

## ABSTRACT

### Using Multiple Parameters to Compare Effluent Quality of Eight Wastewater Treatment Systems

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Wastewater effluent qualities from two study sites in central Texas were compared using whole effluent toxicity (WET) tests and chemical indicator parameters. Three of the effluents were collected from the City of Whitney Wastewater Treatment Facility in Whitney, TX; the remaining five effluents were collected at the Waco Metropolitan Area Regional Sewerage System (WMARSS) and the Baylor Wastewater Research Program site near Waco, TX. The first hypothesis examined at the City of Whitney Wastewater Treatment Facility was that effluent water quality improves through a pond and wetland treatment system. The second hypothesis examined was that there is no difference of effluent water qualities between two seasons. The first hypothesis examined for the Waco effluents was that effluent qualities of the four on-site systems are comparable to a centralized municipal wastewater treatment effluent: WMARSS. The second hypothesis examined was that there is no difference of effluent water qualities between two seasons.

Using Multiple Parameters to Compare Effluent Quality of Eight  
Wastewater Treatment Systems

by

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A Thesis

Approved by the Department of Environmental Science

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## CHAPTER ONE

### Introduction

Wastewater treatment has been practiced for hundreds of years in various forms and with varying efficiencies (Cech 2010). One thing all wastewater treatment has in common, whether it is black water, gray water, industrial wastewater, or some other form, is effluent: the final product after treatment which must be returned to the environment. Depending on the treatment method, effluent may be dispersed in the soil, upon the ground surface or directly into a water source (Cech 2010). Eventually organisms may be exposed to the treated effluent. If wastewater is treated improperly or inefficiently, the remaining water quality characteristics, including, but not limited to, carbonaceous biochemical oxygen demand (CBOD<sub>5</sub>), total suspended solids (TSS), ammonia-nitrogen (NH<sub>3</sub>-N), pH, and dissolved oxygen (DO), may negatively affect organisms within the receiving environment. Some low trophic level aquatic organisms, such as *Daphnia magna*, can be useful indicator species of water quality and can help identify wastewater impacts on water systems (Suter 2007; Ra, et al 2007).

Municipal wastewater treatment systems are required to acquire a National Pollutant Discharge Elimination System (NPDES) permit and operate according to the permit criteria. The common water quality characteristics around which NPDES permits are written include, but are not limited to, CBOD<sub>5</sub>, TSS, NH<sub>3</sub>-N, pH, and DO (TCEQ 2006; TCEQ 2009; TCEQ 2011). These NPDES permits require that effluents being discharged directly into a water body be regularly monitored for their water quality

characteristics of interest, which are set at limits designed to protect the receiving environment. Wastewater effluent is a complex mixture however, and it is infeasible and expensive to test for all constituents of concern (Barata et al 2008). Some NPDES permits, such as those associated with major dischargers, require whole effluent toxicity (WET) tests be performed to verify that the routine monitoring is not missing constituents, or interactions, which may harm the receiving environment (TCEQ 2006). A wastewater treatment facility is considered a major discharger if they discharge more than one-million gallons per day of effluent. Many municipal wastewater treatment facilities, not considered major dischargers, are required to abide by a TPDES permit but do not require performance of WET tests (TCEQ 2009).

Unlike municipal wastewater treatment systems, on-site wastewater treatment systems do not require NPDES permits. Instead of continuous monitoring through an NPDES permit, the on-site system design must be approved prior to installation for private use. Effluent from on-site wastewater treatment systems is usually expected to receive further treatment after disposal and before reaching groundwater or surface water. Septic tank effluent is usually dispersed into a leach field, which is meant to protect the groundwater and runoff to nearby surface water bodies (US EPA 2000). Aerobic system effluent is either dispersed into a leach field, subsurface irrigation system, or disinfected and surface applied. However, according to the U.S. Environmental Protection Agency (US EPA) publication 600/R-00/008, approximately 23% of homes in the United States treat their wastewater through on-site systems, and as many as 70% of those may be failing at any given time (US EPA 2000). On-site system failure is usually defined as sewage backup or surface pooling (US EPA 2000).

Eventually, some effluent from all systems is likely to reach natural waters, surface or subsurface. In surface water bodies are organisms which may be affected by the effluents. Effluent from systems which overlay a water table without a confining layer in between, may reach the groundwater. This groundwater may then be pumped by home owners and municipalities for drinking water; the groundwater also may interact with a stream or lake. The surface applied effluent from aerobic systems may runoff to nearby surface water if the effluent is applied too rapidly for the soil type or if application occurs during or immediately after a rain event when the soil is already saturated. Surface or groundwater contamination is also considered on-site system failure but these events are rarely confirmed or included in statistics regarding on-site system failures (US EPA 2000). Because on-site systems are not required to have NPDES permits, their effluent is not regularly monitored for environmental contaminants. Even when the system design is approved, toxicity testing is not included in the approval process.

*Daphnia magna*, an invertebrate aquatic organism, has been used for toxicity testing throughout the world for decades. Due to its short life cycle it has been found useful for assessing water quality from many sources (Chapman 2000; Rodriguez et al 2006; Suter 2007). Due to limited resources and technologies, it is impractical to attempt measuring all water quality characteristics present in a water sample. Also, the characteristics present may have additive effects which cannot be measured or predicted (Suter 2007). Toxicity tests conducted using live organisms are a way of screening water samples for toxicity which may be missed by routine water quality monitoring (Ra et al 2008; Veen et al 2002). Given their status as low trophic level organisms, if *D. magna* are found to be vulnerable to wastewater effluent, the rest of the food chain, above and

below its trophic level may be affected (Stiling and Rossi 1997; Polis and Strong 1996). The objective of this project was to determine if toxicity differences between the effluent qualities exist among several wastewater treatment systems by comparing water quality parameters and using *D. magna* as an indicator species at two study sites in two seasons.

The first study site was at the City of Whitney, TX at the Whitney Wastewater Treatment Facility (WWTF). This wastewater treatment facility is not considered a major discharger, discharging ~.4 MGD. Two hypotheses were examined at WWTF. The first hypothesis was that effluent water quality improves through a pond and wetland treatment system. The second hypothesis examined was that there is no difference of effluent water qualities between two seasons.

The second study site was located near Waco, TX and consisted of effluents collected from the Waco Metropolitan Regional Sewerage System (WMARSS) and the Baylor Wastewater Research Program (BWRP). The first hypothesis examined was that effluent qualities of the BWRP on-site systems are comparable to a centralized municipal wastewater treatment effluent: WMARSS. The WMARSS facility is a major discharger, discharging ~40 MGD. The second hypothesis examined was that there is no difference of effluent water qualities between two seasons.

### *Setting*

This project was conducted using eight effluents collected from wastewater treatment systems within central Texas. Three of the eight effluents examined were located within the City of Whitney, TX and the remaining five systems were located within the City of Waco, TX (fig. 1). The WWTF utilizes a pond system coupled with constructed wetlands (fig. 2). The Waco treatment systems were the Waco Metropolitan

Area Regional Sewerage System (WMARSS), an activated sludge treatment system (TCEQ 2006) and four onsite systems at the Baylor Wastewater Research Program (BWRP) site, which is adjacent to the WMARSS facility and receives the same wastewater (fig. 3). The Whitney municipal facility utilizes a series of three treatment ponds and two wetlands (one surface and one subsurface) to treat wastewater (TCEQ 2009) (fig. 4). Samples were collected at the end of the third pond, the end of the first wetland cell (surface wetland), and at the final output (fig. 5). The on-site treatment systems sampled at the BWRP site included two aerobic on-site systems, a septic tank, and a submerged bed treatment wetland that received its influent from the septic tank. One of the aerobic systems, the septic tank, and the septic + wetland were dosed 480 gallons/day according to ANSI/Standard 40 design loading (NSF/ANSI 2010) (figs. 6-8). The standard 40 (Std. 40) design loading means the system receives 35% of its influent between 5:00 AM and 8:00 AM, 25% between 11:00 AM and 1:00 PM, and 40% between 5:00 PM and 8:00 PM. The second aerobic system was dosed 480 gallons/day according to an experimental dosing regimen known as influent equalization (I.E.) (TCEQ 2011). This dosing regimen differs from standard 40 in that the system was dosed every fifteen minutes throughout the twenty-four hour period, rather than receiving three large doses in the morning, at noon, and at night. The influent used for the onsite systems at the BWRP site was diverted from the WMARSS facility immediately behind the screens (fig. 9).

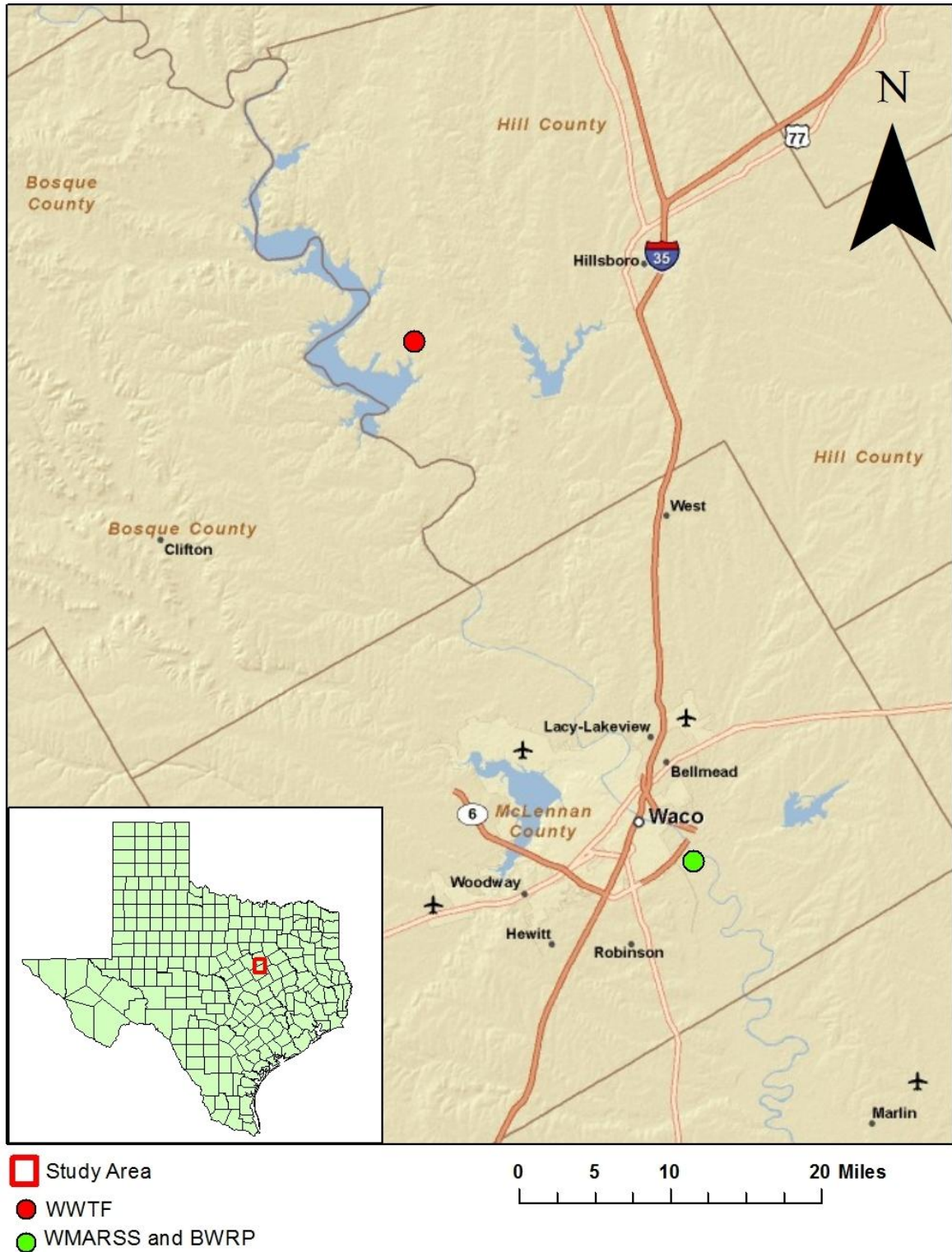


Figure 1. Study locations within Hill and McLennan Counties. The Waco Metropolitan Area Regional Sewerage System facility and Baylor Wastewater Research Program site are located near Waco, TX. The Whitney Wastewater Treatment Facility is located in Whitney, TX.



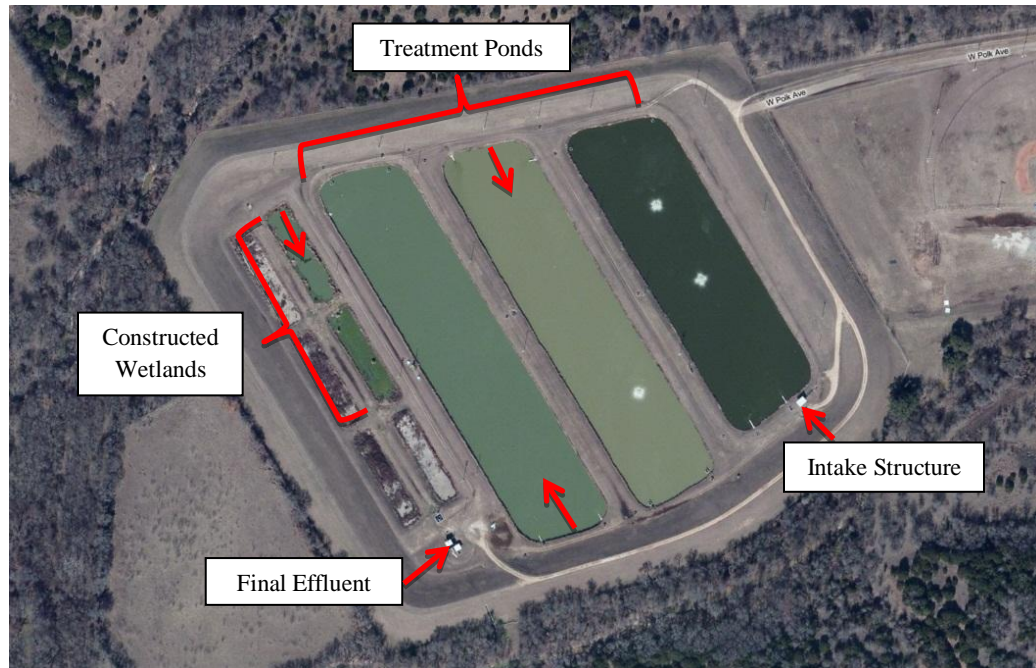


Figure 2. The Whitney Wastewater Treatment Facility, located in Whitney, TX, utilizes a pond and wetland treatment system. Arrows illustrate flow directions through the system.



Figure 3. The Waco Metropolitan Area Regional Sewerage System facility activated sludge municipal wastewater treatment facility and the Baylor Wastewater Research Program site (which receives its influent from WMARSS) are located near Waco, TX.

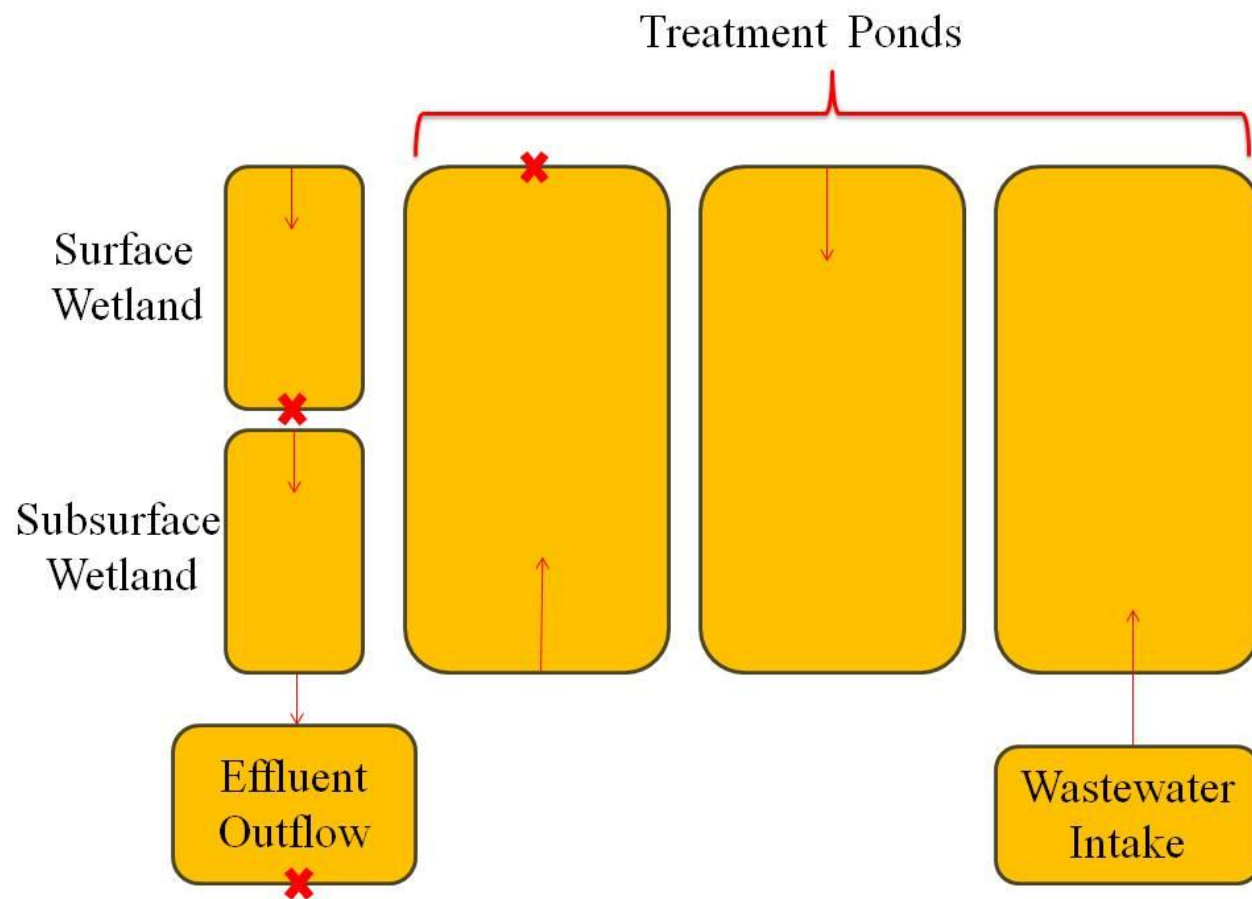


Figure 4. A flow diagram illustrating the path of the wastewater through the Whitney Wastewater Treatment Facility. **X**: sampling locations





Figure 5. The surface wetland cell follows the treatment ponds. The outflow of this wetland flows through a subsurface wetland before being discharged to the environment.

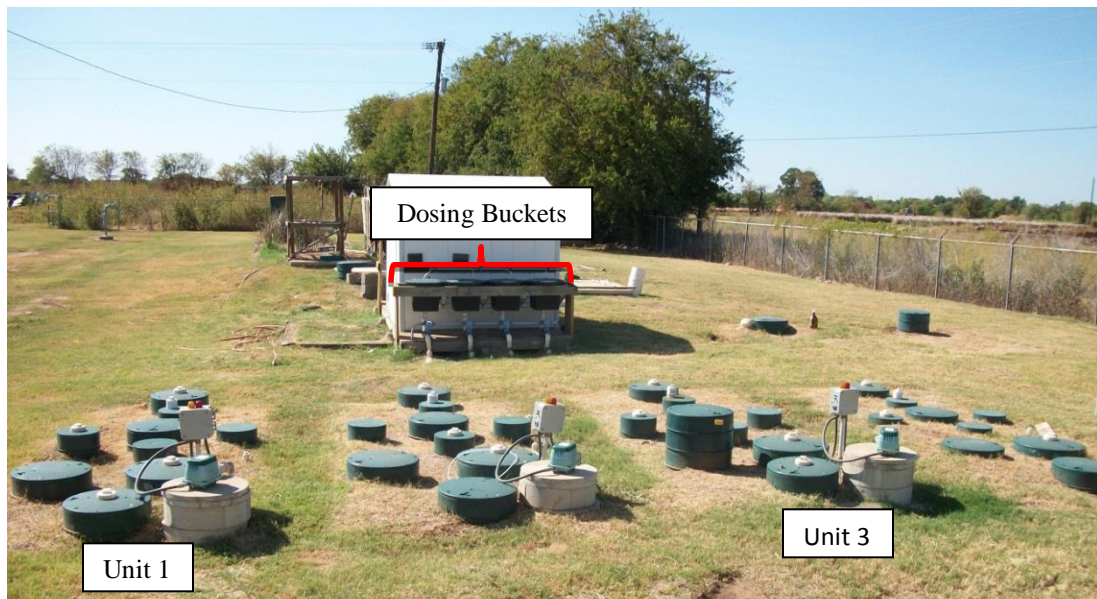


Figure 6. The aerobic units each received 480 gallons wastewater per day through calibrated dosing buckets. Unit 1 was dosed according to the NSF/ANSI standard 40 dosing regimen and unit 3 was dosed according to the influent equalization experimental design.

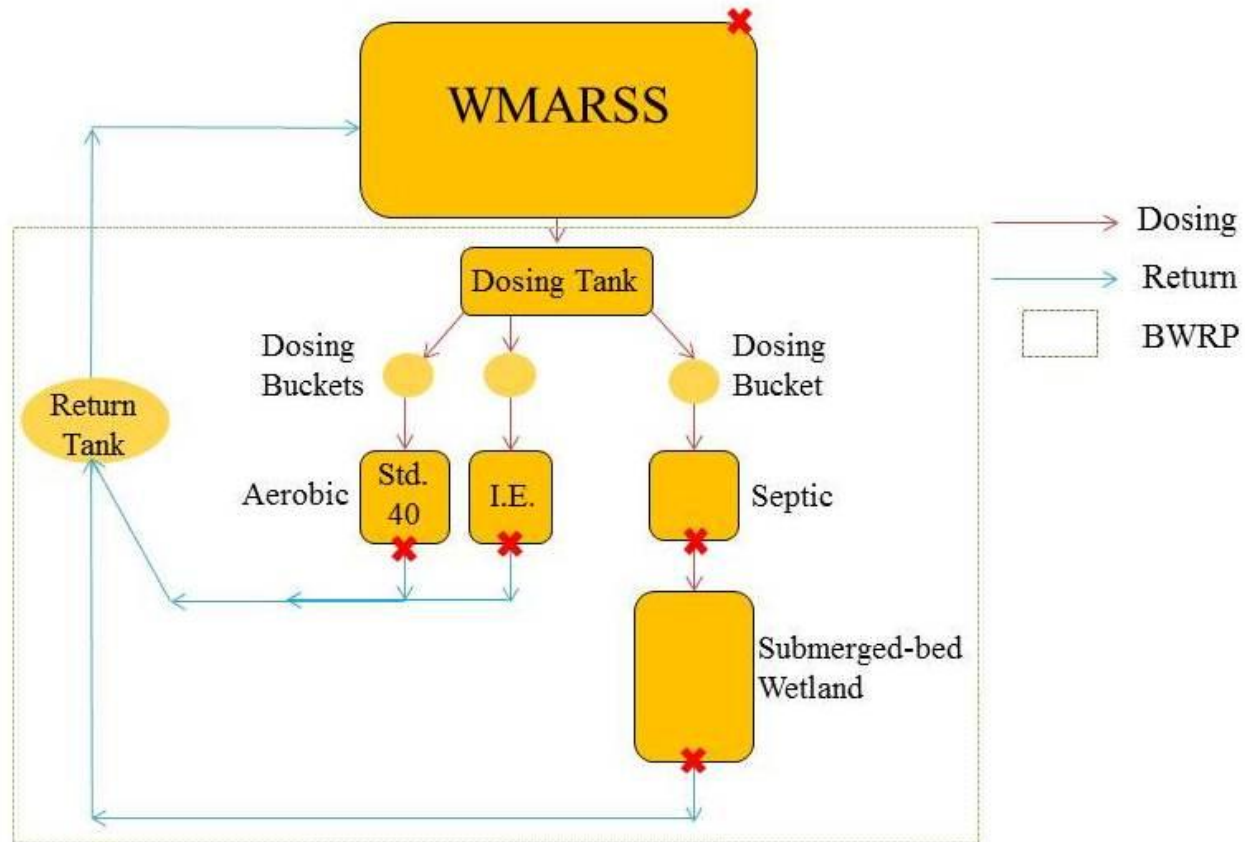


Figure 7. This flow diagram illustrates the path of the wastewater being dosed and the effluent being returned to the Waco Metropolitan Area Regional Sewerage System facility. **X**: sampling locations.





Figure 8. The sub-surface treatment wetland received its influent from the septic tank immediately preceding it, which was dosed 480 gallons per day in accordance with NSF/ANSI standard 40 design loading.

