamentous periphyton was harvested using a 1 mm<sup>2</sup> screen, dried, and weighed using an analytical balance.

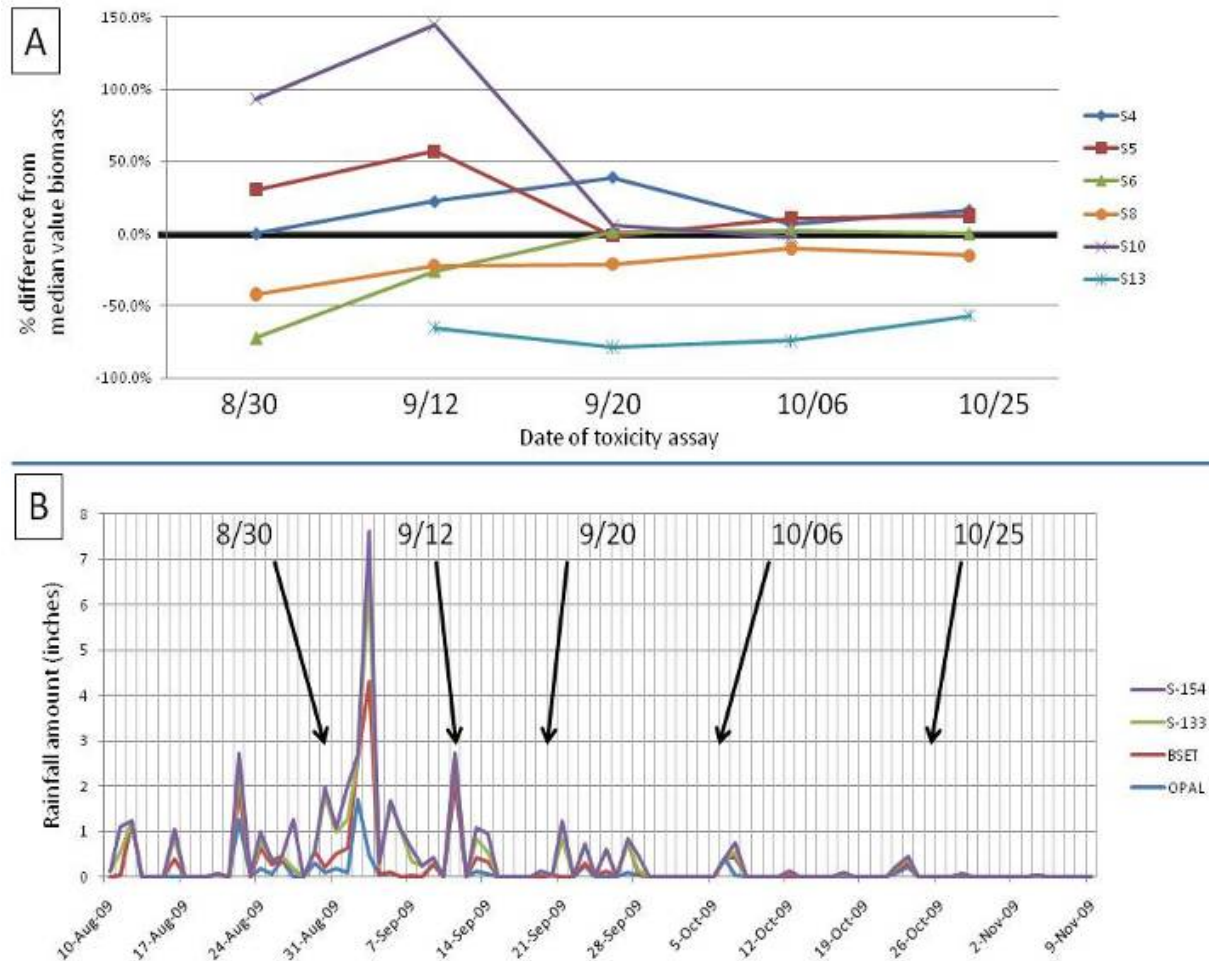
A comparison of rainfall patterns and relative levels of site-specific periphyton development reveals several discernible trends (Figure 332).

- Bioassays sampled during, or after, significant rainfall events (> 1"; see 08/30 and 09/12 bioassays) exhibit dramatic differences in periphyton productivity across sites; with sites S6, S13 and S8 exhibiting the lowest productivity.

- As rainfall frequency and intensity decreases, the differences in periphyton productivity among the sites narrows, although the Taylor Creek (S8), S13 and to a lesser degree S6 sites, still exhibit lower levels of periphyton productivity than the S4 and S5 tributaries.
- The S6 tributary appears to be the most impacted by rain events, as evidenced by the low levels of productivity measured after major rainfall events in the first two experiments, followed by the clear improvement in productivity as rainfall levels and frequency decrease. The improvement at S6 appeared to correspond to a decrease in color of the water (from dark brown to a light tea color).
- The S13 site consistently exhibited the lowest levels of productivity development and also had the darkest water; however the small size of this tributary should be considered when assessing its potential impact on the Taylor Creek ATS.
- The S-10 station, which served as a control outside the Taylor Creek basin, and the extreme eastern S-4 station showed comparatively higher levels of productivity.

The station associated with the western tributaries (S-6 and S-13) show the lowest productivity, and may contain the greatest concentration of the toxic compound(s) responsible for affecting algal turf productivity at the TC-ATS facility. These eastern most tributaries are in a region of the basin in which citrus and potato farming is the most prevalent. Dairy farming is aligned more with the eastern tributaries. The S-8 (Taylor Creek Intake) station also shows comparatively poor productivity. It is not known if this is related to influences from S-13 or from additional toxicity contributed from downstream run-off and intensive herbicide spraying.

Following completion of these static bioassays, attempts were made to set in-field strips of growth matrix on which native populations of periphyton could collect and grow—an apparatus known as a periphytometer. Although there were some difficulties in ensuring the periphytometers would remain effective in the field, the data which was collected again showed the regions of S-6 and S-13 associated with the lowest productivity. Interestingly, the periphytometers placed at the downstream confluence of the tributaries showed much improved productivity. This was a region characterized by extensive growth of floating aquatic vegetation, dominated by water hyacinths. Vascular aquatic plants appeared to thrive in the water in this region, giving indication the toxin(s) associated with this water does not only not affect floating vascular plants, but may be in some manner attenuated by them.



**Figure 32** Comparison of rainfall and site-specific periphyton levels measured with toxicity bioassays. Panel A illustrates relative levels of periphyton harvested from bioassays, expressed as percent relative to the median value<sup>1,2</sup>. Panel B shows the rainfall amounts at recording stations in the Taylor Creek watershed.

<sup>1</sup> Median values calculated within each experiment, from samples common across experiments, with two exceptions; S13 was not analyzed in the 8/30 assay and S10 was not analyzed in 10/25 assay; therefore the median values for these experiments were calculated using 5 samples, as opposed to 6.

<sup>2</sup> With the exception of the 08/30 assay, triplicate analysis was performed for each site-specific water samples.

<sup>3</sup> The growth phases of the 8/30, 9/12, 10/6 and 10/25 bioassays were very similar at 7-8 days and ranging from 104-111 hours of light exposure. In contrast, the 9/20 experiment was performed for 11 days and 170 hours of light exposure.