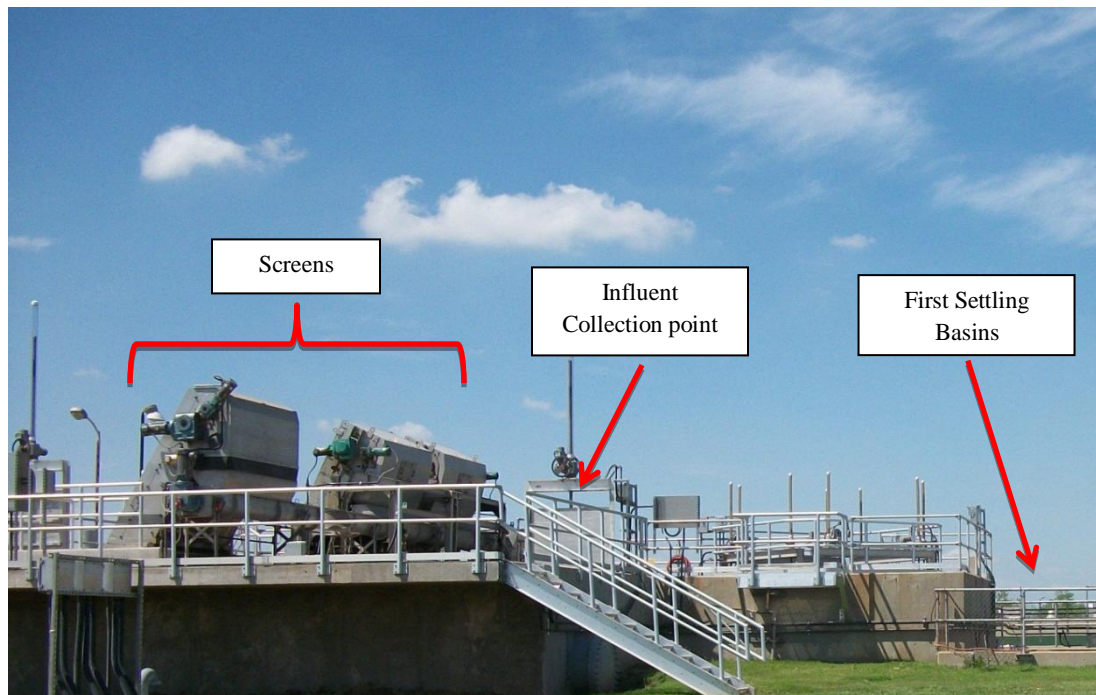


Figure 9. Prior to receiving any treatment, wastewater is flowed through screens to remove large particulate matter. The Baylor Wastewater Research Program receives its influent from immediately behind the screens

## CHAPTER TWO

### Methods and Materials

#### *Experimental Design*

The WWTF effluents were chosen for this study due to their design. The use of constructed wetlands for wastewater treatment is a common practice. This facility provided the opportunity to assess the ability of a surface and subsurface wetland to affect wastewater effluent water quality from a multiple pond wastewater treatment system. The WMARSS and BWRP effluents were chosen for this study because of the unique design of the facilities. Because the on-site systems at the BWRP facility share the same influent as the WMARSS facility, the effluents produced from these on-site systems can be directly compared to the WMARSS municipal effluent.

The climate within central Texas varies between seasons. Therefore this study was designed to examine whether seasonal differences of climate produced detectable differences between any of these effluents. To test the hypothesis that there is no difference between effluent quality for the various effluents examined between seasons, the same water quality parameters were examined and toxicity bioassays were performed, for two seasons at both study sites.

#### *Sample Collection*

Effluent was collected within thirty-six hours of setting up each bioassay in accordance with United States Environmental Protection Agency (US EPA) regulations (US EPA 1994). The water samples could only be held, in the dark at 4° C, for ninety-six

hours, therefore, for a ten-day bioassay, two additional samples were necessary.

Additional effluent samples were collected within thirty-six hours of the first renewal for which it was used and held in the dark at 4°C for no more than ninety-six hours. Each Whitney bioassay was replicated three times in summer (July-September) and winter (December-February) to test for seasonal variation of effluent toxicity (La Point and Waller 2000). Each Waco bioassay was replicated three times in fall (September-November) and winter (December-February) to test for seasonal variation of effluent toxicity.

Effluent collected at the WMARSS site consisted of flow-proportional composite samples which accumulated over the twenty-four hours prior to collection. The effluents from the onsite systems were flow-proportional composite samples which accumulated over the forty-eight hours prior to collection. All composite samples were held in the dark at 4° Celsius until collection. Effluents collected at the City of Whitney Wastewater Treatment Facility (WWTF) were grab samples; grab samples were collected instead of composite in accordance with the NPDES permit associated with the WWTF (TCEQ 2009). In-situ measurements (DO, Temperature, pH, and Ec) were collected from every sample site immediately prior to collecting the sample using YSI handheld sondes. These YSI sondes were calibrated the same day the samples were collected. All in-situ measurements were taken at the same depth, just below the water surface, at all sample locations. At least two liters of water were collected for the whole effluent toxicity (WET) bioassays and two liters were collected for water quality analysis (TSS and CBOD<sub>5</sub>). TSS and CBOD<sub>5</sub> for the Waco samples were analyzed at the WMARSS water quality lab; these constituents for the Whitney samples were analyzed at the

Environmental Monitoring Laboratory in Hillsboro, TX. A portion of the sample collected for the WET tests was submitted to the Center for Reservoir and Aquatic Systems and Research (CRASR) lab at Baylor University for nutrient analysis. Nutrients being tested for were total nitrogen (TN), total phosphorous (TP), and dissolved nitrogen and phosphorous (DNP); the dissolved species of nitrogen and phosphorous were ammonia (NH<sub>3</sub>) and nitrate/nitrite (NO<sub>3</sub>/NO<sub>2</sub>) and phosphate (PO<sub>4</sub>) respectively.

### *Toxicity Bioassays*

Chronic toxicity tests were conducted using effluent from each wastewater treatment system diluted with reconstituted hard water (RHW), using a serial dilution factor of .5 from 100% down to 25% wastewater (APHA et al 1998). The *D. magna* neonates used for all toxicity tests were from cultures maintained at Baylor University. The cultures and bioassays were maintained at 25° C ± 1° C with a photoperiod of sixteen hours light and eight hours dark generally following standard methods (US EPA 1994). Cultures were maintained in 500-ml beakers prior to initiating experiments for which 30-ml plastic cups were used as experimental units (figs. 12 and 13) (Dzialowski et al 2006).

The Whitney bioassays conducted during the summer used five *D. magna*, less than twenty-four hours old, exposed to each concentration for the duration of each study as well as five replicates exposed only to RHW to serve as controls in accordance with US EPA methods for chronic toxicity tests involving effluent (US EPA 1994). For all bioassays thereafter, the Waco bioassays conducted in the fall and the Whitney and Waco bioassays conducted in the winter, five replicates less than twenty-four hours old were exposed to each effluent concentration for the duration of the study, and ten replicates less than twenty-four hours old were exposed to RHW to serve as laboratory controls.



The *D. magna* were fed an algae and cerophyl solution and the bioassay water was renewed every forty-eight hours according to modified EPA methods (Dzialowski et al 2006; Stanley et al 2007). All test organisms were observed at water changes for movement and reproduction. Offspring were counted and removed from the parent container. Renewal involves conducting a serial dilution the same as before with new effluent and RHW. The organisms were then transferred to the renewal media in which food had already been provided (U S EPA 1994).

### *Statistics*

The TOXSTAT, SPSS, and JMP 8 statistical software packages were used for toxicity and water quality analysis. Survival or reproduction data and the whole effluent data were used for comparing the wastewater treatment systems. The dilution data were analyzed using the TOXSTAT statistical software package to determine the lowest observable effect concentration (LOEC) and no observable effect concentration (NOEC) for each effluent type. Water quality data (DO, pH, temperature, TSS, CBOD, TN, NH<sub>3</sub>, NO<sub>3</sub>/NO<sub>2</sub>, TP and PO<sub>4</sub>) were analyzed using SPSS and JMP 8 statistical software packages.

### *Water Quality*

Water quality data consisted of DO, pH, T, TSS, CBOD<sub>5</sub>, and nutrients (TN, NH<sub>3</sub>, NO<sub>2</sub>/NO<sub>3</sub>, TP, and PO<sub>4</sub>). Because three tests were conducted within each of two seasons, there were a total of nine water quality results for each system in both seasons (n=9). There were select incidents when data points were either missing from or in addition to the set, at which point, n≠9 for that parameter (appendices A-X). Nearly all data sets from

both the Waco and Whitney sites failed to meet the assumptions of ANOVA. Therefore, for consistency, all data sets were analyzed using the non-parametric Kruskal-Wallis test, with the pair-wise comparison post-hoc as necessary. The non-parametric tests can be used to analyze data sets whether they meet the assumptions of ANOVA or not, the reverse of which is not true; data which fail to meet the assumptions of ANOVA are best tested using only non-parametric tests (Zar 1999).

#### *Observable Effect Concentrations*

Each effluent dilution series was separately compared to its corresponding lab control using an ANOVA test with a Dunnett's post hoc test, within TOXSTAT, to determine the lowest observable effect concentration (LOEC) and no observable effect concentration (NOEC) for each effluent type (US EPA 1994; Suter 2007). The results used for analysis were average reproduction or proportion of surviving organisms for each test and dilution. Three toxicity bioassays were conducted for each system in both seasons (n=3).

#### *Effluent Comparisons*

For comparison of treatment systems (effluent type) only the 100% effluent concentration data were compared. For the Waco effluents, the WMARSS effluent was first compared to the laboratory control to determine if there was a significant difference. Upon determining there was no significant difference, the WMARSS effluent was used as the "control" to which the on-site systems were compared. For the Whitney analysis, the final effluent (FNEF) was first compared to the laboratory control to determine if there was a significant difference. Upon determining there was no significant difference,

the FNEF was used as the “control” to which the pond 3 (PD3) and wetland cell 1 (WET1) effluent were compared. Survival data were presented as the proportion of surviving organisms at the end of each test (n=3). If all five organisms survived the ten-day exposure, the survival equaled 1; if four of five organisms survived, the survival equaled 0.8, etc. These data were transformed in Microsoft Excel using the arcsine square root transformation, prior to analysis in SPSS. This transformation was used to normalize the data, which would have otherwise likely appeared binomial rather than normally distributed (Zar 1999). To determine the appropriate test to compare the effluents, the data were tested for the assumptions of analysis of variance (ANOVA): normal distribution of the population and homogeneity of variance. Most data sets met the assumptions of ANOVA; however, some data sets failed to meet the assumptions of ANOVA. Data which met the assumptions of ANOVA were analyzed using a one-factor ANOVA with a Tukey’s post-hoc test; the other data sets were analyzed using the non-parametric test Kruskal-Wallace with a pair-wise comparison to identify which effluents, if any, differed from the Final Effluent or WMARSS final effluent for the Whitney and Waco effluents respectively.

Reproduction data were entered as the average number of neonates per *D. magna* for each test (n=3). As with the survival data, it was first determined there was no statistical difference between the WMARSS results and their corresponding control or the FNEF results and their corresponding control. The reproduction data from both Whitney and Waco failed to meet the assumptions of ANOVA, and were therefore analyzed using the Kruskal-Wallace non-parametric test with the pair-wise comparison post-hoc as necessary.

### *Seasonal Comparisons*

To compare the data collected from two seasons a two-factor test was employed. There is not a non-parametric two-factor ANOVA equivalent available in either the JMP 8 or SPSS software packages. Thus, the data were transformed into “average ranked sums”. The ranked data were then analyzed using a two-factor ANOVA within the JMP 8 software package. For these analyses, the effluent type was the first factor, and season was the second factor. After it was determined there was a significant relationship between at least two groups, Tukey’s post-hoc was performed on the results of the interaction. The Tukey’s test was used because it compares the means of the data sets to each other; this test was appropriate because the groups were independent effluents (not dilutions of one effluent type) and they were not being compared to a laboratory control (Zar 1999). For the interaction results, each effluent was compared to itself for both seasons. If the results were in the same group, there was no statistical difference between seasons, but if the effluents were in different groups for each season, there was a significant difference between the seasons for that effluent. A second output of the two-factor ANOVA is the results of a comparison of all the inputs associated with each single factor. The results of the Season factor were taken into consideration if the interaction results were not significant (Zar 1999).

## CHAPTER THREE

### Results and Discussion

#### *Whitney*

##### *Water Quality*

The first hypothesis tested concerning the City of Whitney Municipal Wastewater Treatment Facility was that effluent quality improves with distance through the treatment system. The dissolved oxygen (DO) concentrations measured in-situ fluctuated with distance through the system during both summer and winter. The only significant difference between DO concentrations, however, was in the winter season, when the DO concentration of the WET1 effluent was significantly higher than the concentration in the FNEF (figs. 10 and 11). The higher concentrations of DO measured in the effluents during the winter samples compared to summer samples were to be expected, due to cold water being able to hold more DO than warm water. The higher DO in the WET1 effluent may have been the result of vegetation within the surface wetland (WET1). Algae and wetland plant photosynthesis beneath the surface of the water could have raised the DO (Kadlec and Knight 1996).

The in-situ pH measurements varied between the three effluents in the summer season, with both the WET1 and PD3 effluents having significantly higher median pH measurements than the FNEF (fig. 10). The median pH measurements were 8.4, 9.2, and 9.3 for the FNEF, WET1, and PD3 effluents respectively. There were no significant differences between the median pH measurements of the WET1 or PD3 effluents compared to the FNEF during the winter season (fig. 11). The median pH of the

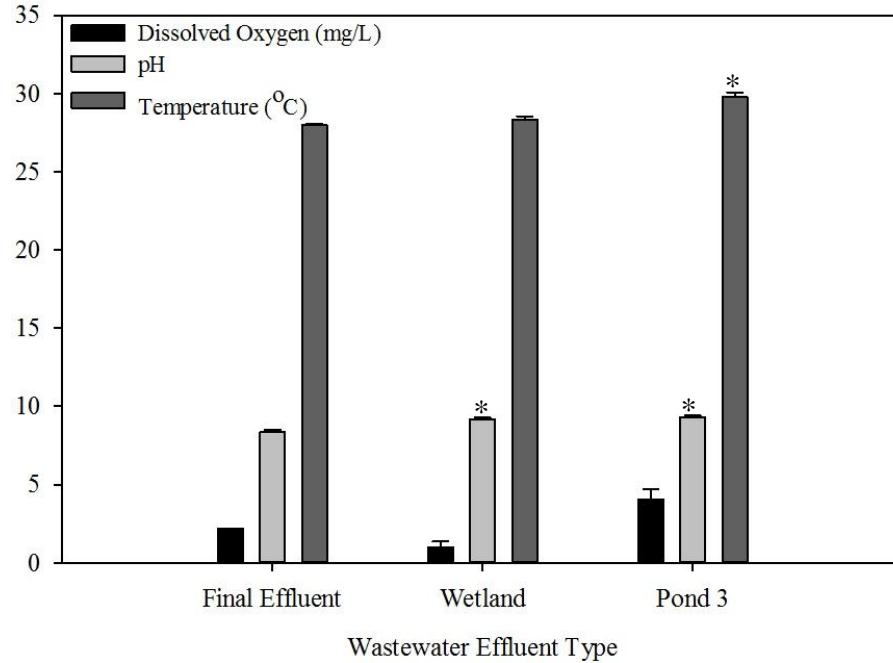


Figure 10. Means ( $n=9$ ;  $\pm$ SD) of the T and DO, medians  $\pm$ SD of pH, measured during summer sample collections at the Whitney Wastewater Treatment Facility. \* denote a significant difference ( $p<0.05$ ) from the final effluent.

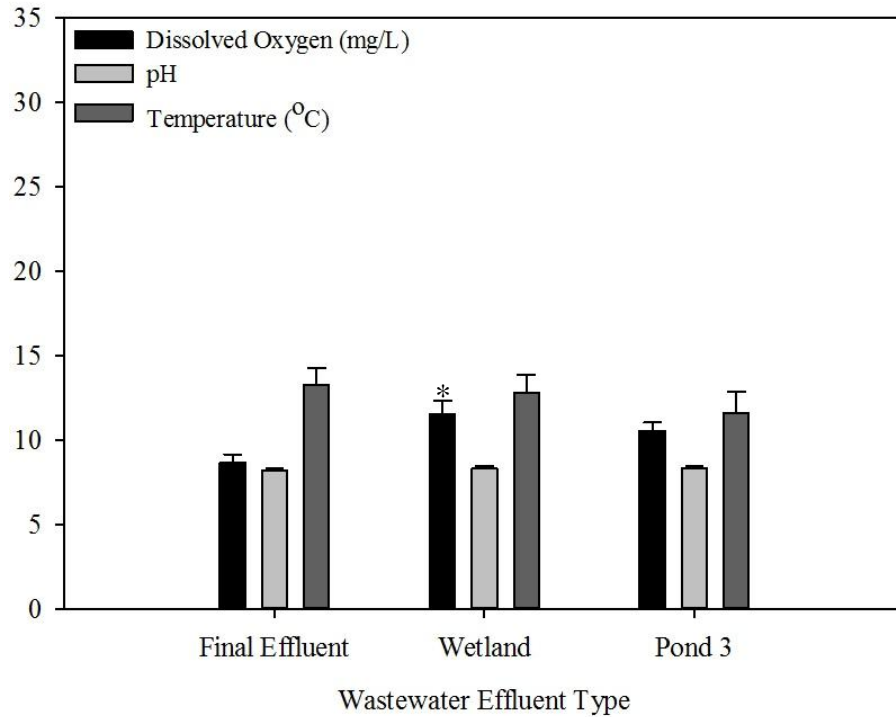


Figure 11. Means ( $n=9$ ;  $\pm$ SD) of the T and DO, medians  $\pm$ SD of pH, measured during winter sample collections at the Whitney Wastewater Treatment Facility.  $N=8$  for PD3 DO. \* denote a significant difference ( $p<0.05$ ) from the final effluent.

final effluent was approximately equal in both summer and winter samples and was always within the permitted range of 6-9 according to the TPDES permit for this wastewater treatment facility (TCEQ 2009) (fig. 12). A green color was observed in the water throughout the WWTF ponds and wetlands suggest an algae bloom was taking place during the summer season, which may explain the median pH measurements within the WET1 and PD3 effluent (Thind and Rowell 1999; Zang et al 2011). The pH which was lower in the winter season than the summer season may have been affected by numerous stimuli, including the lower temperatures and higher concentrations of DO. If the pH measured in the summer was the result of an algae bloom, less algae due to the lower temperatures, could have influenced the lower pH measurements of winter. If nitrification was taking place during the winter season, it could have also decreased the pH (Kadlec and Knight 1996). Nitrification lowers the pH of water by releasing  $H^+$  ions into the water column when the  $O_2$  molecules bind with the N from the  $NH_4$  (fig. 13).

The in-situ temperature measurements varied between effluents in both seasons. There was only a significant difference between effluents in the summer season, when the mean temperature of the PD3 effluent was higher than the mean temperature of the final effluent by  $1.81^{\circ}C$  (figs. 10 and 11) (appendix C). As a pond and wetland system exposed to weather, the treatment efficiency is directly affected by ambient temperatures (Kadlec and Knight 1996). Because the WWTF is an open system, the temperatures of the effluents were found to be similar to each other during both seasons; the significant difference of the temperature of PD3 from the FNEF temperature during the summer season may be explained by the structure of the pond. The pond has a larger surface area

and is exposed to more direct sunlight, potentially allowing it to absorb more heat than the WET1 and FNEF sample locations.

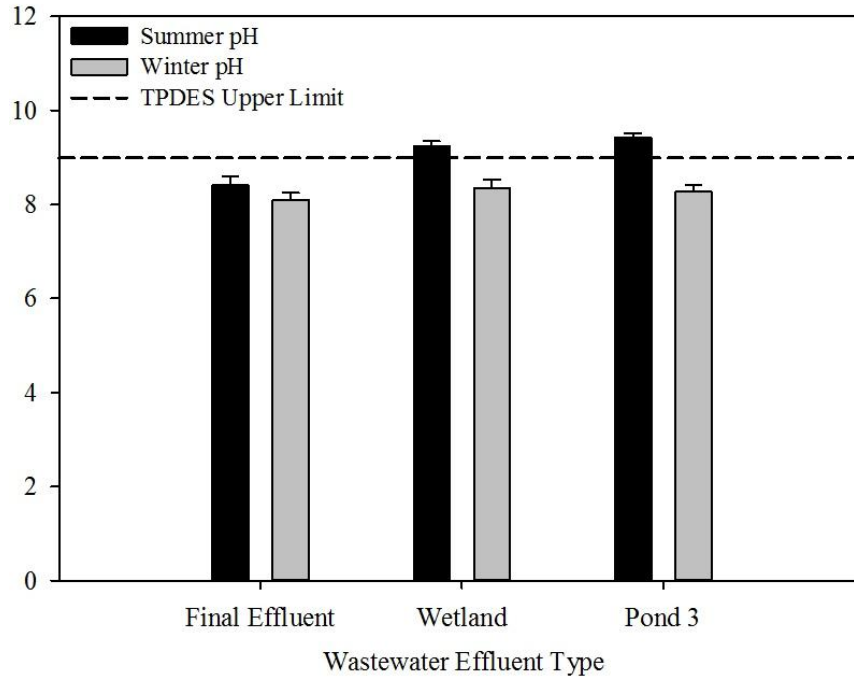


Figure 12. Median pH measurements (n=9;  $\pm$ SD) from summer and winter with the upper pH limit of the Whitney Wastewater Treatment Facility NPDES permit.

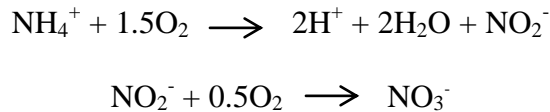


Figure 13. A simplified version of the nitrification process.  $\text{NH}_4^+$  is converted to  $\text{NO}_2^-$  by *Nitrosomonas* bacteria, which is then converted to  $\text{NO}_3^-$  by *Nitrobacter* bacteria. Both processes require oxygen (Kadlec and Knight 1996).

Along with in-situ parameters, TSS and  $\text{CBOD}_5$  are monitored according to the TPDES permit for the WWTF. Both TSS and  $\text{CBOD}_5$  appeared to be reduced through the system in summer and to a lesser extent, in winter. However, only the TSS measured in PD3 was significantly higher than the TSS measured in the final effluent samples during the summer season (fig. 14). There were no significant differences



between the average concentrations of CBOD<sub>5</sub> from the FNEF of either the WET1 or PD3 effluents (fig 14). In the winter season, neither the TSS nor CBOD<sub>5</sub> measured within the WET1 nor PD3 effluents were significantly different from the final effluent (fig. 15). It can be observed however, the average concentrations of TSS were higher in all three effluents in the winter season than the summer, while the average concentrations of CBOD<sub>5</sub> measured in the winter samples, were consistent with the concentrations measured in the summer season (figs. 14 and 15).

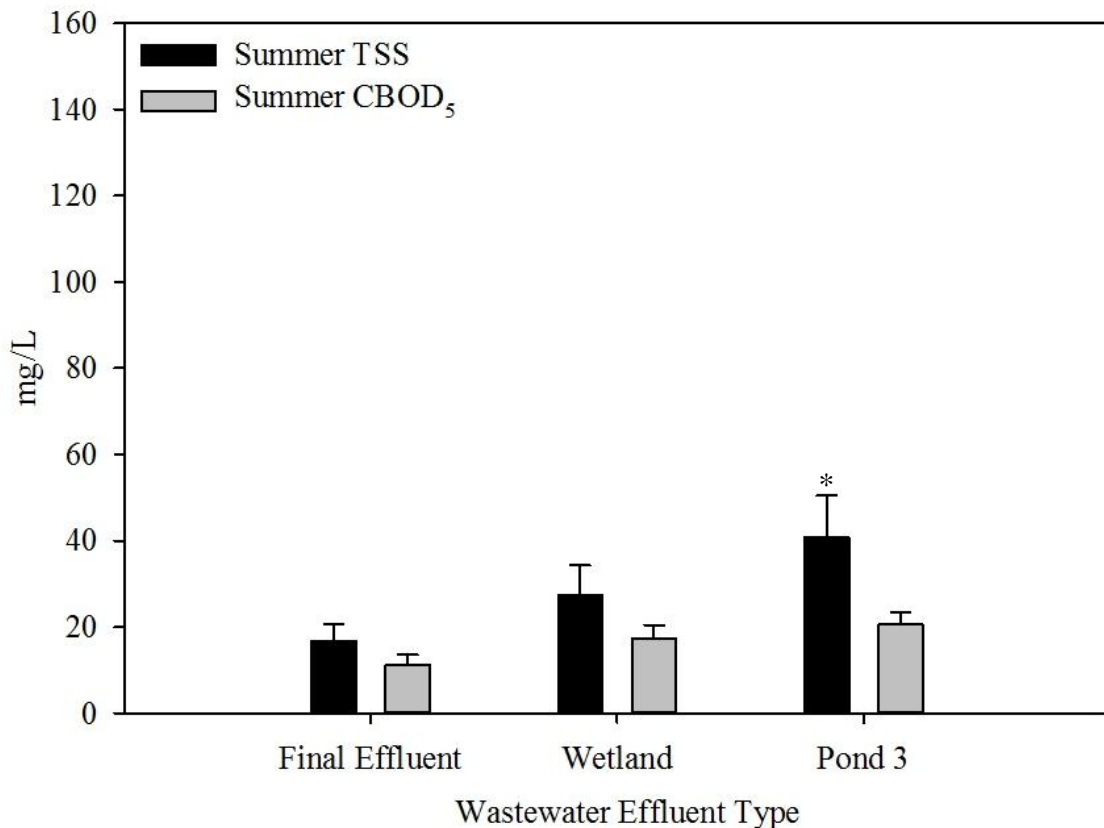


Figure 14. Mean concentrations (n=9;  $\pm$ SD) of TSS and CBOD<sub>5</sub> measured during summer sample collections at the Whitney Wastewater Treatment Facility. \* denote a significant difference ( $p < 0.05$ ) from the final effluent.