## Investigations into Pretreatment Systems Intended to Attenuate Impacts of Toxic Compounds upon Algal Turf Development

### *General Comments*

While identification of the toxic compound(s) responsible for substandard algal turf productivity and poor system performance at the TC-ATS facility is important, it is also important to identify reasonable, cost effective means of treatment such that the impacts of the toxin(s) are abated. Abatement could be a result of enzymatic induced changes to the toxin(s); physical removal through settling, foam fractionation, volatilization, direct uptake within tissue or adsorption; or change in molecular structure or toxicity associated with controlled chemical reactions. During the course of the investigations associated with identification, information was collected which provided some insight into what pretreatment approaches might be most effective. Accordingly, these were investigated through pilot testing in the field. Those pretreatment approaches tested through pilot investigations were:

- **Foam Fractionation**—Selected because of TIE testing which indicated aeration removed the toxin(s), and the possibility of surfactants contributing to toxicity.
- **Water Hyacinth Scrubber (WHS™)**—This would be a replication of the two-stage arrangement associated with the S-154 project which was operated in addition to the single-stage Algal Turf Scrubber® pilot. Also, the periphytometer data indicated toxicity attenuation just downstream of water hyacinth growths within Taylor Creek.
- **STA type wetlands**—Similar consideration as with the WHS™.
- **Alum precipitation**—Treatment with Alum reduces color causing components in the water. There is some indication that there may be a relationship between water color and the presence of toxic compound(s). Alum treatment also reduced pH and alkalinity, while reducing total phosphorus.

Other pretreatment options which may be effective, and could be considered are ozonation; high rate filtration; and high rate aeration.

### *Foam Fractionation*

Because surfactants were suspected as possible contributors to the toxicity of the Taylor Creek water, and because of the evidence provided through TIE analyses and subsequent GC-MS and ESI-MALDI, it seemed reasonable that foam fractionation might provide sufficient removal of the toxin(s) to allow robust algal turf development. Foam fractionation works by sparging air upward through a column, into a counter current flow of the influent water. This creates foaming. The rising foam is then removed through an upper chamber in the column. Foam fractionation is quite effective in removing protein and surfactants from aquaria.

Initially a small foam fractionator was designed and built in-house (Illustration 16). This unit received about 1-2 gpm. The effluent was delivered to a Micro-ATS unit, and the growth was compared to a control. The influent treated with the foam fractionator did not facilitate turf development compared with the control. The turf that developed was composed largely of diatoms mixed with amorphous organic debris. In general, the foam fractionator did not reduce the toxin(s) effects within the Taylor Creek influent.

Recognizing that the in-house foam fractionator may not be as effective as a pre-designed industrial unit, it was decided to purchase and install such a unit (Illustration 17). This unit was designed to manage flows of 100 gpm, which allowed testing of a larger flowway system—namely HydroMentia's 1 foot wide Mobile Pilot Unit (MPU). These units, set up at a length of 50 feet, allowed testing of about 20 gpm taken from the foam fractionator effluent. The foam fractionation did not result in any significant attenuation of toxic influences. In Illustration 17, the control and foam fractionation test unit are compared with the MPU

which received Taylor Creek effluent from a Water Hyacinth Scrubber (WHS™) which shows a healthy algal turf. Pretreatment with a WHS™, as discussed in the following subsection, did prove to be effective in significant de-toxification of the Taylor Creek influent.



(a)

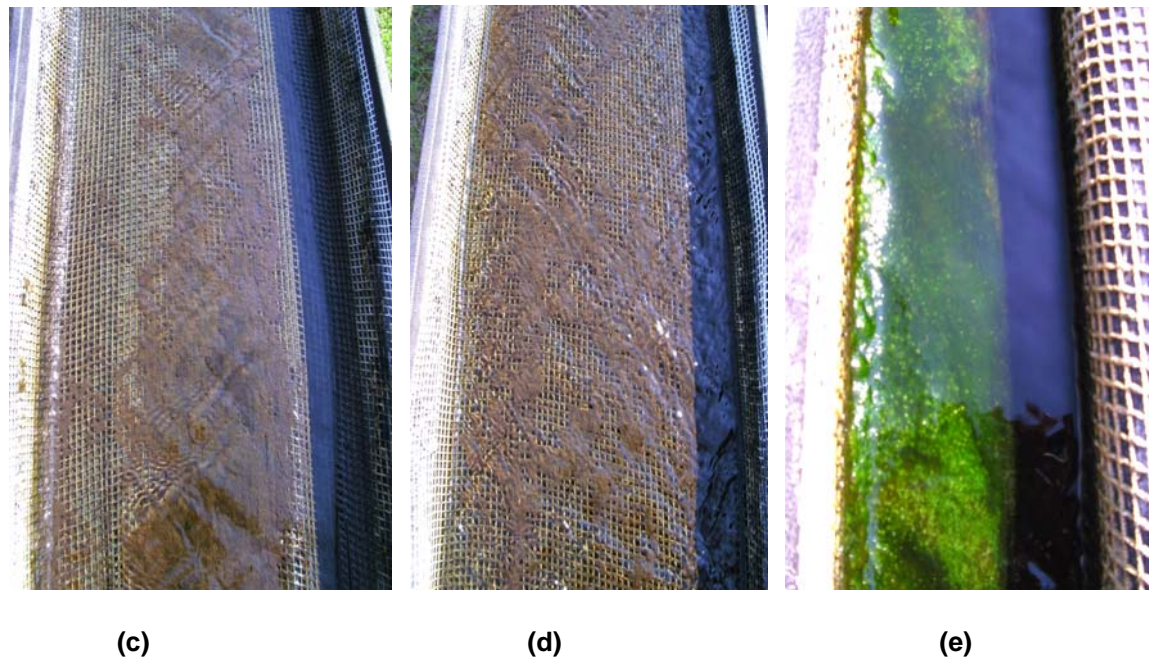
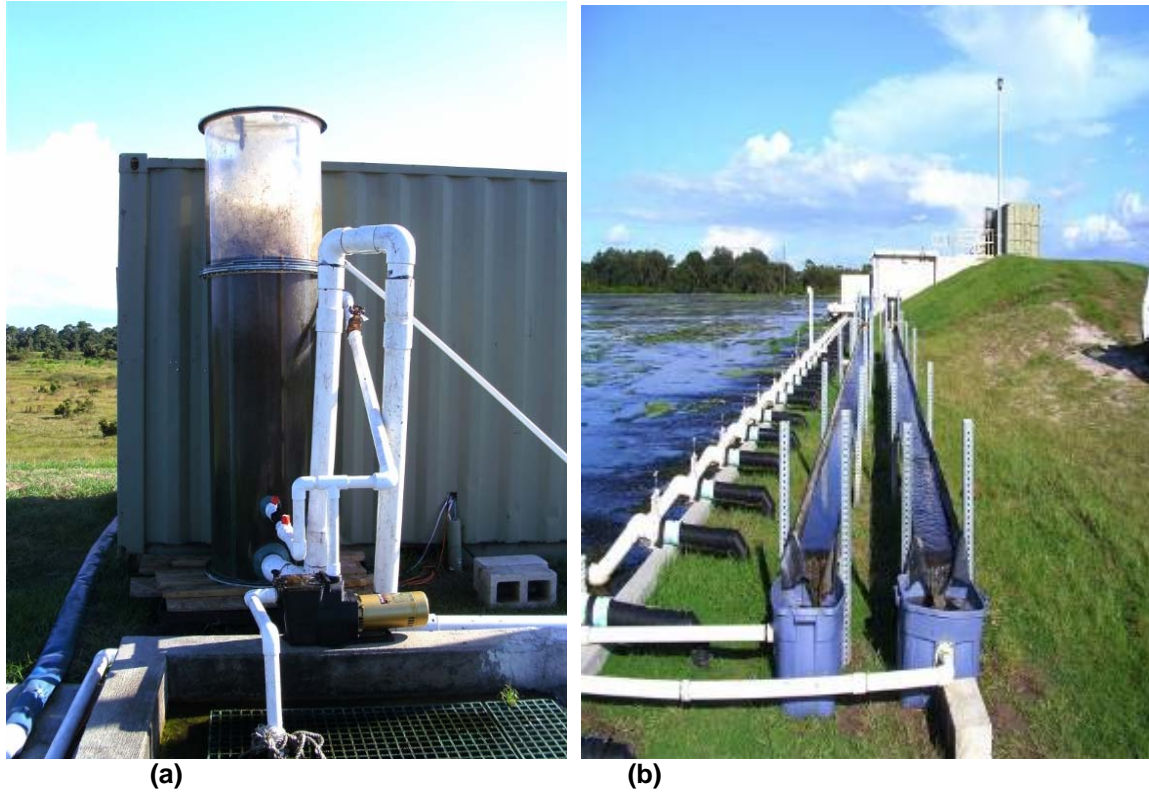


(b)



(c)

**Illustration 16.** (a) In-house designed foam fractionator with Micro-ATS, (b) turf development after foam fractionation (c) turf development control.



**Illustration 17.** (a) Industrial foam fractionator (b) MPU set-up with parallel control and test units (c) turf development control (d) turf development after foam fractionation control (e) turf development after pretreatment with Water Hyacinth Scrubber (WHS™).

### *Water Hyacinth Scrubber*

The pretreatment of influent with a Water Hyacinth Scrubber (WHS™) prior to delivery to an ATS™ system is an approach which was contemplated and evaluated in considerable detail<sup>15</sup>. While the performance of this two-stage system was quite encouraging, the phosphorus and nitrogen areal removal rates were not as high as when the ATS™ was operated as a single stage unit. Consequently, in favor of a reduced area requirement and treatment costs, a single-stage ATS™ system was selected for implementation at the Taylor Creek site (TC-ATS). However, after a prolonged period of poor performance as a result of toxic compound(s) within the TC-ATS facility, it was decided to re-examine pretreatment with a WHS™ unit.

A pilot test unit was established on the TC-ATS site, with a 100 ft x 6 ft lined pond, at an average depth of about 2.5 ft serving as the first stage WHS™ (see Illustration 18). The effluent from this unit was delivered to a 90 foot long MPU unit adjusted in width to establish a Linear Hydraulic Loading Rate of about 20 gpm/lf.

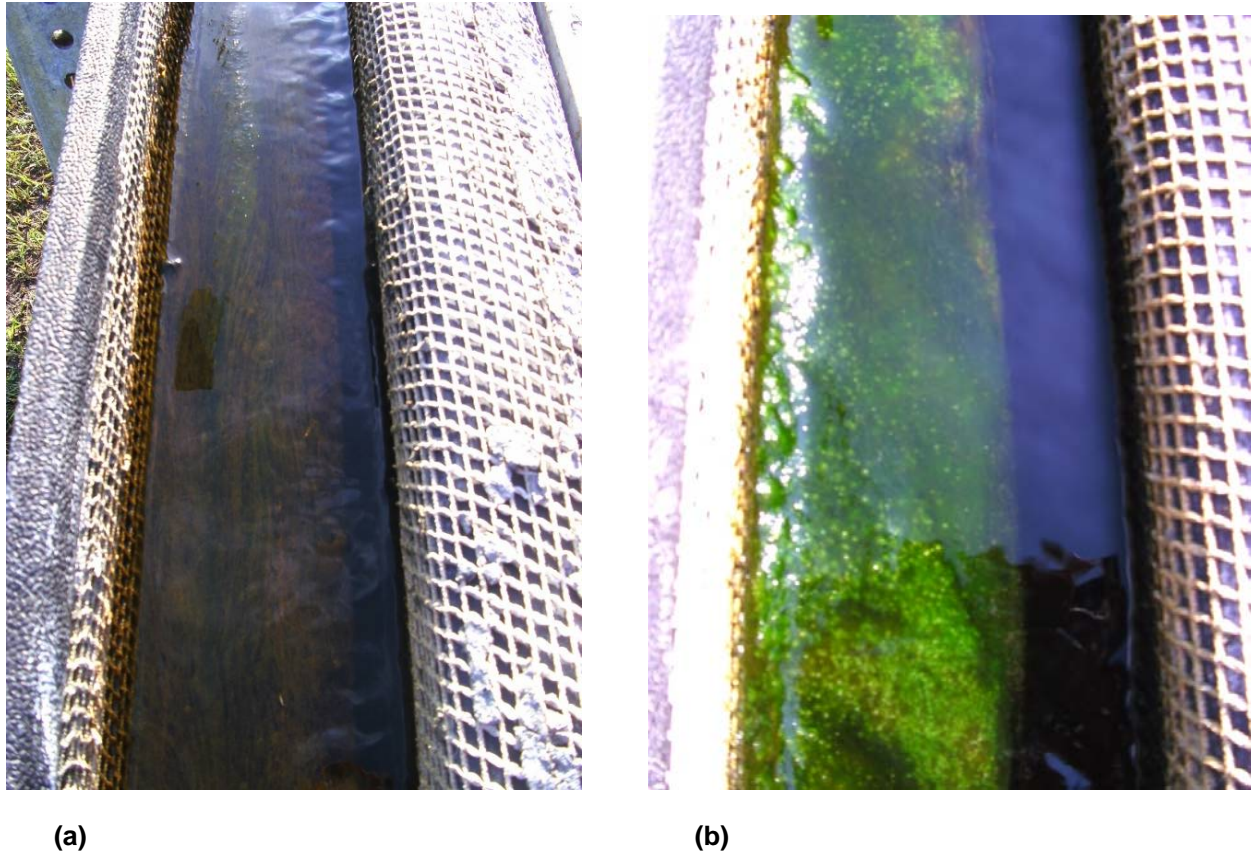


**Illustration 18.** Water Hyacinth Scrubber (WHS™) pretreatment pilot unit at TC-ATS.

Taylor Creek influent was delivered from the TC-ATS Parshall Flume Box to the two-stage WHS-ATS Pilot at a rate of 4 gpm over the period from May 18, 2009 to November 16, 2009. During this period, the water hyacinth density was maintained at about 4 wet lb/ft<sup>2</sup>, or a standing crop of about 2,400 wet pounds or 120 dry pounds. Portions of the crop were harvested weekly, and the harvested tissue analyzed periodically. Water quality of the WHS™ influent and effluent was taken weekly.

<sup>15</sup> HydroMentia (2005) "S-154 Pilot ATS™-WHS™ Aquatic Plant Treatment System Final Report" for SFWMD Contract C-13933

During the course of the testing period, the ATS™ MPU showed development of a healthy algal turf (Illustration 19). As shown, green filamentous algae began developing after just seven days, indicating significant attenuation of toxin(s).