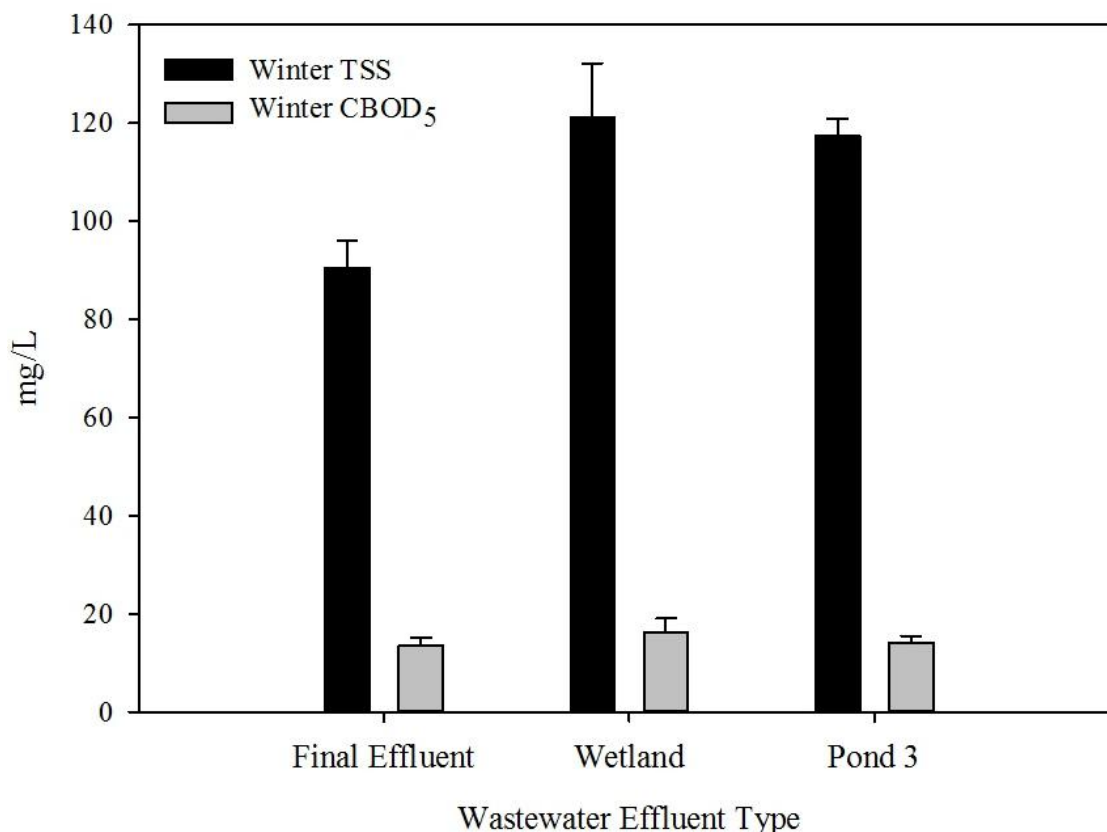


Figure 15. Mean concentrations (n=9;  $\pm$ SD) of TSS and CBOD<sub>5</sub> measured during winter sample collections at the Whitney Wastewater Treatment Facility.

The rise in TSS in the winter season could be caused by several stimuli: plants shedding their organic matter in winter (wetlands) as well as bacterial communities changing with the temperature changes. Higher effluent discharges from the WWTF were observed in the winter season than in the summer season (fig 16). This higher volume of discharge may suggest a higher volume of influent during the winter season. This influent could have contained a higher concentration of TSS or, treating more wastewater could have meant shorter residence times.

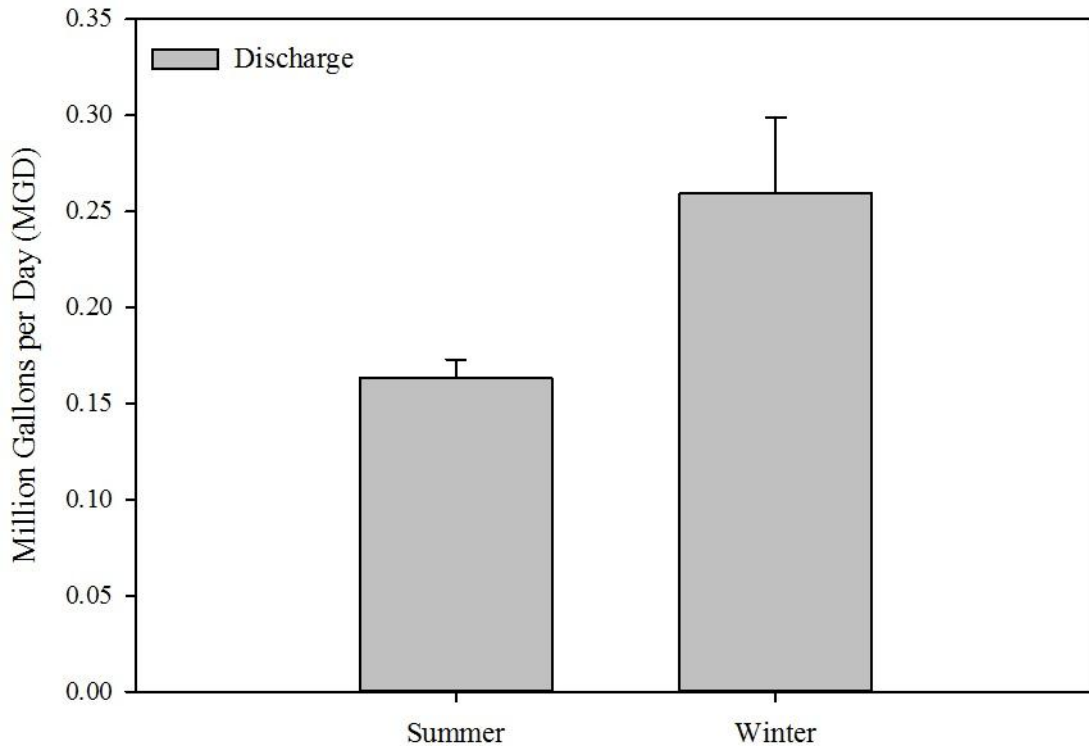


Figure 16. The mean rate ( $n=9$ ;  $\pm$ SD) at which effluent was being discharged from the Whitney Wastewater Treatment Facility was higher in the winter than in summer.

The CBOD<sub>5</sub> was reduced through the system in the summer season, but was approximately equal among effluents in the winter season (figs 14 and 15). For the summer results, which showed a reduction of CBOD<sub>5</sub> through the system, it is possible the microorganism populations were being reduced as the wastewater was treated. The lower CBOD<sub>5</sub> seen in the winter could have been affected by the bacteria in the influent. With the lower temperatures of winter generally come less bacterial activity or different types of bacteria. If the system as a whole contained less carbonaceous bacteria to consume DO, the CBOD<sub>5</sub> could have been reduced, as was seen in the winter samples (fig 15).

Five nutrient species were also measured in both seasons. Three of the nutrient species were total nitrogen (TN), ammonia (NH<sub>3</sub>), and nitrate/nitrite (NO<sub>2</sub>/NO<sub>3</sub>). During

the summer season, the concentrations of TN were reduced through the system, and the concentration measured in the PD3 effluent was significantly higher than the concentration of TN measured in the FNEF. Both the  $\text{NH}_3$  and  $\text{NO}_2/\text{NO}_3$  values measured within the WET1 and PD3 samples collected during the summer season were significantly lower than those measured within the FNEF samples (fig 17). Because the levels of  $\text{NH}_3$  increased through the system, this suggests the organic nitrogen in the wastewater was being converted to  $\text{NH}_3$  through the nitrogen cycle (Kadlec and Knight 1996; Schlesinger 1997). After conversion to  $\text{NH}_3$  the next step in the nitrogen cycle is conversion to  $\text{NO}_2$  and then  $\text{NO}_3$ . Nitrate ( $\text{NO}_3$ ) may then be converted to atmospheric nitrogen ( $\text{N}_2$ ) which escapes the hydrologic system to the atmosphere if conditions are appropriate (Schlesinger 1997; Kadlec and Knight 1996). The nitrogen cycle is temperature dependent; the conversion of  $\text{NO}_3$  to  $\text{N}_2$  is highly affected by temperature, proceeding fastest in warm conditions (Kadlec and Knight 1996; Schlesinger 1997; Mitsch and Gosselink 2007). The reduction of TN through the WWTF system during the summer season may have been the result of nitrification followed by denitrification.

There were no significant differences for any of the measured nitrogen species between the FNEF and PD3 or WET1 during the winter season (fig 18). The average concentration of  $\text{NH}_3$  was lower in the winter samples than in the summer; the concentrations did not appear to change noticeably in the WET1 and PD3 effluents, and there are no significant differences between those effluents and the FNEF. The higher concentrations of DO measured in the winter season may have allowed more  $\text{NH}_3$  to be converted to  $\text{NO}_2/\text{NO}_3$ , accounting for the higher concentrations of those nitrogen species in the winter samples. Also, wetland plants desorb their nutrients back to their

roots and the soil before winter so they are kept within the system; decomposing plant matter also releases nutrients into the soil and water (Kadlec and Knight 1996; Schlesinger 1997; Mitsch and Gosselink 2007). The increased TSS in the winter season may have also contributed to the higher concentrations of TN; organic nitrogen bound up in suspended solids such as plant matter and sediment would have contributed to the concentrations of TN measured.

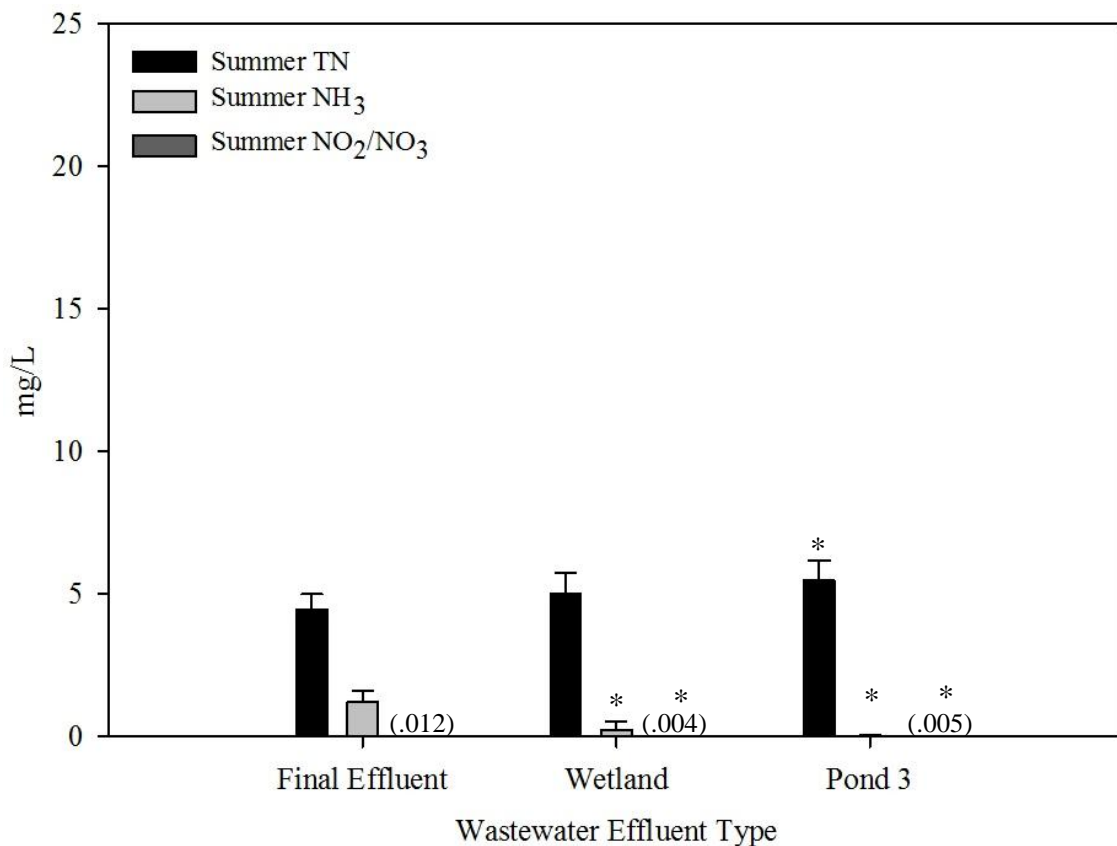


Figure 17. Mean concentrations (n=9;  $\pm$ SD) of TN, NH<sub>3</sub>, and NO<sub>2</sub>/NO<sub>3</sub> measured during summer sample collections at the Whitney Wastewater Treatment Facility. \* denote a significant difference (p<0.05) from the final effluent. NO<sub>2</sub>/NO<sub>3</sub> concentrations are shown in parentheses.

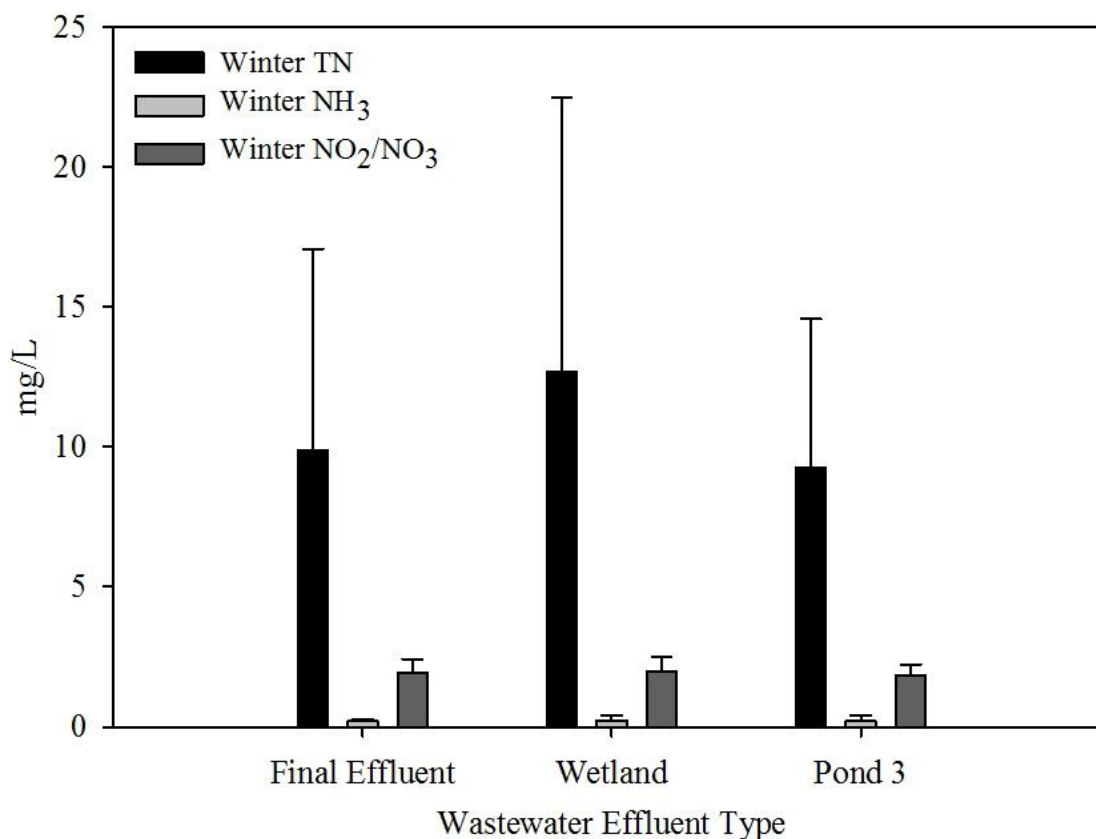


Figure 18. Mean concentrations (n=9;  $\pm$ SD) of TN, NH<sub>3</sub>, and NO<sub>2</sub>/NO<sub>3</sub> measured during winter sample collections at the Whitney Wastewater Treatment Facility.

Along with nitrogen, two species of phosphorous were measured: total phosphorous (TP) and phosphate (PO<sub>4</sub>). During the summer tests, both TP and PO<sub>4</sub> increased through the system; concentrations of both TP and PO<sub>4</sub> measured in the PD3 effluent was significantly lower than the concentrations measured in the final effluent (fig 19). Aerobic, conditions allow for sequestration of phosphorous; likewise, anaerobic, or anoxic conditions, allow the release of sequestered phosphorous (Smil 2000). The DO measured in-situ at the three sampling locations showed the PD3 to have higher DO than the WET1 or FNEF sampling locations in the summer season (fig 10). All samples were collected in the morning hours. The DO measurements (appendix A) suggest the surface and subsurface wetlands were likely going anoxic

during the night, which would have allowed for the release of  $\text{PO}_4$  into the water column from the soil (Smil 2000). This would explain the higher concentrations of TP and  $\text{PO}_4$  measured in the wetland and final effluents (fig 19).

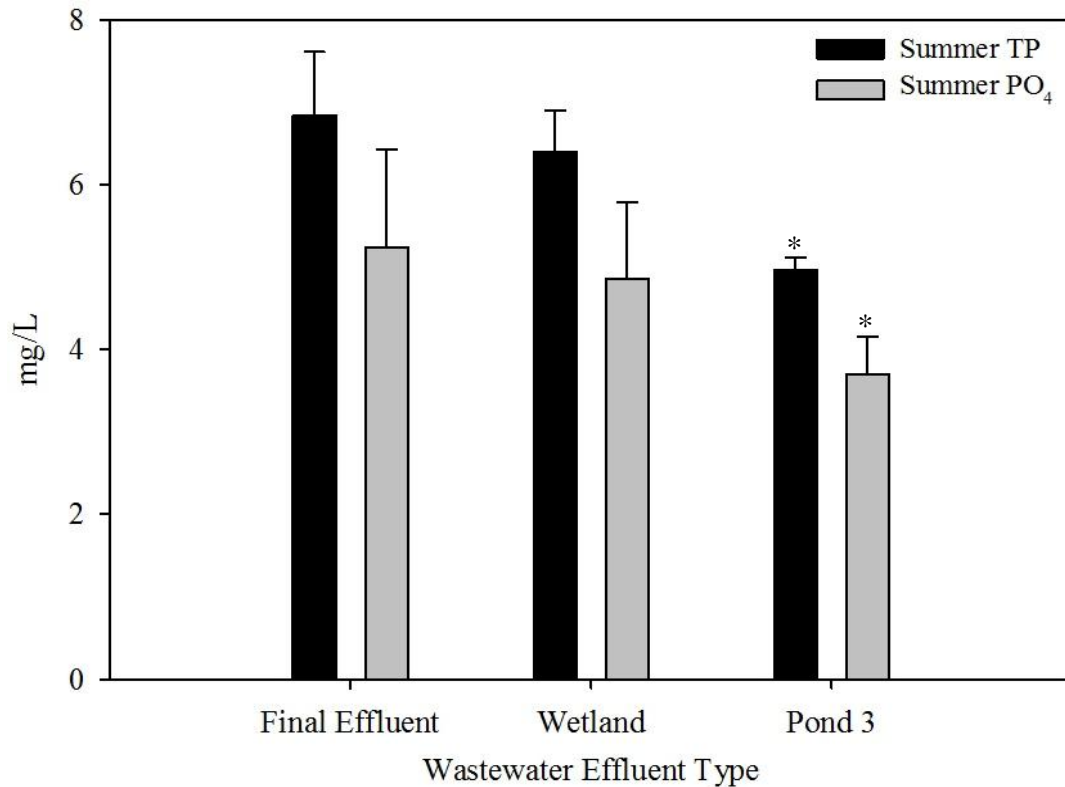


Figure 19. Mean concentrations ( $n=9$ ;  $\pm\text{SD}$ ) of TP and  $\text{PO}_4$  measured during summer sample collections at the Whitney Wastewater Treatment Facility. \* denotes a significant difference ( $p < 0.05$ ) from the final effluent.

Within the samples collected in the winter season the average concentrations of TP and  $\text{PO}_4$  were lower than the average concentrations measured in the summer season (figs 19 and 20); the average concentrations of TP and  $\text{PO}_4$  were similar for all three effluents in the winter and no significant differences were detected (fig 20). The relationship of phosphorous and dissolved oxygen may explain the lower concentrations of TP and  $\text{PO}_4$  measured during the winter season, when mean DO concentrations were

higher in all three effluents than in summer. These measurements and samples were collected during the morning hours, similar to the summer sample collections. The higher DO measurements during the winter sample collections suggest the wetlands (surface and subsurface) were not becoming anoxic during the night, thereby not releasing large amounts of sequestered TP and  $\text{PO}_4$ .

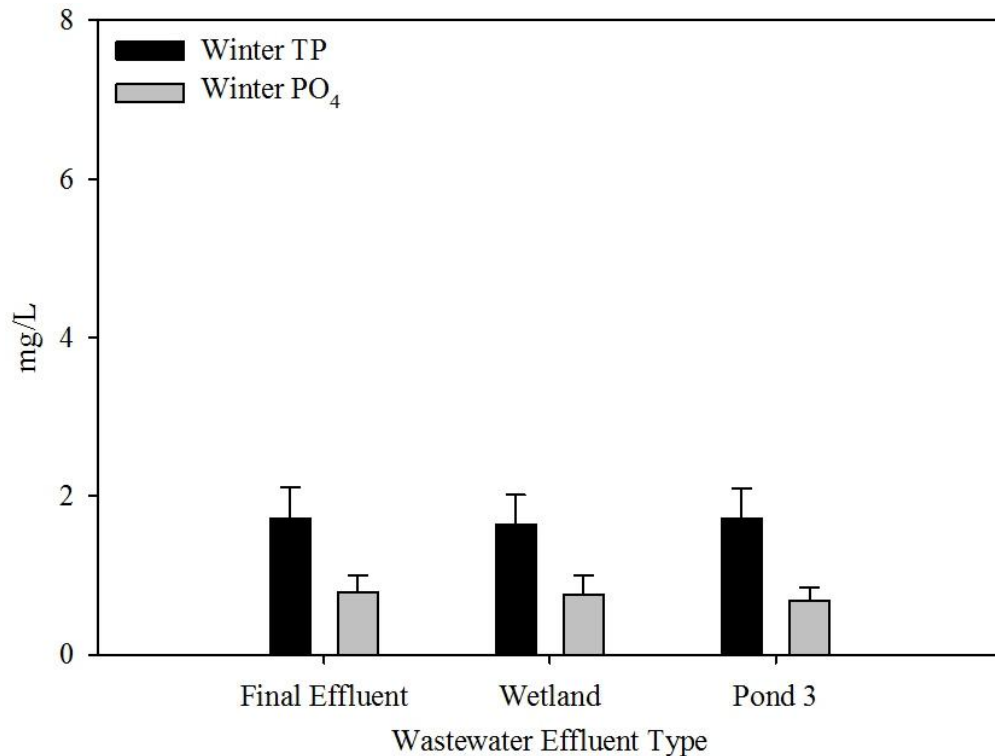


Figure 20. Mean concentrations ( $n=9$ ;  $\pm\text{SD}$ ) of TP and  $\text{PO}_4$  measured during winter sample collections at the Whitney Wastewater Treatment Facility.

As wetlands mature, they accumulate nutrients in the plants and the binding sites on the porous media become saturated (Kadlec and Knight 1996; Schlesinger 1997; Baker et al 1998; Mitsch and Gosselink 2007). When the wetland media is saturated, the system may begin to release phosphorous back to the water column through decomposition of biomass or dissolution of mineral precipitates in which phosphorous is



bound (Kadlec and Knight 1996; Reddy et al 1995). Common precipitating agents include metals such as calcium (Ca), iron (Fe), and aluminum (Al) (Smil 2000). The WWTF aerates the wastewater in ponds 1 and 2, but no additives are used during the treatment process, therefore, any metals present to precipitate phosphorous would be natural present or as part of the influent wastewater. These precipitation and dissolution potentials depend on which elements are involved in the precipitates and the pH and DO concentration of the environment: high pH and anoxic DO conditions promote dissolution (Kadlec and Knight 1996; Smil 2000). This relationship between phosphorous, pH, and DO may partially explain the difference in mean phosphorous concentrations between summer and winter.