**Illustration 19:** Water Hyacinth Scrubber (WHS™) pretreatment pilot unit at TC-ATS—MPU Algal Turf (a) after seven days (b) after seven weeks. Note filamentous algae developing after only seven days.

Performance of the WHS™ unit was in general alignment with expectations, based upon previous experiences with water hyacinth treatment systems (Table 13). Total phosphorus reduction averaged 35.5% with an average TP ARR of 23.81 g/m<sup>2</sup>/yr. Total nitrogen reduction averaged 26.7% with an average ARR of 78.97 g/m<sup>2</sup>/yr. The WHS™ was harvested on six different occasions, with about 30% removal of the standing crop during each harvest. At the end of the testing period 250 lbs of sediments were removed from the WHS™ unit, at 22% solids. Analysis of the water hyacinth tissue and the recovered sediments were conducted (Table 14). The wet hyacinth tissue was about 5% solids. A comparison of TP and TN removed based upon water quality and harvest based calculations were consistent with each other (Table 15).

A first order growth kinetics model, the Hyacinth Design Model or HYADEM, was run for the time period under the water quality conditions experienced. Projections compared to actual results show the model tracks performance closely (Figures 33 and 34). Based upon these results it was concluded that the toxin(s) associated with the Taylor Creek influent did not deleteriously impact productivity of water hyacinths, or the performance of a WHS™ unit.

**Table 13:** WHS™ performance during testing period of WHS-ATS pilot at TC-ATS facility.

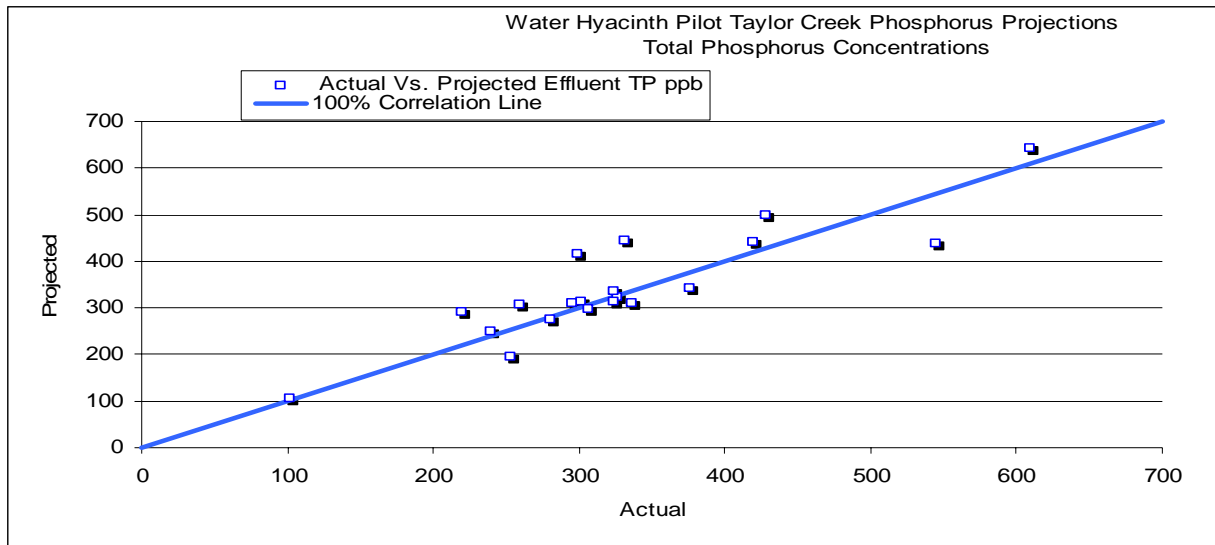
week ending	Influent TP ppb	Hyacinth Effluent TP ppb	Influent TN mg/l	Hyacinth Effluent TN mg/l	WHS™ Total Phosphorus Areal Removal Rate g/m <sup>2</sup> -yr	WHS™ Total Nitrogen Areal Removal Rate g/m <sup>2</sup> -yr	Percent TP Removal	Percent TN Removal
5/18/2009	219	134	1.16	0.86	12.13	42.83	38.8%	25.9%
5/25/2009	261	162	1.14	1.68	14.13	-77.09	37.9%	-47.4%
6/1/2009	200	102	1.09	0.90	13.99	27.12	49.0%	17.4%
6/8/2009	421	239	2.38	1.53	25.98	121.34	43.2%	35.7%
6/15/2009	411	280	1.55	1.19	18.70	51.39	31.9%	23.2%
6/22/2009	598	545	1.69	1.40	7.57	41.40	8.9%	17.2%
6/29/2009	452	336	1.62	1.26	16.56	51.39	25.7%	22.2%
7/6/2009	512	376	2.16	1.58	19.41	82.80	26.6%	26.9%
7/13/2009	806	609	2.19	1.81	28.12	54.25	24.4%	17.4%
7/20/2009	697	428	2.48	1.56	38.40	131.34	38.6%	37.1%
7/27/2009	487	302	2.13	1.30	26.41	118.49	38.0%	39.0%
8/3/2009	480	327	1.82	1.41	21.84	58.53	31.9%	22.5%
8/10/2009	612	299	2.5	1.32	44.68	168.45	51.1%	47.2%
8/17/2009	630	420	2.29	1.80	29.98	69.95	33.3%	21.4%
8/24/2009	460	220	1.96	1.33	34.26	89.94	52.2%	32.1%
8/31/2009	367	253	2.01	1.41	16.27	85.65	31.1%	29.9%
9/7/2009	610	332	2.06	1.28	39.69	111.35	45.6%	37.9%
10/5/2009	480	260	2.3	1.25	31.41	149.90	45.8%	45.7%
10/12/2009	503	324	2.27	1.32	25.55	135.62	35.6%	41.9%
10/20/2010	477	324	1.86	1.22	21.84	91.36	32.1%	34.4%
11/2/2009	442	296	1.56	1.09	20.84	67.10	33.0%	30.1%
11/16/2009	420	307	1.54	1.09	16.13	64.24	26.9%	29.2%
<b>AVERAGE</b>	<b>479</b>	<b>313</b>	<b>1.90</b>	<b>1.35</b>	<b>23.81</b>	<b>78.97</b>	<b>35.5%</b>	<b>26.7%</b>

**Table 14:** Water hyacinth tissue and settled solids analysis WHS-ATS pilot at TC-ATS facility.

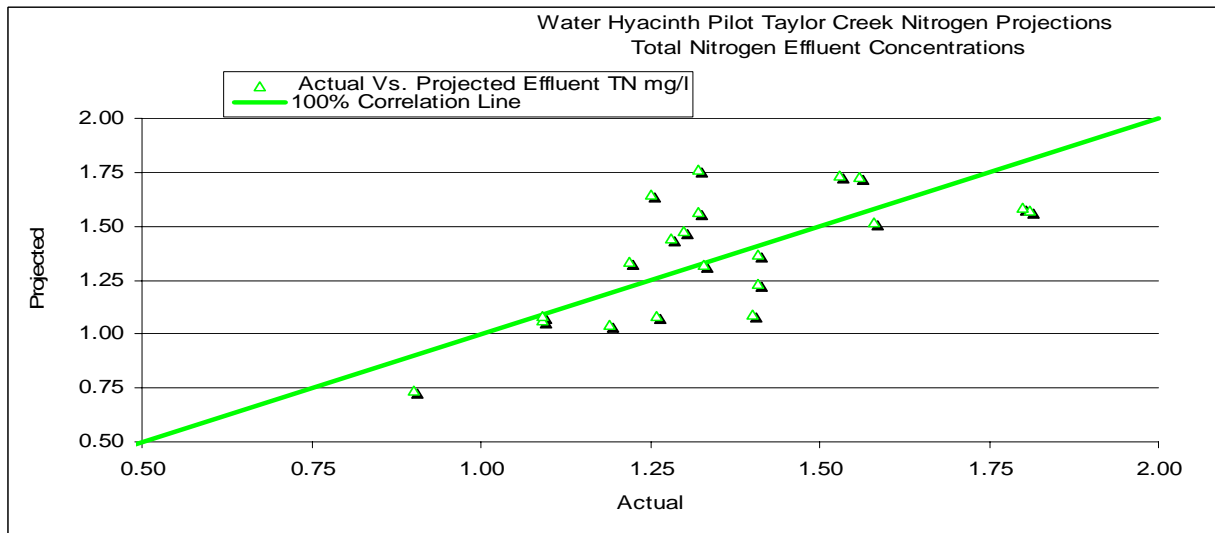
	Hyacinth-1	Hyacinth-2	Hyacinth-3	Hyacinth-4	Average Hyacinth Tissue	Settled Solids
<b>Total N%</b>	1.65	1.59	1.32	1.35	1.48	1.29
<b>Ammonia-N%</b>	0.08	0.01	0.04	0.02	0.04	0.00
<b>Nitrate-N %</b>	nd	nd	nd	nd	nd	nd
<b>TON %</b>	1.55	1.57	1.28	1.34	1.44	1.29
<b>P2O5%</b>	1.13	1.13	0.94	1.09	1.07	0.47
<b>P%</b>	0.54	0.54	0.45	0.52	0.51	0.22
<b>K2O %</b>	0.47	3.32	2.83	4.13	2.69	0.17
<b>S%</b>	0.35	0.36	0.59	0.56	0.47	0.65
<b>Ca%</b>	2.51	1.95	2.51	2.42	2.35	1.38
<b>Mg%</b>	0.71	0.56	0.76	0.73	0.69	0.26
<b>Na%</b>	0.35	0.17	0.34	0.48	0.34	0.06
<b>Cu mg/Kg</b>	nd	nd	nd	nd	nd	nd
<b>Fe mg/Kg</b>	1,513	359	3,179	2,432	1,871	8,527
<b>Mn mg/Kg</b>	891	2,039	1,072	804	1,202	439
<b>Zn mg/Kg</b>	46	62	43	47	50	95
<b>pH</b>	6.20	6.60	6.60	6.60	7	7.00
<b>Total Carbon %</b>	39.75	38.78	38.80	39.80	39.28	15.48
<b>C/N</b>	24.1:1	24.4:1	29.3:1	29.2:1	26.8:1	15.5:1
<b>Cl %</b>	2.46	1.99	1.52	1.58	1.89	0.04

**Table 15:** Comparison of water quality based and harvest based TP and TN removals within the WHS™ unit at WHS-ATS pilot investigation at the TC-ATS facility.

	Water Quality Based Calculations	Hyacinth Harvest and Settled Solids Calculations
TP removed lb	1.5	1.3
TN removed lb	5.5	4.2



**Figure 33:** Water Hyacinth Scrubber (WHS™) pretreatment pilot unit at TC-ATS—actual effluent TP vs HYADEM projected effluent TP.



**Figure 34:** Water Hyacinth Scrubber (WHS™) pretreatment pilot unit at TC-ATS—actual effluent TN vs HYADEM projected effluent TN.

During the course of the WHS-ATS pilot testing period, the productivity of the algal turf associated with the Micro ATS unit was monitored through periodic harvesting, as was the algal turf associated with the Micro-ATS unit receiving flows directly from Taylor Creek (Control); from effluent from the foam fractionator; and from effluent from the Taylor Creek Stormwater Treatment Area (STA) (see next subsection). Tissue samples were also taken from these units.

The algae receiving outflow from the WHS™ and STA pre-treatment units show higher macro-nutrient levels (P, N, and C) in the tissue (Table 16). The comparative algae productivity and harvest based ARR provides clear evidence of improved productivity and performance with both the WHS™ and STA pretreatment (Table 17 and Figure 35).

**Table 16:** Comparison of algae tissue quality water associated with control, WHS™ pretreatment, foam fractionator pretreatment and STA pretreatment.

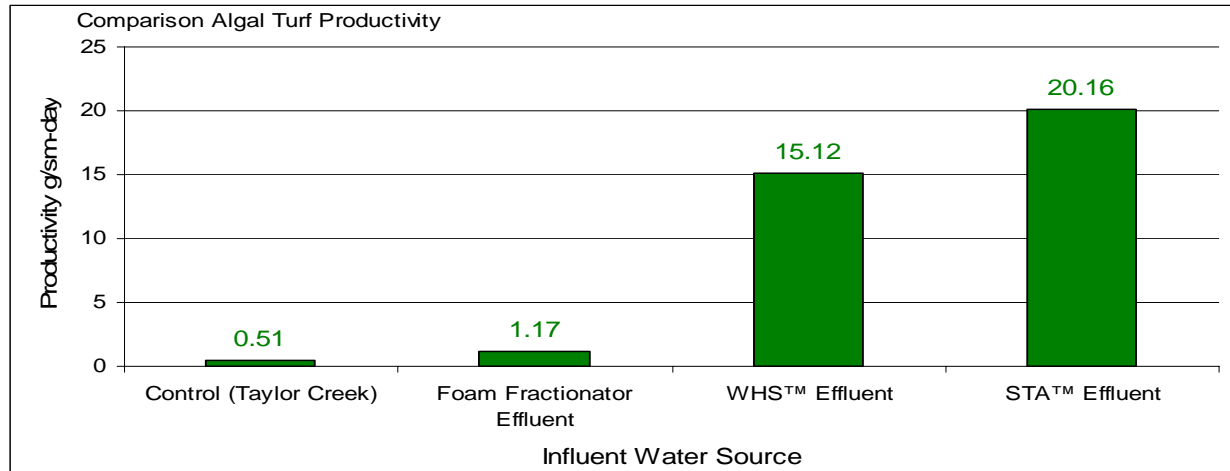
	Algae Control	Algae after Foam Fractionation Pretreatment	Algae after WHS™ Pretreatment	Algae after STA Pretreatment
<b>Total N%</b>	1.95	1.80	2.56	3.30
<b>Ammonia-N%</b>	0.06	0.07	0.02	0.03
<b>Nitrate-N %</b>	nd	nd	nd	nd
<b>TON %</b>	1.90	1.73	2.54	3.27
<b>P2O5%</b>	0.98	0.99	1.28	1.21
<b>P%</b>	0.47	0.47	0.61	0.58
<b>K2O %</b>	0.33	0.33	0.52	1.21
<b>S%</b>	0.51	0.54	0.51	0.69
<b>Ca%</b>	1.97	2.21	1.91	2.73
<b>Mg%</b>	0.52	0.52	0.49	0.36
<b>Na%</b>	0.13	0.15	0.18	0.30
<b>Cu mg/Kg</b>	nd	nd	nd	nd
<b>Fe mg/Kg</b>	11,846	11,230	15,979	7,030
<b>Mn mg/Kg</b>	2,455	2,430	2,267	693
<b>Zn mg/Kg</b>	128	147	90	111
<b>pH</b>	7.20	7.20	7.10	7.00
<b>Total Carbon %</b>	17.72	16.00	27.41	29.05
<b>C/N</b>	9.1:1	8.9:1	10.7:1	8.8:1
<b>Cl %</b>	0.15	0.14	0.23	0.30

**Table 17:** Comparison of algae tissue quality water associated with control, WHS™ pretreatment, foam fractionator pretreatment and STA pretreatment.