#### *Observable Effect Concentrations*

The observable effect concentrations of the WWTF effluents were evaluated, though the WWTF NPDES permit does not require whole effluent toxicity testing. The dilution data collected from the *D. magna* toxicity tests were used to determine the LOEC and NOEC for each effluent type (appendices K and L) (Tables 1 and 2). *D. magna* exposed to undiluted (100%) Wetland 1 and Pond 3 effluents showed no significant differences in survival or reproduction when compared to organisms exposed to undiluted (100%) Final Effluent during the summer or winter tests (figs 21 and 22).

Table 1. The NOEC and LOEC concentrations for the summer *Daphnia magna* toxicity bioassays. These were determined using mean survival and mean reproduction.

Summer						
Effluent	Study 1		Study 2		Study 3	
	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
FNEF	100	—	50	100	100	—
WET1	100	—	—	25	100	—
PD3	100	—	100	—	100	—

Table 2. The NOEC and LOEC concentrations for the winter *Daphnia magna* toxicity bioassays. These were determined using mean survival and mean reproduction.

Winter						
Effluent	Study 1		Study 2		Study 3	
	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
FNEF	100	—	100	—	100	—
WET1	100	—	100	—	100	—
PD3	100	—	100	—	100	—

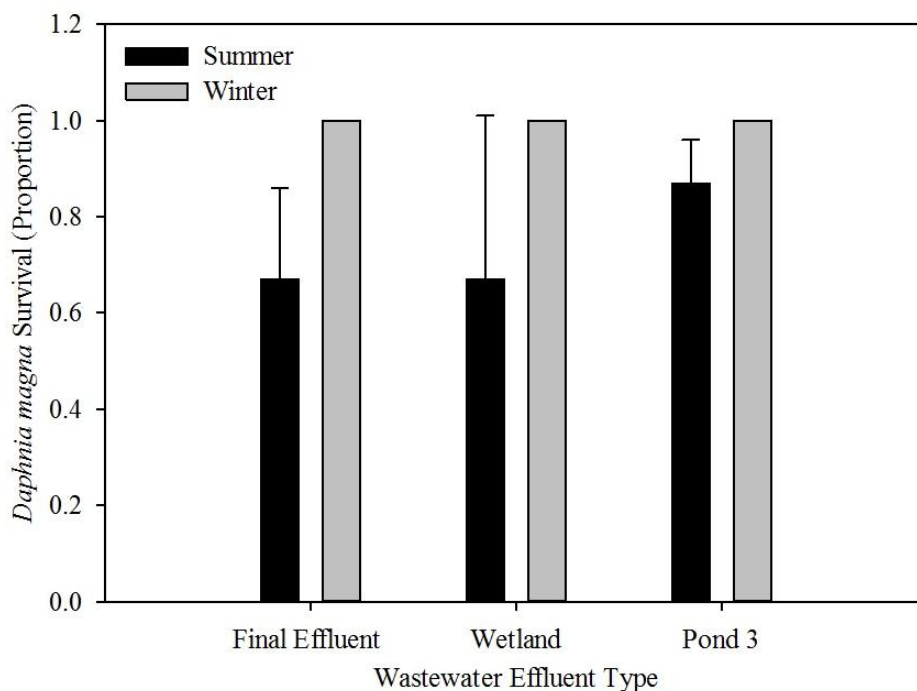


Figure 21. Mean survival ( $n = 3$ ;  $\pm$ SE) of *Daphnia magna* from both the summer and winter toxicity bioassay for various components of the wastewater treatment facility at the City of Whitney, Texas.

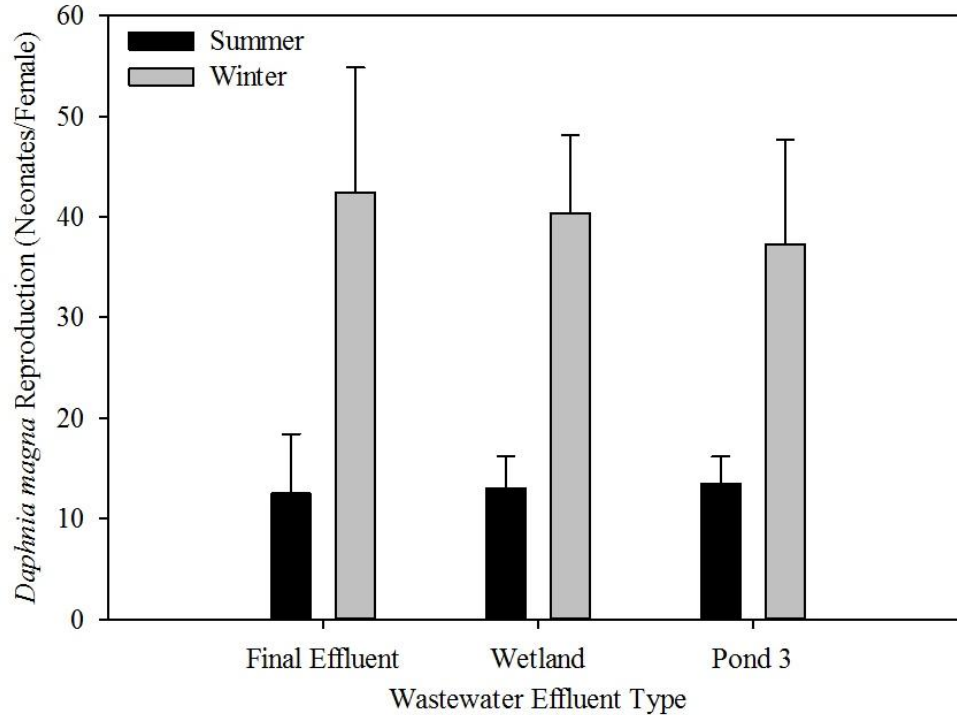


Figure 22. Mean reproduction ( $n = 3$ ;  $\pm$ SE) of *Daphnia magna* from both the summer and winter toxicity bioassay for various components of the wastewater treatment facility at the City of Whitney, Texas.

The *D. magna* exposed to the effluents collected from the same locations in summer and winter tests seemed to have been healthier overall by the end of in the winter tests, based on the higher proportion of surviving organisms and increased reproduction. Some of the water quality differences observed between summer and winter, which may have affected the observed difference in *D. magna* health, included lower pH, reduced  $\text{NH}_3$ , TP and  $\text{PO}_4$  as well as increased DO and TSS in the winter season. However, because the mean reproduction of the replicates exposed to RHW during the summer bioassays was comparable to the mean reproduction of the replicates exposed to the final effluent ( $14.8 \pm \text{SD: } 3.5$  and  $12.5 \pm \text{SD: } 10.2$  respectively), it is possible the culture used to perform the summer studies was compromised.

The composition of the TSS concentrations measured in both seasons is unknown; however, the suspended solids could have contained a beneficial or harmful component, or a mixture of both. Some beneficial components of the TSS may have included, but not been limited too, edible species of algae or bacteria (Infante and Litt 1985). The TSS may have included sediment and organisms which could potentially harm *D. magna*. The higher reproduction observed of *D. magna* exposed to effluent from PD3 than those exposed to the WET1 and FNEF during the summer season suggests there was a beneficial component within that effluent, possibly within in the TSS composition. This possibility also applies to the winter season, when the mean TSS concentrations were higher in all three effluents than in summer. Also, ammonia toxicity to aquatic organisms increases with increased temperature and pH (US EPA 1994). With all three parameters, NH<sub>3</sub>, temperature, and pH, being reduced in winter, the overall toxicity of the water samples to *D. magna* was likely reduced with it, which may partially explain the improved survival and reproduction observed between the summer and winter bioassays. Also, the changes which took place between the end of the pond system (PD3) and the final effluent may have produced the observable differences between the *D. magna* survival and reproduction within each season (figs. 21 and 22; table 3).

Table 3. The effluent water quality changes between the pond 3 and final effluents at the Whitney Wastewater Treatment Facility were different in summer and winter. Arrows indicate whether each parameter increased, decreased, or the same between the pond and final effluents. \* denotes a significant difference between the PD3 and FNEF.

Parameter	Summer		Winter	
	PD3	→ FNEF	PD3	→ FNEF
Dissolved Oxygen	↓		↓	
pH	↓		↓	
Temperature	↓	*	↑	
TSS	↓	*	↓	
CBOD <sub>5</sub>	↓		↓	
TN	↓	*	↑	
NH <sub>3</sub>	↑	*	↑	
NO <sub>2</sub> /NO <sub>3</sub>	↑	*	↑	
TP	↑	*	—	
PO <sub>4</sub>	↑	*	↑	
<i>D. magna</i> Survival	↓		—	
<i>D. magna</i> Reproduction	↓		↓	

### Seasonal Comparisons

The second hypothesis being examined for the City of Whitney Municipal Wastewater Treatment Facility was that there is a significant difference in effluent quality between seasons of the year. The differences observed, between summer and winter for each parameter, were examined according to the interaction results of a two-factor ANOVA. All three effluents sampled showed seasonal differences between concentrations of multiple water quality parameters. The in-situ parameters measured showed significant differences between seasons, with the mean temperature being significantly lower and mean concentrations of DO being significantly higher for all

three effluents during the winter season (table 4). The median pH was significantly lower during the winter season, compared to summer, for both the WET1 and PD3 effluents, but not the Final Effluent (fig 12; table 4).

Table 4. Fall and winter differences of the biological and water quality parameter measurements from the Whitney Wastewater Treatment Facility. A significant difference ( $p < 0.05$ ) between seasons for parameter and effluent type is designated with the letter X..

Effluent	Survival	Reprod.	Temp.	DO	pH	TSS	CBOD	TN	NH <sub>3</sub>	NO <sub>2</sub> /NO <sub>3</sub>	TP	PO <sub>4</sub>
WMARSS			X									
Std. 40 Aerobic			X			X	X					
I.E. Aerobic			X	X	X				X	X		
Septic+Wetland			X				X					
Septic			X									

The mean TSS concentrations measured in the winter samples were significantly higher than those measured in the summer samples for all three effluents. However, the mean concentrations of CBOD<sub>5</sub> measured in the winter season were more similar to each other and to the values measured in the summer tests, with only the CBOD<sub>5</sub> measured in PD3 being significantly different between the two seasons (figs 14 and 15; table 4).

Of the nitrogen species measured, there was not a consistent effect of season on the three effluents sampled. The mean concentrations of TN were significantly higher in winter than summer for all three sample sites. The mean concentration of NH<sub>3</sub> measured in the FNEF in the winter season was significantly lower than that measured in the FNEF in the summer season; however, the other two effluents (WET1 and PD3) did not show a significant difference between seasons. The NO<sub>2</sub>/NO<sub>3</sub> concentrations measured in all three effluents were significantly higher in the winter samples than in the summer samples (figs 17 and 18; table 3). The mean concentrations of both TP and PO<sub>4</sub>

measured in all three effluents were significantly lower in the winter season than in the summer season (figs 19 and 20; table 4).

*D. magna* survival and reproduction in the winter tests both appeared greater when compared to the summer results, but they were not significantly different according to the two-factor ANOVA interaction results (table 4). Because no significant interaction was detected between the two factors (season and effluent type) for the *D. magna* survival and reproduction endpoints, the season comparison results were considered. There was a significant difference between summer and winter for *D. magna* survival and reproduction. This suggests the effluent type did not have a significant effect on the *D. magna*, but the composition of the effluents (effluent quality) between the two seasons, did have a significant effect on the *D. magna*.

The higher mean concentrations of DO, associated with the lower temperatures of winter, were likely the cause of the lower concentrations of  $\text{NH}_3$ , TP, and  $\text{PO}_4$ . The conversion of  $\text{NH}_3$  to  $\text{NO}_2/\text{NO}_3$  may have contributed to the lower pH measurements, thereby possibly reducing the toxic effects of the remaining  $\text{NH}_3$  in all three effluents. The combined factors of higher DO, lower  $\text{NH}_3$ , and lower pH are all improvements of quality in regards to aquatic organisms which may inhabit the receiving waters of these effluents, such as *D. magna*.

*Water Quality*

The first hypothesis being tested concerning the WMARSS and on-site wastewater treatment systems was that the effluent quality of the onsite systems is consistent with that of a 40 MGD centralized municipal wastewater treatment facility. The in-situ dissolved oxygen (DO) concentrations measured within the anaerobic on-site wastewater treatment system effluents (Septic and Septic+Wetland) were significantly lower than the DO measured in the WMARSS effluent in the fall; the DO measured in the aerobic on-site systems in the fall did not show any significant differences from the WMARSS effluent (fig. 23). In the winter season, the average concentrations of DO measured in all on-site systems, except the I.E. Aerobic, was significantly lower than the DO measured within the WMARSS effluent (fig 24). The median in-situ pH measurements collected for the Septic+Wetland and Septic effluents were significantly lower than the median pH of the WMARSS effluent during the fall season; there were no significant pH differences detected between the five effluents during the winter season (figs. 23 and 24). The in-situ temperature measurements were comparable between the four on-site system effluents and the WMARSS effluent during the fall season; however, the on-site systems, except the I.E. Aerobic system, had significantly lower winter temperatures than the WMARSS effluent (figs. 23 and 24).

The increase in mean DO concentrations measured during the winter season may be partially explained by the decrease in temperature, since the capacity of water to hold DO is inversely correlated to temperature (Cech 2010). In both fall and winter the DO concentrations measured in the two aerobic systems were not significantly



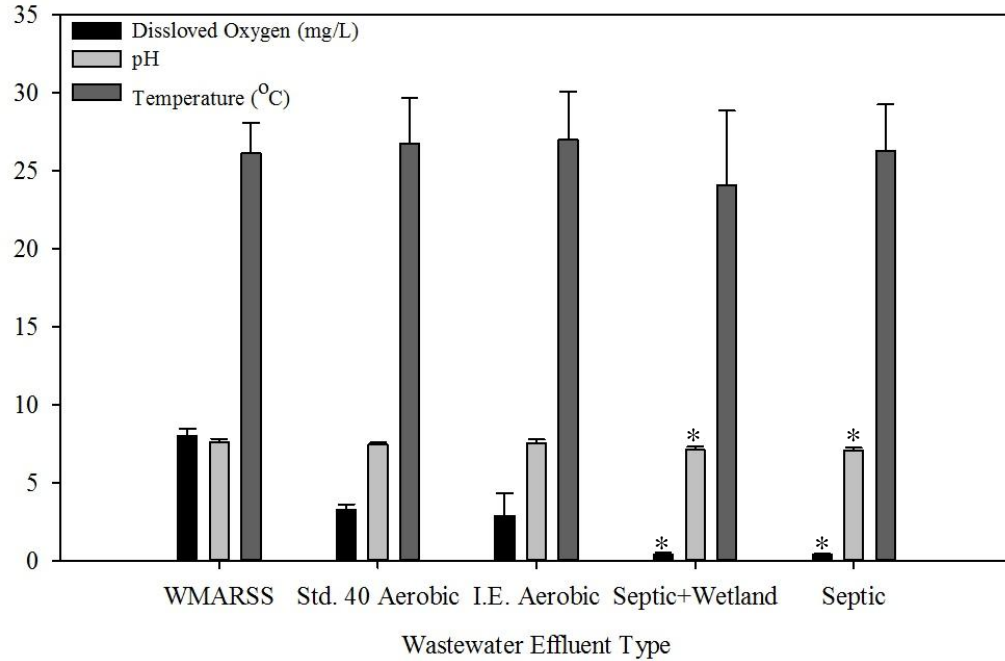


Figure 23. The means ( $n=9$ ;  $\pm$ SD) of the T and DO, medians  $\pm$ SD of the pH, measured during fall sample collections at the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program. BWRP DO  $n=8$ . \* denote a significant difference ( $p<0.05$ ) from the WMARSS effluent.

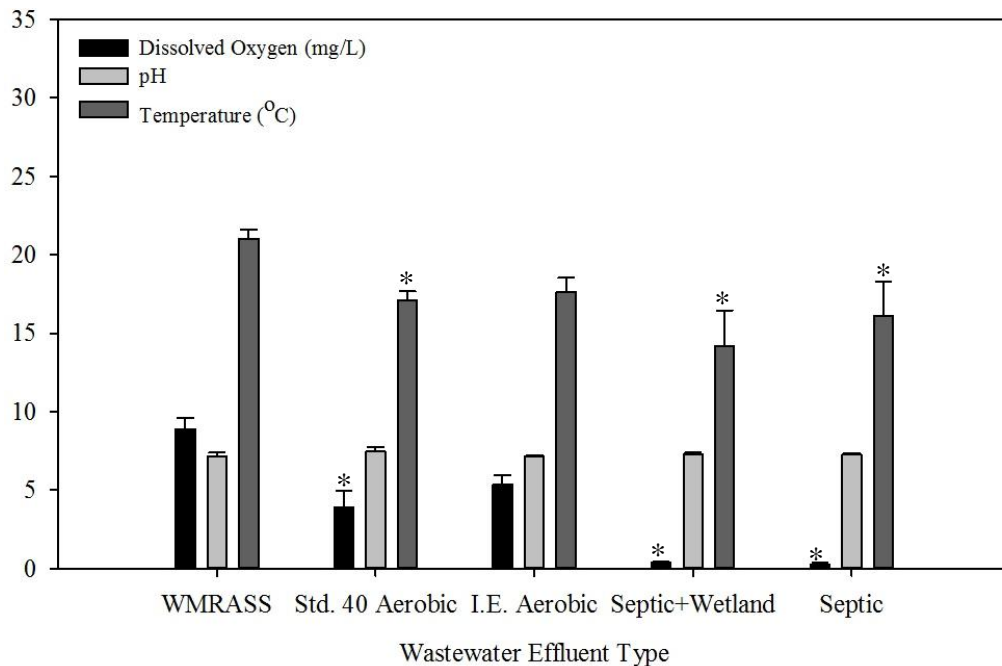


Figure 24. The means ( $n=9$ ;  $\pm$ SD) of the T and DO, medians  $\pm$ SD of the pH, measured during winter sample collections at the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program. Septic+Wetland DO  $n=6$  and Septic DO  $n=7$ . Septic+Wetland and Septic T  $n=7$ . \* denote a significant difference from the WMARSS effluent.

different from each other, but were significantly higher than the two anaerobic on-site systems, which were not different from each other (fig. 25). Oxygen was introduced into the on-site aerobic treatment units, allowing them to maintain higher levels of DO than the Septic and Septic+Wetland on-site systems. No DO was intentionally introduced into the two anaerobic systems (Septic and Septic+Wetland). The observed differences in pH measurements in the winter season is likely correlated with the higher DO and lower temperatures (Cech 2010).

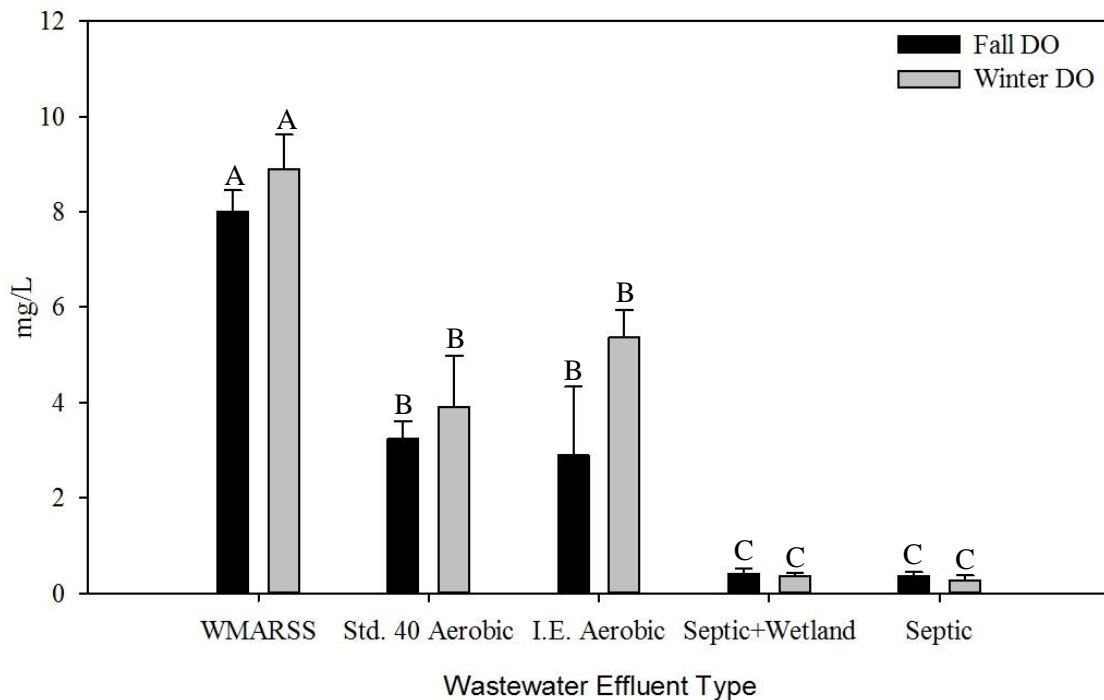


Figure 25. The mean concentrations of DO measured in winter were increased in all systems except Septic. The letters indicate to which population each effluent belongs, per season.

In the fall season, the Septic system was the only on-site system which produced effluent with significantly higher average concentrations of TSS and CBOD<sub>5</sub> than the WMARSS effluent (fig. 26). In the winter season however, the Std. 40 Aerobic and the Septic on-site systems had significantly higher average concentrations of TSS than the

WMARSS effluent (fig. 27). Also in the winter season, the Septic and Septic+Wetland were the only on-site systems to produce effluent with mean CBOD<sub>5</sub> concentrations significantly higher than that measured in the WMARSS effluent (fig 27). The mean concentration of CBOD<sub>5</sub> measured within the Std. 40 Aerobic winter effluent was higher than the WMARSS effluent and comparable to the mean concentration of the Septic+Wetland effluent, which was significantly higher; however, the concentration within the Std. 40 Aerobic effluent was slightly lower than that of the Septic+Wetland effluent (12.7 and 14.72 respectively), so it was not significantly different from the WMARSS effluent.

The increase of TSS and CBOD<sub>5</sub> seen in the winter samples may have been caused by many stimuli; some possible causes include increased TSS and CBOD<sub>5</sub> in the influent or changes in bacterial communities due to the lower temperatures of winter (Kadlec and Knight 1996). Though all systems received the same influent, the Septic on-site system performed less efficiently than the aerobic on-site systems in both seasons, but the difference is more pronounced in the winter than in the fall (fig 28). The wetland which followed the Septic system reduced the TSS and CBOD<sub>5</sub> to levels comparable to the on-site aerobic systems in both fall and winter.

Of the five nutrient forms measured, three were nitrogen species: total nitrogen (TN), ammonia (NH<sub>3</sub>), and nitrate/nitrite (NO<sub>2</sub>/NO<sub>3</sub>). During the fall season, the mean TN concentrations measured within three of the on-site system effluents were significantly higher than the mean TN concentration of the WMARSS effluent: I.E. Aerobic, Septic+Wetland, and Septic. The I.E. Aerobic and Septic+Wetland on-site systems also had higher mean concentrations of NH<sub>3</sub> than that measured in the

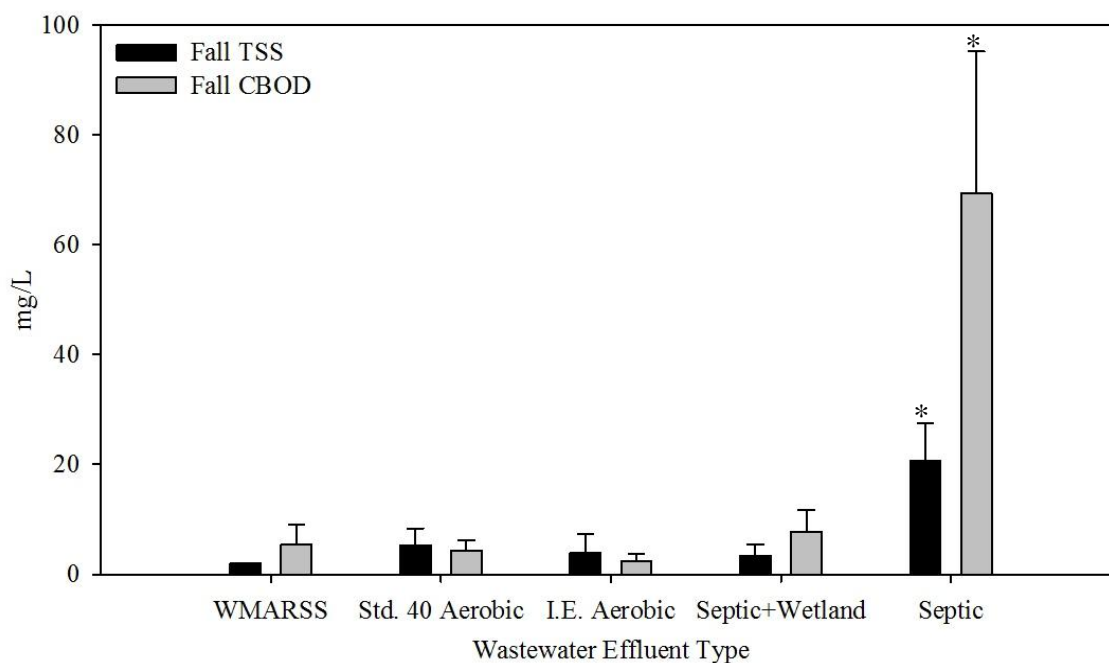


Figure 26. Mean concentrations (n=9;  $\pm$ SD) of TSS and CBOD<sub>5</sub> measured during fall sample collections at the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program. \* denote a significant difference ( $p < 0.05$ ) from the WMARSS effluent.