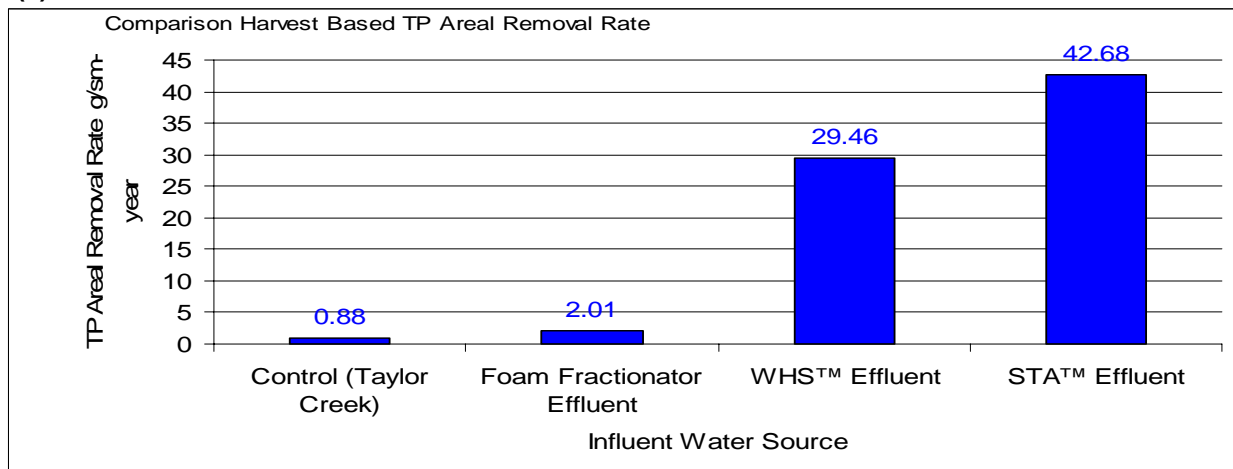
Week	Source	Unit Type	Growing Area m <sup>2</sup>	Harvest Wet Weight g	Percent Solids	Harvest Dry Weight g	Productivity g/m <sup>2</sup> -day	Tissue Phosphorus %	Tissue Nitrogen %	Harvest Based TP Areal Removal Rate g/m <sup>2</sup> -yr
1	Control (Taylor Creek)	MPU	4.65	281.48	6.60%	18.58	0.57	0.47%	1.95%	0.98
	Foam Fractionator Effluent	MPU	4.65	381.36	8.60%	32.80	1.01	0.47%	1.80%	1.73
	WHS™ Effluent	MPU	2.09	3,804.52	4.50%	171.20	11.70	0.61%	2.56%	26.05
	STA™ Effluent	Mini ATS	0.023	273.80	1.65%	4.52	28.06	0.58%	3.30%	59.40
2	Control (Taylor Creek)	MPU	4.65	331.42	5.20%	17.23	0.53	0.47%	1.95%	0.91
	Foam Fractionator Effluent	MPU	4.65	499.40	8.30%	41.45	1.27	0.47%	1.80%	2.18
	WHS™ Effluent	MPU	2.09	3,586.60	5.20%	186.50	12.75	0.61%	2.56%	28.38
	STA™ Effluent	Mini ATS	0.023	149.82	1.85%	2.77	17.22	0.58%	3.30%	36.44
3	Control (Taylor Creek)	MPU	4.65	399.52	4.35%	17.38	0.53	0.47%	1.95%	0.92
	Foam Fractionator Effluent	MPU	4.65	640.14	6.25%	40.01	1.23	0.47%	1.80%	2.11
	WHS™ Effluent	MPU	2.09	3,296.04	5.10%	168.10	11.49	0.61%	2.56%	25.58
	STA™ Effluent	Mini ATS	0.023	145.28	2.05%	2.98	18.50	0.58%	3.30%	39.16
4	Control (Taylor Creek)	MPU	4.65	399.52	4.35%	17.38	0.53	0.47%	1.95%	0.92
	WHS™ Effluent	MPU	2.09	5,575.12	5.10%	284.33	19.43	0.61%	2.56%	43.27
	STA™ Effluent	Mini ATS	0.023	154.36	2.05%	3.16	19.65	0.58%	3.30%	41.61
5	Control (Taylor Creek)	MPU	4.65	354.12	4.65%	16.47	0.51	0.47%	1.95%	0.87
	WHS™ Effluent	MPU	2.09	5,970.10	4.25%	253.73	17.34	0.61%	2.56%	38.61
	STA™ Effluent	Mini ATS	0.023	127.12	2.20%	2.80	17.37	0.58%	3.30%	36.77
6	Control (Taylor Creek)	MPU	4.65	281.48	3.65%	10.27	0.32	0.47%	1.95%	0.54
	WHS™ Effluent	MPU	2.09	5,992.80	4.34%	260.09	17.78	0.61%	2.56%	39.58
7	Control (Taylor Creek)	MPU	4.65	413.14	4.00%	16.53	0.51	0.47%	1.95%	0.87
	WHS™ Effluent	MPU	2.09	6,310.60	4.25%	268.20	18.33	0.61%	2.56%	40.82
8	Control (Taylor Creek)	MPU	4.65	358.66	3.60%	12.91	0.40	0.47%	1.95%	0.68
	WHS™ Effluent	MPU	2.09	5,584.20	3.95%	220.58	15.08	0.61%	2.56%	33.57
9	WHS™ Effluent	MPU	2.09	4,040.60	4.40%	177.79	12.15	0.61%	2.56%	27.06