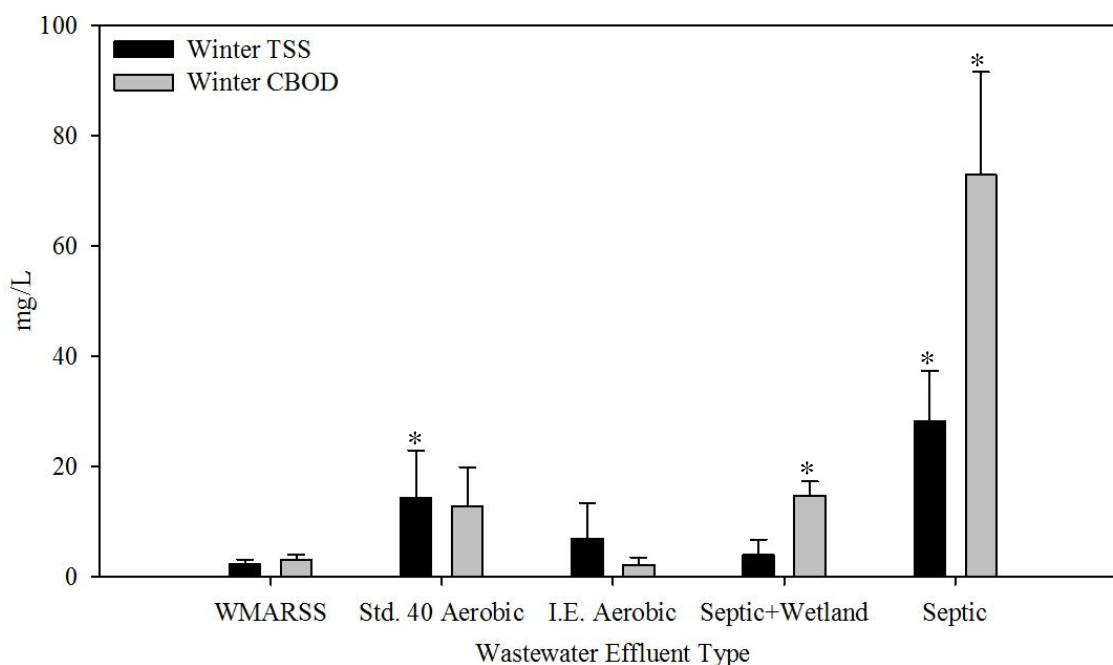


Figure 27. Mean concentrations (n=9;  $\pm$ SD) of TSS and CBOD<sub>5</sub> measured during winter sample collections at the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program. I.E. TSS n=8. \* denote a significant difference ( $p < 0.05$ ) from the WMARSS effluent.

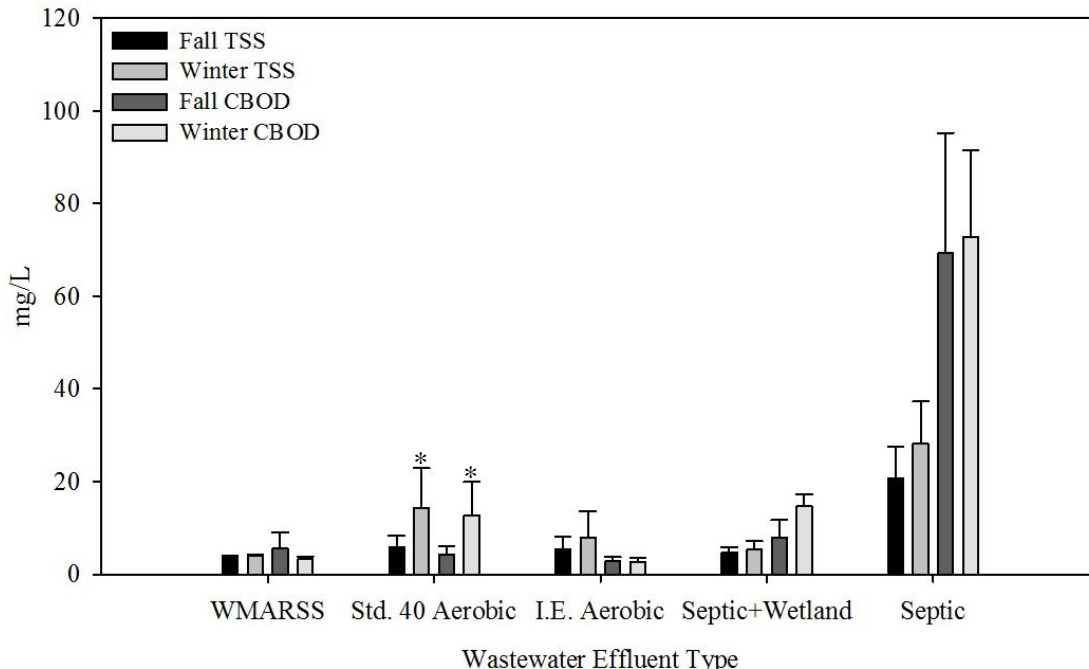


Figure 28. The TSS and CBOD<sub>5</sub> were consistent between seasons for all systems except the Std. 40 Aerobic on-site system, which showed a significant increase of both constituents from fall to winter.

WMARSS effluent. While all four on-site systems had lower average NO<sub>2</sub>/NO<sub>3</sub> concentrations than the WMARSS effluent, only the mean NO<sub>2</sub>/NO<sub>3</sub> concentrations measured in the Septic and Septic +Wetland effluents were significantly lower (fig. 29). During the winter season, all four on-site systems had higher average concentrations of TN than the WMARSS effluent, but only the average concentration of TN measured in the Septic effluent was significantly higher than the average concentration of TN measured in the WMARSS effluent. The average concentrations of NH<sub>3</sub> in all four on-site system effluents during the winter season were higher than the mean NH<sub>3</sub> concentration measured in the WMARSS effluent, however, only the concentrations of the Septic+Wetland and Septic effluents were significantly higher than the WMARSS effluent. As was seen in the fall samples, only the NO<sub>2</sub>/NO<sub>3</sub> in the Septic+Wetland and Septic effluents was significantly lower than that of the WMARSS effluent (fig. 30).

Unlike in the fall season however, the mean concentration of  $\text{NO}_2/\text{NO}_3$  measured in the I.E. aerobic on-site system was higher than the mean concentration of the WMARSS effluent, though not significantly (fig 30).

An inverse relationship between  $\text{NH}_3$  and  $\text{NO}_2/\text{NO}_3$  was maintained for all systems in fall and winter. When conditions allow for nitrification, the concentrations of  $\text{NH}_3$  and  $\text{NO}_2/\text{NO}_3$  will generally maintain an inverse relationship because the transformation of  $\text{NH}_3$  to  $\text{NO}_2/\text{NO}_3$  occurs rapidly (Schlesinger 1997). When mean concentrations of  $\text{NH}_3$  were high, mean concentrations of  $\text{NO}_2/\text{NO}_3$  were low, and the opposite relationship was also observed (figs 29 and 30). Nitrate ( $\text{NO}_3$ ) is expelled from a system through denitrification which converts the  $\text{NO}_3$  to a gaseous species, usually dinitrogen ( $\text{N}_2$ ) (Schlesinger 1997). During the fall season, the WMARSS effluent

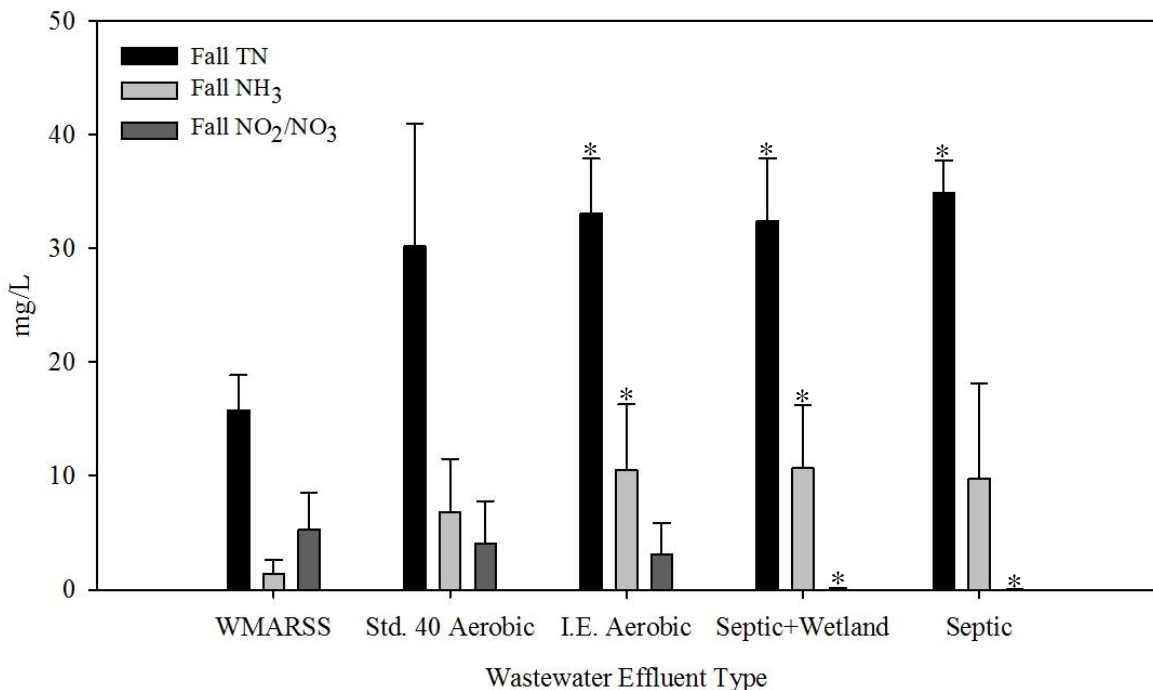


Figure 29. Mean concentrations ( $n=9$ ;  $\pm\text{SD}$ ) of TN,  $\text{NH}_3$ , and  $\text{NO}_2/\text{NO}_3$  measured during fall sample collections at the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program. Std. 40  $\text{NH}_3$   $n=8$ . \* denote a significant difference ( $p<0.05$ ) from the WMARSS effluent.

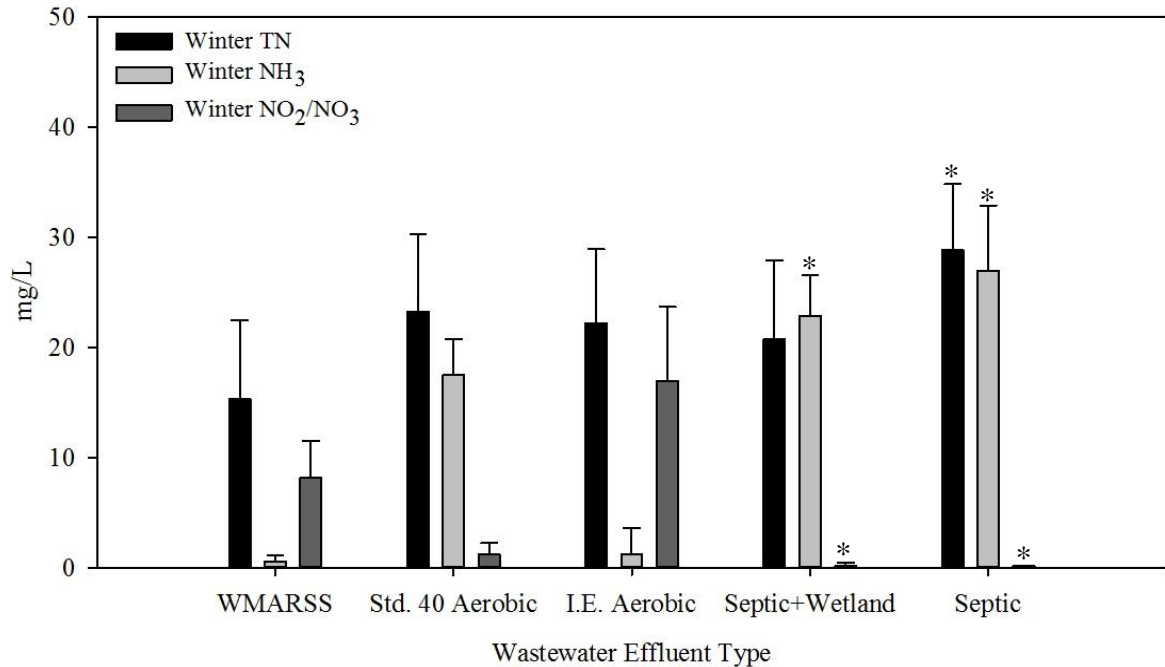


Figure 30. Mean concentrations (n=9;  $\pm$ SD) of TN, NH<sub>3</sub>, and NO<sub>2</sub>/NO<sub>3</sub> measured during winter sample collections at the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program. \* denote a significant difference ( $p < 0.05$ ) from the WMARSS effluent.

was the only system to maintain a higher mean concentration of NO<sub>2</sub>/NO<sub>3</sub> than NH<sub>3</sub>, as well as the lowest mean concentration of TN, suggesting this system was most effective at completing the nitrogen cycle and removing nitrogen from the wastewater (fig. 29). Of the on-site wastewater treatment systems, the Std. 40 and I.E. Aerobic systems had the highest mean concentrations of NH<sub>3</sub> and lowest mean concentrations of TN and NO<sub>2</sub>/NO<sub>3</sub>, outperforming the Septic and Septic+Wetland on-site systems (fig. 29). Nitrification (NH<sub>3</sub>  $\rightarrow$  NO<sub>2</sub>/NO<sub>3</sub>) requires aerobic conditions, therefore, in the anaerobic systems nitrogen can be converted to NH<sub>3</sub> (ammonification), but only negligible amounts of NO<sub>2</sub>/NO<sub>3</sub> can be formed in this environment, explaining why the aerobic onsite systems were able to outperform the Septic and Septic+Wetland on-site systems at converting NH<sub>3</sub> to NO<sub>2</sub>/NO<sub>3</sub> (Schlesinger 1997). Due to their lack of DO, the Septic and Septic+Wetland on-site systems were only able to convert organic nitrogen species

to  $\text{NH}_3$ , but not to  $\text{NO}_2/\text{NO}_3$ , which is an aerobic process. Any  $\text{NO}_2/\text{NO}_3$  which was introduced into the system through the influent was likely lost to denitrification, explaining the high mean  $\text{NH}_3$  and low mean  $\text{NO}_2/\text{NO}_3$  concentrations (Schlesinger 1997).

In the winter, the WMARSS and I.E. effluents contained the lowest concentrations of TN and  $\text{NH}_3$  and the highest concentrations of  $\text{NO}_2/\text{NO}_3$ , suggesting these systems were most effectively removing nitrogen (fig. 30). These were the only two systems which were aerobic twenty-four hours per day; with the increased DO of winter and the consistent aerobic conditions, it should be expected that the WMARSS and I.E. systems be most efficient, of the five systems, at nitrification. The difference observed between the Std. 40 and I.E. systems' ability to convert  $\text{NH}_3$  to  $\text{NO}_2/\text{NO}_3$  may be explained by the dosing and effluent collection schedules. While the Std. 40 on-site aerobic system was aerated twenty-four hours per day, the dosing regimen (three large doses at specific times of day) may have caused the system to be overwhelmed throughout the day, especially when dosing was initiated immediately following the extended periods of no dosing. The samples were collected (twenty-four hours per day) from the effluent chamber. The Std. 40 Aerobic system may have become anaerobic throughout the day, meaning nitrification would stop, and any  $\text{NH}_3$  within the effluent could not be converted to  $\text{NO}_2/\text{NO}_3$  until the system could stabilize and again operate aerobically.

Along with nitrogen, two forms of phosphorous were measured: total phosphorous (TP) and phosphate ( $\text{PO}_4$ ). During both the fall and winter seasons, the average concentrations of TP and  $\text{PO}_4$  measured in all four on-site systems were higher



than the average concentrations of these nutrients measured in the WMARSS effluent. However, in the fall season, only the average concentrations of TP measured in the Septic+Wetland and Septic on-site systems were significantly higher than WMARSS (fig 31).

During the winter season, the Std. 40 Aerobic, Septic+Wetland and Septic systems had significantly higher average concentrations of TP, and the anaerobic systems (Septic+Wetland and Septic) had significantly higher average concentrations of  $\text{PO}_4$  than the WMARSS effluent (fig 32). The I.E. on-site aerobic system was the only on-site system to show no significant differences from the WMARSS effluent for either phosphorous species in either season. Also, the anaerobic on-site systems (Septic+Wetland and Septic) produced effluents with higher average concentrations of TP than either aerobic on-site system in both fall and winter (figs 31 and 32).

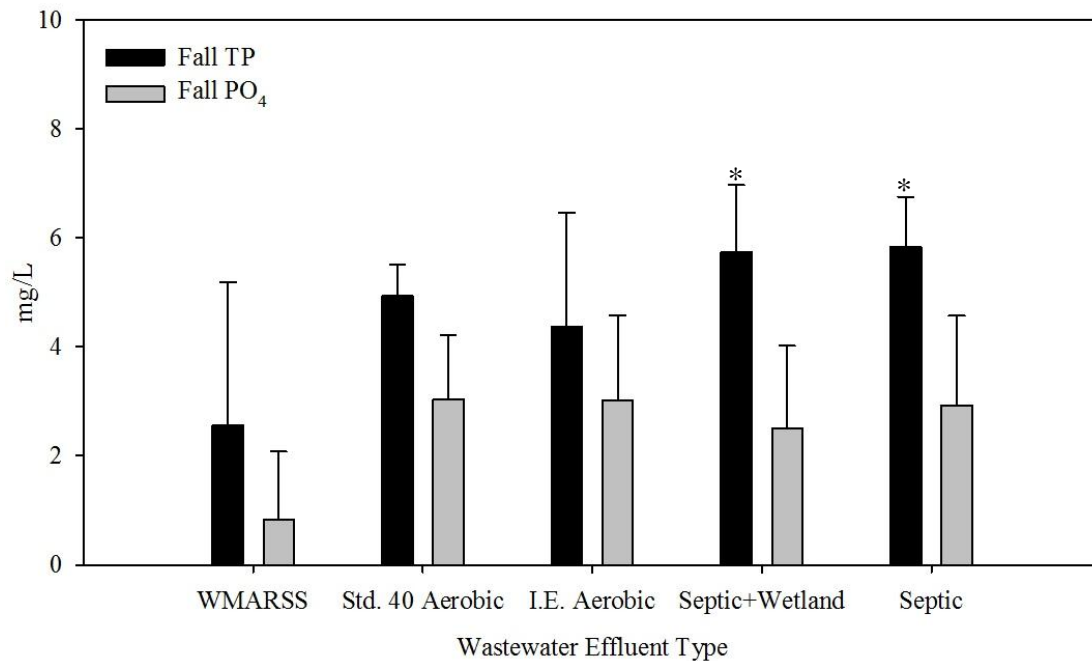


Figure 31. Mean concentrations ( $n=9$ ;  $\pm$ SD) of TP and  $\text{PO}_4$  measured during fall sample collections at the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program. \* denote a significant difference ( $p<0.05$ ) from the WMARSS effluent.

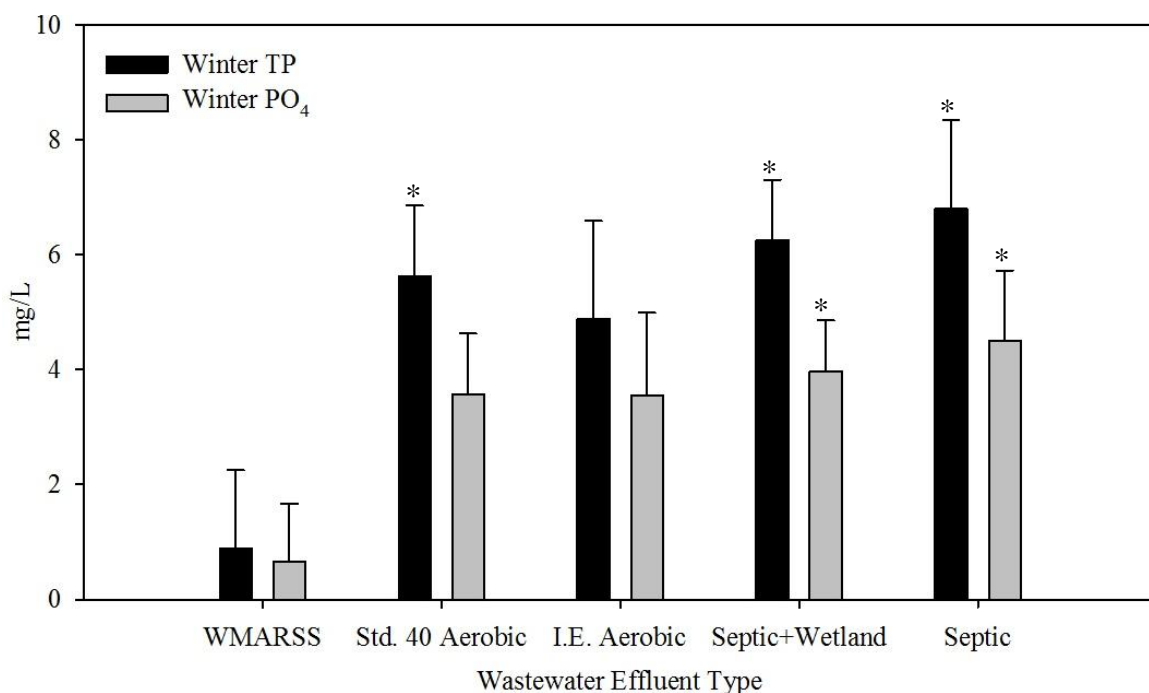


Figure 32. Mean concentrations (n=9;  $\pm$ SD) of TP and PO<sub>4</sub> measured during winter sample collections at the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program.\* denote a significant difference ( $p < 0.05$ ) from the WMARSS effluent.

The differences in phosphorous concentrations between the on-site systems and the WMARSS facility are not surprising since on-site wastewater treatment systems are not designed for nutrient removal and are expected to receive further treatment after the disposal of the effluent, before the effluent reaches a water source (US EPA 2000). Most major municipal wastewater treatment systems, such as the WMARSS facility, are required to monitor and reduce nutrient concentrations in their discharged effluent (de-Bashan and Bashan 2004). As an activated sludge wastewater treatment system, the WMARSS facility has multiple options for phosphorous reduction including, but not limited to: bacterial reduction of phosphorous and precipitation of phosphorous using an additive, such as a salt containing Fe, Ca, or Al (Kadlec and Knight 1996; Smil 2000). Phosphorous can be sequestered into the sludge by allowing the bacterial communities

in the sludge remove the phosphorous, but this method only removes 15-40% of the phosphorous (Smil 2000). By adding salts of Fe, Ca, or Al the phosphorous removal can be increased to 70-90% (Smil 2000). The sludge from these wastewater treatment facilities, WMARSS included, is routinely removed from the system and disposed of, eliminating the possibility of the sequestered phosphorous being reintroduced into the wastewater (TPDES 2006; Smil 2000).

On-site wastewater treatment systems however, do not operate under permits regulating the phosphorous content of their discharges. Also, on-site wastewater treatment systems are not usually designed reduce nutrients; nutrient reduction often happens in the process of treatment, but is not generally the goal for which the systems are designed (US EPA 2000; NSF/ANSI 2010). If properly maintained, on-site systems are supposed to be pumped (sludge removed) routinely. This sludge is then disposed of similarly to the municipal wastewater treatment facility sludge. However, as passive systems, nutrient reducing additives are not part of standard on-site treatment systems; therefore, nutrient reduction is usually expected to be minimal at best.

#### *Observable Effect Concentrations*

In both summer and winter seasons, adverse effects (NOECs and LOECs) were identified in various effluent concentrations within the bioassays performed (Tables 5 and 6). *D. magna* exposed to whole effluent from the four on-site wastewater treatment systems showed no significant difference in mean survival when compared to organisms exposed to undiluted WMARSS effluent for both fall and winter toxicity tests (fig. 33). The WMARSS survival was equal to that of the lab controls, with both having 100% survival for all bioassays performed in summer and winter (appendix W). The survival

was reduced for the *D. magna* exposed to Septic on-site system effluent with 100% mortality in two of the three bioassays conducted during the fall (appendix W), but the reduction was not found to be statistically significant when compared to the survival of organisms exposed to WMARSS effluent. This may have been the result of the large variability within this data set.