0

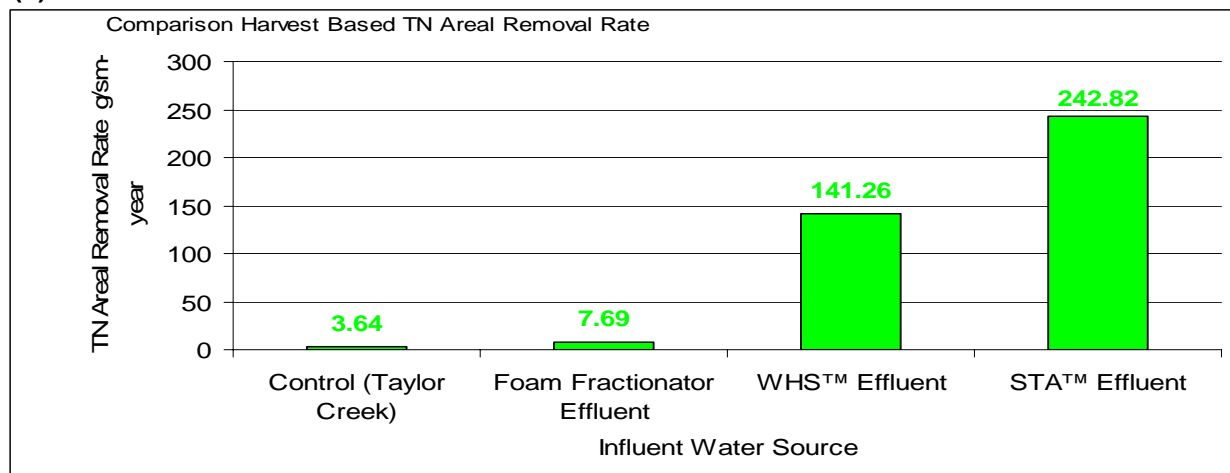
AVERAGES	Productivity g/m <sup>2</sup> -day	Harvest Based TP Areal Removal Rate g/m <sup>2</sup> -yr	Harvest Based TN Areal Removal Rate g/m <sup>2</sup> -yr
Control (Taylor Creek)	0.51	0.88	3.64
Foam Fractionator Effluent	1.17	2.01	7.69
WHS™ Effluent	15.12	29.46	141.26
STA™ Effluent	20.16	42.68	242.82



(a)



(b)



I

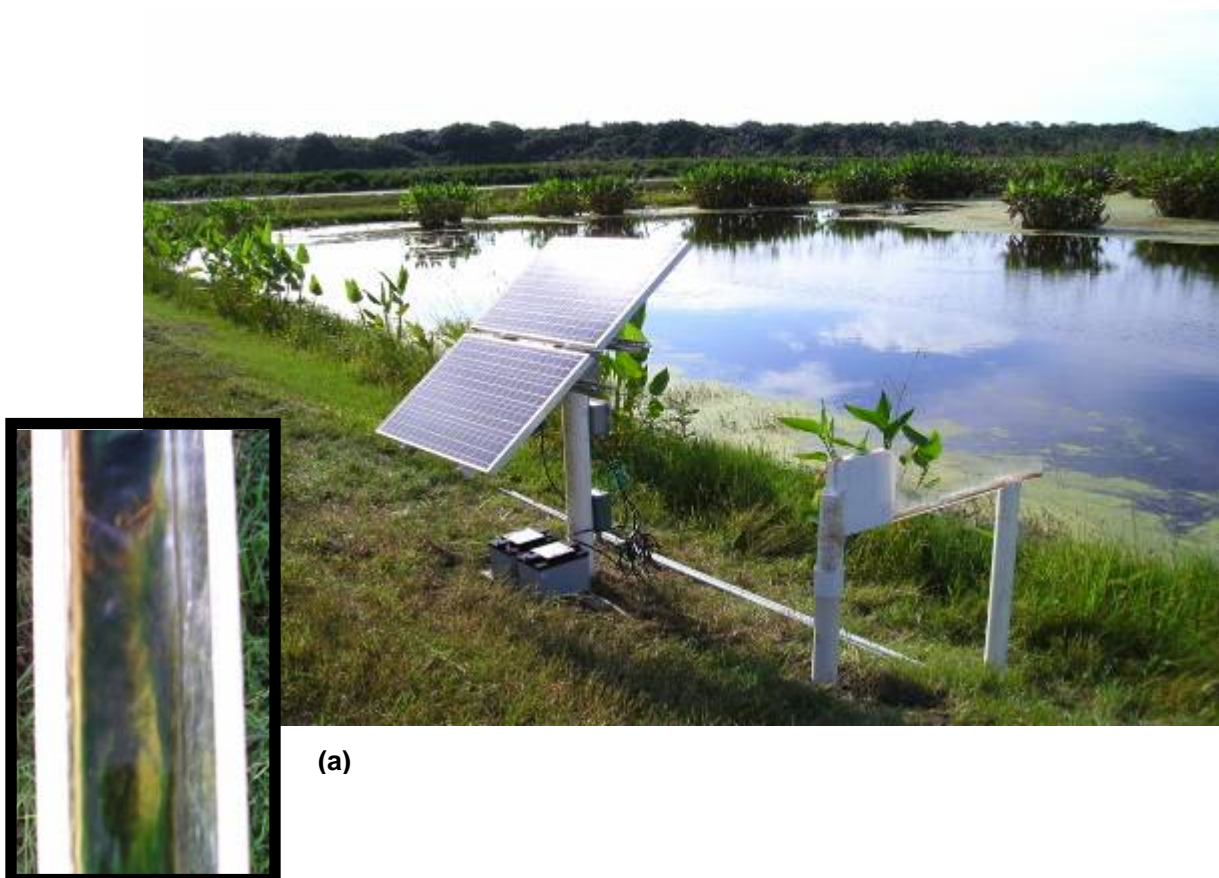
**Figure 35:** Comparative productivity (a), harvest based TP areal removal rate (b) and TN areal removal rate (c) with influent as control; pretreatment by foam fractionator; Water Hyacinth Scrubber (WHS™) pretreatment and STA pretreatment –test units at TC-ATS.

### *Stormwater Treatment Area (STA-Passive Wetland)*

To test the effectiveness of a passive wetland to attenuate the toxin(s) associated with Taylor Creek water, a Micro-ATS unit was set up within the Taylor Creek Stormwater Treatment Area (STA). Because there is no available electrical power within the Taylor Creek STA, a solar unit was used to drive a 1 gpm submersible pump. The solar panel provided power to 12 volt batteries, which permitted the system to operate during the night. The set-up is noted in Illustration 20.

At the time this test was conducted (10/2/09 to 10/16/09) the STA had not been operational for several months, and the water stored within the STA represented Taylor Creek flows which had been detained for at least as long as the down time period—i.e. several months. Consequently, this evaluation did not represent the ability of the STA to attenuate toxins under the STA's normal operational conditions. However, it did reflect the impact of long term detention within a wetland system.

A healthy algal turf developed quickly when the stored STA water was used as an influent to a Micro-ATS unit (Tables 15 and 17 and Figure 35). The productivity and harvest based performance were similar, but higher than when the WHS™ pretreatment effluent was applied to an MPU set-up. As with the WHS™, passive wetlands appear able to significantly attenuate the impact of the toxin(s) associated with Taylor Creek water.



(b)

**Illustration 20.** STA pretreatment Micro-ATS test (a) set-up at Taylor Creek STA; (b) filamentous green algae development after two weeks on Micro-ATS floway.

### *Alum Treatment*

For a two-week period from 11/25/09 to 12/11/09, aluminum sulfate was mixed with the Taylor Creek influent on the TC-ATS facility site at a rate sufficient to establish an aluminum concentration of 15 mg/L. The mixing was done in a 300 gallon tank, with the lined pond previously used for water hyacinth cultivation used for settling. The clarified effluent from this pond was delivered to a Micro-ATS Unit (Illustration 21).



(a)



(b)

**Illustration 21:** Alum pretreatment Micro-ATS test (a) mixing tank at TC-ATS; (b) settling pond with Micro-ATS in background.