Table 5 The NOEC and LOEC concentrations for the fall *Daphnia magna* toxicity bioassays conducted using effluents collected from the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program. These were determined using mean survival and mean reproduction.

Effluent	Fall					
	Study 1		Study 2		Study 3	
	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
WMARSS	100	—	100	—	100	—
Std. 40	100	—	100	—	100	—
I.E.	50	100	100	—	100	—
Septic+Wetland	50	100	50	100	—	25
Septic	50	100	50	100	50	100

Table 6: The NOEC and LOEC concentrations for the winter *Daphnia magna* toxicity bioassays conducted using effluents collected from the Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program. These were determined using mean survival and mean reproduction.

Effluent	Winter					
	Study 1		Study 2		Study 3	
	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
WMARSS	100	—	100	—	100	—
Std. 40	100	—	100	—	100	—
I.E.	100	—	100	—	100	—
Septic+Wetland	100	—	100	—	100	—
Septic	50	100	100	—	50	100

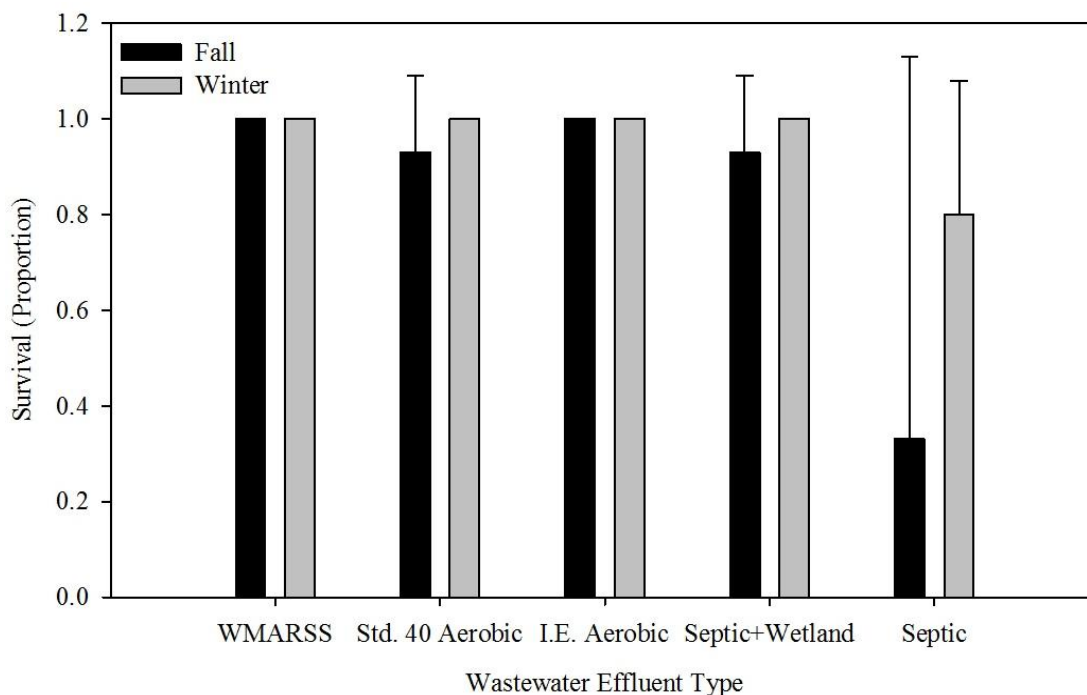


Figure 33. Mean survival ( $n = 3$ ;  $\pm$ SE) of *Daphnia magna* from both the summer and winter toxicity bioassay for Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program on-site units.

*D. magna* reproduction was also not significantly affected by exposure to full strength effluent from the on-site systems in either fall or winter toxicity tests. In the fall tests, the reproduction from organisms exposed to the aerobic on-site systems was comparable to that of the organisms exposed to WMARSS effluent and in the winter season, there was a slight increase of reproduction in the aerobic effluents not seen in the WMARSS effluent. Reproduction was lower in both Septic and Septic+Wetland on-site systems in the fall and winter toxicity tests when compared to WMARSS reproduction, but these reductions were not statistically significant ( $p < 0.05$ ) (fig. 34).

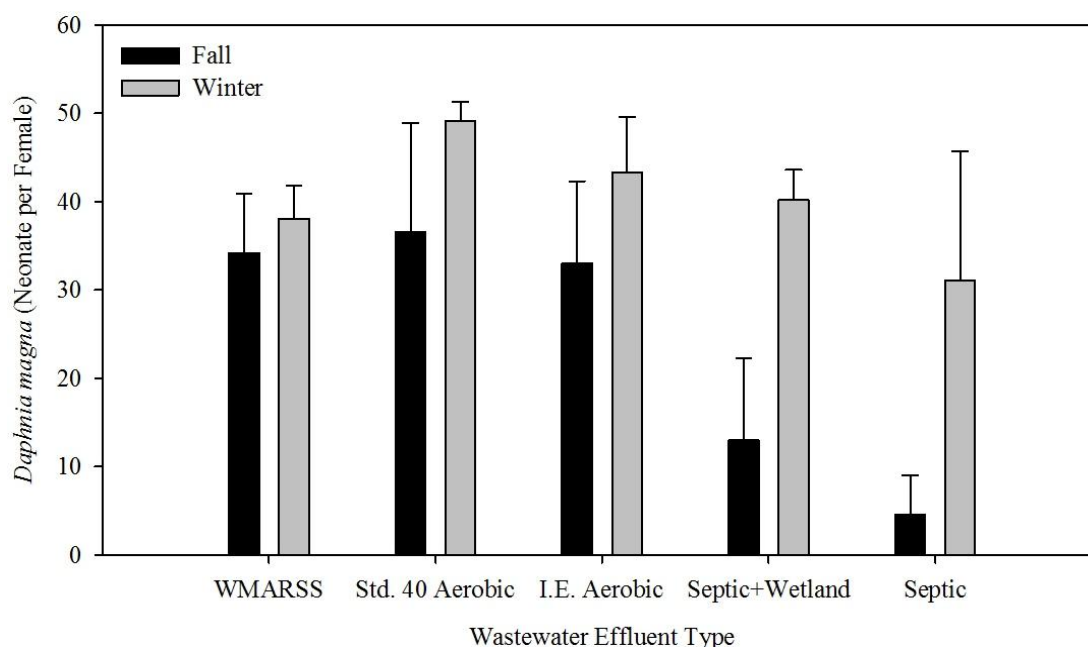


Figure 34. Mean reproduction ( $n = 3$ ;  $\pm$ SE) of *Daphnia magna* from both the summer and winter toxicity bioassay for Waco Metropolitan Area Regional Sewerage System and the Baylor Wastewater Research Program on-site units.

An observable increase in both survival and reproduction of *D. magna* during the winter toxicity tests can be noted for all systems, suggesting the effluent quality was improved in the winter season when compared to fall. Most of the measured water quality characteristics showed minimal differences between seasons; however, aquatic organisms can be affected by slight differences in constituent concentrations even if the differences observed are not statistically significant. Therefore, while *D. magna* suffered no mean significant ill effects from being exposed to 100% effluent from any of these systems in either season, a noticeable improvement in survival and reproduction during the winter tests suggests the *D. magna* may have been sensitive to the effluent quality differences. This improvement can be seen in the NOECs and LOECs as well as the mean survival and reproduction measured for each effluent type (tables 5 and 6; figs. 33 and 34).

### *Seasonal Comparisons*

The second hypothesis regarding the WMARSS and on-site wastewater treatment systems was that there is a difference in effluent quality between seasons. All five systems were affected by the change of season, but not equally. The mean winter temperatures were lower than the mean fall temperatures for all five treatment systems (figs. 23 and 24; table 7). The lower temperatures of winter, allowing more DO to be held within the aerobic systems, likely allowed the increased nutrient reduction observed in the on-site systems in winter; this same decrease in temperature may have adversely affected bacterial colonies responsible for the reduction of TSS and CBOD<sub>5</sub>. The Influent Equalization Aerobic on-site system showed the most significant differences between seasons; along with temperature, the average concentrations of DO, NH<sub>3</sub> and NO<sub>2</sub>/NO<sub>3</sub> as well as the median pH measurements between fall and winter were significantly different (table 7). (The DO was higher in winter and the pH was lower.) The Std. 40 Aerobic on-site system showed a significant difference between seasons for TSS and CBOD<sub>5</sub>. Both constituents were higher in winter compared to fall. Of the two anaerobic on-site systems (Septic and Septic+Wetland), only the Septic+Wetland system showed a significant difference between seasons besides temperature, with the mean concentration of CBOD<sub>5</sub> measured in winter being higher than in fall (figs 26 and 27; table 7). No system showed a significant difference in the mean concentrations of either phosphorous species between seasons.

Table 7. Fall and winter differences of the biological and water quality parameter measurements. A significant difference ( $p < 0.05$ ) between seasons for parameter and effluent type is designated by the letter X.

Effluent	Survival	Reprod.	Temp.	DO	pH	TSS	CBOD	TN	NH <sub>3</sub>	NO <sub>2</sub> /NO <sub>3</sub>	TP	PO <sub>4</sub>
WMARSS			X									
Std. 40 Aerobic			X			X	X					
I.E. Aerobic			X	X	X				X	X		
Septic+Wetland			X				X					
Septic			X									

No significant interaction was detected between the two factors (season and effluent type) for five of the parameters examined. Therefore, for these five parameters (*D. magna* survival and reproduction, TN, TP, and PO<sub>4</sub>), the one-factor (season) results were considered. However, no significant difference was detected between fall and winter for *D. magna* survival or reproduction, TN, TP, or PO<sub>4</sub> according to the one-factor results.



## CHAPTER FOUR

### Conclusions

#### *Whitney*

1. The hypothesis that effluent water quality is improved through the pond and wetland system at the WWTF is rejected. While water quality of the pond and wetland effluents appeared to generally improve through the system in regards to the parameters regulated by this facility's NPDES permit, various chemical parameters did not improve through the system in one or both seasons. The only parameters regulated by TCEQ for this facility which either did not improve, or did not meet the limits outlined by the WWTF NPDES permit, were the mean concentration of DO in the summer season and the mean concentration of TSS in the winter season (TCEQ 2009). However, TP, PO<sub>4</sub>, NH<sub>3</sub>, and NO<sub>2</sub>/NO<sub>3</sub> increased through the system during the summer season.
2. Because there were no observed toxic effects to *D. magna* from any effluent in either season, the hypothesis that that effluent quality improves through the pond and wetland system, considering the effects on *D. magna*, is rejected. Of the three locations sampled along the treatment train at the City of Whitney Wastewater Treatment Facility, none showed a significant difference in toxicity ( $p < 0.05$ ) for either endpoint assessed: survival and reproduction. While the wastewater received further treatment beyond the three ponds, the water had already been treated to a level tolerable by *D. magna* by the end of the third pond.

3. The second hypothesis examined, that there is no difference in effluent qualities between seasons, was rejected. While effluent quality appeared to generally improve in winter when compared to summer, for all effluents examined, there was a significant difference between seasons for multiple water quality parameters.

#### *Waco*

1. Regarding the water chemistry examined within the five Waco effluents, the first hypothesis that the effluent quality of four on-site wastewater treatment systems is comparable to a large municipal wastewater effluent, is rejected for all four on-site systems. Every on-site system effluent showed a significant difference ( $p < 0.05$ ) from the WMARSS effluent for at least one water chemistry parameter in both fall and winter studies. The aerobic on-site systems however, produced effluent of comparable quality to WMARSS effluent for majority of the parameters being examined in both seasons. Because these systems are designed to receive further treatment beyond the effluent tank, their effluents may be of comparable quality to the WMARSS effluent by the time the effluent reaches a receiving water body.
2. Concerning only the *D. magna* toxicity results, the first hypothesis examined for the Waco effluents, that the effluent quality of four on-site wastewater treatment systems is comparable to a large municipal wastewater effluent, is accepted for the two aerobic on-site system effluents, but rejected for the two anaerobic on-site system effluents. Though there were no statistical differences ( $p < 0.05$ ) found between any of the on-site system effluents and the WMARSS effluent,

reduction of survival was noted in both seasons examined for the Septic on-site wastewater treatment system; also, reduction of reproduction was apparent for both Septic+Wetland and Septic on-site wastewater treatment systems in both seasons examined.

3. The second hypothesis examined, that there is no statistical difference of effluent qualities between seasons is rejected for all five effluents. Every effluent examined, including the WMARSS municipal treatment system effluent, showed a significant difference ( $p < .05$ ) between seasons for at least one of the water chemistry parameters monitored.
4. Because of the of observable effects to *D. magna* exposed to the anaerobic on-site system effluents, and the significant differences of effluent quality between these systems and that of the effluent produced by the WMARSS municipal treatment facility, the receiving environment may be at risk from these effluents without further treatment.

#### *General Conclusions*

1. The WWTF (pond with surface wetland system) appeared more affected by season than the WMARSS or BWRP on-site systems. This can be explained by the WWTF being an open system, with minimal manipulation performed during the course of treatment. The WMARSS facility, while open, is continuously monitored and manipulated; the BWRP on-site systems are closed and buried, and thereby insulated.
2. Six of eight effluents tested from properly maintained systems did not show toxic effects within two seasons using *D. magna* as an indicator species. Only the two

on-site anaerobic wastewater treatment systems, Septic+Wetland and Septic, showed any adverse effects on either survival or reproduction

## APPENDICES

## Appendix A

### City of Whitney Wastewater Treatment Facility In-situ Dissolved Oxygen Measurements

Fall				Winter							
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
FNEF 7/11	2.46	WET1 7/11	3.05	PD3 7/11	5.14	FNEF 12/12	9.48	WET1 12/12	12.79	PD3 12/12	12.82
FNEF 7/15	2.08	WET1 7/15	1.13	PD3 7/15	4.11	FNEF 12/15	6.7	WET1 12/15	8.15	PD3 12/15	8.06
FNEF 7/18	2.11	WET1 7/18	0.85	PD3 7/18	2.0	FNEF 12/20	7.75	WET1 12/20	9.4	PD3 12/20	9.09
FNEF 8/12	1.85	WET1 8/12	0.89	PD3 8/12	5.27	FNEF 12/27	9.92	WET1 12/27	12.2	PD3 12/27	11.17
FNEF 8/16	2.28	WET1 8/16	0.86	PD3 8/16	5.22	FNEF 1/1	9.09	WET1 1/1	13.51	PD3 1/1	11.74
FNEF 8/21	2.34	WET1 8/21	0.86	PD3 8/21	5.45	FNEF 1/5	9.49	WET1 1/5	12.55	PD3 1/5	11.6
FNEF 8/25	2.62	WET1 8/25	0.49	PD3 8/25	2.45	FNEF 2/1	8.07	WET1 2/1	11.4	PD3 2/1	10.28
FNEF 8/29	1.78	WET1 8/29	0.3	PD3 8/29	3.17	FNEF 2/5	8.24	WET1 2/5	10.9	PD3 2/5	
FNEF 9/2	2.22	WET1 9/2	0.28	PD3 9/2	3.72	FNEF 2/8	9.43	WET1 2/8	13.05	PD3 2/8	10.11
Mean	2.19		0.97		4.06		8.69		11.55		10.61
Std. Dev.	0.26		0.79		1.23		1.00		1.69		1.44

## Appendix B

### City of Whitney Wastewater Treatment Facility In-situ pH Measurements

Summer						Winter					
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
FNEF 7/11	8.35	WET1 7/11	9.15	PD3 7/11	9.3	FNEF 12/12	7.8	WET1 12/12	8.1	PD3 12/12	8.1
FNEF 7/15	8.14	WET1 7/15	9	PD3 7/15	9.24	FNEF 12/15	7.82	WET1 12/15	8.12	PD3 12/15	8.1
FNEF 7/18	7.87	WET1 7/18	8.9	PD3 7/18	9.21	FNEF 12/20	7.79	WET1 12/20	7.95	PD3 12/20	7.9
FNEF 8/12	8.31	WET1 8/12	9.27	PD3 8/12	9.43	FNEF 12/27	7.9	WET1 12/27	8.1	PD3 12/27	8.13
FNEF 8/16	8.23	WET1 8/16	9.15	PD3 8/16	9.29	FNEF 1/1	8.3	WET1 1/1	8.4	PD3 1/1	8.3
FNEF 8/21	8.56	WET1 8/21	9.34	PD3 8/21	9.56	FNEF 1/5	8.2	WET1 1/5	8.3	PD3 1/5	8.3
FNEF 8/25	8.55	WET1 8/25	9.17	PD3 8/25	9.3	FNEF 2/1	8.51	WET1 2/1	8.8	PD3 2/1	8.42
FNEF 8/29	8.78	WET1 8/29	9.5	PD3 8/29	9.72	FNEF 2/5	8.17	WET1 2/5	8.5	PD3 2/5	8.55
FNEF 9/2	8.93	WET1 9/2	9.55	PD3 9/2	9.72	FNEF 2/8	8.35	WET1 2/8	8.77	PD3 2/8	8.63
Median	8.35		9.17		9.30		8.17		8.30		8.30
Std. Dev.	0.31		0.20		0.19		0.26		0.29		0.22

## Appendix C

### City of Whitney Wastewater Treatment Facility In-situ Temperature Measurements

Summer						Winter					
Effluent	°C	Effluent	°C	Effluent	°C	Effluent	°C	Effluent	°C	Effluent	°C
FNEF 7/11	27.9	WET1 7/11	29.1	PD3 7/11	30.1	FNEF 12/12	10.6	WET1 12/12	10.2	PD3 12/12	9.6
FNEF 7/15	28.1	WET1 7/15	28.5	PD3 7/15	30.5	FNEF 12/15	14.2	WET1 12/15	14.0	PD3 12/15	13.8
FNEF 7/18	28.1	WET1 7/18	28.7	PD3 7/18	30.4	FNEF 12/20	11.9	WET1 12/20	12.4	PD3 12/20	12.0
FNEF 8/12	28.3	WET1 8/12	28.3	PD3 8/12	29.5	FNEF 12/27	10.6	WET1 12/27	10.2	PD3 12/27	8.0
FNEF 8/16	27.9	WET1 8/16	28	PD3 8/16	29.3	FNEF 1/1	14.0	WET1 1/1	12.5	PD3 1/1	11.0
FNEF 8/21	28.1	WET1 8/21	28.3	PD3 8/21	30.2	FNEF 1/5	12.4	WET1 1/5	11.4	PD3 1/5	9.0
FNEF 8/25	28.2	WET1 8/25	28.7	PD3 8/25	29.9	FNEF 2/1	19.8	WET1 2/1	18.1	PD3 2/1	16.4
FNEF 8/29	27.8	WET1 8/29	28.2	PD3 8/29	29.6	FNEF 2/5	14.5	WET1 2/5	14.4	PD3 2/5	13.0
FNEF 9/2	27.3	WET1 9/2	27.2	PD3 9/2	28.5	FNEF 2/8	11.5	WET1 2/8	12.1	PD3 2/8	11.6
Mean	28.0		28.3		29.8		13.3		12.8		11.6
Std. Dev.	0.28		0.51		0.59		2.70		2.32		2.45



## Appendix D

### City of Whitney Wastewater Treatment Facility TSS measurements

Summer						Winter					
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
FNEF 7/11	24	WET1 7/11	36	PD3 7/11	48	FNEF 12/12	80	WET1 12/12	100	PD3 12/12	96
FNEF 7/15	16	WET1 7/15	22	PD3 7/15	34	FNEF 12/15	52	WET1 12/15	164	PD3 12/15	92
FNEF 7/18	18	WET1 7/18	26	PD3 7/18	28	FNEF 12/20	156	WET1 12/20	148	PD3 12/20	180
FNEF 8/12	16	WET1 8/12	22	PD3 8/12	36	FNEF 12/27	128	WET1 12/27	104	PD3 12/27	104
FNEF 8/16	16	WET1 8/16	28	PD3 8/16	54	FNEF 1/1	80	WET1 1/1	158	PD3 1/1	148
FNEF 8/21	10	WET1 8/21	16	PD3 8/21	26	FNEF 1/5	80	WET1 1/5	116	PD3 1/5	104
FNEF 8/25	14	WET1 8/25	40	PD3 8/25	38	FNEF 2/1	108	WET1 2/1	116	PD3 2/1	132
FNEF 8/29	22	WET1 8/29	30	PD3 8/29	50	FNEF 2/5	66	WET1 2/5	80	PD3 2/5	84
FNEF 9/2	14	WET1 9/2	26	PD3 9/2	52	FNEF 2/8	64	WET1 2/8	104	PD3 2/8	116
Mean	16.67		27.33		40.67		90.44		121.11		117.33
Std. Dev.	4.00		6.93		9.98		31.83		27.28		29.15

## Appendix E

### City of Whitney Wastewater Treatment Facility CBOD<sub>5</sub> measurements

Summer						Winter					
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
FNEF 7/11	15	WET1 7/11	14	PD3 7/11	20	FNEF 12/12	11	WET1 12/12	12	PD3 12/12	12
FNEF 7/15	11	WET1 7/15	15	PD3 7/15	19	FNEF 12/15	12	WET1 12/15	13	PD3 12/15	12
FNEF 7/18	15	WET1 7/18	24	PD3 7/18	18	FNEF 12/20	11	WET1 12/20	12	PD3 12/20	14
FNEF 8/12	12	WET1 8/12	17	PD3 8/12	15	FNEF 12/27	17	WET1 12/27	19	PD3 12/27	17
FNEF 8/16	11	WET1 8/16	16	PD3 8/16	25	FNEF 1/1	17	WET1 1/1	23	PD3 1/1	17
FNEF 8/21	9	WET1 8/21	16	PD3 8/21	23	FNEF 1/5	16	WET1 1/5	23	PD3 1/5	17
FNEF 8/25	8	WET1 8/25	22	PD3 8/25	22	FNEF 2/1	14	WET1 2/1	15	PD3 2/1	14
FNEF 8/29	10	WET1 8/29	17	PD3 8/29	23	FNEF 2/5	12	WET1 2/5	15	PD3 2/5	12
FNEF 9/2	9	WET1 9/2	15	PD3 9/2	20	FNEF 2/8	12	WET1 2/8	14	PD3 2/8	12
Mean	11.11		17.33		20.56		13.56		16.22		14.11
Std. Dev.	2.38		3.20		2.87		2.36		4.13		2.18

## Appendix F

### City of Whitney Wastewater Treatment Facility TN measurements

Summer				Winter			
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
FNEF 7/11	3.975	WET1 7/11	5.22	PD3 7/11	5.36	FNEF 12/12	7.7
FNEF 7/15	4.18	WET1 7/15	4.32	PD3 7/15	4.88	FNEF 12/15	30.2
FNEF 7/18	4.66	WET1 7/18	5.3	PD3 7/18	5.18	FNEF 12/20	6.88
FNEF 8/12	4.29	WET1 8/12	4.62	PD3 8/12	4.72	FNEF 12/27	8.62
FNEF 8/16	4.24	WET1 8/16	4.74	PD3 8/16	5.34	FNEF 1/1	7.6
FNEF 8/21	3.86	WET1 8/21	4.52	PD3 8/21	4.76	FNEF 1/5	7.61
FNEF 8/25	4.19	WET1 8/25	3.88	PD3 8/25	5.64	FNEF 2/1	6.44
FNEF 8/29	5.44	WET1 8/29	6.42	PD3 8/29	6.72	FNEF 2/5	6.92
FNEF 9/2	5.28	WET1 9/2	5.93	PD3 9/2	6.63	FNEF 2/8	6.86
Mean	4.46		4.99		5.47		9.87
Std. Dev.	0.53		0.76		0.70		7.21

## Appendix G

### City of Whitney Wastewater Treatment Facility NH<sub>3</sub> measurements

Summer				Winter			
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
FNEF 7/11	0.45	WET1 7/11	0.007	PD3 7/11	0.03	FNEF 12/12	0.09
FNEF 7/15	0.84	WET1 7/15	0.006	PD3 7/15	0.003	FNEF 12/15	0.16
FNEF 7/18	1.01	WET1 7/18	0.005	PD3 7/18	0.004	FNEF 12/20	0.28
FNEF 8/12	1.38	WET1 8/12	0.01	PD3 8/12	0.005	FNEF 12/27	0.17
FNEF 8/16	1.07	WET1 8/16	0.008	PD3 8/16	0.005	FNEF 1/1	0.11
FNEF 8/21	1.33	WET1 8/21	0.15	PD3 8/21	0.09	FNEF 1/5	0.22
FNEF 8/25	1.49	WET1 8/25	0.52	PD3 8/25	0.009	FNEF 2/1	0.21
FNEF 8/29	1.9	WET1 8/29	0.69	PD3 8/29	0.02	FNEF 2/5	0.35
FNEF 9/2	1.35	WET1 9/2	0.66	PD3 9/2	0.02	FNEF 2/8	0.23
Mean	1.20		0.23		0.02		0.20
Std. Dev.	0.39		0.29		0.03		0.08

## Appendix H

### City of Whitney Wastewater Treatment Facility NO<sub>2</sub>/NO<sub>3</sub> measurements

Summer				Winter			
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
FNEF 7/11	0.013	WET1 7/11	0.001	PD3 7/11	0.008	FNEF 12/12	1.97
FNEF 7/15	0.005	WET1 7/15	0.001	PD3 7/15	0.001	FNEF 12/15	1.84
FNEF 7/18	0.004	WET1 7/18	0.003	PD3 7/18	0.002	FNEF 12/20	1.6
FNEF 8/12	0.008	WET1 8/12	0.002	PD3 8/12	0.002	FNEF 12/27	2.02
FNEF 8/16	0.013	WET1 8/16	0.002	PD3 8/16	0.002	FNEF 1/1	1.57
FNEF 8/21	0.014	WET1 8/21	0.002	PD3 8/21	0.004	FNEF 1/5	1.352
FNEF 8/25	0.022	WET1 8/25	0.009	PD3 8/25	0.009	FNEF 2/1	1.704
FNEF 8/29	0.014	WET1 8/29	0.009	PD3 8/29	0.011	FNEF 2/5	2.7
FNEF 9/2	0.011	WET1 9/2	0.009	PD3 9/2	0.011	FNEF 2/8	2.8
Mean	0.01		0.004		0.01		1.95
Std. Dev.	0.005		0.003		0.004		0.47

## Appendix I

### City of Whitney Wastewater Treatment Facility TP measurements

Summer				Winter					
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
FNEF 7/11	5.61	WET1 7/11	5.6	PD3 7/11	5.28	FNEF 12/12	2.28	WET1 12/12	2.18
FNEF 7/15	5.79	WET1 7/15	5.88	PD3 7/15	4.96	FNEF 12/15	2.07	WET1 12/15	2.12
FNEF 7/18	6.2	WET1 7/18	5.77	PD3 7/18	4.85	FNEF 12/20	2.01	WET1 12/20	1.9
FNEF 8/12	7.94	WET1 8/12	7.02	PD3 8/12	4.86	FNEF 12/27	2.0	WET1 12/27	1.76
FNEF 8/16	6.96	WET1 8/16	6.37	PD3 8/16	5.19	FNEF 1/1	1.75	WET1 1/1	1.71
FNEF 8/21	7.71	WET1 8/21	6.64	PD3 8/21	4.78	FNEF 1/5	1.65	WET1 1/5	1.6
FNEF 8/25	7.0	WET1 8/25	6.87	PD3 8/25	4.86	FNEF 2/1	1.27	WET1 2/1	1.17
FNEF 8/29	7.33	WET1 8/29	6.47	PD3 8/29	4.94	FNEF 2/5	1.13	WET1 2/5	1.19
FNEF 9/2	6.99	WET1 9/2	6.94	PD3 9/2	4.9	FNEF 2/8	1.19	WET1 2/8	1.17
Mean	6.84		6.40		4.96		1.706		1.64
Std. Dev.	0.77		0.50		0.16		0.399		0.37

## Appendix J

### City of Whitney Wastewater Treatment Facility PO<sub>4</sub> measurements

Summer				Winter							
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
FNEF 7/11	3.59	WET1 7/11	4.09	PD3 7/11	3.37	FNEF 12/12	1.26	WET1 12/12	1.21	PD3 12/12	0.92
FNEF 7/15	4.25	WET1 7/15	3.94	PD3 7/15	3.37	FNEF 12/15	0.85	WET1 12/15	1.11	PD3 12/15	0.62
FNEF 7/18	4.38	WET1 7/18	3.84	PD3 7/18	2.93	FNEF 12/20	0.65	WET1 12/20	0.95	PD3 12/20	0.97
FNEF 8/12	5.72	WET1 8/12	5.97	PD3 8/12	4.33	FNEF 12/27	0.95	WET1 12/27	0.53	PD3 12/27	0.68
FNEF 8/16	5.85	WET1 8/16	5.80	PD3 8/16	4.10	FNEF 1/1	0.76	WET1 1/1	0.69	PD3 1/1	0.67
FNEF 8/21	7.32	WET1 8/21	6.24	PD3 8/21	4.28	FNEF 1/5	0.69	WET1 1/5	0.62	PD3 1/5	0.66
FNEF 8/25	5.9	WET1 8/25	3.85	PD3 8/25	3.67	FNEF 2/1	0.57	WET1 2/1	0.60	PD3 2/1	0.76
FNEF 8/29	6.2	WET1 8/29	5.37	PD3 8/29	3.87	FNEF 2/5	0.62	WET1 2/5	0.56	PD3 2/5	0.44
FNEF 9/2	3.83	WET1 9/2	4.57	PD3 9/2	3.4	FNEF 2/8	0.64	WET1 2/8	0.62	PD3 2/8	0.43
Mean	5.23		4.85		3.70		0.78		0.76		0.68
Std. Dev.	1.19		0.93		0.45		0.21		0.24		0.17

## Appendix K

### City of Whitney Wastewater Treatment Facility *D. magna* Survival

		Summer				Winter			
		<i>Test 1</i>	<i>Test 2</i>	<i>Test 3</i>	<i>Average</i>	<i>Test 1</i>	<i>Test 2</i>	<i>Test 3</i>	<i>Average</i>
Control		1	0.8	1	0.93	0.9	0.9	0.8	0.87
FNEF	100%	0.8	0.4	0.8	0.67	1	1	1	1
	50%	1	0.4	0.2	0.53	1	0.8	1	0.93
	25%	1	0.8	0.8	0.87	1	1	1	1
WET1	100%	1	0.2	0.8	0.67	1	1	1	1
	50%	1	0.2	0.2	0.47	1	1	1	1
	25%	1	0.6	0.4	0.67	1	1	1	1
PD3	100%	0.8	0.8	1	0.87	1	1	1	1
	50%	1	0.6	0.8	0.80	1	1	1	1
	25%	1	0.4	0.6	0.67	1	1	1	1



## Appendix L

### City of Whitney Wastewater Treatment Facility *D. magna* Reproduction

		Summer				Winter			
		<i>Test 1</i>	<i>Test 2</i>	<i>Test 3</i>	<i>Average</i>	<i>Test 1</i>	<i>Test 2</i>	<i>Test 3</i>	<i>Average</i>
Control		19.8	12	12.6	14.8	22.9	14.1	18.4	18.5
FNEF	100%	24.4	5.2	8.2	12.6	35.2	25.6	66.6	42.5
	50%	35	6.4	5.6	15.7	50.4	47.4	51.6	49.8
	25%	29.4	8.4	7.8	15.2	31.2	43.4	53.8	42.8
WET1	100%	18.4	7.2	13.4	13.0	25	46.2	50	40.4
	50%	36	1	6.8	14.6	38	49.8	41.2	43.0
	25%	29.8	4	3	12.3	23.4	40.6	43.4	35.8
PD3	100%	8.2	16	16.4	13.5	45.2	16.8	50	37.3
	50%	17.2	10.6	11.8	13.2	19.4	54	60.6	44.7
	25%	9.4	2.8	11.4	7.9	34.6	43.5	56.2	44.8

## Appendix M

### WMARSS and BWRP In-situ Dissolved Oxygen Measurements

Fall									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 9/16	7.93	Std. 40 9/16	3.44	I.E. 9/16	2.87	Sep+Wet 9/16	0.31	Septic 9/16	0.38
WMARSS 9/19	7.91	Std. 40 9/19	3.32	I.E. 9/19	2.43	Sep+Wet 9/19	0.26	Septic 9/19	0.38
WMARSS 9/23	7.86	Std. 40 9/23	3.7	I.E. 9/23	3.16	sep+wet 9/23	0.32	Septic 9/23	0.3
WMARSS 10/10	8.07	std. 40 10/10	3.4	I.E. 10/10	4.31	Sep+wet 10/10	0.37	Septic 10/10	0.26
WMARSS 10/14	7.04	std. 40 10/14	2.93	I.E. 10/14	4.12	Sep+wet 10/14	0.59	Septic 10/14	0.33
WMARSS 10/17	7.85	std. 40 10/17		I.E. 10/17		Sep+wet 10/17		Septic 10/17	
WMARSS 11/2	8.8	std. 40 11/2	2.42	I.E. 11/2	0.85	Sep+Wet 11/2	0.45	Septic 11/2	0.37
WMARSS 11/4	8.37	std. 40 11/4	3.06	I.E. 11/4	0.61	Sep+Wet 11/4	0.49	Septic 11/4	0.33
WMARSS 11/7	8.16	std. 40 11/7	3.59	I.E. 11/7	4.73	Sep+Wet 11/7	0.49	Septic 11/7	0.56
Mean	8.00		3.23		2.89		0.41		0.36
Std. Dev.	0.45		0.39		1.44		0.11		0.08

Appendix M continued

Winter									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 12/12	10.63	Std. 40 12/12	2.79	I.E. 12/12	4.94	Sep+Wet 12/12	0.32	Septic 12/12	0.19
WMARSS 12/16	8.15	Std. 40 12/16	3.32	I.E. 12/16	4.82	Sep+Wet 12/16	0.33	Septic 12/16	0.33
WMARSS 12/19	9.67	Std. 40 12/19	3.33	I.E. 12/19	5.07	Sep+Wet 12/19	0.36	Septic 12/19	0.31
WMARSS 1/20	8.58	Std. 40 1/20	5.1	I.E. 1/20	6.34	Sep+Wet 1/20	0.48	Septic 1/20	0.26
WMARSS 1/23	8.82	Std. 40 1/23	5.45	I.E. 1/23	6.32	Sep+Wet 1/23		Septic 1/23	
WMARSS 1/27	8.6	Std. 40 1/27	4.69	I.E. 1/27	5.34	Sep+Wet 1/27		Septic 1/27	
WMARSS 1/30	8.47	Std. 40 1/30	4.56	I.E. 1/30	5.37	Sep+Wet 1/30		Septic 1/30	0.02
WMARSS 2/3	8.55	Std. 40 2/3	2.04	I.E. 2/3	4.68	Sep+Wet 2/3	0.32	Septic 2/3	0.28
WMARSS 2/6	8.64	Std. 40 2/6	3.93	I.E. 2/6	5.44	Sep+Wet 2/6	0.43	Septic 2/6	0.41
Mean	8.90		3.91		5.37		0.37		0.26
Std. Dev.	0.72		1.07		0.57		0.06		0.12

## Appendix N

### WMARSS and BWRP In-situ pH Measurements

Fall									
Effluent		Effluent		Effluent		Effluent		Effluent	
WMARSS 9/16	7.44	Std. 40 9/16	7.58	I.E. 9/16	7.82	Sep+Wet 9/16	7.1	Septic 9/16	7.16
WMARSS 9/19	7.17	Std. 40 9/19	7.43	I.E. 9/19	7.74	Sep+Wet 9/19	7.21	Septic 9/19	7.01
WMARSS 9/23	7.86	Std. 40 9/23	7.27	I.E. 9/23	7.34	sep+wet 9/23	7.09	Septic 9/23	7.09
WMARSS 10/10	7.73	std. 40 10/10	7.44	I.E. 10/10	7.56	Sep+wet 10/10	7.71	Septic 10/10	7.08
WMARSS 10/14	7.38	std. 40 10/14	7.38	I.E. 10/14	7.49	Sep+wet 10/14	7.01	Septic 10/14	6.96
WMARSS 10/17	7.41	std. 40 10/17	7.25	I.E. 10/17	7.48	Sep+wet 10/17	7.03	Septic 10/17	6.98
WMARSS 11/2	7.61	std. 40 11/2	7.53	I.E. 11/2	7.55	Sep+Wet 11/2	7.29	Septic 11/2	7.23
WMARSS 11/4	7.61	std. 40 11/4	7.41	I.E. 11/4	7.64	Sep+Wet 11/4	7.27	Septic 11/4	7.11
WMARSS 11/7	7.72	std. 40 11/7	7.66	I.E. 11/7	6.99	Sep+Wet 11/7	7.1	Septic 11/7	7.5
Median	7.61		7.43		7.55		7.10		7.09
Std. Dev.	0.20		0.13		0.23		0.20		0.16

Appendix N continued

Winter									
Effluent		Effluent		Effluent		Effluent		Effluent	
WMARSS 12/12	7.23	Std. 40 12/12	7.46	I.E. 12/12	7.29	Sep+Wet 12/12	7.16	Septic 12/12	7.62
WMARSS 12/16	7.79	Std. 40 12/16	7.44	I.E. 12/16	7.25	Sep+Wet 12/16	7.26	Septic 12/16	7.38
WMARSS 12/19	7.67	Std. 40 12/19	7.47	I.E. 12/19	7.21	Sep+Wet 12/19	7.52	Septic 12/19	7.33
WMARSS 1/20	7.13	Std. 40 1/20	7.60	I.E. 1/20	7.15	Sep+Wet 1/20	7.11	Septic 1/20	7.23
WMARSS 1/23	7.10	Std. 40 1/23	7.02	I.E. 1/23	7.02	Sep+Wet 1/23	7.29	Septic 1/23	7.23
WMARSS 1/27	7.17	Std. 40 1/27	7.38	I.E. 1/27	7.27	Sep+Wet 1/27	7.30	Septic 1/27	7.23
WMARSS 1/30	7.05	Std. 40 1/30	6.79	I.E. 1/30	7.10	Sep+Wet 1/30	7.27	Septic 1/30	7.22
WMARSS 2/3	7.1	Std. 40 2/3	7.57	I.E. 2/3	7.10	Sep+Wet 2/3	7.07	Septic 2/3	7.25
WMARSS 2/6	7.03	Std. 40 2/6	7.54	I.E. 2/6	7.12	Sep+Wet 2/6	7.10	Septic 2/6	7.27
Median	7.13		7.46		7.15		7.26		7.25
Std. Dev.	0.26		0.26		0.09		0.13		0.12

## Appendix O

### WMARSS and BWRP In-situ Temperature Measurements

Fall									
Effluent	°C	Effluent	°C	Effluent	°C	Effluent	°C	Effluent	°C
WMARSS 9/16	28.4	Std. 40 9/16	30.8	I.E. 9/16	31	Sep+Wet 9/16	30.3	Septic 9/16	30.4
WMARSS 9/19	27.4	Std. 40 9/19	31	I.E. 9/19	31	Sep+Wet 9/19	30.1	Septic 9/19	30.3
WMARSS 9/23	27.4	Std. 40 9/23	29.9	I.E. 9/23	30.1	Sep+Wet 9/23	28.4	Septic 9/23	29.1
WMARSS 10/10	25.8	Std. 40 10/10	26	I.E. 10/10	27.7	Sep+Wet 10/10	23.7	Septic 10/10	26.5
WMARSS 10/14	27.1	Std. 40 10/14	26.4	I.E. 10/14	26.8	Sep+Wet 10/14	24	Septic 10/14	25.9
WMARSS 10/17	27.9	Std. 40 10/17	26.1	I.E. 10/17	26.7	Sep+Wet 10/17	24.4	Septic 10/17	25.9
WMARSS 11/2	25.2	Std. 40 11/2	23.8	I.E. 11/2	23.9	Sep+Wet 11/2	20.3	Septic 11/2	23.4
WMARSS 11/4	23.8	Std. 40 11/4	23.1	I.E. 11/4	22.8	Sep+Wet 11/4	14.8	Septic 11/4	21.8
WMARSS 11/7	22.1	Std. 40 11/7	23.6	I.E. 11/7	23	Sep+Wet 11/7	20.6	Septic 11/7	23
Mean	26.1		26.7		27.0		24.1		26.3
Std. Dev.	1.97		2.93		3.08		4.79		2.99

Appendix O continued

Winter									
Effluent	°C	Effluent	°C	Effluent	°C	Effluent	°C	Effluent	°C
WMARSS 12/12	21.5	Std. 40 12/12	17.1	I.E. 12/12	17.7	Sep+Wet 12/12	11.7	Septic 12/12	16.2
WMARSS 12/16	20.7	Std. 40 12/16	18	I.E. 12/16	18.9	Sep+Wet 12/16	14.3	Septic 12/16	17.4
WMARSS 12/19	21.4	Std. 40 12/19	18	I.E. 12/19	18.4	Sep+Wet 12/19	13.4	Septic 12/19	17.2
WMARSS 1/20	20.9	Std. 40 1/20	16.7	I.E. 1/20	16.6	Sep+Wet 1/20	13.2	Septic 1/20	16.5
WMARSS 1/23	20.8	Std. 40 1/23	16.8	I.E. 1/23	17.3	Sep+Wet 1/23		Septic 1/23	
WMARSS 1/27	20.6	Std. 40 1/27	16.9	I.E. 1/27	17.1	Sep+Wet 1/27		Septic 1/27	
WMARSS 1/30	20.8	Std. 40 1/30	16.1	I.E. 1/30	16.1	Sep+Wet 1/30	14.7	Septic 1/30	11
WMARSS 2/3	22.3	Std. 40 2/3	17.1	I.E. 2/3	18.5	Sep+Wet 2/3	19.3	Septic 2/3	18.3
WMARSS 2/6	20.4	Std. 40 2/6	17.2	I.E. 2/6	18.2	Sep+Wet 2/6	12.7	Septic 2/6	16.2
Mean	21.04		17.10		17.64		14.19		16.11
Std. Dev.	0.56		0.57		0.89		2.28		2.20

## Appendix P

### WMARSS and BWRP TSS Measurements

Fall									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 9/16	2	Std. 40 9/16	2	I.E. 9/16	2	Sep+Wet 9/16	5.5	Septic 9/16	24
WMARSS 9/19	2	Std. 40 9/19	4.4	I.E. 9/19	2	Sep+Wet 9/19	7	Septic 9/19	25
WMARSS 9/23	2	Std. 40 9/23	2	I.E. 9/23	2	Sep+Wet 9/23	6	Septic 9/23	32
WMARSS 10/10	2	Std. 40 10/10	2	I.E. 10/10	11	Sep+Wet 10/10	2	Septic 10/10	17
WMARSS 10/14	2	Std. 40 10/14	4.7	I.E. 10/14	2	Sep+Wet 10/14	2	Septic 10/14	15
WMARSS 10/17	2	Std. 40 10/17	4.6	I.E. 10/17	2	Sep+Wet 10/17	2	Septic 10/17	14
WMARSS 11/2	2	Std. 40 11/2	10.7	I.E. 11/2	2	Sep+Wet 11/2	2	Septic 11/2	16
WMARSS 11/4	2	Std. 40 11/4	8.2	I.E. 11/4	2	Sep+Wet 11/4	2	Septic 11/4	30
WMARSS 11/7	2	Std. 40 11/7	8.8	I.E. 11/7	9.7	Sep+Wet 11/7	2	Septic 11/7	13
Mean	2.00		5.27		3.86		3.39		20.67
Std. Dev.	0.00		3.06		3.48		2.00		6.80



Appendix P continued

Winter									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 12/12	2	Std. 40 12/12	19	I.E. 12/12	2	Sep+Wet 12/12	2	Septic 12/12	21.3
WMARSS 12/16	2	Std. 40 12/16	25	I.E. 12/16	2	Sep+Wet 12/16	2	Septic 12/16	17
WMARSS 12/19	2	Std. 40 12/19	32	I.E. 12/19		Sep+Wet 12/19	2	Septic 12/19	18
WMARSS 1/20	2	Std. 40 1/20	9.3	I.E. 1/20	2	Sep+Wet 1/20	8.5	Septic 1/20	38
WMARSS 1/23	2	Std. 40 1/23	12	I.E. 1/23	2	Sep+Wet 1/23	2	Septic 1/23	41
WMARSS 1/27	4.6	Std. 40 1/27	6.8	I.E. 1/27	6.6	Sep+Wet 1/27	7.5	Septic 1/27	24
WMARSS 1/30	2	Std. 40 1/30	8.4	I.E. 1/30	6.6	Sep+Wet 1/30	7.5	Septic 1/30	38
WMARSS 2/3	2	Std. 40 2/3	6	I.E. 2/3	14	Sep+Wet 2/3	2	Septic 2/3	35
WMARSS 2/6	2	Std. 40 2/6	10.7	I.E. 2/6	20.3	Sep+Wet 2/6	2	Septic 2/6	21
Mean	2.29		14.36		6.94		3.94		28.14
Std. Dev.	0.82		8.52		6.39		2.76		9.12

## Appendix Q

### WMARSS and BWRP CBOD<sub>5</sub> Measurements

Fall									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 9/16	3.09	Std. 40 9/16	2.76	I.E. 9/16	1	Sep+Wet 9/16	8.68	Septic 9/16	94.4
WMARSS 9/19	2.51	Std. 40 9/19	2.84	I.E. 9/19	3.33	Sep+Wet 9/19	8.69	Septic 9/19	63
WMARSS 9/23	2.62	Std. 40 9/23	2.6	I.E. 9/23	4.02	Sep+Wet 9/23	5.22	Septic 9/23	44.6
WMARSS 10/10	8.69	Std. 40 10/10	2.18	I.E. 10/10	4.42	Sep+Wet 10/10	7.41	Septic 10/10	28.1
WMARSS 10/14	4.3	Std. 40 10/14	4.32	I.E. 10/14	2.05	Sep+Wet 10/14	5.79	Septic 10/14	72.1
WMARSS 10/17	13.5	Std. 40 10/17	3.53	I.E. 10/17	1	Sep+Wet 10/17	6.79	Septic 10/17	83.4
WMARSS 11/2	7.88	Std. 40 11/2	7.17	I.E. 11/2	1	Sep+Wet 11/2	1	Septic 11/2	38.2
WMARSS 11/4	3.17	Std. 40 11/4	6.3	I.E. 11/4	2.17	Sep+Wet 11/4	17.1	Septic 11/4	102
WMARSS 11/7	3.87	Std. 40 11/7	6.78	I.E. 11/7	2.84	Sep+Wet 11/7	8.76	Septic 11/7	98.1
Mean	5.51		4.28		2.43		7.72		69.32
Std. Dev.	3.53		1.85		1.24		4.04		25.93

Appendix Q continued

Winter									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 12/12	4.02	Std. 40 12/12	16.9	I.E. 12/12	1	Sep+Wet 12/12	18.7	Septic 12/12	77
WMARSS 12/16	2.96	Std. 40 12/16	30.8	I.E. 12/16	1	Sep+Wet 12/16	16.4	Septic 12/16	112
WMARSS 12/19	1	Std. 40 12/19	14.1	I.E. 12/19	1	Sep+Wet 12/19	15.5	Septic 12/19	89.4
WMARSS 1/20	3	Std. 40 1/20	8.24	I.E. 1/20	1	Sep+Wet 1/20	14.7	Septic 1/20	50.5
WMARSS 1/23	3.2	Std. 40 1/23	7.68	I.E. 1/23	1	Sep+Wet 1/23	14.2	Septic 1/23	62.7
WMARSS 1/27	3.3	Std. 40 1/27	7.26	I.E. 1/27	2.76	Sep+Wet 1/27	15.4	Septic 1/27	54.7
WMARSS 1/30	3.6	Std. 40 1/30	7.5	I.E. 1/30	2.8	Sep+Wet 1/30	15.4	Septic 1/30	54.7
WMARSS 2/3	2.4	Std. 40 2/3	12.8	I.E. 2/3	4.42	Sep+Wet 2/3	8.84	Septic 2/3	80
WMARSS 2/6	4.2	Std. 40 2/6	9.04	I.E. 2/6	4.14	Sep+Wet 2/6	13.3	Septic 2/6	74.9
Mean	3.08		12.70		2.12		14.72		72.88
Std. Dev.	0.90		7.17		1.35		2.52		18.68

## Appendix R

### WMARSS and BWRP TN Measurements

Fall									
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
WMARSS 9/16	18.04	Std. 40 9/16	30.6	I.E. 9/16	35.6	Sep+Wet 9/16	35.3	Septic 9/16	33
WMARSS 9/19	19.16	Std. 40 9/19	30	I.E. 9/19	41.2	Sep+Wet 9/19	32.6	Septic 9/19	38.9
WMARSS 9/23	13.12	Std. 40 9/23	31.4	I.E. 9/23	31.8	sep+wet 9/23	34.8	Septic 9/23	36.8
WMARSS 10/10	8.78	std. 40 10/10	14.52	I.E. 10/10	27	Sep+wet 10/10	23	Septic 10/10	30.4
WMARSS 10/14	15.5	std. 40 10/14	15.66	I.E. 10/14	24.4	Sep+wet 10/14	25.6	Septic 10/14	30.8
WMARSS 10/17	18.17	std. 40 10/17	20.4	I.E. 10/17	31.8	Sep+wet 10/17	27	Septic 10/17	33.6
WMARSS 11/2	14.88	std. 40 11/2	46.2	I.E. 11/2	34	Sep+Wet 11/2	37.4	Septic 11/2	38.4
WMARSS 11/4	16.74	std. 40 11/4	41.6	I.E. 11/4	34.8	Sep+Wet 11/4	38.8	Septic 11/4	36.6
WMARSS 11/7	17.82	std. 40 11/7	41.5	I.E. 11/7	37.4	Sep+Wet 11/7	37.6	Septic 11/7	35.2
Mean	15.80		30.21		33.11		32.46		34.86
Std. Dev.	3.06		10.90		4.83		5.49		2.93