Treatment with alum reduced the total phosphorus level significantly, while also reducing color and pH. What appeared to be a healthy algal turf developed on the Micro-ATS unit within two weeks, indicates that alum treatment may attenuate the influence of the toxin(s) associated with the Taylor Creek water. Previous work with static bioassays of alum treated water also provided indication that alum treatment may attenuate the influence of the toxin(s) associated with the Taylor Creek water.

## SUMMARY DISCUSSION AND RECOMMENDATIONS

### Summary of Evidence

Over the three-year period of operation of the Taylor Creek ATS™ facility, efforts to identify and mitigate for the compound(s) and factor(s) associated with the system's below projection performance and algal turf productivity resulted in both conclusive and indicative evidence.

#### *Conclusive Evidence*

1. Waters associated with Taylor Creek in the vicinity of the TC-ATS facility pump intake are intermittently toxic as determined by EPA's TIE methodology and as verified by in-field evidence.
2. The intermittent toxicity resulted in significantly reduced treatment performance and periphyton growth and periodic algal die-offs.
3. The TIE testing shows the toxicity associated with this water is not associated with heavy metals.
4. Toxic compound(s) within this Taylor Creek water accumulates within the foam generated from the agitation of this water.
5. Treatment of this water with activated carbon significantly attenuates the deleterious impacts of the toxin(s) associated with this water.
6. Treatment of this water through a water hyacinth scrubber designed for a two day detention period can significantly attenuate the deleterious impacts of the toxin(s) associated with this water.
7. Treatment of this water through extended detention within a passive wetland system (STA) can significantly attenuate the deleterious impacts of the toxin(s) associated with this water.
8. Treatment of this water through supplementation of nitrogen, iron, magnesium, boron and sodium bicarbonate did not attenuate the deleterious impacts of the toxin(s) associated with this water.
9. Adjustment of hydraulic loading rates, flowway slope, grid type, and surge cycle did not attenuate the deleterious impacts of the toxin(s) associated with this water.
10. Metolachlor ESA and Metolachlor OXA, metabolic products associated with the herbicide Metolachlor, were found in the Taylor Creek water.
11. Deisopropylatrazin and Didealklyatrazin were found in the Taylor Creek water.

#### *Indicative Evidence*

1. The toxic compound(s) associated with Taylor Creek are likely to be organic in nature, as they are readily removed by activated carbon.
2. Treatment of this water with 15 mg/L of aluminum applied as aluminum sulfate may attenuate the deleterious impacts of the toxin(s) associated with this water.

3. Treatment of water associated with Taylor Creek in the vicinity of the TC-ATS facility pump through foam fractionation did not significantly attenuate the deleterious impacts of the toxin(s) associated with this water.
4. GC-MS analysis of the water revealed within some samples a spike whose closest match per the NIST MS spectra library included the compounds, dipropylene glycol monomethylether and 1-[1-methyl-2-[2-propenyloxy]ethoxy]-2-propanol, which are commonly used in agriculture as adjuvants and solvents.
5. Analysis of the foam associated with the Taylor Creek influent using negative electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) mass spectrometry, a method used to detect anionic, cationic, and/or non-ionic surfactants in Taylor Creek foam samples resulted in the detection of unknown chemical components, including compounds with masses of 214.7 Da or 226 Da, 228 Da and 703.8 Da.
6. Diluted foam was analyzed using the Methylene Blue Active Substance (MBAS) screening procedure, a means of detecting anionic surfactants at levels ranging from 0.25 – 3.0 mg/L. Results indicated the presence of 'MB active substances' (an indirect measure of anionic surfactants) at an estimated anionic surfactant levels in the undiluted foam at about 40 mg/L.
7. Analytical evidence indicates surfactants may be involved in rendering the Taylor Creek water toxic.
8. Analysis through static bioassays of waters from the Taylor Creek influent pump station and tributaries upstream provided indication that a) the highest levels of toxicity are associated with two tributaries in the northwestern reaches of the watershed, which generally are associated with citrus and potato farming and b) toxicity appears to be associated with periods of heavy rainfall and run-off.

With the disclosure that Taylor Creek water within the vicinity of the TC-ATS pump station intake is intermittently toxic to algae, the possibility exists of regional deleterious impacts upon the aquatic ecology associated with both Taylor Creek and its receiving water—Lake Okeechobee.

While there is evidence that the influence of the toxic element(s) can be significantly abated by treatment systems including WHS™, STA type wetlands, and possibly alum addition, the nature of the attenuation of this toxin(s) within these processes is unknown. It is possible, for example, that removal is through physical processes such as settling or volatilization, rather than metabolic destruction through biochemical reactions. It is also possible that the toxin(s) is not removed but rather its toxicity sequestered via chemical changes in the water (e.g. pH, DO or temperature shifts). If removal is by settling or sequestration, rather than chemical destruction, then the toxin(s) could at some time again assert its toxicity, as settled solids release the substance(s) back into the water column, or if chemical conditions change such that sequestration is reversed.

Certainly questions that need to be addressed relate to the need to determine if this toxicity has had deleterious impacts upon Lake Okeechobee or upon human health, and if so what is the nature of these deleterious impacts, and does the possibility of accumulation of the toxin(s) within the lake represent a serious threat to its eco-structure or to human health.

Considering the seriousness of these concerns it is recommended that those entities responsible for protecting the State's water, agricultural and human resources pursue a comprehensive program to identify the involved toxin(s), identify the source of the involved toxin(s), and determine the long term efficacy of processes used in abatement of the impacts of this toxin(s), including identification of the fate of the toxin(s),

## Recommendations

Based on the findings and evidence associated with the Taylor Creek Algal Turf Scrubber® Nutrient Recovery Project, the following is a list of program recommendations:

### *Toxicity Identification*

1. Develop a geographic toxicity testing and sampling program to narrow the source of the toxicity.
2. Through analytical means as described herein and additional means as required, including but not necessarily limited to in-field periphytometers; static bioassays; TIE; GC-MS; NMR; ESI-MALDI; and extraction, isolation and bioassay verification, establish the specific identification and source of the compound(s) responsible for toxicity. Such analytical efforts shall include both water and sediment samples from Taylor Creek; and any water treatment systems employed within the Taylor Creek basin.
3. Once the toxin(s) is identified, assess the past and potential future impacts upon Taylor Creek and Lake Okeechobee.

### *Toxicity Treatment Evaluation*

1. Once the toxin(s) have been identified, present findings to the Florida Department of Environmental Protection and Florida Department of Agriculture and Consumer Services so that efforts can be made to eliminate or reduce toxic impacts to periphyton within the Taylor Creek basin.
2. Once the toxin(s) are identified and the source of contamination is removed from the watershed, evaluation of the ATS™ technology should be conducted if desired phosphorus load reductions have not been met within the basin.
3. Evaluate all treatment systems examined for reduction of phosphorus including their respective capabilities in eliminating over a long term, the deleterious impacts of the identified toxin(s), and their cost effectiveness based upon a fifty years present worth evaluation, evaluate all systems establish a reasonable basin wide plan for reduction of phosphorus and elimination of toxicity.
4. If identification and elimination of the toxin is not pursued, it is recommended that pilot testing a periphyton based ATS™ be employed to include pre-treatment via STA, Alum and/or WHS systems. The ATS™ pilot units will serve as long-term bioassays of the STA, Alum and WHS effluent and the Taylor Creek influent.