Appendix R continued

Winter									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 12/12	13.74	Std. 40 12/12	29.6	I.E. 12/12	24	Sep+Wet 12/12	8.42	Septic 12/12	33.6
WMARSS 12/16	19.04	Std. 40 12/16	7.96	I.E. 12/16	8	Sep+Wet 12/16	7.9	Septic 12/16	36.4
WMARSS 12/19	14.3	Std. 40 12/19	32.4	I.E. 12/19	16.34	Sep+Wet 12/19	28.6	Septic 12/19	29.4
WMARSS 1/20	33.1	Std. 40 1/20	22.8	I.E. 1/20	26.8	Sep+Wet 1/20	26.4	Septic 1/20	14.7
WMARSS 1/23	15.3	Std. 40 1/23	21.4	I.E. 1/23	23.4	Sep+Wet 1/23	27	Septic 1/23	29.8
WMARSS 1/27	9.18	Std. 40 1/27	20.8	I.E. 1/27	20	Sep+Wet 1/27	21.3	Septic 1/27	25.8
WMARSS 1/30	13.58	Std. 40 1/30	18.26	I.E. 1/30	20.6	Sep+Wet 1/30	20.8	Septic 1/30	25.6
WMARSS 2/2	6.38	Std. 40 2/3	25.8	I.E. 2/3	30.6	Sep+Wet 2/3	23	Septic 2/3	33
WMARSS 2/6	13.48	Std. 40 2/6	30.2	I.E. 2/6	30.2	Sep+Wet 2/6	23.4	Septic 2/6	31.3
Mean	15.34		23.25		22.22		20.76		28.84
Std. Dev.	7.13		7.05		6.70		7.17		6.00

## Appendix S

### WMARSS and BWRP NH<sub>3</sub> Measurements

Fall									
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
WMARSS 9/16	0.07	Std. 40 9/16	6.65	I.E. 9/16	17.4	Sep+Wet 9/16	18.15	Septic 9/16	21.6
WMARSS 9/19	0.12	Std. 40 9/19	8.28	I.E. 9/19	17.6	Sep+Wet 9/19	13.5	Septic 9/19	21.4
WMARSS 9/23	0.08	Std. 40 9/23	1.19	I.E. 9/23	15.9	Sep+Wet 9/23	18.4	Septic 9/23	15.7
WMARSS 10/10	0.28	Std. 40 10/10	2.34	I.E. 10/10	12.1	Sep+Wet 10/10	8.08	Septic 10/10	15.8
WMARSS 10/14	3.90	Std. 40 10/14	0.17	I.E. 10/14	3.7	Sep+Wet 10/14	0.38	Septic 10/14	0.44
WMARSS 10/17	1.63	Std. 40 10/17		I.E. 10/17	0.42	Sep+Wet 10/17	4.29	Septic 10/17	0.40
WMARSS 11/2	1.65	Std. 40 11/2	13.3	I.E. 11/2	10.4	Sep+Wet 11/2	11	Septic 11/2	2.41
WMARSS 11/4	2.75	Std. 40 11/4	10.1	I.E. 11/4	11.25	Sep+Wet 11/4	12.05	Septic 11/4	2.31
WMARSS 11/7	1.72	Std. 40 11/7	12.1	I.E. 11/7	5.66	Sep+Wet 11/7	10.1	Septic 11/7	7.53
Mean	1.36		6.77		10.49		10.66		9.73
Std. Dev.	1.28		4.73		5.79		5.57		8.41

Appendix S continued

Winter									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 12/12	1.17	Std. 40 12/12	18.1	I.E. 12/12	0.01	Sep+Wet 12/12	22.2	Septic 12/12	30.4
WMARSS 12/16	0.11	Std. 40 12/16	24.8	I.E. 12/16	7.82	Sep+Wet 12/16	22.6	Septic 12/16	32.8
WMARSS 12/19	0.2	Std. 40 12/19	20	I.E. 12/19	1.71	Sep+Wet 12/19	24.8	Septic 12/19	24.8
WMARSS 1/20	1.09	Std. 40 1/20	14	I.E. 1/20	0.3	Sep+Wet 1/20	23.2	Septic 1/20	27.6
WMARSS 1/23	1.73	Std. 40 1/23	16.3	I.E. 1/23	0.29	Sep+Wet 1/23	20.6	Septic 1/23	22
WMARSS 1/27	0.45	Std. 40 1/27	14.3	I.E. 1/27	0.26	Sep+Wet 1/27	17.3	Septic 1/27	21.4
WMARSS 1/30	0.13	Std. 40 1/30	14.2	I.E. 1/30	0.18	Sep+Wet 1/30	24.4	Septic 1/30	16.8
WMARSS 2/2	0.11	Std. 40 2/3	18.8	I.E. 2/3	0.21	Sep+Wet 2/1	17.9	Septic 2/3	29.4
WMARSS 2/6	0.18	Std. 40 2/6	16.7	I.E. 2/6	0.49	Sep+Wet 2/3	24.4	Septic 2/6	37.2
						Sep+Wet 2/6	31.2		
Mean	0.57		17.47		1.25		22.86		26.93
Std. Dev.	0.57		3.28		2.37		3.73		5.97

## Appendix T

### WMARSS and BWRP NO<sub>2</sub>/NO<sub>3</sub> Measurements

Fall									
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
WMARSS 9/16	7.13	Std. 40 9/16	9.61	I.E. 9/16	6.14	Sep+Wet 9/16	0.01	Septic 9/16	0.01
WMARSS 9/19	8.02	Std. 40 9/19	8.42	I.E. 9/19	2.27	Sep+Wet 9/19	0.33	Septic 9/19	0.02
WMARSS 9/23	5.59	Std. 40 9/23	9.28	I.E. 9/23	3.6	Sep+Wet 9/23	0.01	Septic 9/23	0.01
WMARSS 10/10	2.78	Std. 40 10/10	3.81	I.E. 10/10	3.15	Sep+Wet 10/10	0.05	Septic 10/10	0.02
WMARSS 10/14	0.02	Std. 40 10/14	2.05	I.E. 10/14	2.1	Sep+Wet 10/14	0.02	Septic 10/14	0.02
WMARSS 10/17	0.03	Std. 40 10/17	0.08	I.E. 10/17	0.06	Sep+Wet 10/17	0.22	Septic 10/17	0.08
WMARSS 11/2	8.28	Std. 40 11/2	0.73	I.E. 11/2	0.57	Sep+Wet 11/2	0.01	Septic 11/2	0.01
WMARSS 11/4	7.18	Std. 40 11/4	2.14	I.E. 11/4	0.85	Sep+Wet 11/4	0.01	Septic 11/4	0.02
WMARSS 11/7	8.36	Std. 40 11/7	0.36	I.E. 11/7	9.1	Sep+Wet 11/7	.0004	Septic 11/7	0.01
Mean	5.27		4.05		3.09		0.07		0.02
Std. Dev.	3.24		3.73		2.75		0.11		0.02



Appendix T continued

Winter									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 12/12	9.5	Std. 40 12/12	0.89	I.E. 12/12	12.12	Sep+Wet 12/12	0.09	Septic 12/12	0.18
WMARSS 12/16	2.98	Std. 40 12/16	0.83	I.E. 12/16	8.54	Sep+Wet 12/16	0.12	Septic 12/16	0.15
WMARSS 12/19	10.22	Std. 40 12/19	0.9	I.E. 12/19	9.53	Sep+Wet 12/19	0.07	Septic 12/19	0.14
WMARSS 1/20	10.52	Std. 40 1/20	3.76	I.E. 1/20	19.66	Sep+Wet 1/20	0.99	Septic 1/20	0.10
WMARSS 1/23	8.82	Std. 40 1/23	1.95	I.E. 1/23	16.08	Sep+Wet 1/23	0.02	Septic 1/23	0.18
WMARSS 1/27	5.33	Std. 40 1/27	1.06	I.E. 1/27	14.82	Sep+Wet 1/27	0.08	Septic 1/27	0.14
WMARSS 1/30	12	Std. 40 1/30	0.35	I.E. 1/30	16.46	Sep+Wet 1/30	0.13	Septic 1/30	0.06
WMARSS 2/2	11.4	Std. 40 2/3	0.92	I.E. 2/3	23.8	Sep+Wet 2/1	0.25	Septic 2/3	0.08
WMARSS 2/6	2.8	Std. 40 2/6	0.27	I.E. 2/6	31.3	Sep+Wet 2/3	0.09	Septic 2/6	0.11
						Sep+Wet 2/6	0.05		
Mean	8.17		1.21		16.92		0.19		0.13
Std. Dev.	3.35		1.01		6.79		0.27		0.04

## Appendix U

### WMARSS and BWRP TP Measurements

Fall									
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
WMARSS 9/16	1.36	Std. 40 9/16	4.69	I.E. 9/16	5.12	Sep+Wet 9/16	5.44	Septic 9/16	5.69
WMARSS 9/19	5.71	Std. 40 9/19	5.14	I.E. 9/19	6.13	Sep+Wet 9/19	4.9	Septic 9/19	6.65
WMARSS 9/23	1.56	Std. 40 9/23	6.08	I.E. 9/23	6.64	Sep+Wet 9/23	6.91	Septic 9/23	7.78
WMARSS 10/10	0.62	Std. 40 10/10	4.56	I.E. 10/10	3.33	Sep+Wet 10/10	3.0	Septic 10/10	4.81
WMARSS 10/14	2.34	Std. 40 10/14	4.06	I.E. 10/14	3.55	Sep+Wet 10/14	7.31	Septic 10/14	4.68
WMARSS 10/17	8.64	Std. 40 10/17	4.61	I.E. 10/17	4.51	Sep+Wet 10/17	6.13	Septic 10/17	5.64
WMARSS 11/2	0.67	Std. 40 11/2	5.52	I.E. 11/2	0.04	Sep+Wet 11/2	6.51	Septic 11/2	6.41
WMARSS 11/4	0.31	Std. 40 11/4	5.22	I.E. 11/4	2.96	Sep+Wet 11/4	6.23	Septic 11/4	5.49
WMARSS 11/7	0.31	Std. 40 11/7	4.58	I.E. 11/7	7.11	Sep+Wet 11/7	5.29	Septic 11/7	5.37
Mean	2.39		4.94		4.38		5.75		5.84
Std. Dev.	2.72		0.57		2.08		1.22		0.92

Appendix U continued

Winter									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 12/12	0.42	Std. 40 12/12	6.34	I.E. 12/12	5.7	Sep+Wet 12/12	6.85	Septic 12/12	7.22
WMARSS 12/16	0.23	Std. 40 12/16	6.43	I.E. 12/16	4.22	Sep+Wet 12/16	6.6	Septic 12/16	6.6
WMARSS 12/19	0.27	Std. 40 12/19	5.16	I.E. 12/19	2.68	Sep+Wet 12/19	5.6	Septic 12/19	4.79
WMARSS 1/20	4.65	Std. 40 1/20	4.15	I.E. 1/20	5.4	Sep+Wet 1/20	3.59	Septic 1/20	6.45
WMARSS 1/23	1.24	Std. 40 1/23	7.33	I.E. 1/23	8.56	Sep+Wet 1/23	6.41	Septic 1/23	10.6
WMARSS 1/27	0.34	Std. 40 1/27	7.51	I.E. 1/27	4.7	Sep+Wet 1/27	7.64	Septic 1/27	7.64
WMARSS 1/30	0.24	Std. 40 1/30	4.94	I.E. 1/30	2.47	Sep+Wet 1/30	7.0	Septic 1/30	6.18
WMARSS 2/2	0.23	Std. 40 2/3	4.29	I.E. 2/3	4.86	Sep+Wet 2/1	6.85	Septic 2/3	5.95
WMARSS 2/6	0.39	Std. 40 2/6	4.4	I.E. 2/6	5.4	Sep+Wet 2/3	6.28	Septic 2/6	5.74
						Sep+Wet 2/6	5.81		
Mean	0.89		5.62		4.89		6.26		6.80
Std. Dev.	1.36		1.24		1.70		1.05		1.55

## Appendix V

### WMARSS and BWRP PO<sub>4</sub> Measurements

Fall									
Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L	Effluent	mg/L
WMARSS 9/16	0.86	Std. 40 9/16	3.62	I.E. 9/16	4.02	Sep+Wet 9/16	3.45	Septic 9/16	4.49
WMARSS 9/19	4.25	Std. 40 9/19	3.66	I.E. 9/19	4.38	Sep+Wet 9/19	2.3	Septic 9/19	4.3
WMARSS 9/23	1.12	Std. 40 9/23	3.66	I.E. 9/23	4.68	Sep+Wet 9/23	4.05	Septic 9/23	3.75
WMARSS 10/10	0.22	Std. 40 10/10	3	I.E. 10/10	2.03	Sep+Wet 10/10	1.52	Septic 10/10	2.84
WMARSS 10/14	0.02	Std. 40 10/14	2.05	I.E. 10/14	2.1	Sep+Wet 10/14	0.02	Septic 10/14	0.02
WMARSS 10/17	0.03	Std. 40 10/17	0.08	I.E. 10/17	0.06	Sep+Wet 10/17	0.22	Septic 10/17	0.08
WMARSS 11/2	0.49	Std. 40 11/2	3.85	I.E. 11/2	2.39	Sep+Wet 11/2	4.27	Septic 11/2	4.27
WMARSS 11/4	0.21	Std. 40 11/4	3.98	I.E. 11/4	2.31	Sep+Wet 11/4	2.75	Septic 11/4	2.75
WMARSS 11/7	0.21	Std. 40 11/7	3.45	I.E. 11/7	5.19	Sep+Wet 11/7	3.87	Septic 11/7	3.87
Mean	0.82		3.04		3.02		2.50		2.93
Std. Dev.	1.26		1.18		1.56		1.52		1.64

Appendix V continued

Winter									
Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l	Effluent	mg/l
WMARSS 12/12	0.26	Std. 40 12/12	4.02	I.E. 12/12	3.22	Sep+Wet 12/12	3.53	Septic 12/12	4.26
WMARSS 12/16	0.15	Std. 40 12/16	3.38	I.E. 12/16	3.18	Sep+Wet 12/16	3.4	Septic 12/16	4.26
WMARSS 12/19	0.17	Std. 40 12/19	2.28	I.E. 12/19	1.73	Sep+Wet 12/19	2.82	Septic 12/19	3.04
WMARSS 1/20	3.44	Std. 40 1/20	2.64	I.E. 1/20	3.92	Sep+Wet 1/20	2.56	Septic 1/20	3.66
WMARSS 1/23	0.95	Std. 40 1/23	5.5	I.E. 1/23	6.6	Sep+Wet 1/23	5.02	Septic 1/23	7.36
WMARSS 1/27	0.15	Std. 40 1/27	5.12	I.E. 1/27	3.34	Sep+Wet 1/27	4.36	Septic 1/27	5.28
WMARSS 1/30	0.29	Std. 40 1/30	3.62	I.E. 1/30	1.84	Sep+Wet 1/30	5.48	Septic 1/30	4.46
WMARSS 2/2	0.15	Std. 40 2/3	2.72	I.E. 2/3	3.12	Sep+Wet 2/1	4.46	Septic 2/3	3.28
WMARSS 2/6	0.35	Std. 40 2/6	2.88	I.E. 2/6	5.06	Sep+Wet 2/3	4.34	Septic 2/6	5.02
						Sep+Wet 2/6	3.85		
Mean	0.65		3.57		3.56		3.98		4.51
Std. Dev.	1.01		1.06		1.43		0.88		1.22

# Appendix W

## WMARSS and BWRP *Daphnia magna* Survival

		Fall				Winter			
		<i>Test 1</i>	<i>Test 2</i>	<i>Test 3</i>	<i>Average</i>	<i>Test 1</i>	<i>Test 2</i>	<i>Test 3</i>	<i>Average</i>
Control		1	1	1	1	1	1	1	1
WMARSS	100%	1	1	1	1	1	1	1	1
	50%	1	1	1	1	1	0.8	1	0.93
	25%	1	1	1	1	1	1	1	1
Std. 40	100%	1	1	0.8	0.93	1	1	1	1
	50%	1	0.8	0.8	0.87	1	1	1	1
	25%	1	1	0.8	0.93	1	1	1	1
I.E.	100%	1	1	1	1	1	1	1	1
	50%	1	1	0.8	0.93	1	1	1	1
	25%	1	1	1	1	1	1	1	1
Septic+Wetland	100%	1	1	0.8	0.93	1	1	1	1
	50%	1	1	1	1	1	1	1	1
	25%	1	1	1	1	1	1	1	1
Septic	100%	1	0	0	0.33	0.8	1	0.6	0.80
	50%	1	1	1	1	0.8	1	1	0.93
	25%	1	1	1	1	1	1	1	1

## Appendix X

### WMARSS and BWRP *Daphnia magna* Reproduction

		Fall				Winter			
		<i>Test 1</i>	<i>Test 2</i>	<i>Test 3</i>	<i>Average</i>	<i>Test 1</i>	<i>Test 2</i>	<i>Test 3</i>	<i>Average</i>
Control		34.3	45.5	36.2	38.7	19.3	13.8	25.8	19.6
WMARSS	100%	26.4	47.6	28.6	34.2	39	31.2	44	38.1
	50%	25.8	46.6	40	37.5	32.4	30.2	37.2	33.3
	25%	32	29.8	43.2	35.0	26.8	21.8	47.8	32.1
Std. 40	100%	45.6	51.8	12	36.5	48.8	45.4	53	49.1
	50%	33.6	38.6	27.2	33.1	42.8	46.4	44.8	44.7
	25%	31.6	39.6	24.6	31.9	40.4	26	46.8	37.7
I.E.	100%	23.6	51.6	23.8	33.0	30.8	48.2	51	43.3
	50%	29.8	40.8	25.6	32.1	28	47.2	53	42.7
	25%	25.6	36	30.2	30.6	27.6	44.4	48.6	40.2
Septic+Wetland	100%	5.6	31.4	2	13.0	33.4	43.4	43.8	40.2
	50%	30.4	40	1.2	23.9	28.4	56.2	51	45.2
	25%	31	54.6	5	30.2	46.4	53.4	44.2	48.0
Septic	100%	13.6	0	0	4.5	11.8	59.8	21.8	31.1
	50%	42.8	51	17.8	37.2	32.6	55.6	65.6	51.3
	25%	55.2	41.4	3.2	33.3	50.2	59.2	61.8	57.1

## BIBLIOGRAPHY

- American Public Health Association, American Water Works Association, Water Environment Foundation. "Standard Methods for the Examination of Water and Wastewater." 20th ed. American Public Health Association, Washington, DC, USA (1998).
- Baker, Michael J., David W. Blowes, and Carol J. Ptacek. "Laboratory Development of Permeable Reactive Mixtures for the Removal of Phosphorous from Onsite Wastewater Disposal Systems." *Environ. Sci. and Technol.* 32, no. 15 (1998): 2308-2316.
- Barata, C., P. Alañon, S. Gutierrez-Alonso, M.C. Riva, C. Fernández, and J.V. Tarazona. "A *Daphnia magna* feeding bioassay as a cost effective and ecological relevant sublethal toxicity test for environmental risk assessment of toxic effluents." *Science of the Total Environment* 405 (2008): 78-86.
- Cech, Thomas V. *Principles of Water Resources*. 3<sup>rd</sup> ed. Hoboken: John Wiley & Sons, 2010.
- Chapman, Peter M. "Annual Review: Whole Effluent Toxicity Testing-Usefulness, Level of Protection, and Risk Assessment." *Environmental Toxicology and Chemistry* 19, no. 1 (2000): 3-13.
- De-Bashan, Luz E. and Yoav Bashan. "Recent advances in removing phosphorous from wastewater and its future use as fertilizer (1997-2003)." *Water Research* 38(2004): 4222-4246.
- Dzialowski, E.M., P.K. Turner, and B.W. Brooks. "Physiological and reproductive effects of beta adrenergic receptor antagonists in *Daphnia magna*." *Arch. Environ. Contam. Toxicol.* 50(2006):503-510.
- Infante, Aida and Arni H. Litt. "Differences between two species of *Daphnia* in the use of 10 species of algae in Lake Washington." *Limnol. Oceanogr.* 30, no. 5 (1985): 1053-1059.
- Kadlec, Robert H. and Robert L. Knight. *Treatment Wetlands*. Boca Raton: CRC Press, 1996.
- La Point, Thomas W. and William T. Waller. "Annual Review: Field Assessments in Conjunction with Whole Effluent Toxicity Testing." *Environmental Toxicology and Chemistry* 19 (2000): 14-24.



- Mitsch, William J. and James G. Gosselink. *Wetlands*. Hoboken: John Wiley & Sons, 2007.
- NSF International Standard/American National Standard. *Residential Wastewater Treatment Systems*. Ann Arbor: NSF International, 2010.
- NSF International Standard/American National Standard. Residential wastewater treatment systems. NSF/ANSI 40, 2010.
- Polis, Gary A. and Donald R. Strong. "Food Web Complexity and Community Dynamics." *The American Naturalist* 147, no. 5 (May 1996): 813-846.
- Ra, Jin Sung, Byoung Cheun Lee, Nam Ik Chang, and Sang Don Kim. "Comparative Whole Effluent Toxicity Assessment of Wastewater Treatment Plant Effluents using *Daphnia magna*." *Bull Environ Contam Toxicol* 80 (2008): 196-200.
- Ra, Jin Sung, Hyun Koo Kim, Nam Ik Chang, and Sang Don Kim. "Whole Effluent Toxicity (WET) Tests on Wastewater Treatment Plants with *Daphnia magna* and *Selenastrum capricornutum*." *Environ Monit Assess* 129 (2007): 107-113.
- Reddy, K.R., O.A. Diaz, L.J. Scinto, and M. Agami. "Phosphorous dynamics in selected wetlands and streams of the lake Okeechobee Basin." *Ecological Engineering* 5 (1995): 183-207.
- Rodriguez, Pilar, Maite Martinez-Madrid, and Adolfo Cid. "Ecotoxicological Assessment of Effluents in the Basque Country (Northern Spain) by Acute and Chronic Toxicity Tests Using *Daphnia magna* straus." *Ecotoxicology* 15 (2006): 559-572.
- Schlesinger, William H. *Biogeochemistry: An Analysis of Global Change*. San Diego: Academic Press, 1997.
- Smil, Vaclav. "Phosphorous in the environment: natural flows and human interferences." *Annu. Rev. Energy Environ.* 30 (2000): 53-88.
- Stanley, Jacob K., Alejandro J. Ramirez, C. Kevin Chambliss and Bryan W. Brooks. "Enantiospecific Sublethal Effects of the Antidepressant Fluoxetine to a Model Aquatic Vertebrate and Invertebrate." *Chemosphere* 69, no. 1 (2007): 9-16.
- Stilling, Peter and Anthony M. Rossi. "Experimental Manipulations of Top-Down and Bottom-Up Factors in a Tri-Trophic System." *Ecology* 78, no. 5 (1997): 1602-1606.
- Suter III, Glenn W. *Ecological Risk Assessment*. 2<sup>nd</sup> ed. Boca Raton: CRC Press, 2007.
- Texas Commission on Environmental Quality. Changes in On-Site Wastewater Treatment and Evaluation: Influent Equalization. Contract No. 582-11-99565. 2011

- Texas Commission on Environmental Quality. Permit to discharge wastes. TPDES Permit no. WQ0011071001, 2006.
- Texas Commission on Environmental Quality. Permit to discharge wastes. TPDES Permit no. WQ0011408002, 2009.
- Thind, H. S., D. L. Rowell. "Effects of Algae and Fertilizer-nitrogen on pH, Eh, and Depth of Aerobic Soil in Laboratory Columns of a Flooded Sandy Loam." *Biol. Fertil. Soils*. 28 (1999): 162-168.
- U.S. Environmental Protection Agency. *Chapter 1: Background and use of onsite wastewater treatment systems*. US EPA 600/R-00/008, 2000.
- U.S. Environmental Protection Agency. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*. 2nd Ed. US EPA, Office of Water, 1994.
- Veen, Eleanor Van, Nicola Burton, Sean Comber, and Michael Gardner. "Speciation of Copper in Sewage Effluents and its Toxicity to *Daphnia magna*." *Environ. Sci. and Technol.* 21, no. 2 (2002): 275-280.
- Zang, Changjuan, Suiliang Huang, Min Wu, Shenglan Du, Miklas Scholz, Feng Gao, Chao Lin, Yong Guo, and Yu Dong. "Comparison of Relationships Between pH, Dissolved Oxygen, and Chlorophyll a for Aquaculture and Non-aquaculture Waters." *Water Air Soil Pollut.* 219 (2011): 157-174.
- Zar, Jerrold H. *Biostatistical Analysis*. 4<sup>th</sup> ed. Upper Saddle River: Prentice Hall, 1999.